

**Drug Master File of Finerenone**

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

# **Drug Master File of Finerenone**

**DMF-001**

Signature/Stamp:

Date:

Place: Changsha City, China

**HinYe Pharmaceutical Co., Ltd.**

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## **3.2.S.1 General Information**

### **3.2.S.1.1 Nomenclature**

#### **Recommended International Nonproprietary Name (INN)**

Finerenone

#### **European Pharmacopoeia monograph name**

N/A

#### **United States Adopted Name (USAN)**

Finerenone

#### **Chemical name**

(4S)-4-(4-cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthyridine-3-carboxamide

#### **Company code**

YA2304

#### **Chemical Abstracts Service (CAS) registry number**

1050477-31-0

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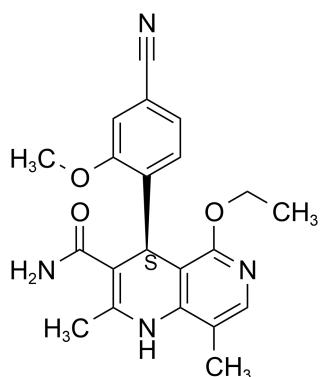
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### 3.2.S.1.2 Structure

#### Structure formula



#### Molecular formula

C<sub>21</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>

#### Relative molecular mass (M<sub>r</sub>)

378.43

#### Stereochemistry

The molecule of Finerenone contains one chiral centers at C-9. The active substance has absolute configuration (S) for the C-9 positions.

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### **3.2.S.1.3 General Properties**

<b>Items</b>	<b>Characteristics or properties</b>
Appearance	White to yellow powder
Melting point	Approximately 252 °C
Solubility	Dissolved in methanol, slightly soluble in ethanol, acetonitrile, and acetone, slightly soluble in isopropanol, almost insoluble in water
Hygroscopicity	It is non-hygrosopic.
hydrate	This product does not contain crystal water.
Dissociation constant [(pKa)]	4.39
partition coefficient	Log D (1-Octanol/buffer solution pH 2.4) =0.4 Log D (1-Octanol/buffer solution pH 7.4) =2.8
BCS classification	Class II

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### **3.2.S.2 Manufacture**

#### **3.2.S.2.1 Manufacturer**

##### **Manufacturer**

Name: Hinye Pharmaceutical Co., Ltd.

DUNS Number: 526215965

Address: No.109, Kangtian Road, Changsha National Biological Industrial Base, Hunan Province, China

Telephone Number: +86-731-82728186

Fax Number: +86-731-82728064

Post Code: 410331

##### **Manufacturing Site**

Name: Hinye Pharmaceutical Co., Ltd.

DUNS Number: 526215965

Address: No.109, Kangtian Road, Changsha National Biological Industrial Base, Hunan Province, China

Telephone Number: +86-731-82728186

Fax Number: +86-731-82728064

Post Code: 410331

##### **Responsibility of manufacturer**

Hinye Pharmaceutical Co., Ltd. has the full responsibility of the manufacture, packaging and testing of Finerenone.

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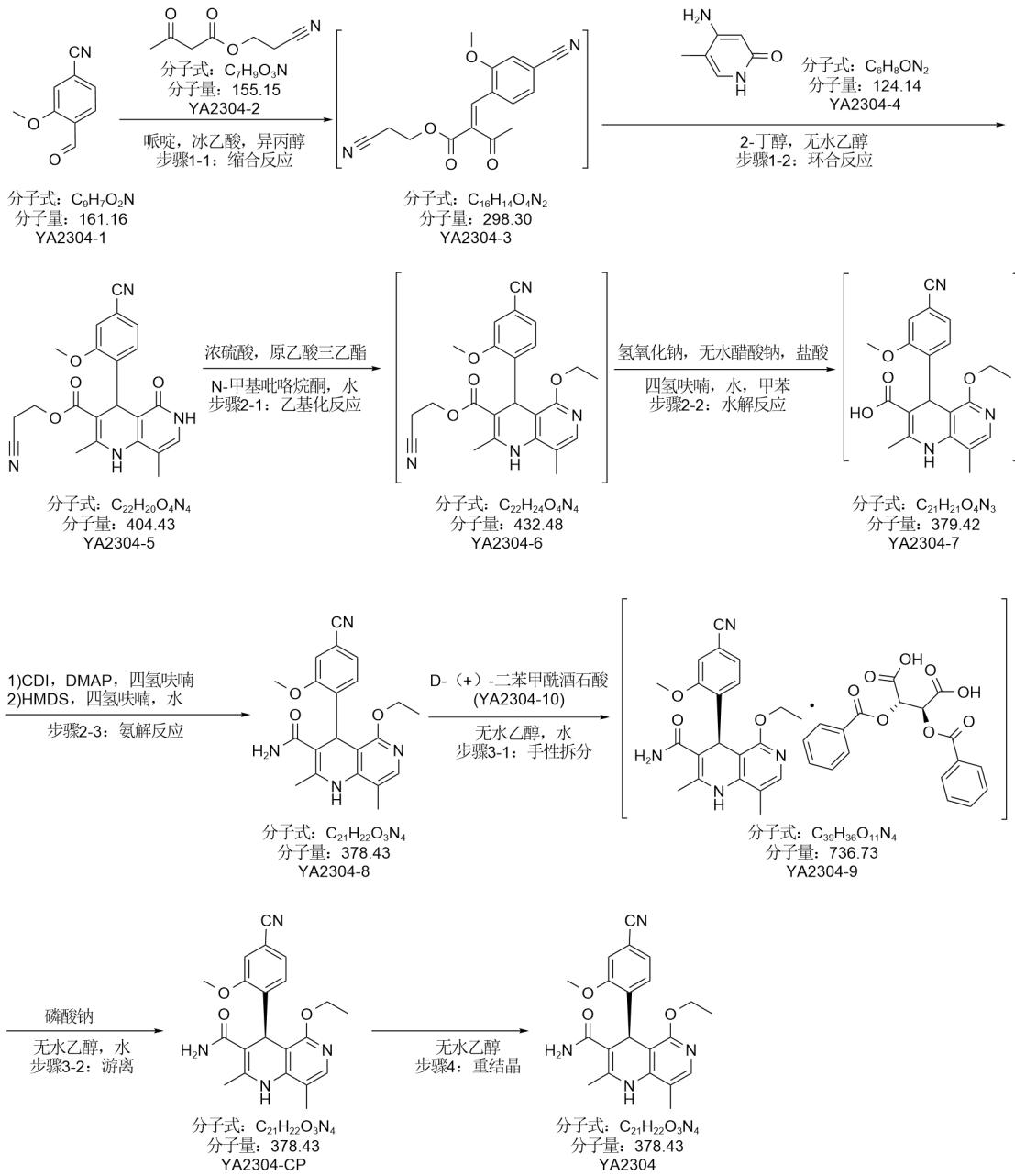
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### 3.2.S.2.2 Description of Manufacturing Process and Process Controls

#### 3.2.S.2.2.1 Synthetic Process of the Manufacturing Process



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<b>Materia l Code</b>	<b>Chemical Name</b>	<b>CAS No.</b>
CDI	N. N-carbonyldiimidazole	530-62-1
DMAP	4-Dimethylaminopyridine	1122-58-3
HMDS	Hexamethyldisilazane	999-97-3
YA2304-1	4-cyano-2-methoxybenzaldehyde	21962-45-8
YA2304-2	Ethyl 2-cyanoacetoacetate	65193-87-5
YA2304-4	4-amino-5-methyl-2-hydroxypyridine	95306-64-2
YA2304-10	D - (+) - Dibenzoyl Tartaric Acid	17026-42-5
YA2304-3	2-cyanoethyl 2- (4-cyano-2-methoxybenzylidene)-3-oxobutyric acid ester	1050477-3 9-8
YA2304-5	4- (4-cyano-2-methoxyphenyl)-2,8-dimethyl-5-oxo-1,4,5,6-tetrahydro-1,6-naphthalene-3-carboxylic acid 2-cyanoethyl ester	1050477-4 3-4
YA2304-6	4- (4-cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthalene-3-carboxylic acid 2-cyanoethyl ester	1050477-4 4-5
YA2304-7	4- (4-cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthalene-3-carboxylic acid	1050477-4 5-6
YA2304-8	4- (4-cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthalene-3-carboxamide	1050477-2 7-4
YA2304-9	Non maleketone D - (+) - dibenzoyl tartrate	N/A
YA2304-CP	(4S) -4- (4-cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthalene-3-carboxamide	1050477-3 1-0
YA2304	(4S) -4- (4-cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthalene-3-carboxamide	1050477-3 1-0

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### 3.2.S.2.3 Control of Materials

#### 3.2.S.2.3.1 Materiased in the Manufacturing of the Drug Substance

Table 3.2.S.2.3-1 Materials Used in the Manufacturing of the Drug Substance

Materials	Name	Grade
Starting materials	4-cyano-2-methoxybenzaldehyde (YA2304-1)	Industrial chemicals
	Ethyl 2-cyanoacetoacetate (YA2304-2)	Industrial chemicals
	4-amino-5-methyl-2-hydroxypyridine (YA2304-4)	Industrial chemicals
Reagents	piperidine	Industrial chemicals
	Glacial acetic Acid	Industrial chemicals
	Concentrated sulfuric acid	Industrial chemicals
	Triethyl orthoacetate	Industrial chemicals
	Sodium Hydroxide	Industrial chemicals
	Anhydrous sodium acetate	Industrial chemicals
	Concentrated hydrochloric Acid	Industrial chemicals
	N, N-carbonyl diimidazole	Industrial chemicals
	4-dimethylaminopyridine	Industrial chemicals
	Hexamethyldisilazane	Industrial chemicals
Solvents	D-(+)-dibenzoyltartaric acid	Industrial chemicals
	Sodium phosphate	Industrial chemicals
	Isopropyl alcohol	Industrial chemicals
	2-butanol	Industrial chemicals
	N-methylpyrrolidone	Industrial chemicals
	tetrahydrofuran	Industrial chemicals
	Toluene	Industrial chemicals
	Anhydrous ethanol	Industrial chemicals

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### **3.2.S.2.6 Manufacturing Process Development**

Hinye Pharmaceutical Co., Ltd. began to develop Finerenone on the basis of publicly available patent. Three consecutive commercial-scale validation batches were manufactured in Dec. 2023 to Feb. 2024.

Based on the literature (Authorization Notice No. CN101641352A), The patent describes that Starting from 4-cyano-2-methoxybenzaldehyde, it undergoes a Krebs condensation reaction with ethyl 2-cyanoacetoacetate, followed by a cyclization reaction with 4-amino-5-methyl-2-hydroxypyridine. The racemate is then obtained through ethylation, hydrolysis, and amidation reactions, and finally separated and transformed into the product.

This route involves 5 chemical reaction steps, 1 splitting step, and 1 crystallization step, with moderate reaction steps and easy availability of raw materials. Compared with other synthetic routes, it avoids the use of expensive catalysts, has relatively high yields, is easy to operate, and is suitable for commercial production. Therefore, the applicant develops and improves the process based on this route, and proposes this route as the declared process route.

The preparation of this product is divided into 7 steps: in the first step, YA2304-1 and YA2304-2 are used as raw materials, and pyridine and acetic acid are used as catalysts to prepare YA2304-3 through condensation reaction; The second step is to prepare intermediate YA2304-5 by cyclization reaction between YA2304-3 and raw material YA2304-4. In the third step, intermediate YA2304-5 is catalyzed by concentrated sulfuric acid to undergo ethylation reaction with triethyl acetate to obtain YA2304-6; Step four, YA2304-6 is hydrolyzed under the action of sodium hydroxide to obtain YA2304-7; The fifth step is to activate YA2304-7 with CDI and prepare intermediate YA2304-8 by ammonification reaction with Finerenone.

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hexamethyldisilazane. Step 6: Intermediate YA2304-8 and YA2304-10 are chiral separated to obtain YA2304-9; Step seven, YA2304-9 was prepared using sodium phosphate to obtain intermediate YA2304-CP. YA2304 was prepared by recrystallization of YA2304-CP with anhydrous ethanol.

In Apr. Dec. 2023 to Feb. 2024, the manufacturing process of Finerenone had been successfully validated by running three consecutive commercial-scale batches (Batch No.: 231201, 240101, 240102). The batch size of Commercial scale batches and the validation batches were 10 kg/batch. The manufacturing site and process of Commercial scale batches and the validation batches were same. Refers to 3.2.S.4.4, the analyses results show that the Commercial scale batches and the validation batches Finerenone have the same quality.

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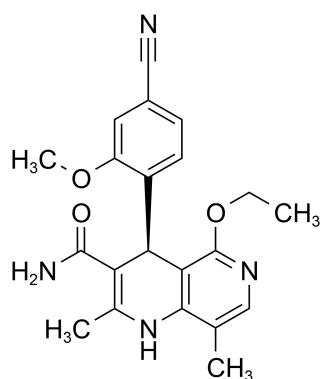
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### 3.2.S.3 Characterization

#### 3.2.S.3.1 Elucidation of Structure and other Characteristics

##### I. Evidences of Chemical Structure

The drug substance described in the present dossier is Finerenone which has the following structure formula:



Molecular formula: C<sub>21</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>

Molecular weight: 378.43 g/mol

Chemical name: (4S)-4-(4-cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthyridine-3-carboxamide

The applicant conducted Elemental analysis, <sup>1</sup>H nuclear magnetic resonance spectroscopy, <sup>13</sup>C nuclear magnetic resonance spectroscopy, 2D nuclear magnetic resonance, infrared spectroscopy, mass spectrometry, single crystal X-ray powder diffraction, thermogravimetric analysis, differential scanning calorimetry, and X-ray powder diffraction on the sample of Finerenone (batch No. 231101) prepared in commercial batches according to the registration process described in 3.2.S.2.2, and confirmed the chemical structure of the product as shown in Table 3.2.S.3.1-1.

The applicant conducted physical and chemical property analysis such as Raman spectroscopy, differential thermal analysis, and thermogravimetric analysis on commercially produced samples with batch No. 231101, confirming that there is no crystallization water or crystallization solvent in the homemade Finerenone, and that the crystal form of this product is

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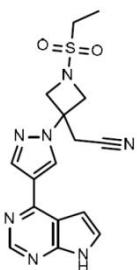
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consistent with the crystal form of the active pharmaceutical ingredient used in the original manufacturer's formulation.

Table 3.2.S.3.1-1 Sample Information for Structure Elucidation

Structure	Sample information
 Molecular formula: C <sub>21</sub> H <sub>22</sub> N <sub>4</sub> O <sub>3</sub> Molecular weight: 378.43 g/mol	BatchNo.: 231101 Assay: 100.1% Source of sample: Hinye Pharmaceutical Co., Ltd. Test items: LC-MS/MS, IR, NMR, XRD, TGA and DSC.

The following investigations were carried out to confirm the chemical structure:

- a. liquid chromatography tandem mass spectrometry (LC-MS/MS)
- b. Infrared Absorption Spectroscopy (IR)
- c. Nuclear Magnetic Resonance Spectroscopy (NMR)
- d. Thermogravimetric Analysis (TGA)
- e. Differential Scanning Calorimetry (DSC)
- f. X-ray Powder Diffraction (XRD)
- g. Elemental Analysis
- h. single crystal x-ray diffraction (single crystal XRD)

Details and data concerning these analyses or tests are presented as follows.

### Ia. liquid chromatography tandem mass spectrometry (LC-MS/MS)

Sample Batch No.: 231101

Instrument: Vanquish-TSQ QUANTIS liquid chromatography-mass spectrometry

Test method: China Pharmacopoeia 2020 Edition four General Rules 0512

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and 0431.

Test result:

Table 3.2.S.3.1-2 Mass Spectrum Data of the Substance to be Examined

Batch No.	Accurate measured mass value	Theoretical value	Elemental composition
231101	379.18	378.43	[C <sub>21</sub> H <sub>22</sub> N <sub>4</sub> O <sub>3</sub> +H] <sup>+</sup>

Interpretation:

The molecular weight of Finerenone is 378.43. In the positive ion mode, the mass-charge ratio of 379.18 peak is detected, which is attributed to the molecular ion peak in the positive ion mode, which is consistent with the molecular weight of Finerenone.

The spectrum is provided in: 3.2.S.3.1 Annex 1 *Mass spectrum of Finerenone (batch No.: 231101)*

### Ib. Infrared Absorption Spectroscopy (IR)

Sample Batch No.: 231101

Instrument: Shimadzu IR Affinity-1s Fourier Transform Infrared Spectrometer

Sample preparation: KBr pellet pressing method

Table 3.2.S.3.1-3 Infrared Spectrum Data of the Substance to be Examined

Absorption peak posit ion (cm <sup>-1</sup> )	Type of vibr ation	Possible Gro ups	Remarks
3475.73	$\nu_{\text{NH}_2}$	-CONH <sub>2</sub>	Asymmetric stretching vibr ation of nitrogen and hydr ogen
3415.93	$\nu_{\text{NH}}$	-CONH <sub>2</sub>	Nitrogen hydrogen symmetr ic stretching vibration
3365.78	$\nu_{\text{NH}}$	-NH	Nitrogen hydrogen stretchi ng vibration
3115.04, 3079.53	$\nu_{\text{CH}}$	Pyridine rin g, benzene r ing	Carbon hydrogen stretchin g vibration

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2974.23, 2953.02, 28 35.36	$\nu_{\text{CH}}$	-CH <sub>3</sub> , -CH <sub>2</sub> , -C H	Carbon hydrogen stretchin g vibration
2229.71	$\nu_{\text{C}=\text{N}}$	-CN	Carbon nitrogen triple bon d stretching vibration
1683.86	$\nu_{\text{C}=\text{O}}$	-CONH <sub>2</sub>	Carbon oxygen double bon d stretching vibration
1660.71	$\nu_{\text{C}=\text{C}}$	-C=C	Carbon carbon double bon d stretching vibration
1606.70, 1573.91, 14 89.05	$\nu_{\text{C}=\text{N}}, \nu_{\text{C}=\text{C}}$	Pyridine rin g, benzene r ing	Expansion and contraction vibrations of carbon nitro gen and carbon carbon do uble bonds
1463.97	$\delta_{\text{CH}}$	-CH <sub>2</sub>	In-plane shear vibration
1454.33, 1431.18, 14 08.04, 1381.03	$\delta_{\text{CH}}$	-CH <sub>3</sub>	Out of plane deformation v ibration
1267.23, 1257.59	$\nu_{\text{C}-\text{O}-\text{C}}$	-OCH <sub>3</sub> , -OCH <sub>2</sub>	Asymmetric stretching vibr ation of ether bond
1138.00, 1031.92	$\nu_{\text{C}-\text{O}-\text{C}}$	-OCH <sub>3</sub> , -OCH <sub>2</sub>	Symmetric stretching vibrat ion of ether bond

### Analysis and results:

IR  $\nu$  cm<sup>-1</sup> (KBr) : 3475.73cm<sup>-1</sup>, 3415.93cm<sup>-1</sup> are the stretching vibration peaks of the primary amide group, 1683.86cm<sup>-1</sup> are the stretching vibration peaks of the carbonyl group in the amide, indicating that the compound contains an amide structure. 3365.78cm<sup>-1</sup> is the nitrogen and hydrogen expansion vibration of secondary amine, indicating that the compound contains a secondary amine structure. 3115.04cm<sup>-1</sup> and 3079.53cm<sup>-1</sup> are the hydrocarbon stretching vibration of pyridine ring and benzene ring, and 1606.70cm<sup>-1</sup>, 1573.91cm<sup>-1</sup> and 1489.05cm<sup>-1</sup> are the skeleton vibration of pyridine ring and benzene ring, indicating that the compound contains pyridine ring and benzene ring. 2229.71cm<sup>-1</sup> is cyanogroup stretching vibration, indicating that the compound contains a cyanogroup. 2974.23cm<sup>-1</sup>, 2953.02cm<sup>-1</sup>, 2835.36cm<sup>-1</sup> and 2964.59cm<sup>-1</sup> are the hydrocarbon stretching vibrations of multiple methyl and methylene groups, and 1463.97cm<sup>-1</sup>, 1454.33cm<sup>-1</sup>, 1431.18, 1408.04 and 1381.03 are bending vibrations.

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Indicating that the compound contains methylene and multiple methyl groups.  $1267.23\text{cm}^{-1}$  and  $1257.59\text{cm}^{-1}$  are asymmetric stretching vibrations of alkyl aromatic ether bonds, and  $1138.00\text{cm}^{-1}$  and  $1031.92\text{cm}^{-1}$  are symmetric stretching vibrations of ether bonds, indicating that the compound contains two alkyl aromatic ether bonds. According to the above IR data, the compound contains amide bond, secondary amine, pyridine ring, phenyl ring, carbon-carbon double bond, alkyl aromatic ether bond, methylene and several methyl groups, which is consistent with the structure of Finerenone.

The spectrum is provided in:

*3.2.S.3.1 Annex 2 Infrared (IR) spectrum of Finerenone. (batch No.: 231101).*

### Ic. Nuclear Magnetic Resonance Spectroscopy (NMR)

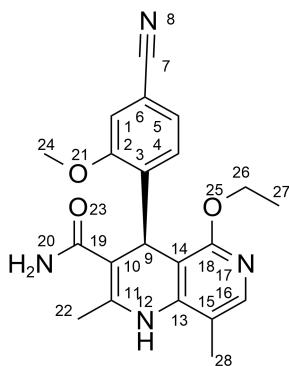
Sample Batch No.: 231101

Instrument: Bruker Avance AV 400 Superconducting Fourier-Transform NMR Spectrometer

Test conditions:

- solvent: DMSO- $d_6$
- inner standard: TMS ( $^1\text{H}$  NMR)
- resonant frequency for the  $^1\text{H}$  NMR: 400MHz
- resonant frequency for the  $^{13}\text{C}$  NMR: 100MHz

The serial number of carbon atoms and hydrogen atoms in the structure (not IUPAC nomenclature, only for NMR spectrometry):



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Table 3.2.S.3.1-4  $^1\text{H}$  NMR Spectrum Data of the Substance to be Examined

Peak No.	Chemical shift (ppm)	Multiplicity	Number of protons	Attributed to
1	1.029-1.064	t	3H	27-CH <sub>3</sub>
2	2.118	s	3H	28-CH <sub>3</sub>
3	2.190	s	3H	22-CH <sub>3</sub>
4	3.824	s	3H	24-CH <sub>3</sub>
5	3.986-4.030	m	2H	26-CH <sub>2</sub>
6	5.384	s	1H	9-CH
7	6.714-6.804	bs	2H	20-NH <sub>2</sub>
8	7.144-7.163	d	1H	4-CH
9	7.274-7.293	d	1H	5-CH
10	7.376	s	1H	1-CH
11	7.551	s	1H	16-CH
12	7.711	s	1H	12-NH

According to H-H COSY, DEPT135, DEPT90,  $^{13}\text{C}$ - $^1\text{H}$  HSQC $^{13}\text{C}$ ,  $^1\text{H}$  HMBC spectrum, the hydrogen spectrum of the tested sample is assigned as follows:

On  $^1\text{H}$  NMR spectra,  $\delta_{\text{H}} 2.500$  is the DMSO- $d_6$  solvent peak,  $\delta_{\text{H}} 3.383$  ppm is the water peak, both of which are the solvent peaks in deuterium reagents.

Peak 1:  $\delta_{\text{H}}$  1.029-1.064, 3H, t, 6.8Hz, CH<sub>3</sub>, should be coupled with methylene. COSY shows correlation with  $\delta_{\text{H}} 3.986$  (26-CH<sub>2</sub>), HSQC shows correlation with  $\delta_{\text{C}} 14.353$  (27-C). It can be determined as 27-CH<sub>3</sub>.

Peak 2:  $\delta_{\text{H}} 2.118$ , 3H, t, is CH<sub>3</sub>. COSY shows a weak correlation with  $\delta_{\text{H}} 7.551$  (16-CH), HSQC shows a strong correlation with  $\delta_{\text{C}} 13.846$ , HMBC shows a strong correlation with  $\delta_{\text{C}} 111.538$ ,  $\delta_{\text{C}} 144.271$  (16-CH) and a weak correlation with  $\delta_{\text{C}} 103.245$ ,  $\delta_{\text{C}} 159.464$ . It can be determined as 28-CH<sub>3</sub>.

Peak 3:  $\delta_{\text{H}} 2.190$ , 3H, t, is CH<sub>3</sub>. COSY shows a weak correlation with  $\delta_{\text{H}} 5.384$  (9-CH), HSQC shows a weak correlation with  $\delta_{\text{C}} 18.146$ , HMBC shows a strong correlation with  $\delta_{\text{C}} 105.399$ ,  $\delta_{\text{C}} 138.229$  and a weak correlation with  $\delta_{\text{C}} 169.843$ , and a very weak correlation with  $\delta_{\text{C}} 32.442$  (9-CH). It can be determined as 22-CH<sub>3</sub>.

Peak 4:  $\delta_{\text{H}} 3.824$ , 3H, s, is CH<sub>3</sub>, HSQC shows a correlation with  $\delta_{\text{C}} 56.098$ , HMBC shows a correlation with  $\delta_{\text{C}} 155.720$  (3-C) and a weak correlation with  $\delta_{\text{C}} 114.63$ , can be determined as 24-CH<sub>3</sub>.

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Peak 5: δ<sub>H</sub> 63.986-4.030, 2H, m, 6.8Hz, CH, coincidences with δ<sub>H</sub> 1.029 (27-CH<sub>3</sub>). COSY shows a correlation with δ<sub>H</sub> 1.029 (27-CH<sub>3</sub>), HSQC shows a correlation with δ<sub>C</sub> 60.624, and HMBC shows a strong correlation with δ<sub>C</sub> 14.353 and δ<sub>C</sub> 159.464(18-C), which can be determined as 26-CH<sub>2</sub>.

Peak 6: δ<sub>H</sub> 5.384, 1H, s, COSY shows a weak correlation with δ<sub>H</sub> 2.190, HSQC shows a correlation with δ<sub>C</sub> 32.442, HMBC shows a correlation with δ<sub>C</sub> 103.245, δ<sub>C</sub> 105.399, δ<sub>C</sub> 130.989, δ<sub>C</sub> 138.229, δ<sub>C</sub> 141.761, δ<sub>C</sub> 144.271, δ<sub>C</sub> 155.720, δ<sub>C</sub> 159.464, δ<sub>C</sub> 169.843, which can be identified as 9-CH.

Peak 7: δ<sub>H</sub> 66.714-6.804, 2H, bs, HSQC shows no correlation peak and can be determined as 20-NH<sub>2</sub>.

Peak 8: δ<sub>H</sub> 7.144-7.163, 1H, d, 7.6Hz, COSY showed correlation with δ<sub>H</sub> 7.274, HSQC showed correlation with δ<sub>C</sub> 130.989, HMBC showed correlation with δ<sub>C</sub> 32.442, δ<sub>C</sub> 109.596, δ<sub>C</sub> 119.057, δ<sub>C</sub> 155.720 (3-C). So it can be determined as 4-CH.

Peak 9: δ<sub>H</sub> 7.274-7.293, 1H, d, 7.6Hz, COSY display is associated with δ<sub>H</sub> 7.144, HSQC display is associated with δ<sub>C</sub> 124.852, HMBC display is associated with δ<sub>C</sub> 114.182, δ<sub>C</sub> 119.057, δ<sub>C</sub> 141.761. Can be identified as 5-CH.

Peak 10: δ<sub>H</sub> 7.376, 1H, s, HSQC shows correlation with δ<sub>C</sub> 114.182, HMBC shows correlation with δ<sub>C</sub> 109.596, δ<sub>C</sub> 119.057, δ<sub>C</sub> 124.852, δ<sub>C</sub> 141.761, δ<sub>C</sub> 155.720. It can be determined as 1-CH.

Peak 11: δ<sub>H</sub> 7.551, 1H, s, HSQC shows correlation with δ<sub>C</sub> 144.271, HMBC shows correlation with δ<sub>C</sub> 159.464 (18-C) and can be determined as 16-CH.

Peak 12: δ<sub>H</sub> 7.711, s, 1H, HSQC shows no correlation peak and can be determined as 12-NH.

Based on the above analysis, all the 22 H signals in the structure of the tested sample are clearly attributed, which is consistent with its structure.

Table 3.2.S.3.1-5 <sup>13</sup>C NMR Spectrum Data of the Substance to be Examined

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Peak No.	Chemical shift (ppm)	Number of C atoms	Attributed to
1	13.846	1	28-C
2	14.353	1	27-C
3	18.146	1	22-C
4	32.442	1	9-C
5	56.098	1	24-C
6	60.624	1	26-C
7	103.245	1	14-C
8	105.399	1	10-C
9	109.596	1	2-C
10	111.538	1	15-C
11	114.182	1	1-C
12	119.057	1	6-C
13	124.852	1	5-C
14	130.989	1	4-C
15	138.229	1	11-C
16	141.761	1	7-C
17	144.271	1	13-C
18	144.271	1	16-C
19	155.720	1	3-C
20	159.464	1	18-C
21	169.843	1	19-C

According to DEPT135, DEPT90,  $^{13}\text{C}$ - $^1\text{H}$  HSQC,  $^{13}\text{C}$ - $^1\text{H}$  HMBC spectrum, the carbon spectrum of the tested sample is classified as follows:

638.905 ~ 40.121 ppm on the  $^{13}\text{C}$  NMR spectra are the multiple peaks of the solvent DMSO- $d_6$ .

Peak 1:  $\delta_{\text{C}} 13.846$ , DEPT135, DEPT90 spectrum shows first order carbon, HSQC shows direct correlation with  $\delta_{\text{H}} 2.118$  ( $28-\text{CH}_3$ ), HMBC shows remote correlation with  $\delta_{\text{H}} 7.551$  ( $16-\text{CH}$ ). It can be determined as 28-C.

Peak 2:  $\delta_{\text{C}} 14.353$ , DEPT135, DEPT90 spectrum shows first order carbon, HSQC shows direct correlation with  $\delta_{\text{H}} 1.029$  ( $27-\text{CH}_3$ ), HMBC shows remote correlation with  $\delta_{\text{H}} 3.986$  ( $26-\text{CH}_2$ ). It can be determined as 27-C.

Peak 3:  $\delta_{\text{C}} 18.146$ , DEPT135, DEPT90 spectrum shows first order carbon,

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HSQC shows direct correlation with  $\delta_{\text{H}} 2.190$  (22-CH<sub>3</sub>), HMBC shows remote correlation with  $\delta_{\text{H}} 7.711$  (12-NH). It can be determined as 22-C.

Peak 4:  $\delta_{\text{H}} 32.442$ , DEPT135, DEPT90 spectrum shows tertiary carbon, HSQC shows direct correlation with  $\delta_{\text{H}} 5.384$  (9-CH), HMBC shows remote correlation with  $\delta_{\text{H}} 7.144$  (4-CH),  $\delta_{\text{H}} 7.376$  (1-CH),  $\delta_{\text{H}} 2.190$  (22-CH<sub>3</sub>). It can be determined as 9-C.

Peak 5:  $\delta_{\text{H}} 56.098$ , DEPT135, DEPT90 spectrum shows first order carbon, HSQC shows direct correlation with  $\delta_{\text{H}} 3.824$  (24-CH<sub>3</sub>), HMBC shows no remote correlation. It can be determined as 24-C.

Peak 6:  $\delta_{\text{H}} 60.624$ , DEPT135, DEPT90 spectrum shows secondary carbon, HSQC shows direct correlation with  $\delta_{\text{H}} 3.986$  (26-CH<sub>2</sub>), HMBC shows remote correlation with  $\delta_{\text{H}} 1.029$  (27-CH<sub>3</sub>). It can be determined as 26-C.

Peak 7:  $\delta_{\text{H}} 103.245$ , DEPT135, DEPT90 spectrum shows quaternary carbon, HSQC shows no direct correlation, HMBC shows remote correlation with  $\delta_{\text{H}} 5.384$  (9-CH),  $\delta_{\text{H}} 7.711$  (12-NH),  $\delta_{\text{H}} 7.551$  (16-CH),  $\delta_{\text{H}} 2.118$  (28-CH<sub>3</sub>). It can be determined to be 14-C.

Peak 8:  $\delta_{\text{H}} 105.399$ , DEPT135, DEPT90 spectrum shows quaternary carbon, HSQC shows no direct correlation, HMBC shows remote correlation with  $\delta_{\text{H}} 5.384$  (9-CH),  $\delta_{\text{H}} 7.711$  (12-NH),  $\delta_{\text{H}} 2.190$  (22-CH<sub>3</sub>). It can be determined as 10-C.

Peak 9:  $\delta_{\text{H}} 109.596$ , DEPT135, DEPT90 spectrum shows quaternary carbon, HSQC shows no direct correlation, HMBC shows remote correlation with  $\delta_{\text{H}} 7.144$  (4-CH),  $\delta_{\text{H}} 7.376$  (1-CH). It can be determined as 2-C.

Peak 10:  $\delta_{\text{H}} 111.538$ , DEPT135, DEPT90 spectrum shows quaternary carbon, HSQC shows no direct correlation, HMBC shows remote correlation with  $\delta_{\text{H}} 2.118$  (28-CH<sub>3</sub>),  $\delta_{\text{H}} 7.551$  (16-CH),  $\delta_{\text{H}} 7.711$  (12-NH). It can be determined as 15-C.

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Peak 11:  $\delta_{\text{H}} 14.182$ , DEPT135, DEPT90 spectrum shows quaternary carbon, HSQC shows direct correlation with  $\delta_{\text{H}} 7.376$  (1-CH), HMBC shows remote correlation with  $\delta_{\text{H}} 7.274$  (5-CH),  $\delta_{\text{H}} 3.824$  (24-CH<sub>3</sub>). It can be determined as 1-C.

Peak 12:  $\delta_{\text{H}} 19.057$ , DEPT135, DEPT90 spectrum shows quaternary carbon, HSQC shows no direct correlation, HMBC shows remote correlation with  $\delta_{\text{H}} 7.376$  (1-CH),  $\delta_{\text{H}} 7.274$  (5-CH),  $\delta_{\text{H}} 7.144$  (4-CH). It can be determined as 6-C.

Peak 13:  $\delta_{\text{H}} 24.852$ , DEPT135, DEPT90 spectrum shows tertiary carbon, HSQC shows direct correlation with  $\delta_{\text{H}} 7.274$  (5-CH), HMBC shows remote correlation with  $\delta_{\text{H}} 7.376$  (1-CH),  $\delta_{\text{H}} 7.144$  (4-CH). It can be determined as 5-C.

Peak 14:  $\delta_{\text{H}} 30.989$ , DEPT135, DEPT90 spectrum shows tertiary carbon, HSQC shows direct correlation with  $\delta_{\text{H}} 7.144$  (4-CH), HMBC shows remote correlation with  $\delta_{\text{H}} 5.384$  (9-CH). Can be determined as 4-C.

Peak 15:  $\delta_{\text{H}} 38.229$ , DEPT135, DEPT90 spectrum shows quaternary carbon, HSQC shows no direct correlation, HMBC shows remote correlation with  $\delta_{\text{H}} 2.190$  (22-CH<sub>3</sub>),  $\delta_{\text{H}} 5.384$  (9-CH),  $\delta_{\text{H}} 7.711$  (12-NH). It can be determined as 11-C.

Peak 16:  $\delta_{\text{H}} 41.761$ , DEPT135, DEPT90 spectrum shows quaternary carbon, HSQC shows no direct correlation, HMBC shows remote correlation with  $\delta_{\text{H}} 5.384$  (9-CH),  $\delta_{\text{H}} 7.274$  (5-CH),  $\delta_{\text{H}} 7.376$  (1-CH). It can be determined as 7-C.

Peak 17:  $\delta_{\text{H}} 44.271$ , DEPT135, DEPT90 spectrum shows quaternary carbon, HSQC shows direct correlation with  $\delta_{\text{H}} 7.551$  (16-CH), And HMBC also shows a remote correlation with  $\delta_{\text{H}} 2.118$  (28-CH<sub>3</sub>),  $\delta_{\text{H}} 5.384$  (9-CH),  $\delta_{\text{H}} 7.551$  (16-CH),  $\delta_{\text{H}} 7.711$  (12-NH). They can be identified as 13-C and 16-C carbons.

Peak 18:  $\delta_{\text{H}} 55.720$ , DEPT135, DEPT90 spectrum shows quaternary carbon, HSQC shows no direct correlation, HMBC showed a remote correlation with  $\delta_{\text{H}}$

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$\delta_{\text{H}} 3.824$  (24-CH<sub>3</sub>),  $\delta_{\text{H}} 7.144$  (4-CH),  $\delta_{\text{H}} 5.384$  (9-CH),  $\delta_{\text{H}} 7.376$  (1-CH),  $\delta_{\text{H}} 7.274$  (5-CH). Can be determined as 3-C.

Peak 19:  $\delta_{\text{C}} 159.464$ , HSQC shows no direct correlation, HMBC shows correlation with  $\delta_{\text{H}} 2.118$  (28-CH<sub>3</sub>),  $\delta_{\text{H}} 3.986$ - $4.030$  (26-CH<sub>2</sub>),  $\delta_{\text{H}} 7.551$  (16-CH),  $\delta_{\text{H}} 7.711$  (12-NH), so it can be determined that the peak is 18-C.

Peak 20:  $\delta_{\text{C}} 169.843$ , DEPT135, DEPT90 spectrum shows quaternary carbon, HMBC shows a remote correlation with  $\delta_{\text{H}} 5.384$  (27-CH<sub>3</sub>),  $\delta_{\text{H}} 2.118$  (28-CH<sub>3</sub>),  $\delta_{\text{H}} 7.711$  (12-NH), we can determine that the peak is 19-C.

According to the above analysis, the carbon spectrum of the tested sample shows 20 groups of carbon peaks, a total of 21 carbon atoms, and all of them are clearly attributed.

The NMR data were consistent with the target compounds.

The spectra are provided in:

*3.2.S.3.1 Annex 3 NMR spectrum of Finerenone (batch No.: 231101)*

### **Id. Thermogravimetric Analysis (TGA)**

*Sample Batch No.: 231101.*

*Instrument: : TG 209 F1 Thermogravimetric Analyzer*

Test conditions:

- 35-500 °C, heating rate of 10 °C/min;
- nitrogen, flow rate 20ml/min

Test result:

Table 3.2.S.3.1-6 TGA Result of the Substance to be Examined

Lot No.	Endothermic process	
	Temperature	Mass loss
231101	35.0 °C ~ 184.2 °C	0.60%

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	242.7 °C ~ 438.8 °C	97.30%
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### Interpretation:

TGA shows that the weight loss of the self-made sample is 0.60% at 35°C~184.2°C. Combined with DSC detection spectrum, the sample does not absorb heat before melting (about 254°C), indicating that the sample does not contain crystal water or crystalline solvent.

The TGA thermogram is provided in:

3.2.S.3.1 Annex 4 TGA thermogram report of Finerenone (batch No.: 231101 ).

3.2.S.3.1 Annex 5 TGA thermogram report of Finerenone (batch No.: 231201, 240101, 240102)

### Ie. Differential Scanning Calorimetry (DSC)

Sample Batch No.: 231101.

Instrument: DSC 25 Differential Scanning Calorimeter

Test conditions:

- experimental atmosphere: N<sub>2</sub>, 50 mL/min.
- scanning procedure: Heat up from 30 °C to 350 °C at 20 °C/min rate.

Test result:

The samples submitted for inspection were detected by differential scanning calorimetry, and there was a multiple endothermic process within the detection temperature range (30-400°C). The peak temperature is shown in Table 3.2.S.3.1-7:

Table 3.2.S.3.1-7 DSC Result of the Substance to be Examined

Batch No	endothermic process	
	The initial temperature of extrapolation/ °C	Peak temperature/ °C

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231101	254.38	257.04
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Interpretation:

DSC showed that the self-made sample had an endothermic peak at 254°C, without other endothermic peaks, indicating that the endothermic peak was the melting point, consistent with the melting point reported in the original research patent (about 252°C).

The DSC pattern is provided in:

3.2.S.3.1 Annex 6 DSC pattern on Finerenone (batch No.: 231101, 231201, 240101, 240102)

### **If. X-ray Diffraction Pattern (XRD)**

Sample Batch No.: 231101.

Instrument: Japanese Institute of Technology (Smartlab 3kW) X-ray diffractometer

Test method: Light tube voltage 40kW, filament current 15mA, Cu radiation, Ni filtering, Hypix 3000 two-dimensional detector.

Detection and analysis: The sample has the maximum peak value (error range is  $\pm 0.2^\circ$ ) at 2θ angles of 8.5, 14.1, 17.2, 19.0, 20.5, 25.6 and 26.5, which is consistent with the characteristic peak of polycrystalline substance I reported in the original research patent.

The XRD pattern is provided in:

3.2.S.3.1 Annex 7 XRD pattern on Finerenone (batch No.: 231101)

3.2.S.3.1 Annex 8 XRD pattern on Finerenone (batch No.: 231201)

3.2.S.3.1 Annex 9 XRD pattern on Finerenone (batch No.: 240101)

3.2.S.3.1 Annex 10 XRD pattern on Finerenone (batch No.: 240102)

### **Ig. Elemental Analysis**

Instrument model: Elementar Vario EL cube element analyzer

Finerenone

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Test method: JY/T 0580-2020 element analyzer analysis method general rules

Table 3.2.S.3.1-8 Elemental analysis data of the tested sample

Elements	C	H	N	O
Test results	66.57%	5.95%	14.96%	12.52% (This value is calculated from the first three test values)
Theoretical result	66.65%	5.86%	14.81%	12.68%

Analytical results: According to the test results of C, H and N, the mass fraction of oxygen element can be calculated as 12.52%. The error between the test results of C, H, O and N and the theoretical results is less than 0.3%, which can be determined to be consistent with the molecular composition C21H22N4O3 of Finerenone.

The Elemental Analysis report is provided in:

3.2.S.3.1 Annex 11 Elemental Analysis report on Finerenone (batch No.: 231101)

### **Ih. Single crystal X-ray diffraction**

Finerenone contains a chiral center, and the stereoscopic configuration can be confirmed by single crystal X-ray diffraction. See the attached single crystal test report for details.

Monocrystalline culture: 160mg of Finerenone and 6ml of anhydrous ethanol were heated and dissolved, then filtered with precision. The filtrate was placed in a vial and incubated at 25°C for 3~4 days to obtain monocrystalline.

Instrument: D8 Venture

Testing parameters:

- Light source: Cu target
- X-ray: CU-k (= 1.54178 Å)
- Detector: CMOS surface detector

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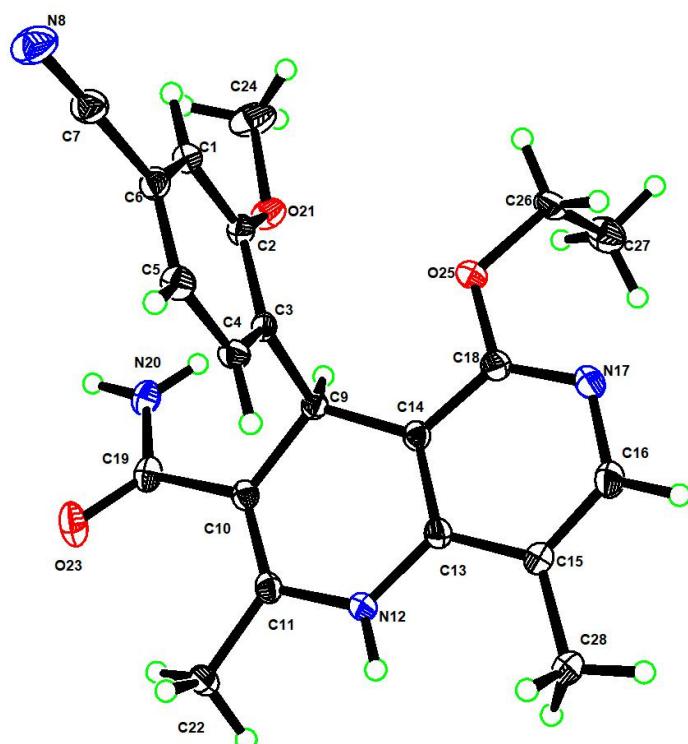
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- Resolution: 0.80 Å
- Current voltage: 50 kV, 1.2mA
- exposure time: 2 s
- Face detector to sample distance: 40 mm
- Test temperature: 150(2)K.

#### Structure analysis and finishing process:

After integral reduction of diffraction data by SAINT program, empirical absorption correction of data by SADABS program; SHELXT2014 was used to analyze the single crystal structure by direct method, and the structure was refined by least square method. The hydrogen atom refining process was obtained by isotropic calculation, the hydrogen atom on N was obtained by residual electron density, and the hydrogen atom on C-H was obtained by calculation and hydrogenation, and it was refined by riding model. The Flack constant is -0.01(11), and the chirality of C9 is S configuration. See Figure 3.2.S.3.1-1 for an ellipsoid diagram of the molecular stereostructure of Finerenone.



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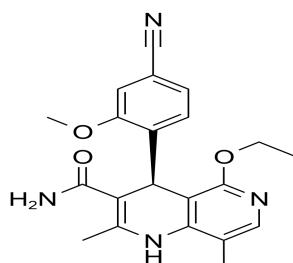
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Fig. 3.2.S.3.1-1 Ellipsoid of the three-dimensional structure of Finerenone

### **Comprehensive analysis and conclusion**

The molecular formula of Finerenone is  $C_{21}H_{22}N_4O_3$ , there is a chiral center, and the chiral configuration of C9 is S configuration (the atomic number here is the nuclear magnetic structure analysis number). The applicant confirmed the chemical plane structure of the product by elemental analysis, mass spectrometry, infrared spectroscopy, nuclear magnetic resonance  $^1H$  spectrum, nuclear magnetic resonance  $^{13}C$  spectrum and two-dimensional nuclear magnetism; In order to confirm the three-dimensional configuration of Finerenone, the applicant cultured single crystals with 231101 batches of samples, commissioned the Research Center for Drug Quality Control and Solid State Chemistry, Shanghai Institute of Pharmaceutical Sciences, Chinese Academy of Sciences to test them, and confirmed that C9 in the molecular structure of the product is the S configuration. TGA proved that this product does not contain crystal water and crystalline solvent, and the structural formula is as follows:



The applicant used DSC and XRD methods to identify the crystal shape of the self-made Finerenone, and confirmed that the crystal shape of the product is consistent with that reported in the original patent.

## **II. Physicochemical Characteristics**

### **IIa. Appearance**

According to the evaluation report of the original research formulation launched by the original manufacturer in the European Union, the appearance of the original product is White to yellow crystalline non-hygrosopic powder. The applicant conducted a visual inspection on the

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self-made commercial batch of Finerenone, and the test results are shown in the table below.

Table 3.2.S.3.1-8 Results of Appearance Studies of Finerenone

Batch No.	Appearance
EMA Assessment report	White to yellow crystalline non-hygroscopic powder
231201	White powder
240101	White powder
240102	White powder

### **IIb. Solubility**

Referring to the IF document of the original preparation agent listed in Japan, it can be seen that the solubility of the original preparation API is "dissolved in methanol, slightly soluble in ethanol, acetonitrile and acetone, slightly soluble in isopropyl alcohol, and almost insoluble in water". The applicant has studied the solubility of three batches of commercially produced finelidone in methanol, ethanol, acetonitrile, acetone, isopropyl alcohol and water in accordance with the General rules of the Chinese Pharmacopoeia 2020 Edition. The test results are basically consistent with the description of solubility of the original research API.

Table 3.2.S.3.1-9 Results of Solubility Studies of Finerenone

Batch No.	Solubility
231201	This product is dissolved in methanol, slightly soluble in ethanol, acetonitrile and acetone, slightly soluble in isopropyl alcohol, and almost insoluble in water
240101	This product is dissolved in methanol, slightly soluble in ethanol, acetonitrile, and acetone, slightly soluble in isopropyl alcohol, and almost insoluble in water
240102	This product is dissolved in methanol, slightly soluble in ethanol, acetonitrile, and acetone, slightly soluble in isopropyl alcohol, and almost insoluble in water

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### **IIC. Hygroscopicity**

Refer to the IF file of the original development agent listed in Japan, it can be seen that the original research product has no moisture induction. According to the General Rule 9103 of the Fourth Part of the 2020 edition of Chinese Pharmacopoeia, the applicant conducted a humidification test on three batches of commercial-scale mass-produced Finerenone. The experimental conditions were  $25\pm1^{\circ}\text{C}$  and the relative humidity was  $80\%\pm2\%$ . The test results showed that the product had non-hygroscopic, which was consistent with that of the original product.

Table 3.2.S.3.1-10 Results of Hygroscopicity Determination of Finerenone

Batch No.	Weight of blank vessel (g)	Sample (g)	Weight of blank vessel and sample(g) after standing for 24h	Mass increasing percentage (%)	Results
231201	20.63545	1.01424	21.64979	0.01%	non-hygroscopic
240101	22.77341	1.02158	23.79492	-0.01%	non-hygroscopic
240102	20.08668	1.19142	21.27804	-0.01%	non-hygroscopic
Original formulation IF file			non-hygroscopic		

### **IID. Melting Point**

The crystal form of Finerenone was reported in the patent CN106795155 A applied by the original research manufacturer in China. The crystal form was measured by DSC and melted at about  $252^{\circ}\text{C}$ . The curve diagram is as follows:

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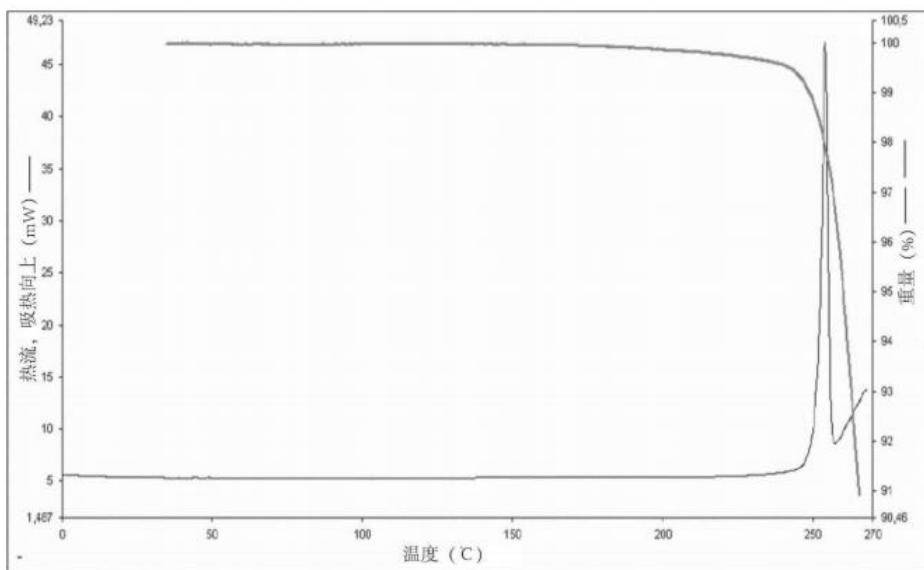


图1：以多晶型物I的晶体形式的式(I)化合物的DSC (20 Kmin<sup>-1</sup>) 和TGA

Fig. 3.2.S.3.1-2 patent CN106795155A reported the DSC map of Fine  
renone crystal type

The applicant's evaluation of a pilot lot (Batch No.: 231101) and three commercial-scale batch production batches of Finerenone (Batch No.: 231201, 240101 and 240102). Differential scanning calorimetry (DSC) was performed on 231201, 240101 and 240102. The results showed melting at about 252 °C, and no other absorption and exothermic peaks were observed, indicating that no phase changes occurred other than melting. The DSC maps of the four batches of samples are consistent with those of the crystal type reported in patent CN106795155A. The specific data are shown in below Table.

Table 3.2.S.3.1-11 Results of Melting Point Determination of Finerenone

Batch No.	Endothermic process	
	Extrapolation starting temperature/ °C	Peak temperature/ °C
231101	254.38	257.04
231201	253.88	256.33
240101	254.02	256.74
240102	255.16	256.93

The DSC thermogram on Finerenone is provided in:

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3.2.S.3.1 Annex 6 DSC pattern of Finerenone (batch No.: 231101, 231201, 240101, 240102)

### **IIe. Thermogravimetric Analysis (TGA)**

Combined with the chemical structure formula and molecular formula of Finerenone published in the evaluation report of the original preparation agent listed in the EU (see 3.2.S.1.2 for details), it can be seen that the Finerenone used in the original research does not contain crystal water or crystallization solvent. The applicant conducted thermogravimetric analysis of a pilot batch (Batch No.: 231101) and three commercial-scale batches of Finerenone (Batch No.: 231201, 240101 and 240102). The specific data are shown in below Table.

Table 3.2.S.3.1-12 TGA Result of the Substance to be Examined

Batch No.	Endothermic process	
	Temperature/C	Mass loss %
231101	184 °C	0.60%
231201	175 °C	0.16%
240101	175 °C	0.24%
240102	175 °C	0.16%

See 3.2.S.2.2.3 for the crystalline solvent of this product, it can be seen that the crystalline solvent of this product is anhydrous ethanol, which may bring a small amount of water, so the crystalline solvent most likely to be contained in this product is ethanol and water. From the analysis of the test results, it can be seen that there is basically no weight loss when the sample is heated to 175°C, indicating that the sample does not contain crystal water or crystalline solvent.

The TGA thermogram is provided in:

3.2.S.3.1 Annex 4 TGA thermogram report of Finerenone (batch No.: 231101).

3.2.S.3.1 Annex 5 TGA thermogram report of Finerenone (batch No.: 231201, 240101, 240102).

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### IIf. Polymorphism

In the patent CN106795155A applied by the original research manufacturer in China, the polycrystalline form I of Finerenone was reported. The crystal form by powder X-ray diffraction pattern (that is, XRD pattern) has a peak maximum at the 2 $\theta$  Angle of 8.5, 14.1, 17.2, 19.0, 20.5, 25.6, 26.5. The relevant XRD pattern is shown in below Fig. 3.2.S.3.1-3.

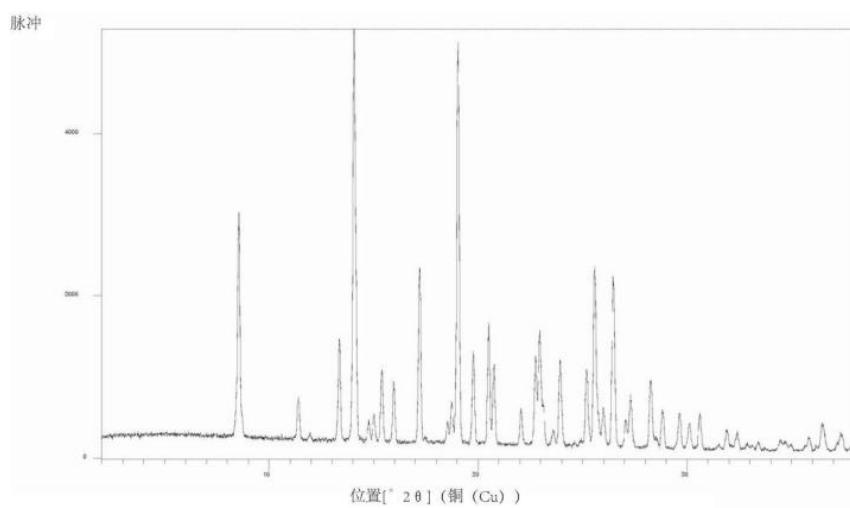


图3: 以多晶型物I的晶体形式的式(I)化合物的X射线衍射图

Figure 3.2.S.3.1-3 patent CN106795155A reported the XRD pattern of Finerenone polycrystalline form I

Applicant for a pilot lot (Batch No.: 231101) and three commercial-scale batches of Finerenone (Batch No.: 231201, 240101 and 240102) were tested by XRD. The XRD patterns of the four batches of samples were consistent with those of polycrystalline I crystal as reported in patent CN106795155A. At the same time, combined with the DSC and XRD test results, it can be seen that the applicant's self-made Finerenone crystal pattern is consistent with the Finerenone polycrystalline pattern I reported in patent CN106795155A, and the registration process can ensure that the same crystal pattern of the product is consistently produced.

The XRD pattern is provided in:

3.2.S.3.1 Annex 7 XRD pattern of Finerenone (batch No.: 231101)

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*3.2.S.3.1 Annex 8 XRD pattern of Finerenone (batch No.: 231201)*

*3.2.S.3.1 Annex 9 XRD pattern of Finerenone (batch No.: 240101)*

*3.2.S.3.1 Annex 10 XRD pattern of Finerenone (batch No.: 240102)*

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### **III. Material Safety Data Sheet**

#### **SECTION 1: Identification**

##### 1.1 Product identifier

Product name: Finerenone

##### 1.2 Recommended use of the chemical and restrictions on use

Identified uses: Industrial and scientific research use.

##### 1.3 Supplier's details

Company: Hinye Pharmaceutical Co., Ltd.

Address: No.109, Kangtian Road, Changsha National Biological Industrial Base, Hunan Province

Telephone: 86-10-69703845

#### **SECTION 2: Hazard identification**

##### 2.1 GHS Classification

Not a hazardous substance or mixture.

##### 2.2 GHS Label elements, including precautionary statements

Not a hazardous substance or mixture.

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### **2.3 Physical and chemical hazards**

Referring to current information, no physical or chemical hazard.

### **2.4 Health hazards**

Referring to current information, no health hazard.

### **2.5 Environmental hazards**

Referring to current information, no environmental hazard.

### **2.6 Other hazards**

Caution: Physiologically highly active, therapeutically usable substance. The substance must be handled with the care required for hazardous materials.

## **SECTION 3: Composition/information on ingredients**

### **3.1 Substances**

Product name	CAS No.	Formula	Molecular weight
Finerenone	1050477-31-0	C <sub>21</sub> H <sub>22</sub> N <sub>4</sub> O <sub>3</sub>	378.43 g/mol

## **SECTION 4: First-aid measures**

If inhaled

After inhalation: fresh air. Consult doctor if feeling unwell.

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In case of skin contact

In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower. Consult a physician.

In case of eye contact

After eye contact: rinse out with plenty of water. Remove contact lenses.

If swallowed

After swallowing: immediately make victim drink water (two glasses at most). Consult a physician.

### **4.2 Most important symptoms/effects, acute and delayed**

The most important known symptoms and effects are described in the labelling (refers to section 2.2) and/or in section 11

### **4.3 Indication of any immediate medical attention and special treatment needed**

no data available

### **4.4 Notes to physician**

No data available

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### **SECTION 5: Firefighting measures**

#### 5.1 Extinguishing media

Suitable extinguishing media

Water Foam Carbon dioxide (CO<sub>2</sub>) Dry powder

Unsuitable extinguishing media

For this substance/mixture no limitations of extinguishing agents are given.

#### 5.2 Special hazards arising from the substance or mixture

Carbon oxides

Nitrogen oxides (NO<sub>x</sub>)

Combustible.

Development of hazardous combustion gases or vapours possible in the event of fire.

#### 5.3 Advice for firefighters

Stay in danger area only with self-contained breathing apparatus. Prevent skin contact by keeping a safe distance or by wearing suitable protective clothing.

Suppress (knock down) gases/vapors/mists with a water spray jet. Prevent fire extinguishing water from contaminating surface water or the ground water system.

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### **SECTION 6: Accidental release measures**

#### 6.1 Personal precautions, protective equipment and emergency procedures

Advice for non-emergency personnel: Avoid inhalation of dusts. Avoid substance contact.

Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, consult an expert.

For personal protection refers to section 8.

#### 6.2 Environmental precautions

Do not let product enter drains.

#### 6.3 Methods and materials for containment and cleaning up

Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (refers to sections 7 and 10). Take up dry. Dispose of properly.

Clean up affected area. Avoid generation of dusts.

#### 6.4 Reference to other sections

For disposal refers to section 13.

### **SECTION 7: Handling and storage**

#### 7.1 Precautions for safe handling

For precautions refers to section 2.2.

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### 7.2 Conditions for safe storage, including any incompatibilities

Storage conditions

Tightly closed. Dry.

Storage stability

Recommended storage temperature

2 - 8 °C

Storage class

Storage class (TRGS 510): 11: Combustible Solids.

## **SECTION 8: Exposure controls/personal protection**

### 8.1 Control parameters

Ingredients with workplace control parameters

Contains no substances with occupational exposure limit values.

### 8.2 Exposure controls

Appropriate engineering controls

Change contaminated clothing. Preventive skin protection recommended.

Wash hands after working with substance.

Personal protective equipment

Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

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### Skin protection

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: [www.kcl.de](http://www.kcl.de)).

### Full contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: KCL 741 Dermatril® L

### Splash contact

Material: Nitrile rubber

Minimum layer thickness: 0.11 mm

Break through time: 480 min

Material tested: KCL 741 Dermatril® L

### Body Protection

#### protective clothing

### Respiratory protection

required when dusts are generated.

Our recommendations on filtering respiratory protection are based on the

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following standards: DIN EN 143, DIN 14387 and other accompanying standards relating to the used respiratory protection system.

Control of environmental exposure

Do not let product enter drains.

### **SECTION 9: Physical and chemical properties**

#### 9.1 Information on basic physical and chemical properties

Appearance

Form:solid

Odor

no data available

Odor Threshold

no data available

pH

no data available

Melting point/freezing point

no data available

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Initial boiling point and boiling range

no data available

Flash point

No data available

Evaporation rate

No data available

Flammability (solid,gas)

no data available

Upper/lower flammability or explosive limits

no data available

Vapor pressure

no data available

Vapor density

no data available

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### Density

no data available

### Relative density

no data available

### Water solubility

no data available

### Partition coefficient n-octanol/water

no data available

### Autoignition temperature

no data available

### Decomposition temperature

no data available

### Viscosity

no data available

### Explosive properties

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no data available

Oxidizing properties

none

### 9.2 Other safety information

no data available

## **SECTION 10: Stability and reactivity**

### 10.1 Chemical stability

The product is chemically stable under standard ambient conditions (room temperature) .

### 10.2 Possibility of hazardous reactions

no data available

### 10.3 Conditions to avoid

no information available

### 10.4 Incompatible materials

Strong oxidizing agents

## **Drug Master File of Finerenone**

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10.5 Hazardous decomposition products

In the event of fire: refers to section 5

## **SECTION 11: Toxicological information**

11.1 Information on toxicological effects

Acute toxicity

Oral: no data available

Inhalation: no data available

Dermal: no data available

Skin corrosion/irritation

no data available

Serious eye damage/eye irritation

No data available

Respiratory or skin sensitization

No data available

Germ cell mutagenicity

No data available

Carcinogenicity

No data available

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Reproductive toxicity

No data available

Specific target organ toxicity - single exposure

No data available

Specific target organ toxicity - repeated exposure

No data available

Aspiration hazard

No data available

### **11.2 Additional Information**

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

## **SECTION 12: Ecological information**

### **12.1 Toxicity**

no data available

### **12.2 Persistence and degradability**

no data available

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### 12.3 Bioaccumulative potential

no data available

### 12.4 Mobility in soil

no data available

### 12.5 Results of PBT and vPvB assessment

no data available

### 12.6 Endocrine disrupting properties

No data available

### 12.7 Other adverse effects

no data available

## **SECTION 13: Disposal considerations**

### 13.1 Waste treatment methods

Product

Offer surplus and non-recyclable solutions to a licensed disposal company.

## **SECTION 14: Transport information**

### 14.1 UN No.

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ADR/RID: - IMDG: - IATA-DGR: -

### 14.2 UN Proper Shipping Name

ADR/RID: Not dangerous goods

IMDG: Not dangerous goods

IATA: Not dangerous goods

### 14.3 Transport hazard class(es)

ADR/RID: - IMDG: - IATA-DGR: -

### 14.4 Packing group

ADR/RID: - IMDG: - IATA-DGR: -

### 14.5 Environmental hazards

ADR/RID: no IMDG: no IATA: no

### 14.6 Special precautions for user

no data available

### 14.7 Incompatible materials

Strong oxidizing agents

### Further information

Not classified as dangerous in the meaning of transport regulations.

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### **SECTION 15: Regulatory information**

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

National regulatory information

Other regulations

Please pay attention on the waste treatment should also comply with local regulations requirement.

### **SECTION 16: Other information**

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product.

## **Drug Master File of Finerenone**

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### **Annexes to 3.2.S.3.1**

3.2.S.3.1 Annex 1 Mass spectrum of Finerenone (batch No.: 231101)

3.2.S.3.1 Annex 2 Infrared (IR) spectrum of Finerenone (batch No.: 231101)

180301)

3.2.S.3.1 Annex 3 NMR spectrum of Finerenone (batch No.: 231101)

3.2.S.3.1 Annex 4 Elemental analysis report of Finerenone (batch No.: 231101)

3.2.S.3.1 Annex 5 TGA thermogram report of Finerenone (batch No.: 231101)

3.2.S.3.1 Annex 6 XRD pattern on Finerenone (batch No.: 231101)

3.2.S.3.1 Annex 7 DSC pattern of Finerenone (batch No.: 210601)

3.2.S.3.1 Annex 8 DSC pattern of Finerenone (batch No.: 210602)

3.2.S.3.1 Annex 9 DSC pattern of Finerenone (batch No.: 210603)

3.2.S.3.1 Annex 10 XRD pattern of Finerenone (batch No.: 210601)

3.2.S.3.1 Annex 11 XRD pattern of Finerenone (batch No.: 210602)

3.2.S.3.1 Annex 12 XRD pattern of Finerenone (batch No.: 210603)

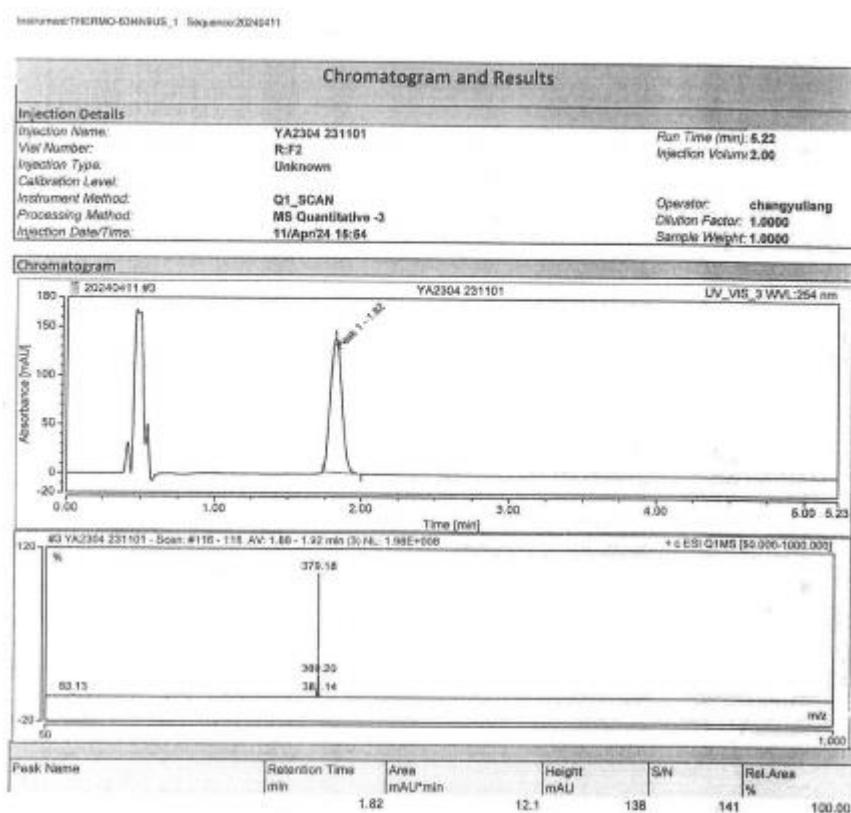
## **Drug Master File of Finerenone**

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### **3.2.S.3.1 Annex 1 Mass spectrum of Finerenone (batch No.: 231101)**



Default MS Report Information

Choochison (et al. 2008).  
Yardline 7.2-31.2%.

## Finerenone

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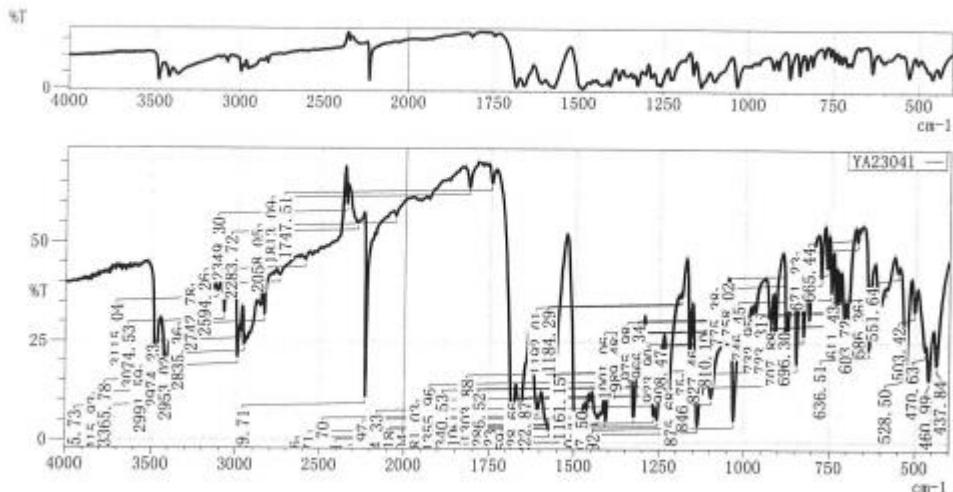
Version: 001

Date: Sep. 2024

## 3.2.S.3.1 Annex 2 Infrared (IR) spectrum of Finerenone (batch No.: 231101)

 SHIMADZU

YA2304-231101



項目	内容
分析日期	2023/12/17 12:57:19
分析者	裴东
文件名	药物研究院-2023年-YA2202 - 1-48-2 - YA23041.ispc
扫描参数文件	药物研究院-2023年-YA2202 - YA2304.iscp
样品名称	YA2304
样品ID	231101
速率	
扫描次数	10
分辨率	4 [cm⁻¹]
变换函数	Haus-Genzel

峰	高度	校正强度	基点(高)	基点(低)	面积	校正面积	描述
1 437.84	19.492	11.909	445.56	403.12	2839.131	160.361	
2 460.99	15.725	9.604	466.77	445.56	1657.322	92.561	
3 470.63	23.148	3.155	463.78	466.77	1881.864	32.060	
4 503.42	33.057	7.078	511.14	493.78	1098.841	57.256	
5 526.50	17.316	25.367	547.78	511.14	2472.827	375.251	
6 551.64	43.016	2.031	561.29	547.78	748.894	14.504	
7 586.36	38.826	0.741	588.29	561.29	1557.429	17.122	
8 603.72	34.830	4.223	609.51	588.29	1335.866	42.407	
9 611.43	38.480	1.820	619.15	609.51	589.002	11.015	
10 636.51	23.382	26.284	655.80	619.15	2078.304	242.760	
11 655.44	52.954	1.125	667.37	655.80	534.609	5.850	
12 671.23	50.635	3.338	677.01	667.37	457.774	13.886	
13 696.30	31.665	7.877	702.09	677.01	1429.737	40.444	
14 707.88	31.473	6.631	717.52	702.09	988.002	47.794	
15 723.31	34.603	8.766	729.09	717.52	699.913	44.521	

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16	732.95	34.911	10.485	738.74	729.09	574.337	51.552	
17	746.45	37.985	12.148	752.24	738.74	746.821	69.541	
18	758.02	44.010	9.114	763.81	752.24	588.638	46.145	
19	775.38	41.562	11.655	785.03	763.81	1075.330	84.737	
20	810.10	31.087	14.484	815.89	785.03	1709.308	109.197	
21	827.46	26.966	17.153	835.18	815.89	1211.581	133.732	
22	846.75	19.783	25.501	856.39	835.18	1369.819	206.967	
23	875.68	16.179	30.956	887.26	856.39	1963.796	326.556	
24	908.47	26.908	12.731	914.26	887.26	1626.300	75.453	
25	923.90	27.113	11.880	939.33	914.26	1624.125	106.058	
26	966.34	33.050	2.517	970.19	939.33	1894.510	-15.832	
27	975.88	32.704	1.693	979.84	970.19	639.253	7.098	
28	989.48	29.849	2.302	995.27	979.84	1053.004	12.309	
29	1001.06	30.067	0.888	1008.77	985.27	938.000	5.832	
30	1031.92	5.495	31.711	1047.35	1008.77	2934.259	471.934	
31	1097.50	11.222	15.494	1111.00	1047.35	4859.907	535.778	
32	1138.00	4.133	26.419	1153.43	1111.00	3567.844	560.179	
33	1161.15	19.704	20.250	1170.79	1153.43	1178.104	146.508	
34	1184.29	36.577	2.859	1188.15	1170.79	1023.608	13.896	
35	1192.01	36.344	0.769	1195.87	1188.15	488.023	2.838	
36	1222.87	13.603	2.718	1224.80	1195.87	2126.710	-20.419	
37	1228.66	13.477	5.438	1236.37	1224.80	943.609	28.815	
38	1257.59	5.619	5.081	1263.37	1236.37	2310.922	90.231	
39	1267.23	6.528	5.453	1276.88	1263.37	1197.615	44.263	
40	1296.52	15.718	11.058	1294.24	1276.88	1370.487	88.710	
41	1303.88	19.144	5.216	1307.74	1294.24	1028.629	37.036	
42	1325.10	6.444	14.378	1334.74	1313.52	1816.204	140.589	
43	1340.53	15.355	4.488	1346.31	1334.74	954.105	26.455	
44	1355.96	16.847	5.603	1369.46	1346.31	1871.714	83.873	
45	1381.03	12.810	12.624	1390.68	1369.46	1709.305	125.749	
46	1408.04	5.360	9.554	1413.82	1390.68	1963.678	80.819	
47	1431.18	5.916	2.917	1448.54	1423.47	2314.324	53.516	
48	1454.33	10.173	1.952	1458.18	1448.54	856.697	9.741	
49	1463.97	7.787	2.936	1459.75	1458.18	1048.686	15.499	
50	1489.05	1.443	22.745	1525.69	1469.76	4549.019	677.247	
51	1573.91	2.892	22.339	1597.06	1525.69	5631.830	793.091	
52	1606.70	8.216	9.036	1631.78	1597.06	2909.844	172.589	
53	1660.71	4.963	13.823	1672.28	1631.78	3435.827	281.896	
54	1683.86	3.375	21.379	1732.08	1672.28	3595.976	84.452	
55	1747.51	64.901	3.938	1759.08	1737.88	695.078	35.604	
56	1813.09	63.735	5.793	1828.52	1789.94	1250.104	78.755	
57	2058.05	56.592	1.586	2067.69	1996.32	2936.741	34.800	
58	2229.71	11.177	44.919	2245.14	2179.56	3625.654	663.408	
59	2283.72	54.861	5.234	2341.58	2245.14	4090.883	311.622	
60	2349.30	59.551	6.814	2358.94	2341.58	632.922	53.298	
61	2594.26	45.551	1.479	2619.33	2571.11	2584.302	29.329	
62	2742.78	41.739	1.752	2763.99	2679.13	4808.550	58.672	
63	2835.36	31.830	6.636	2843.07	2787.14	3391.333	33.719	
64	2953.02	24.232	5.203	2962.66	2945.30	1259.617	41.883	
65	2974.23	27.535	2.481	2978.09	2962.66	1080.618	19.154	
66	2991.59	21.035	11.736	3007.02	2978.09	2090.946	154.007	
67	3074.53	32.300	5.894	3082.25	3049.46	2059.246	58.696	
68	3115.04	37.746	1.515	3126.61	3097.68	1777.945	21.344	
69	3365.78	17.462	8.750	3396.64	3126.61	19219.617	816.647	
70	3415.93	14.360	12.636	3444.87	3396.64	3739.923	249.205	

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71	3475.73	10.830	25.479	3522.02	3444.87	5262.880	457.573	
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3/3

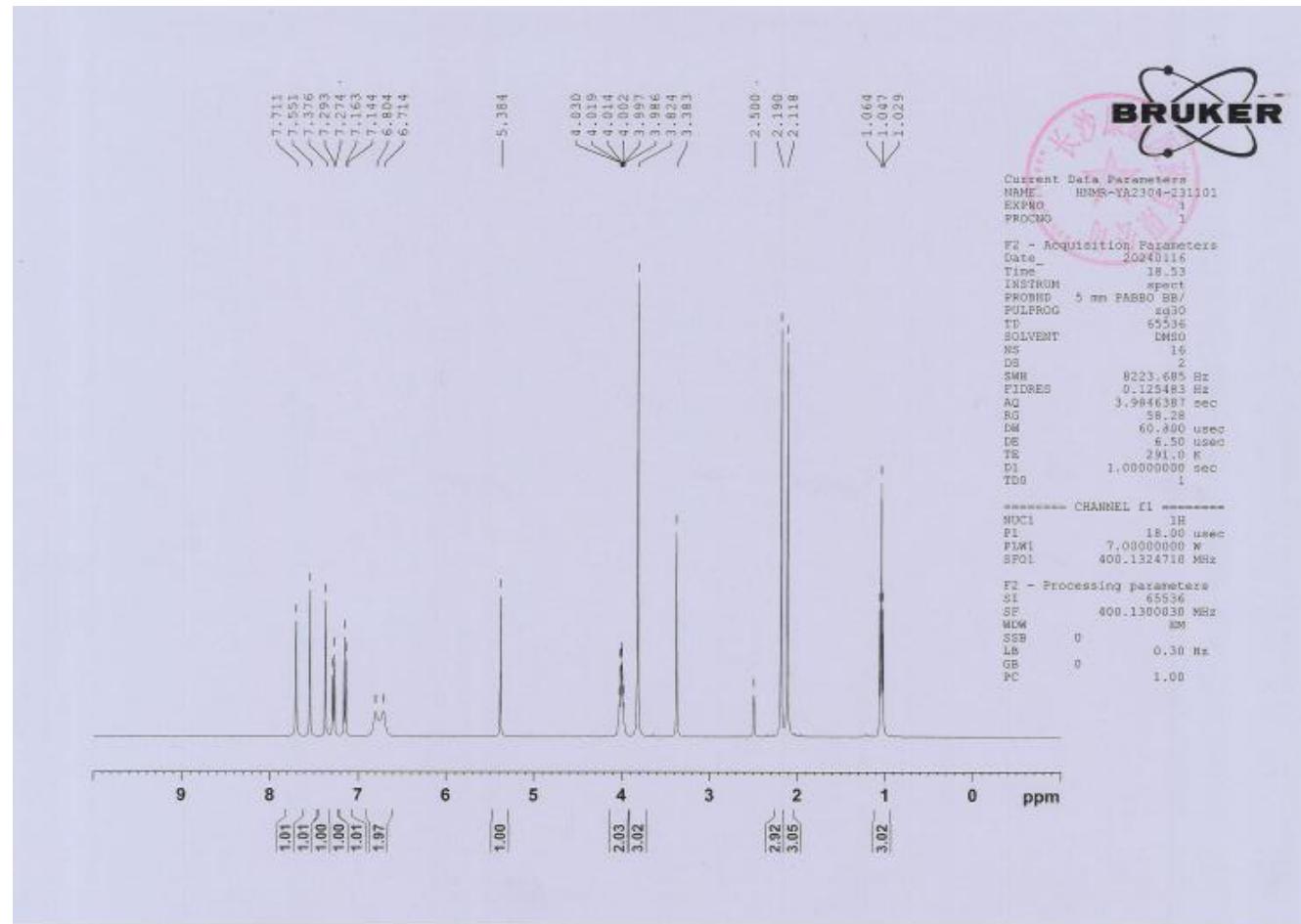
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### 3.2.S.3.1 Annex 3 NMR spectrum of Finerenone (batch No.: 231101)



<sup>1</sup>H-NMR 谱

Finerenone

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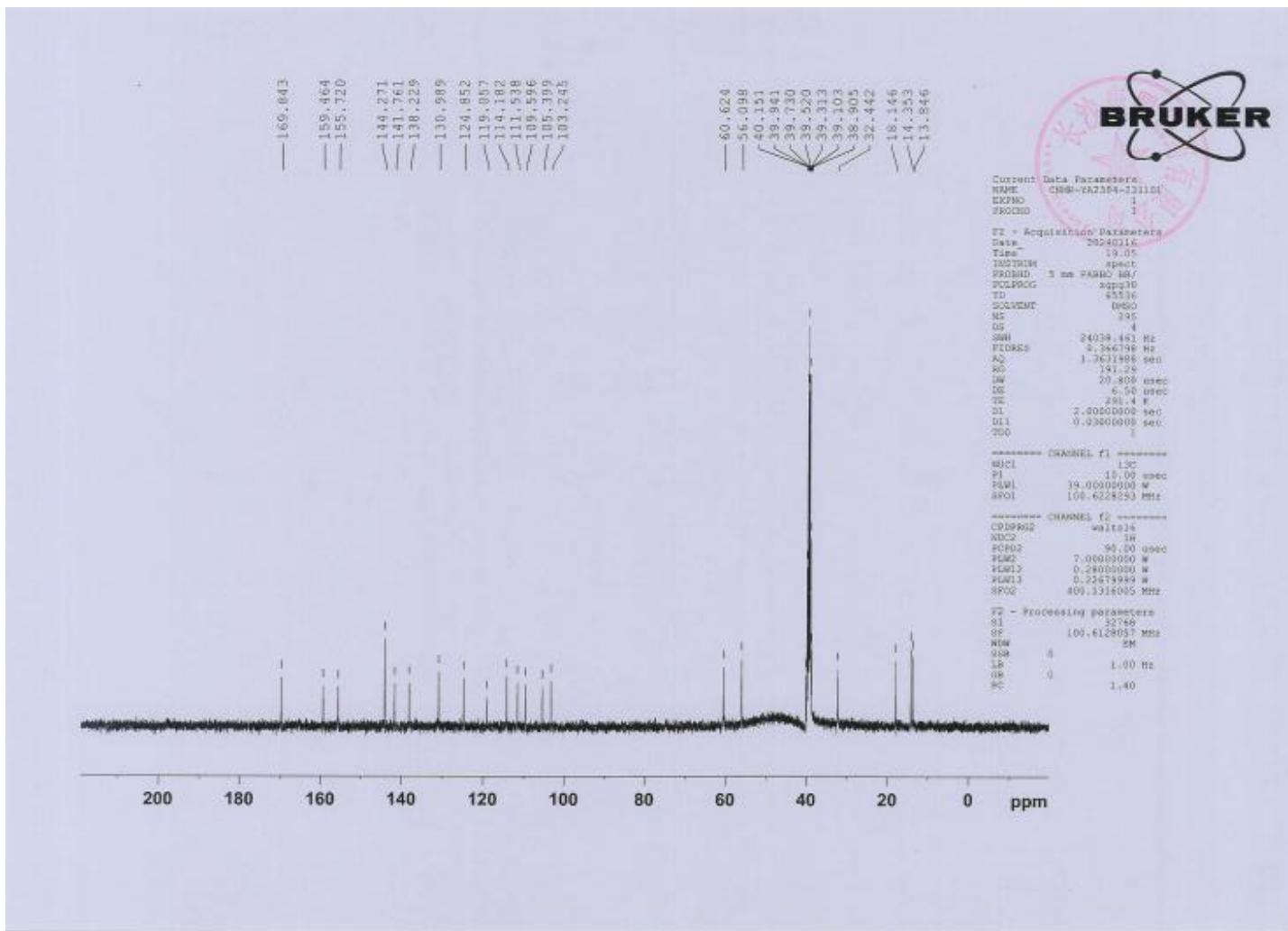
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<sup>13</sup>C-NMR 谱

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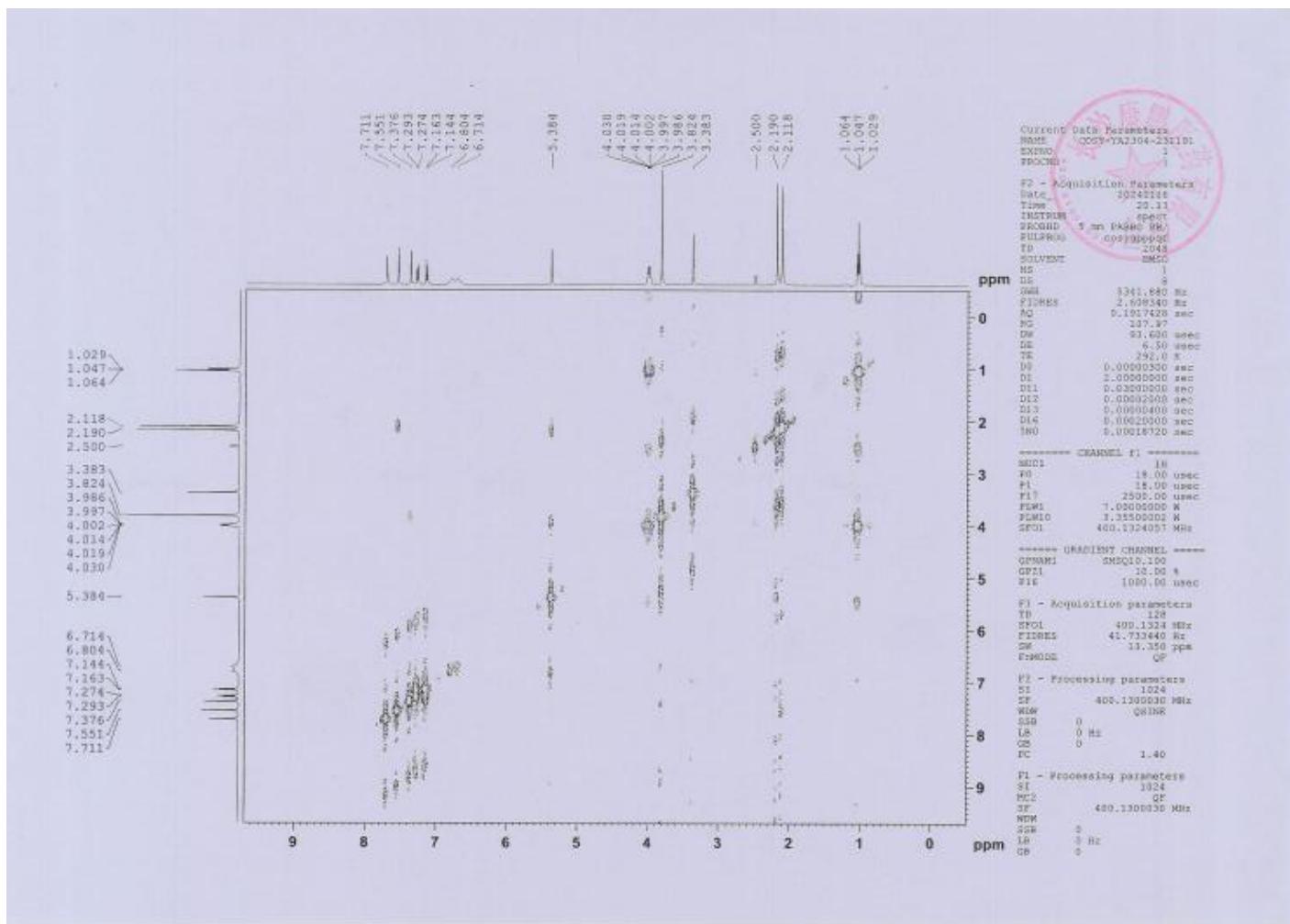
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COSY 谱

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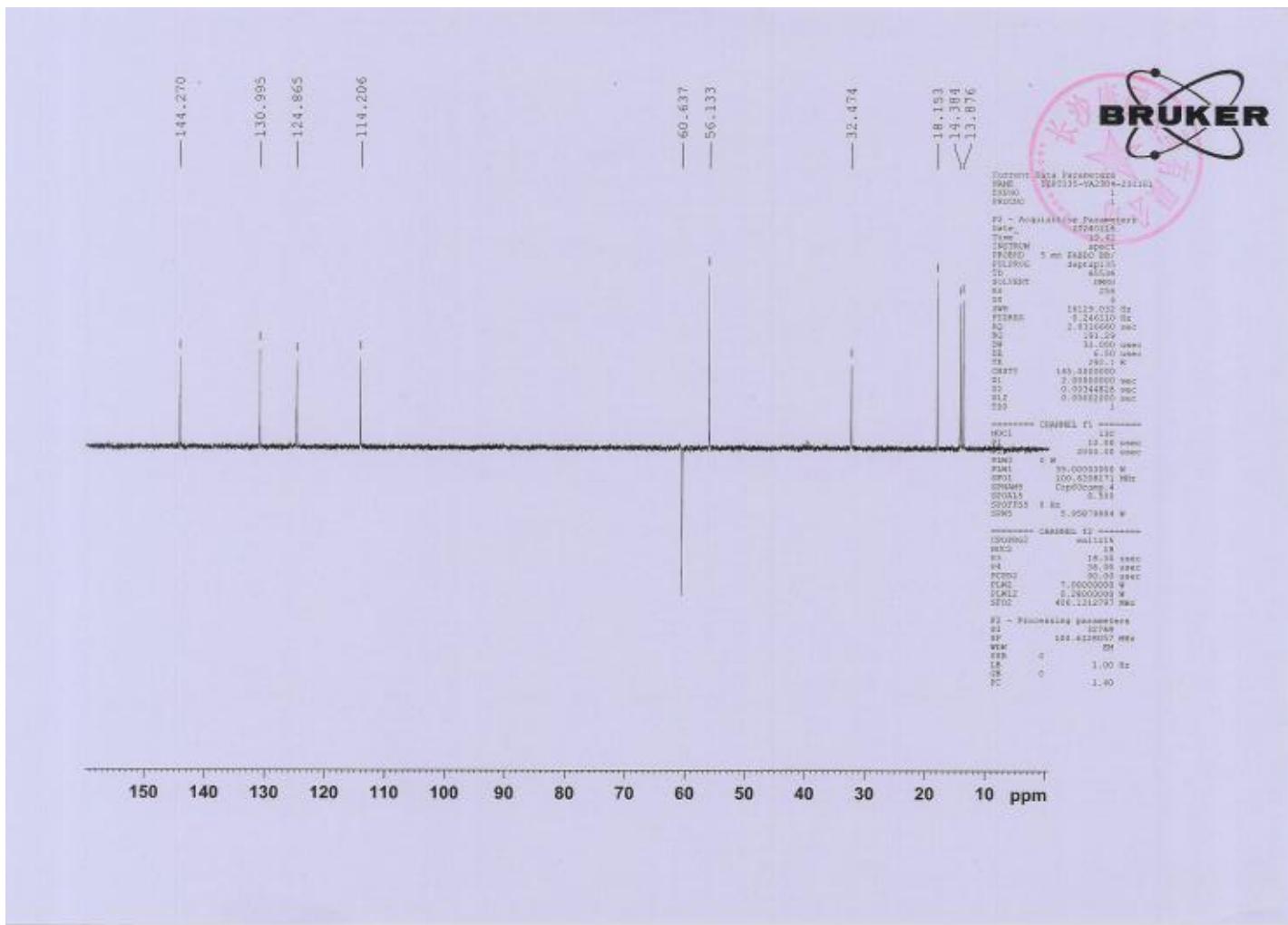
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## **Drug Master File of Finerenone**

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DEPT135 谱

## Finerenone

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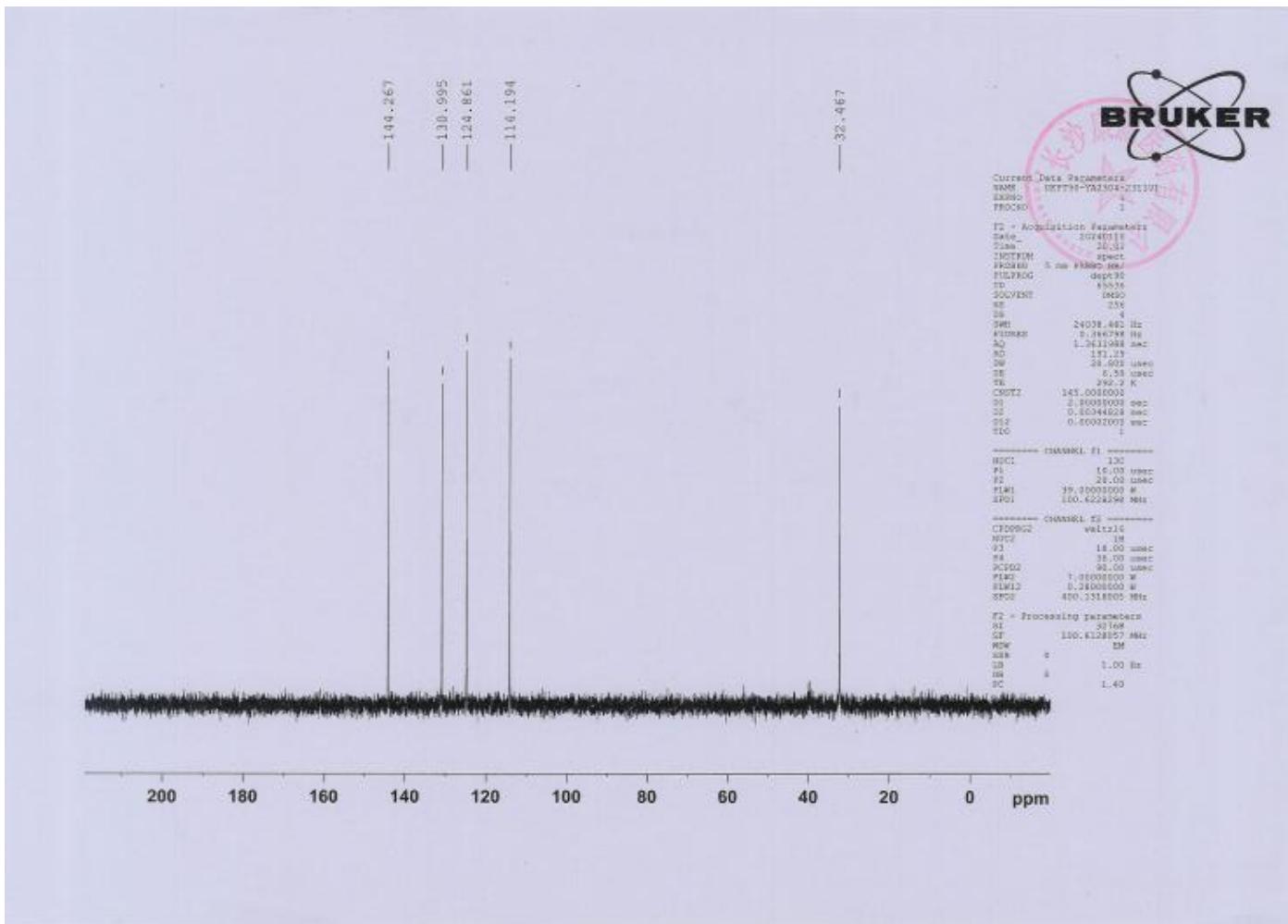
Hinye Pharmaceutical Co., Ltd.

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### DEPT90 谱

### Finerenone

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HSQC 谱

Finerenone

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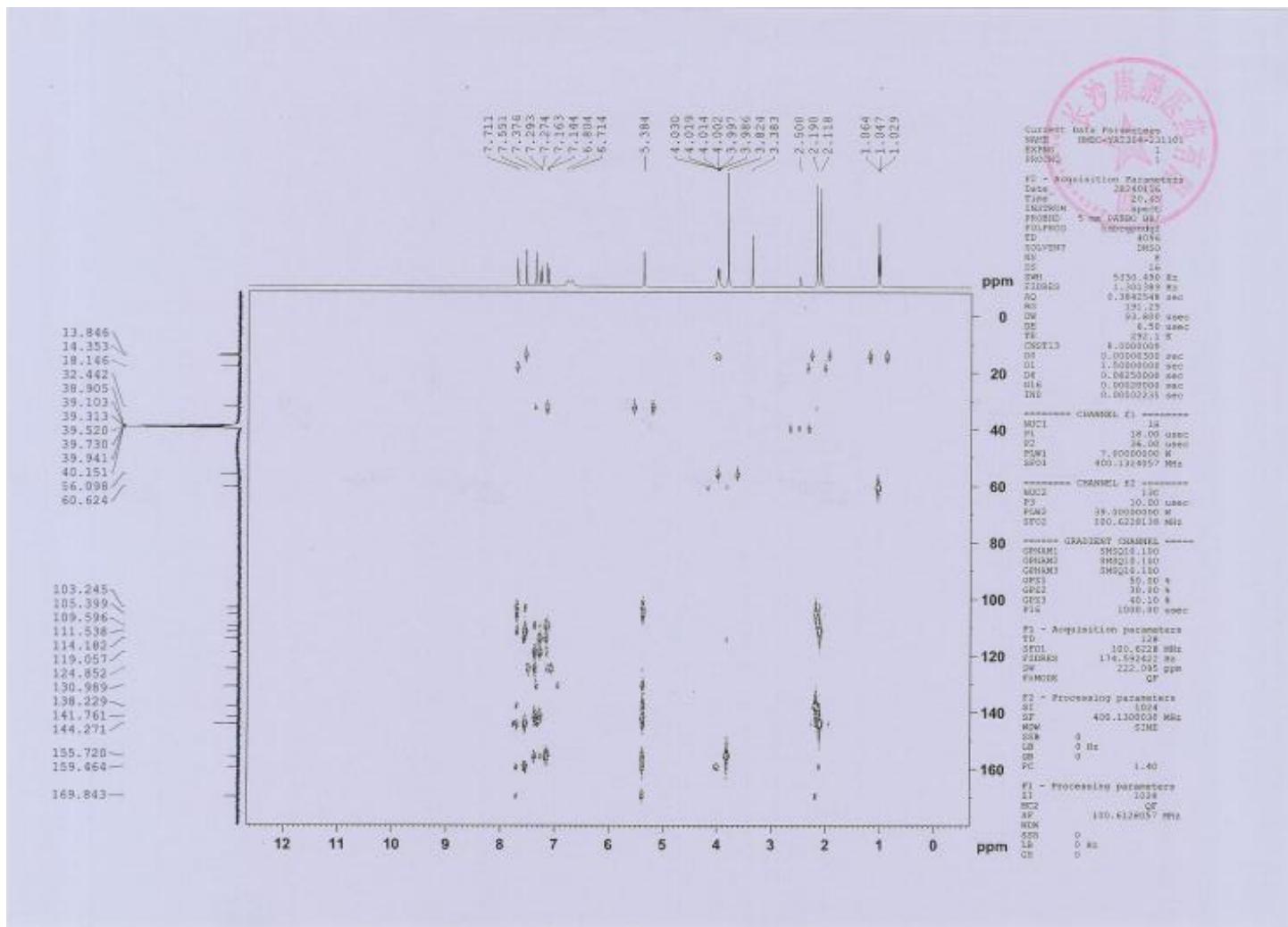
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## **Drug Master File of Finerenone**

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HMBC 谱

## Finerenone

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# Drug Master File of Finerenone

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## 3.2.S.3.1 Annex 4 TGA thermogram report of Finerenone (batch No.: 231101)



湖南航天天麓新材料检测有限责任公司

Hunan Aerospace TianLu advanced material testing Co.,Ltd

# 检 测 报 告

## TEST REPORT

(报告编号: TL-BG 20240115-02DT-01)

*委托单位	天地恒一制药股份有限公司
*委托单位地址	湖南省长沙市浏阳市洞阳镇康天路 109 号

湖南航天天麓新材料检测有限责任公司

(加盖 检测专用章)

检测专用章

第 1 页 共 4 页

Finerenone

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TL-BG 20240115-02DT-01



## 检 测 说 明 TEST OF CALIBRATION

1. 报告无“检测专用章”，骑缝章无效；
2. 复制报告无效；
3. 报告无编制、审核、批准签字无效；
4. 报告涂改无效，报告页数不全无效；
5. 若对检测报告有异议，请在收到报告之日起七日内向检测单位提出，无法保存、复现的样品，不受理申诉；
6. 检测结果仅适用于收到的样品；
7. 检测报告的编号具有唯一性，后缀“-01”为报告版本号，首版版本号为 01，第一次改版版本号为 02，依次类推。自发出后原报告即刻作废；
8. 报告中带“\*”处由委托单位提供，公司对委托方提供信息不负法律责任；当委托方提供的信息影响检测结果的有效性时，公司不负法律责任；
9. 报告中带“△”的项目检测结果来自外部供应商，为分包项目；
10. 未加盖 CMA 资质认定标志的不具有社会证明作用，数据仅用于科研、教学或内部质量控制；
11. 若将本报告用于广告的目的需经本公司书面确认。

宁乡实验室地址：湖南省长沙市宁乡高新技术产业园区金洲北路 001 号

望城实验室地址：湖南省长沙市望城经济开发区金星北路 1106 号 1 栋 2 层

株洲实验室地址：湖南省株洲市芦淞区董家塅高科园航空路 88 号

互联网检测云平台： [www.casic-t.com](http://www.casic-t.com)

联系电话：0731-88391857

第 2 页 共 4 页

# Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

TL-BG 20240115-02DT-01



## 检测报告

(Test Report)

### 一、基础信息

*委托单位	天地恒一制药股份有限公司									
*生产单位	/									
*受检单位	/									
*送检样品基本信息	样品名称	批号/编号/生产日期	规格/包装规格	剂型/类别	样品数量	产地/厂家	分装情况	容器介质	效期	备注
YA2304-231101	/	/	/	100mg	/	/	/	/	/	
*检测项目	热重分析									
检测类别	委托送检									
收样日期	2024.01.12	检测日期	2024.01.15							
结论	依据热分析法 中国药典 2020 年版四部通则(0661)对所送样品进行检测，提供实测结果。 签发日期：2024年01月15日 									
编制	李昊	审核	许思瑞	批准	冉恭昊					

### 二、检测分析方法及仪器

*检测项目	*检测依据	*判定依据	主要检测设备名称
热重分析	热分析法 中国药典 2020 年版四部通则(0661)	/	热重分析仪\TGA209F1\

### 三、检测结果

样品名称或编号	检测项目	测量单位	检测结果		
			起始点(℃)	失重温度(℃)	残留质量(%)
YA2304-231101	TG	/	/	184.2	99.40
备注：	1. 测试条件： 1) 35℃~500℃，升温速率 10℃/min； 2) 吹扫气：氮气，流量 50ml/min； 3) 保护气：氮气，流量 20ml/min。				

# Drug Master File of Finerenone

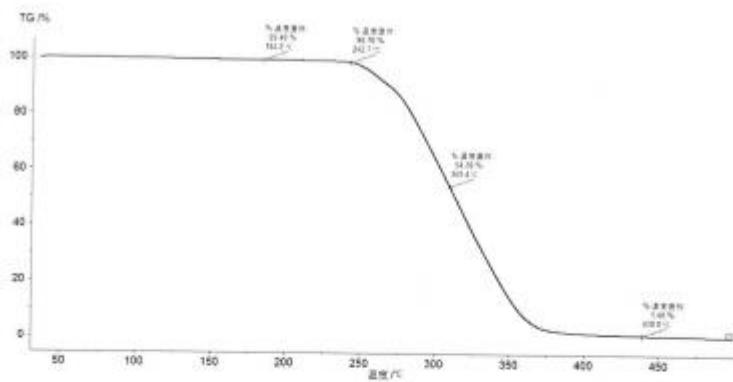
Document: YA2304-Quality

Version: 001

Date: Sep. 2024

TL-BG 20240115-02DT-01

## 四、附图



YA2304-231101 热重分析图

备注：本报告检测地址为湖南省长沙市宁乡高新技术产业园区金洲北路 001 号

以下空白

第 4 页 共 4 页

# Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

## 3.2.S.3.1 Annex 5 TGA thermogram report of Finerenone (batch No.: 231201, 240101 and 240102)



湖南航天天麓新材料检测有限责任公司

Hunan Aerospace TianLu advanced material testing Co.,Ltd

# 检 测 报 告

## TEST REPORT

(报告编号: TL/BG-20240403-03DT)

\*委托单位

天地恒一制药股份有限公司

\*委托单位地址

湖南省长沙市浏阳市洞阳镇康天路 109 号

湖南航天天麓新材料检测有限责任公司

(加盖 检测专用章)

检测专用章

第 1 页 共 5 页

Finerenone

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Hinye Pharmaceutical Co., Ltd.

# Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

TL/BG-20240403-03DT



## 检 测 说 明 TEST OF CALIBRATION

1. 报告无“检测专用章”，骑缝章无效；
2. 复制报告无效；
3. 报告无编制、审核、批准签字无效；
4. 报告涂改无效，报告页数不全无效；
5. 若对检测报告有异议，请在收到报告之日起七日内向检测单位提出，无法保存、复现的样品，不受理申诉；
6. 检测结果仅适用于收到的样品；
7. 检测报告的编号具有唯一性，后缀“-01”为报告版本号，首版版本号为 01，第一次改版版本号为 02，依次类推。自发出后原报告即刻作废；
8. 报告中带“\*”处由委托单位提供，公司对委托方提供信息不负法律责任；当委托方提供的信息影响检测结果的有效性时，公司不负法律责任；
9. 报告中带“△”的项目检测结果来自外部供应商，为分包项目；
10. 未加盖 CMA 资质认定标志的不具有社会证明作用，数据仅用于科研、教学或内部质量控制；
11. 若将本报告用于广告的目的需经本公司书面确认。

宁乡实验室地址：湖南省长沙市宁乡高新技术产业园区金洲北路 001 号  
望城实验室地址：湖南省长沙市望城经济开发区金星北路 1106 号 1 栋 2 层  
株洲实验室地址：湖南省株洲市芦淞区董家塅高科园航空路 88 号  
互联网检测云平台：[www.casic-t.com](http://www.casic-t.com)  
联系电话：0731-88391857

第 2 页 共 5 页

# Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

TL/BG-20231109-03DT-02



## 检测报告

(Test Report)

### 一、基础信息

*委托单位	天地恒一制药股份有限公司									
*生产单位	/									
*受检单位	/									
*送检样品 基本信息	样品名称	批号/编号/生 产日期	规格/包 装规格	剂型/ 类别	样品数 量	产地/ 厂家	分装 情况	容器 介质	效 期	备注
	YA2304-231 201	/	/	/	100mg	/	/	/	/	最高温度 测到 500°C
	YA2304-240 101	/	/	/	100mg	/	/	/	/	最高温度 测到 500°C
	YA2304-240 102	/	/	/	100mg	/	/	/	/	最高温度 测到 500°C
*检测项目	热重分析									
检测类别	委托送检									
收样日期	2024-04-03		检测日期		2024-04-03					
结论	依据热分析法 中国药典 2020 年版四部通则(0661)对所送样品进行检测，提供实测结果。  签发日期：2024 年 04 月 11 日  (检测专用章)									
编制	许思琦	审核	李红	检测专用章	批准	李红				

### 二、检测分析方法及仪器

*检测项目	*检测依据	*判定依据	主要检测设备名称
热重分析	热分析法 中国药典 2020 年 版四部通则(0661)	/	NETZSCH TG 209 F1 Libra 热重分析仪

# Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

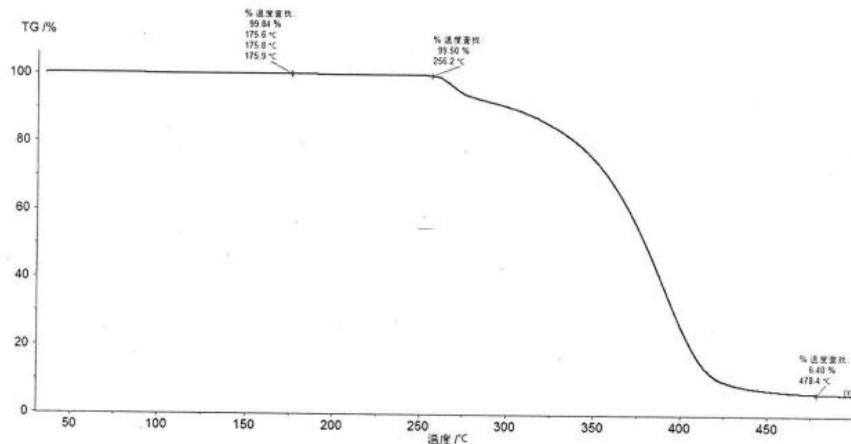
TL/BG-20231109-03DT-02



## 三、检测结果

样品名称 或编号	检测项目	测量单位	检测结果		
			起始点 (°C)	5%失重温度 (°C)	残留质量 (%)
YA2304-231201	热重分析	/	/	/	/
YA2304-240101	热重分析	/	/	/	/
YA2304-240102	热重分析	/	/	/	/
备注:	1. 测试条件: 1) 35°C~500°C, 升温速率 20°C/min; 2) 吹扫气: 氮气, 流量 50ml/min; 3) 保护气: 氮气, 流量 20ml/min。				

## 四、附图



YA2304-231201 热重分析图

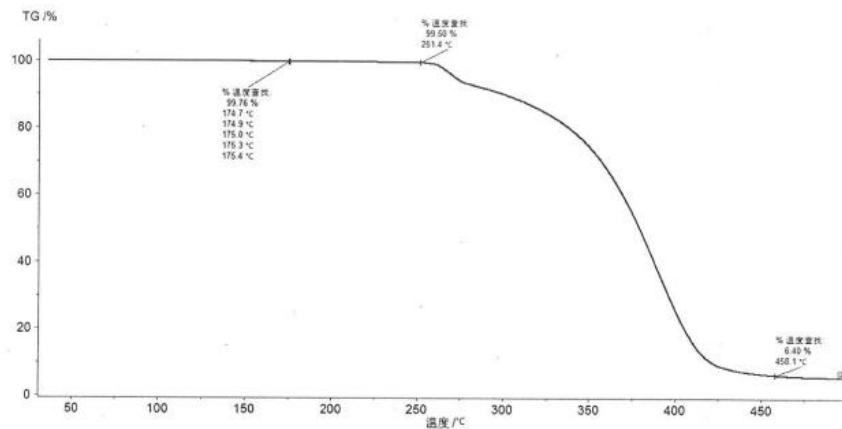
# Drug Master File of Finerenone

Document: YA2304-Quality

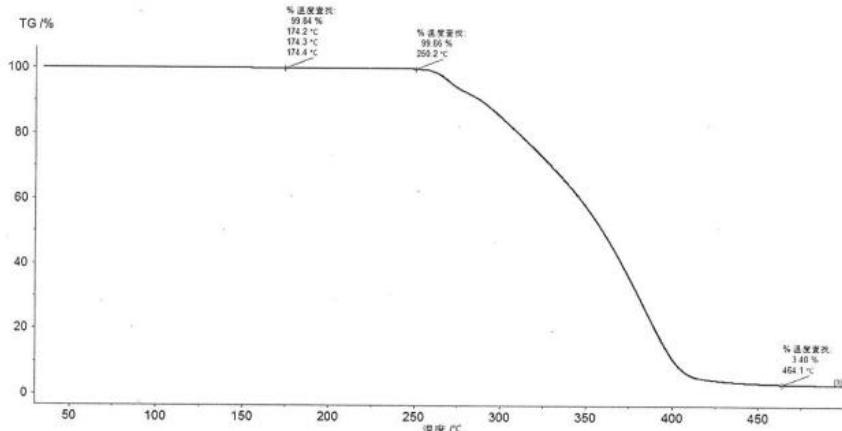
Version: 001

Date: Sep. 2024

TL/BG-20231109-03DT-02



YA2304-240101 热重分析图



YA2304-240102 热重分析图

备注：本报告检测地址为湖南省长沙市宁乡高新技术产业园区金洲北路 001 号

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第 5 页 共 5 页

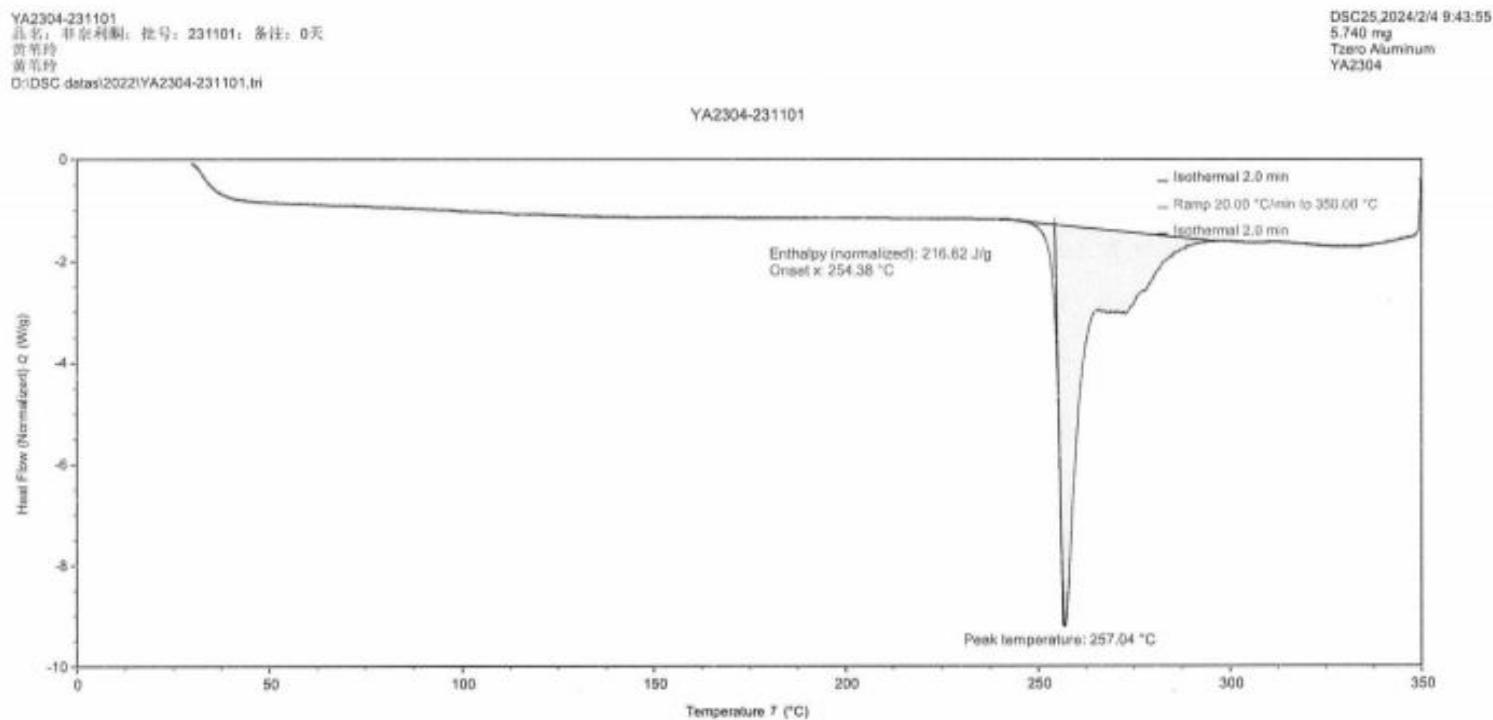
## Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

### 3.2.S.3.1 Annex 6 DSC pattern of Finerenone (batch No.: 231101, 231201, 240101, 240102)



TA Instruments Trios V5.1.1.46572

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## Drug Master File of Finerenone

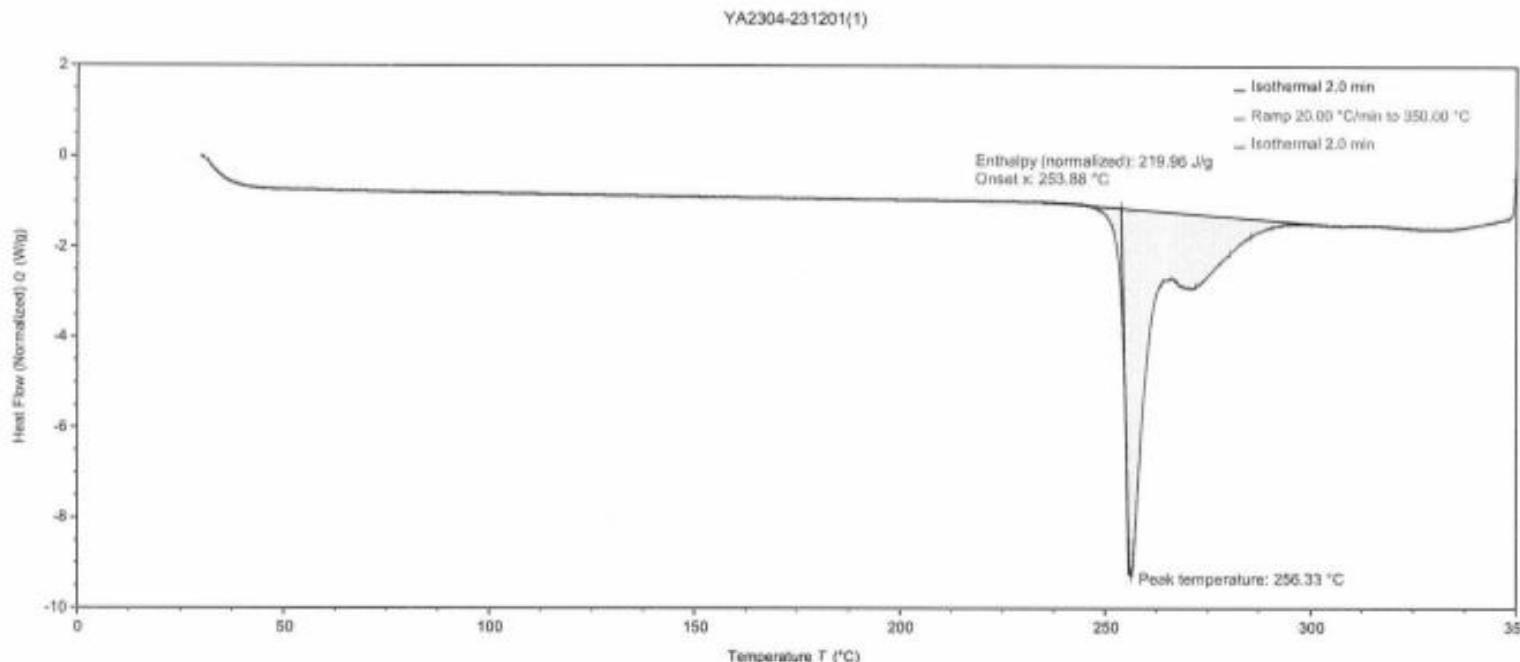
Document: YA2304-Quality

Version: 001

Date: Sep. 2024

YA2304-231201  
品名：非奈利酮 批号：231201 备注：0天  
黄伟玲  
黄伟玲  
D:\DSC\datas\2022\YA2304-231201(1).mz

DSC25,2024/2/6 10:35:34  
5.740 mg  
Tzero Aluminum  
YA2304



TA Instruments Trios V5.1.1.46572

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Hinye Pharmaceutical Co., Ltd.

## Drug Master File of Finerenone

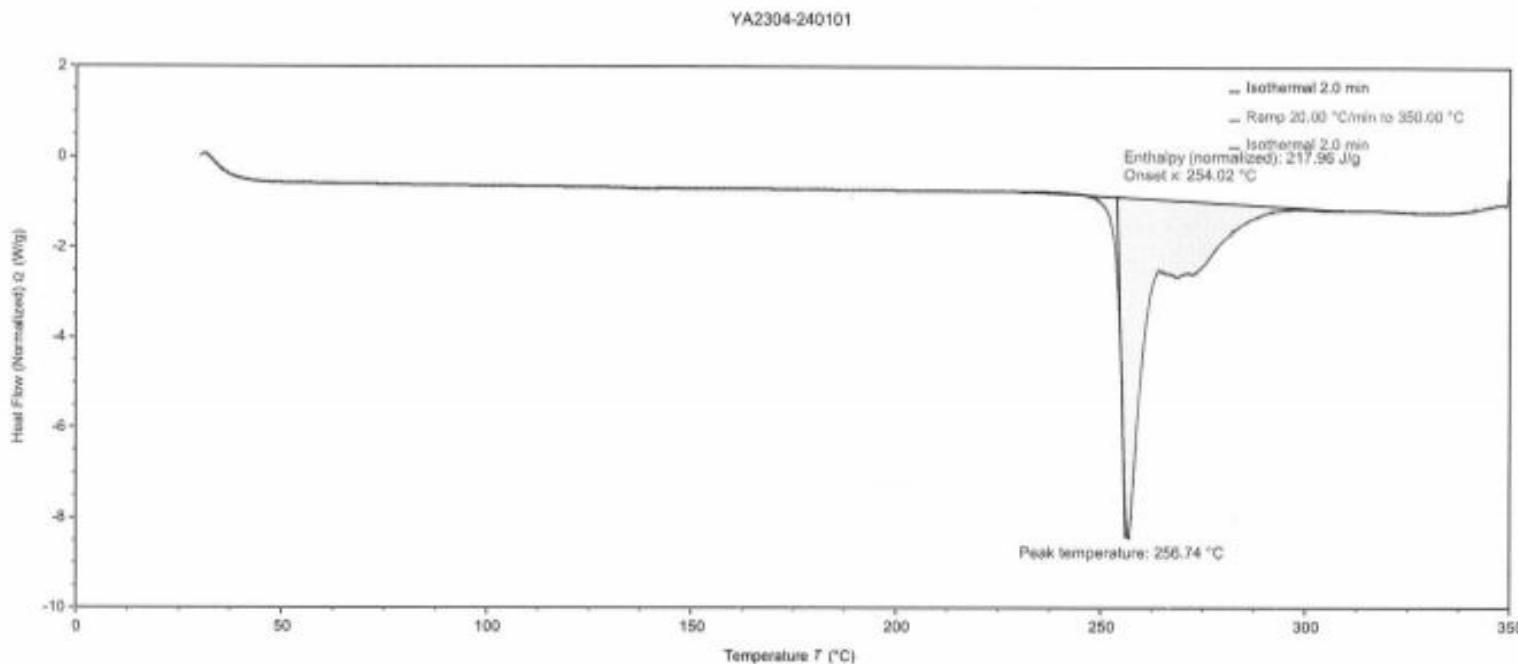
Document: YA2304-Quality

Version: 001

Date: Sep. 2024

YA2304-240101  
品名：非奈利酮 批号：240101 备注：0美  
黄伟玲  
黄伟玲  
D:\DSC\datas\2022\YA2304-240101.tri

DSC25,2024/2/4 11:03:06  
5.787 mg  
Tzero-Aluminum  
YA2304



TA Instruments Trios V5.1.1.46572

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## Drug Master File of Finerenone

Document: YA2304-Quality

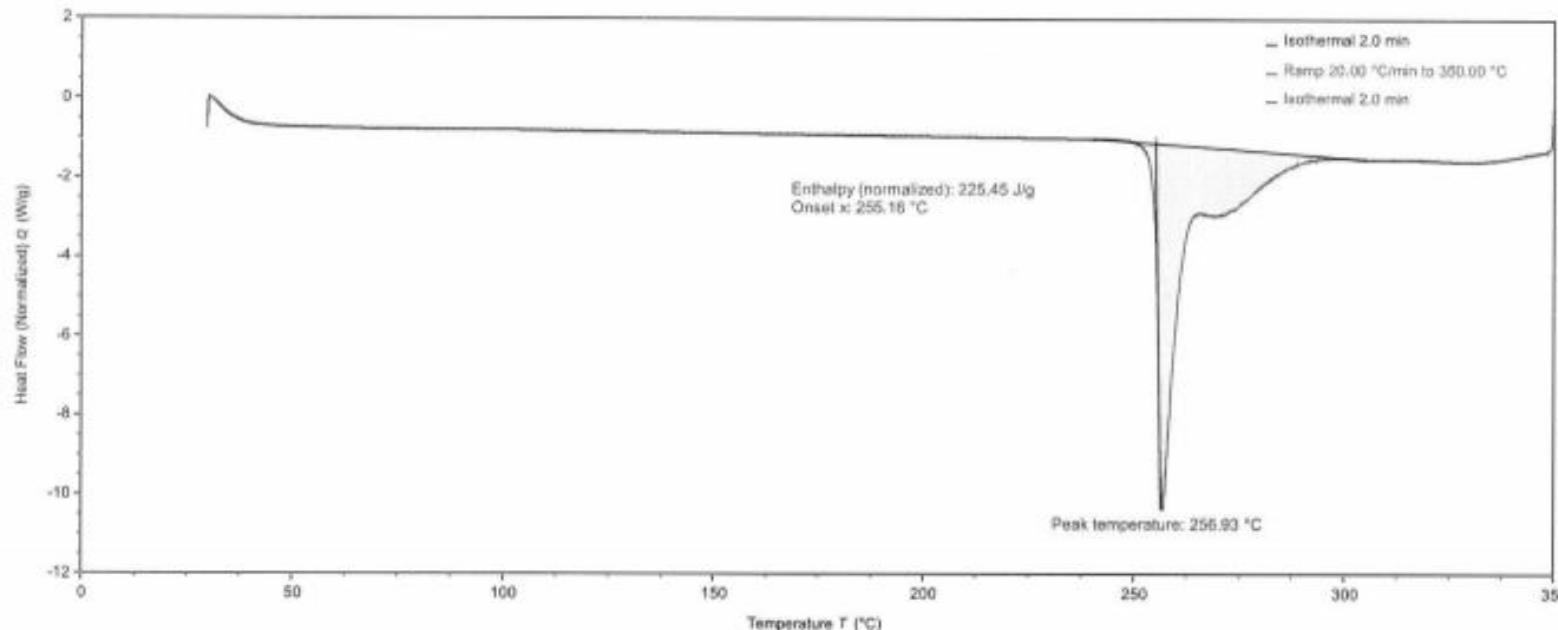
Version: 001

Date: Sep. 2024

YA2304-240102  
品名：卡托利酮；批号：240102；备注：0天  
黄晶玲  
黄晶玲  
D:\DSC\datas\2022\YA2304-240102.tr

DSC25,2024/2/4 11:31:51  
5.466 mg  
Tzero Aluminum  
YA2304

YA2304-240102



TA Instruments Trios V5.1.1.46572

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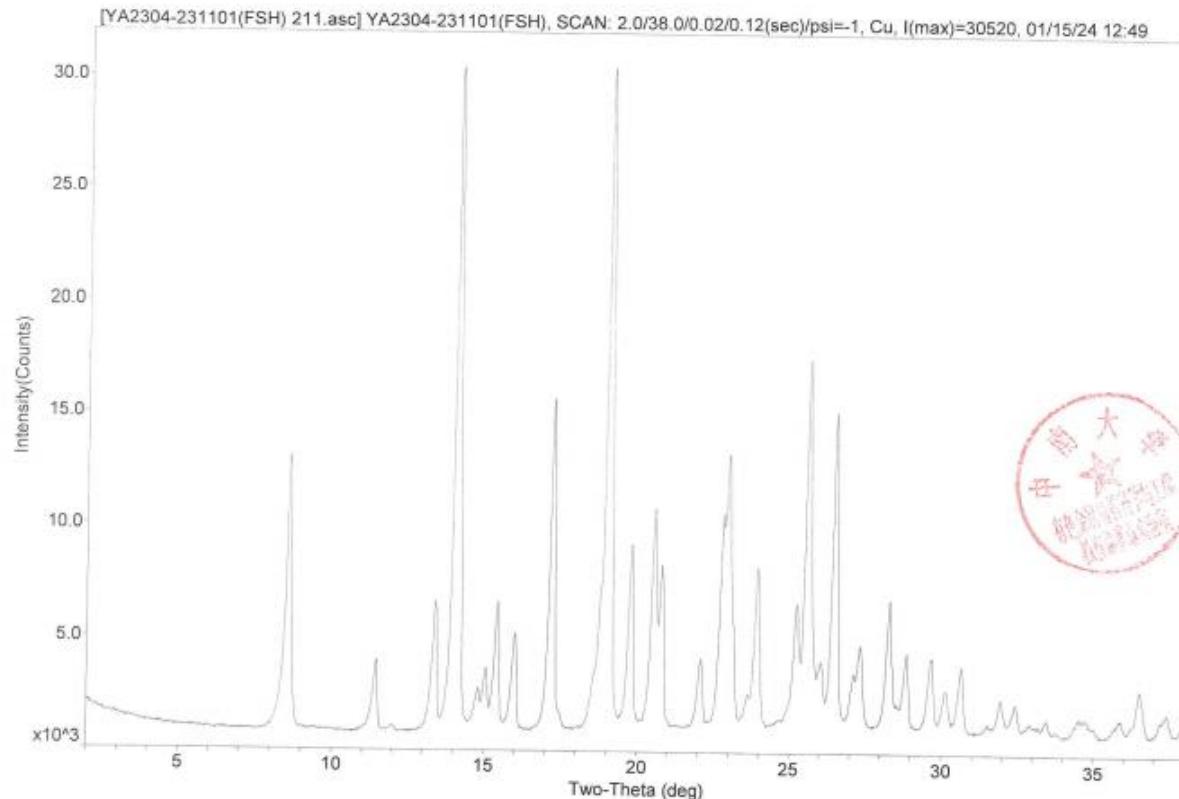
## Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

### 3.2.S.3.1 Annex 7 XRD pattern on Finerenone (batch No.: 231101)



Finerenone

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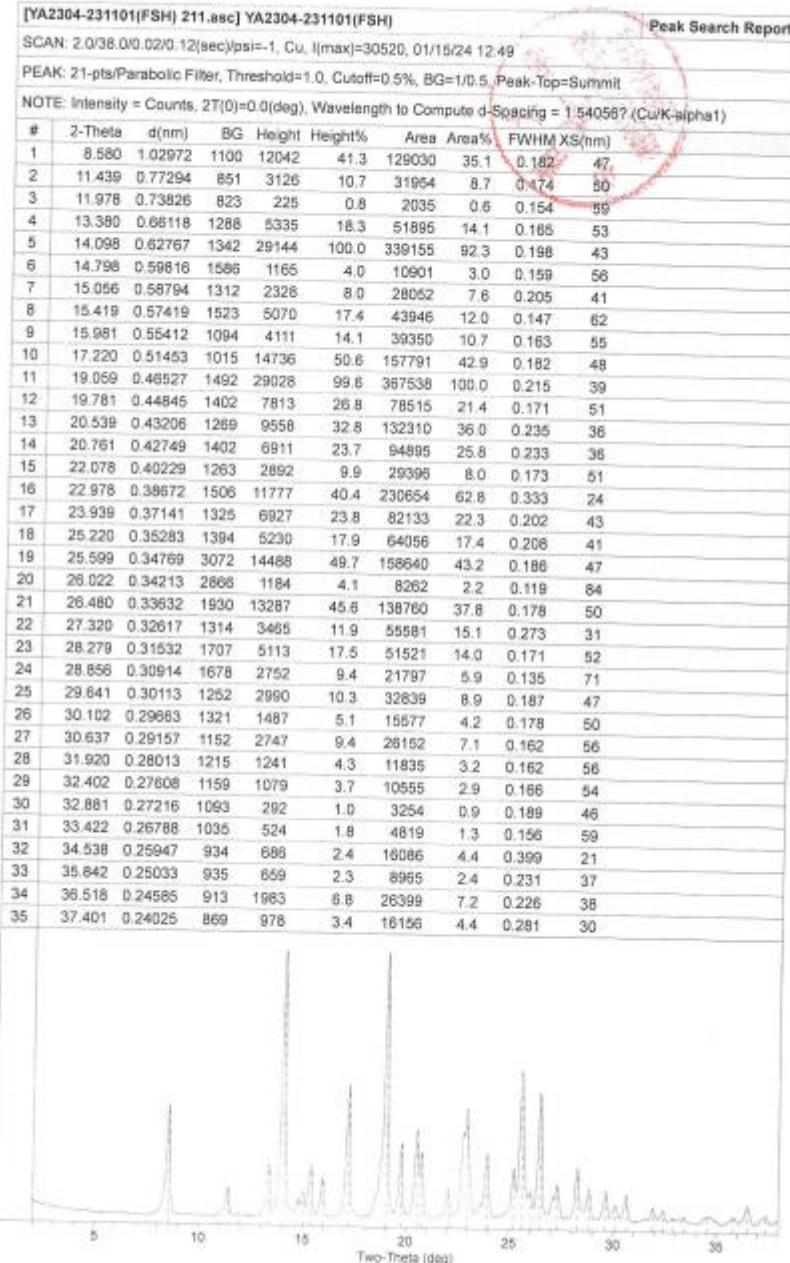
Hinye Pharmaceutical Co., Ltd.

# Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024



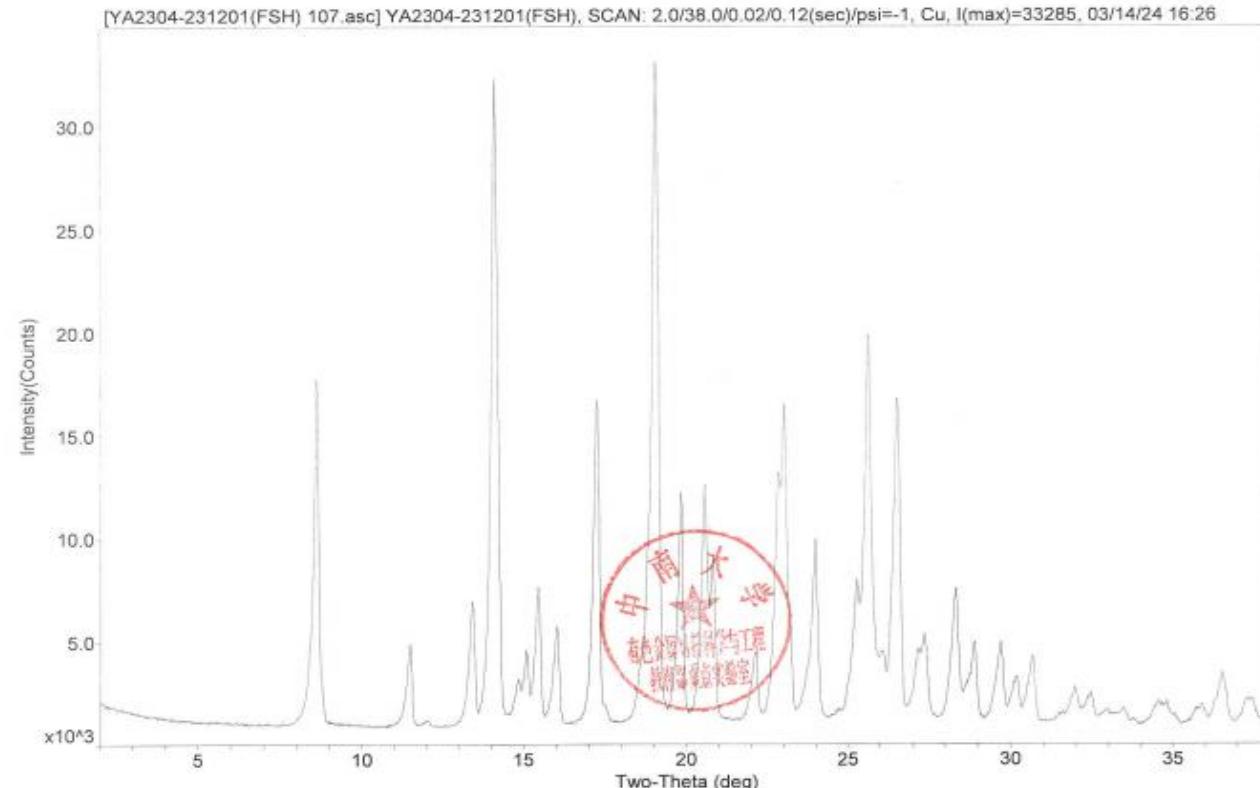
## Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

### 3.2.S.3.1 Annex 8 XRD pattern on Finerenone (batch No.: 231201)



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# Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024



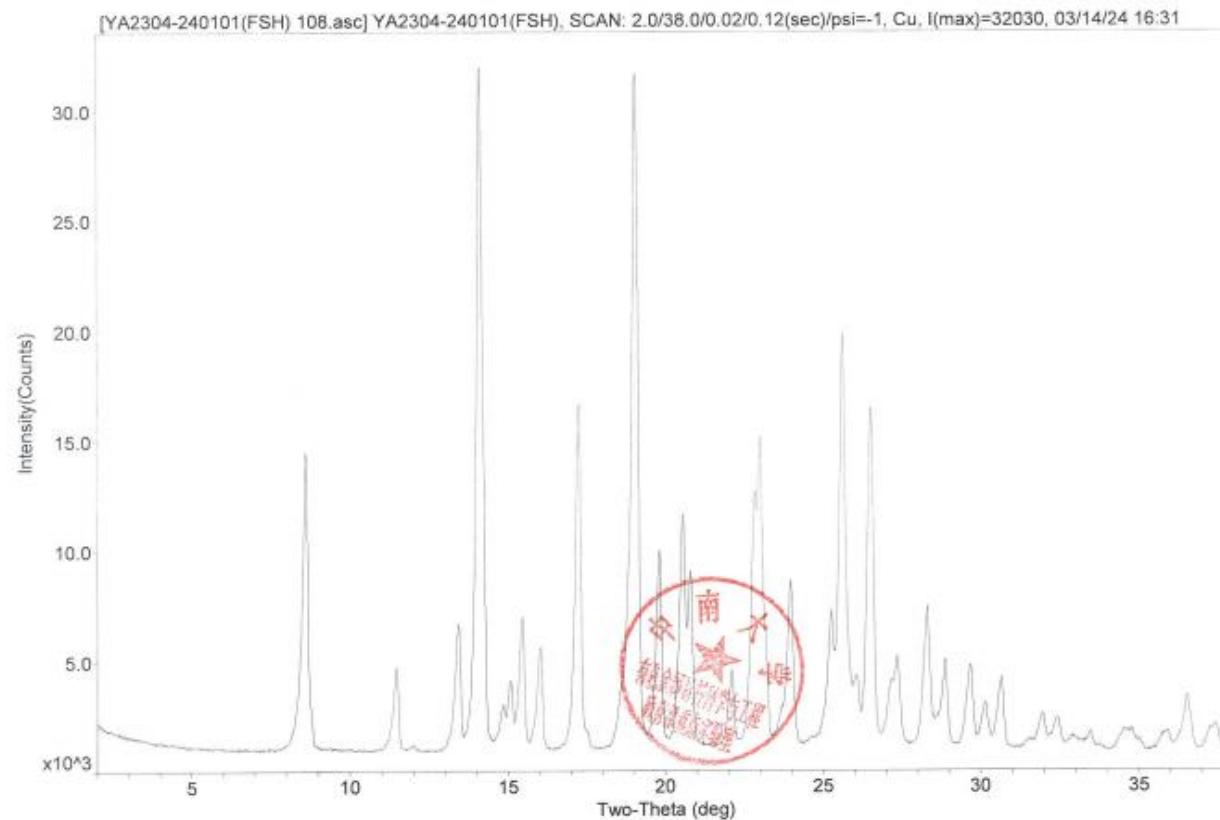
## Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

### 3.2.S.3.1 Annex 9 XRD pattern on Finerenone (batch No.: 240101)



Finerenone

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Hinye Pharmaceutical Co., Ltd.

# Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

[YA2304-240101(FSH) 108.asc] YA2304-240101(FSH)										Peak Search Report			
SCAN: 2.0/38.0/0.02/0.12(sec)\psi=1, Cu, I(max)=32039, 03/14/24 16:31													
PEAK: 23-pts/Quartic Filter, Threshold=3.0, Cutoff=0.1%, BG=3/1.2, Peak-Top=Summit													
NOTE: Intensity = Counts, 2T(0)=0.0(deg), Wavelength to Compute d-Spacing = 1.540567 (Cu/K-alpha1)													
#	2-Theta	d(?)	BG	Height	Height%	Area	Area%	FWHM	XSi(?)				
1	8.620	10.2491	928	13563	44.1	166844	34.0	0.209	407				
2	11.462	7.7140	869	3806	12.4	43325	8.8	0.194	446				
3	11.735	7.5349	868	124	0.4	2442	0.5	0.335	244				
4	12.002	7.3681	887	261	0.8	3168	0.6	0.208	414				
5	13.420	6.5922	1080	5581	18.2	68052	13.9	0.207	412				
6	14.138	6.2590	1297	30733	100.0	380829	77.7	0.211	405				
7	14.840	5.9648	1399	1578	5.1	31298	6.4	0.337	243				
8	15.062	5.8772	1291	2762	9.0	35935	7.3	0.221	383				
9	15.459	5.7271	1201	5740	18.7	63533	13.0	0.188	461				
10	16.037	5.5218	1198	4394	14.3	42826	8.7	0.166	537				
11	17.260	5.1335	968	15722	51.2	184940	37.7	0.200	430				
12	17.479	5.0697	968	895	2.9	37032	7.6	0.703	115				
13	18.622	4.7608	1308	2668	8.7	38688	7.9	0.247	341				
14	19.080	4.6478	1224	30563	99.4	463226	94.5	0.258	325				
15	19.332	4.5876	1288	499	1.6	41544	8.5	1.415	57				
16	19.838	4.4718	1559	8412	27.4	87136	17.8	0.176	500				
17	20.579	4.3123	1319	10289	33.5	209020	42.6	0.345	239				
18	20.801	4.2668	1264	7787	25.3	76853	15.7	0.158	531				
19	22.101	4.0186	1301	3207	10.4	32616	6.7	0.173	518				
20	22.840	3.8903	1414	11259	36.6	172213	35.1	0.260	324				
21	23.001	3.8635	1340	13801	44.9	490362	100.0	0.094	135				
22	23.683	3.7538	1458	1364	4.4	48064	9.8	0.509	136				
23	23.981	3.7108	1361	7215	23.5	93254	19.0	0.220	390				
24	25.242	3.5253	1217	6040	19.7	125007	25.5	0.352	236				
25	25.639	3.4716	1484	18395	59.9	275701	56.2	0.255	332				
26	26.080	3.4185	1880	2637	8.6	68775	14.2	0.450	183				
27	26.520	3.3583	1218	15281	49.7	194748	39.7	0.217	398				
28	27.161	3.2805	1508	2590	8.4	62208	12.7	0.408	203				
29	27.341	3.2593	1415	3743	12.2	63468	12.9	0.288	292				
30	28.319	3.1489	1191	6288	20.4	92354	18.8	0.251	340				
31	28.880	3.0890	1168	3863	12.6	57815	11.8	0.254	335				
32	29.698	3.0057	1136	3802	11.7	49714	10.1	0.235	367				
33	30.159	2.9808	1064	1978	6.4	32710	8.7	0.281	302				
34	30.659	2.9136	1373	2796	9.1	25903	5.3	0.157	582				
35	31.578	2.8309	1028	374	1.2	6597	1.3	0.300	283				
36	31.978	2.7964	1098	1413	4.8	29061	5.9	0.350	241				
37	32.440	2.7577	1039	1258	4.1	24519	5.0	0.331	255				
38	32.936	2.7172	1140	388	1.3	8013	1.6	0.351	241				
39	33.461	2.6758	1010	893	2.3	10915	2.2	0.268	321				
40	34.521	2.5560	864	949	3.1	28486	5.8	0.510	185				
41	34.780	2.5773	865	998	3.2	28485	5.8	0.485	173				
42	35.018	2.5603	867	512	1.7	12586	2.6	0.418	202				
43	35.917	2.4982	890	844	2.7	19365	3.9	0.390	217				
44	36.521	2.4583	911	2436	7.9	39048	8.0	0.273	317				
45	37.458	2.3989	1047	972	3.2	18234	3.7	0.319	269				

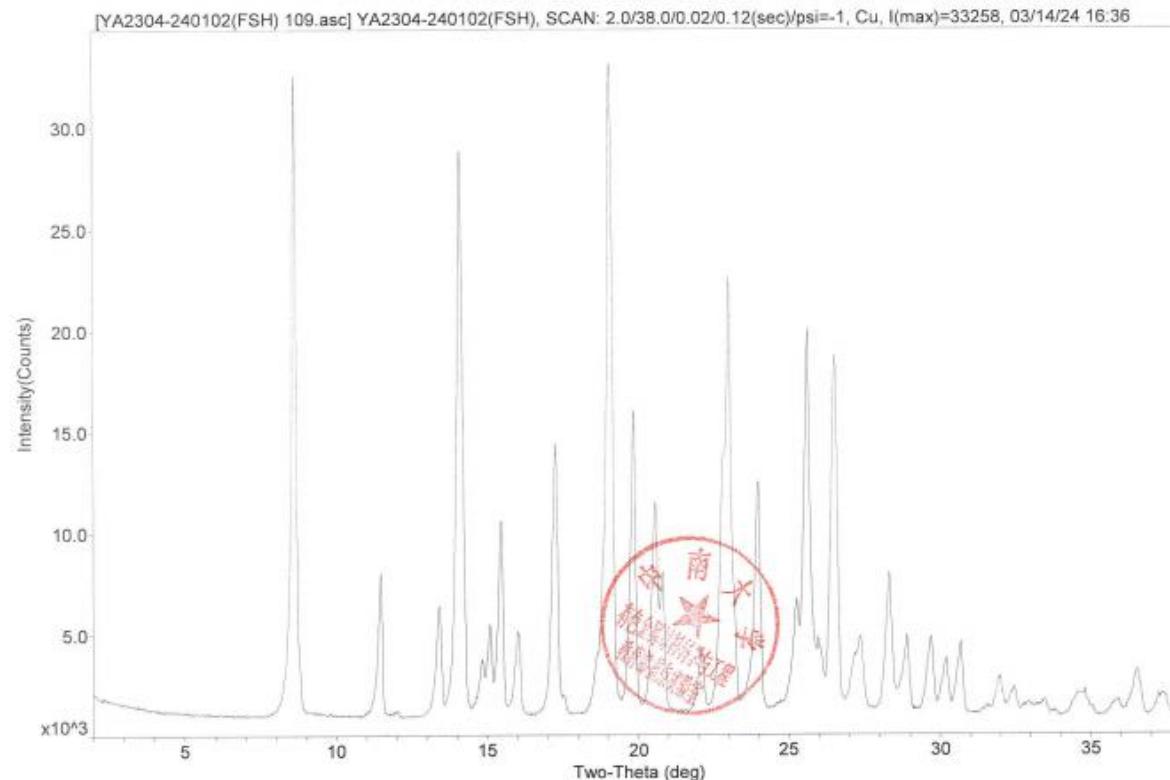
## Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

### 3.2.S.3.1 Annex 10 XRD pattern on Finerenone (batch No.: 240102)



Finerenone

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Hinye Pharmaceutical Co., Ltd.

# Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

[YA2304-240102(FSH) 109.asc] YA2304-240102(FSH)										Peak Search Report
SCAN: 2.0/38.0/0.02/0.12(sec)\psi=-1, Cu,  max =33258, 03/14/24 18:36										
PEAK: 23-pts/Quartic Filter, Threshold=3.0, Cutoff=0.1%, BG=3/1.2, Peak-Top=Summit										
NOTE: Intensity = Counts, 2T(0)=0.0(deg), Wavelength to Compute d-Spacing = 1.540567 (Cu/K-alpha1)										
#	2-Theta	d(?)	BG	Height	Height%	Area	Area%	FWHM	XS(?)	
1	8.656	10.2071	957	31702	99.2	313437	66.5	0.168	528	
2	8.825	10.0122	933	1506	4.7	21169	4.5	0.239	351	
3	8.971	9.8490	978	200	0.6	9757	2.1	0.829	96	
4	11.498	7.6898	902	7088	22.2	68486	14.5	0.164	543	
5	11.803	7.4919	898	155	0.5	4073	0.9	0.447	181	
6	12.038	7.3461	894	297	0.9	3777	0.8	0.216	393	
7	13.420	6.5922	1107	5251	16.4	60302	12.8	0.195	441	
8	14.139	6.2588	1272	27609	86.4	343033	72.8	0.211	403	
9	14.579	6.0709	1355	126	0.4	4595	1.0	0.620	130	
10	14.839	5.9849	1377	2317	7.2	46825	9.9	0.344	239	
11	15.100	5.8626	1236	4197	13.1	55946	11.9	0.227	373	
12	15.478	5.7201	1185	9404	29.4	100797	21.4	0.182	479	
13	16.022	5.5273	1232	3862	12.1	38874	8.2	0.170	520	
14	17.260	5.1333	1008	13393	41.9	162702	34.5	0.207	415	
15	17.520	5.0579	1012	913	2.9	34731	7.4	0.847	125	
16	18.622	4.7609	1403	2441	7.6	30433	6.5	0.212	403	
17	19.081	4.6475	1294	31964	100.0	471296	100.0	0.251	335	
18	19.332	4.5875	1344	573	1.8	45099	9.8	1.368	60	
19	19.859	4.4670	1665	14386	45.0	141932	30.1	0.168	531	
20	20.198	4.3928	1486	149	0.5	15188	3.2	1.733	47	
21	20.580	4.3121	1409	10083	31.5	194653	41.3	0.329	251	
22	20.819	4.2632	1350	6682	20.9	64424	13.7	0.184	546	
23	22.119	4.0155	1354	2817	8.8	31540	6.7	0.190	458	
24	22.860	3.8869	1552	12234	38.3	205327	43.6	0.285	293	
25	23.038	3.8573	1416	21251	66.5	359056	78.2	0.287	291	
26	23.999	3.7049	1441	11034	34.5	134671	28.6	0.207	416	
27	25.279	3.5202	1283	5389	16.9	104974	22.3	0.331	252	
28	25.640	3.4715	1523	18570	58.1	266549	56.6	0.244	348	
29	25.980	3.4261	1670	3108	9.7	115712	24.6	0.633	130	
30	26.540	3.3558	1288	17451	54.6	233673	49.6	0.228	377	
31	27.181	3.2780	1575	2409	7.5	70881	15.0	0.500	165	
32	27.360	3.2571	1492	3311	10.4	57816	12.3	0.297	283	
33	28.319	3.1488	1258	6688	20.9	90782	19.3	0.231	372	
34	28.898	3.0871	1246	3604	11.3	53104	11.3	0.250	341	
35	29.681	3.0074	1213	3580	11.2	47409	10.1	0.225	384	
36	30.198	2.9571	1139	2607	8.2	40489	8.6	0.264	323	
37	30.677	2.9120	1471	3077	9.6	26709	5.7	0.148	631	
38	31.597	2.8293	1096	287	0.9	6022	1.3	0.357	236	
39	31.978	2.7984	1160	1721	5.4	29179	6.2	0.288	295	
40	32.459	2.7580	1061	1264	4.0	21759	4.6	0.293	291	
41	32.940	2.7169	1196	345	1.1	12422	2.6	0.612	136	
42	33.479	2.6744	1047	629	2.0	12534	2.7	0.339	250	
43	34.580	2.5917	900	1110	3.5	32862	6.9	0.500	168	
44	34.817	2.5747	897	1288	4.0	32836	7.0	0.433	195	
45	35.918	2.4982	916	729	2.3	17253	3.7	0.402	211	
46	36.540	2.4571	943	2210	6.9	39855	8.5	0.307	280	
47	37.419	2.4013	1014	808	2.8	17690	3.8	0.331	259	

## Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

### 3.2.S.3.1 Annex 11 Elemental analysis on Finerenone (batch No.: 231101)



## 分析检测报告

### REPORT FOR ANALYZING & TESTING

报告编号: 20240044E

Report No.

样品名称: YA2304-231101

Sample Name

委托单位: 天地恒一制药股份有限公司

Customer

委托单位地址: 湖南省长沙国家生物产业基地康天路  
Address 109 号

检测类别: 委托  
Testing Type



中山大学分析测试中心

INSTRUMENTAL ANALYSIS & RESEARCH CENTER

SUN YAT-SEN UNIVERSITY

地址(address): 中国广州市新港西路 135 号 (135 Xingangxi Road, Guangzhou, China)

电话(Tel): 020-84113210

电子邮箱(E-mail): iarc@mail.sysu.edu.cn

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# Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024



样品名称 Sample Name	YA2304-231101	样品数量 Quantity	1个
样品外观 Physical form	白色粉末	接样日期 Date for acceptance	2024.01.30
分析项目 Items for analyzing	元素分析	检测日期 Date for testing	2024.02.27

## 分析检测结果 Results

送检样品 YA2304-231101 的 C、H、N 元素含量测定结果如下：

元素	C	H	N
质量分数 (%)	66.57	5.95	14.96
RSD (%)	0.1	1.5	0.2

(以下空白)

分析方法(引用标准) Methods (Standards) for analyzing	JY/T 0580-2020 元素分析仪分析方法通则 检测仪器: Elementar Vario EL cube 元素分析仪
备注 Remarks	/

检测人  
Operator

王海波

签发人  
Approver

李红伟

日期  
Date

2024 年 02 月 29 日

### 声明:

- 本分析检测报告样品名称、批号(标识)由送样单位提供,本中心不负责其真伪;检测结果仅对送检样负责。如对报告内容有异议,请在报告发出之日起(以邮戳日期为准)15个工作日内向本中心提出,过期恕不受理。
- 本分析检测报告复印件未加盖本中心红色分析测试专用章无效,任何涂改增删无效。单独抽出某些页导致误解或用于其他用途而造成的后果,本中心不承担责任。
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IARC/SYSU-410-54

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## **Drug Master File of Finerenone**

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Date: Sep. 2024

### **3.2.S.3.2 Impurities**

The applicant combined the registration process to analyze the impurity spectrum of Finerenone, in which there may be organic impurities, inorganic impurities, residual solvents, metal impurities and potential genotoxic impurities, five categories of impurities, and the source, destination and related control strategies of the above five categories of impurities have been analyzed and studied as follows:

#### **3.2.S.3.2.1 Organic Impurities**

The organic impurities in Finerenone mainly come from the starting materials and intermediates, side reaction products and degradation products that are not completely reacted into the starting materials during the reaction process. For the information of the impurities, see Table 3.2.S.3.2.1-1 Organic impurities spectrum of Finerenone. Of the impurities, YA2304-23, YA2304-24, YA2304-33, YA2304-43, YA2304-38, YA2304-40, YA2304-41, YA2304-1, YA2304-4, 4-dimethylamino pyridine, YA2304-47, sulfate impurities and nitrosamines impurities With a cautionary structure, the applicant does not make a specific analysis in this section, but a separate description and analysis in section 3.2.S.3.2.5 of Genotoxic Impurities.

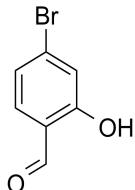
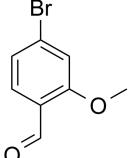
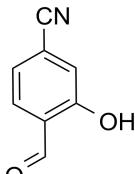
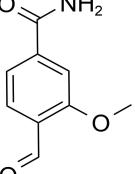
## Drug Master File of Finerenone

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Table 3.2.S.3.2-1 Potential Organic Impurities in Drug Substance Finerenone

<b>Names of impurities</b>	<b>Chemical name</b>	<b>Chemical structure formula</b>	<b>Impurity source</b>	<b>Control limits</b>	<b>Whether to set quality standards</b>
YA2304-23	4-bromo-2-hydroxybenzaldehyde		Impurity introduced by starting material YA2304-1	N/A	no (Including warning structure, see "3.2.S.3.2.5 Genotoxic Impurities" section for relevant control strategies)
YA2304-24	4-bromo-2-methoxybenzaldehyde		Impurity introduced by starting material YA2304-1	N/A	no (Including warning structure, see "3.2.S.3.2.5 Genotoxic Impurities" section for relevant control strategies)
YA2304-33	4-cyano-2-hydroxybenzaldehyde		Impurity introduced by starting material YA2304-1	N/A	no (Including warning structure, see "3.2.S.3.2.5 Genotoxic Impurities" section for relevant control strategies)
YA2304-43	4-aminoformyl-2-methoxybenzaldehyde		Impurity introduced by starting material YA2304-1	N/A	no (Including warning structure, the relevant control strategies are detailed in section)

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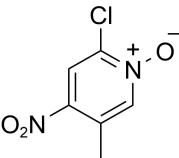
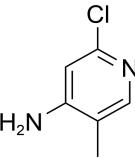
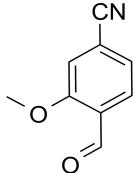
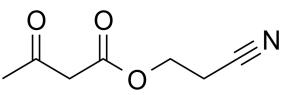
					3.2.S.3.2.5 Genotoxic Impurities)
YA2304-38	4-methylene-2-oxacyclobutanone		Impurity introduced by starting material YA2304-2	N/A	no (Including warning structure, see "3.2.S.3.2.5 Genotoxic Impurities" section for relevant control strategies)
YA2304-39	3-hydroxypropionitrile		Impurity introduced by starting material YA2304-2	N/A	no (The impurity has been controlled by no more than 5.0% in the starting material YA2304-2 and is no longer controlled in the finished product)
YA2304-57	2-cyanoethyl acetate		Impurity introduced by starting material YA2304-2	N/A	no (The impurity has been controlled by no more than 2.0% in the starting material YA2304-2 and is no longer controlled in the finished product)

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YA2304-40	2-chloro-5-methyl-4-nitropyridine-n-oxide		Impurities introduced by starting material YA2304-4	NA	no (Including warning structure, see "3.2.S.3.2.5 Genotoxic Impurities" section for relevant control strategies)
YA2304-41	2-chloro-4-amino-5-methylpyridine		Impurity introduced by starting material YA2304-4	N/A	no (Including warning structure, see "3.2.S.3.2.5 Genotoxic Impurities" section for relevant control strategies)
YA2304-1	4-cyano-2-methoxybenzaldehyde		Incompletely reacted starting material in the preparation of intermediate YA2304-5	N/A	no (Including warning structure, see "3.2.S.3.2.5 Genotoxic Impurities" section for relevant control strategies)
YA2304-2	2-cyanoacetoacetate ethyl ester		Incompletely reacted starting material in the preparation of intermediate YA2304-5	N/A	no (The impurity has been controlled not more than 0.5% in the intermediate YA2304-5 and is no longer controlled in the finished product)

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YA2304-3	2-cyanoethyl 2-(4-cyano-2-methoxybenzyl) -3-oxy-butyrate		An incomplete intermediate product in the preparation of intermediate YA2304-5	N/A	no (The impurity has been controlled less than 1.0% in the intermediate YA2304-5 and is no longer controlled in the finished product)
YA2304-4	4-amino-5-methyl-2-hydroxypyridine		Incomplete starting material for preparation of intermediate YA2304-5	N/A	no (Including warning structure, see "3.2.S.3.2.5 Genotoxic Impurities" section for relevant control strategies)
YA2304-47	4-amino-5-methyl-2-methoxypyridine		Derived impurity of starting material YA2304-4	N/A	no (Including warning structure, see "3.2.S.3.2.5 Genotoxic Impurities" section for relevant control strategies)
YA2304-5	4 - (4 - cyano - 2 - methoxy phenyl) - 2, 8 - dimethyl - 5 - ,4,5,6 oxygen generation - 1-4 h - 1, 6 - nalidixic - 3-2 - ethyl cyano formate		During the preparation of intermediate YA2304-8, the intermediate was not completely reacted	N/A	no (The impurity has been controlled not more than 0.3% in the intermediate YA2304-8, and is no longer controlled in the finished product)

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YA2304-6	4 - (4 - cyano - 2 - methoxy phenyl) - 5 - ethoxy - 2, 8 - dimethyl - 1, 4-2 h - 1, 6 - nalidixic - 3-2 - ethyl cyano formate		Incomplete intermediate product in the preparation of intermediate YA230 4-8	N/A	no (The impurity has been controlled not more than 0.3% in the intermediate YA2304-8, and is no longer controlled in the finished product)
YA2304-7	4-(4-cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-nalidixic-3-carboxylic acid		Incomplete intermediate product in the preparation of intermediate YA230 4-8	N/A	no (The impurity has been controlled not more than 0.15% in the intermediate YA2304-8, and is no longer controlled in the finished product)
YA2304-55	4 - (4 - cyano - 2 - methoxy phenyl) - 2, 8 - dimethyl - 5 - ,4,5,6 oxygen generation - 1-4 h - 1, 6 - nalidixic - 3 - carbonyl imidazole		Incomplete intermediate product in the preparation of intermediate YA230 4-8	N/A	no (The impurity has been controlled not more than 0.20% in the intermediate YA2304-8, and is no longer controlled in the finished product)
4-dimethylaminopyridine (DMAP)	4-dimethylaminopyridine (DMAP)		Reaction reagent in the synthesis of intermediate YA2304-8	N/A	no (Including warning structure, see "3.2.S.3.4.2.5 Genotoxic Impurities" section for relevant control strategies)

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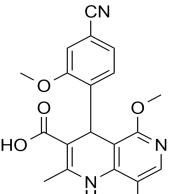
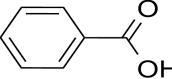
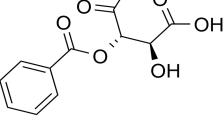
					s)
YA2304-10	D- (+) -dibenzoyltartaric acid		Resolution reagent in the synthesis of intermediate YA2304-CP	≤0.10%	is (Finished products are controlled according to specific impurities)
YA2304-12	4 - (4 - amino formyl - 2 - methoxy phenyl) - 5 - ethoxy - 2, 8 - dimethyl - 1, 4-2 h - 1, 6 - nalidixic - 3 - formamide		Side reaction product in the synthesis of the intermediate YA2304-CP	≤0.10%	is (Controlled by non-specific impurities in finished product)
YA2304-14	4 - (4 - cyano - 2 - methoxy phenyl) - 5 - methoxy - 2, 8 - dimethyl - 1, 4-2 h - 1, 6 - nalidixic - 3 - formamide		Side reaction products	≤0.15%	is (Finished products are controlled by specific impurities)
YA2304-15	4 - (4 - cyano - 2 - methoxy phenyl) - 5-2 - isopropyl oxygen radicals, 8 - dimethyl - 1, 4-2		Side reaction products	≤0.15%	is (Finished products are controlled by specific impurities)

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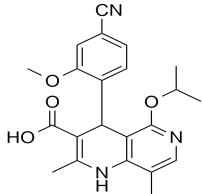
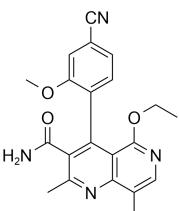
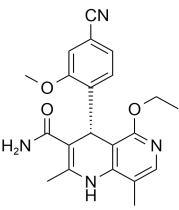
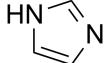
	h - 1, 6 - nalidixic - 3 - formamide				
YA2304-16	4-(4-cyano-2-methoxyphenyl)-5-methoxy-2,8-dimethyl-1,4-dihydro-1,6-nalididine-3-carboxylic acid		Side reaction products	≤0.10%	is (Controlled by non-specific impurities in finished product)
YA2304-17	Benzoic acid		A by-product in the synthesis of the intermediate YA2304-CP	N/A	no (The impurity is controlled as a specific impurity in the crude product, the limit is not more than 0.15%, and the finished product is no longer controlled)
YA2304-52	D-(+)-monobenzoyl tartaric acid		A by-product in the synthesis of the intermediate YA2304-CP	N/A	no (Has been in the finished Finerenone through multiple batches of statistical proof can be completely removed, the finished product is no longer controlled)

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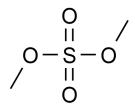
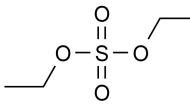
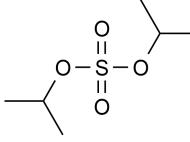
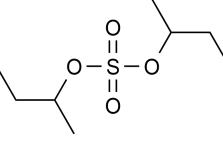
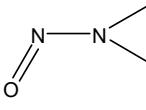
YA2304-18	4 - (4 - cyano - 2 - methoxy phenyl) - 5-2 - isopropyl oxygen radicals, 8 - dimethyl - 1, 4-2 h - 1, 6 - nalidixic - 3 - formic acid		Side reaction products	≤0.10%	is (Controlled by non-specific impurities in finished product)
YA2304-19	4-(4-cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,6-nalididine-3-formamide		Side reaction products	≤0.15%	is (Finished products are controlled by specific impurities)
YA2304-20	4 - (4 - (4 r) - cyano - 2 - methoxy phenyl) - 5 - ethoxy - 2, 8 - dimethyl - 1, 4-2 h - 1, 6 - nalidixic - 3 - formamide		enantiomers	≤0.15%	is (Finished products are controlled by specific impurities)
imidazole	Imidazole		Reaction byproducts and hydrolysates of CDI	N/A	no (has been completely removed in the finished finerenone through multiple batches of statistical proof, no longer controlled in the finished product)

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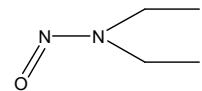
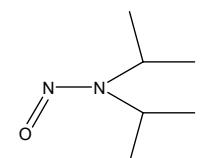
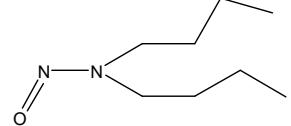
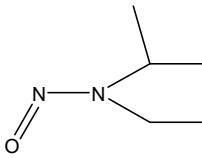
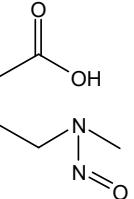
Dimethyl sulfate	Dimethyl sulfate		Side reaction products	≤75ppm	is (Including the warning structure, the relevant control strategy is detailed in the section "3.2.S.3.4.2.5 Genotoxic Impurities")
Diethyl sulfate	Diethyl sulfate		Side reaction products	≤75ppm	is (Including the warning structure, the relevant control strategy is detailed in the section "3.2.S.3.4.2.5 Genotoxic Impurities")
Diisopropyl sulfate	Diisopropyl sulfate		Side reaction products	≤75ppm	is (Including the warning structure, the relevant control strategy is detailed in the section "3.2.S.3.4.2.5 Genotoxic Impurities")
Di-sec-butyl sulfate	Di-sec-butyl sulfate		Side reaction products	≤75ppm	is (Including the warning structure, the relevant control strategy is detailed in the section "3.2.S.3.4.2.5 Genotoxic Impurities")
NDMA	N-nitrosodimethyl amine		Side reaction product	N/A	no (No longer controlled in finished finerenone after

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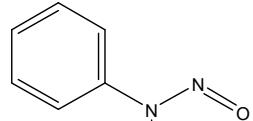
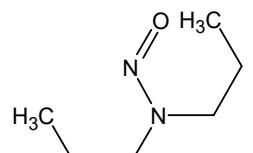
					multiple batch statistics prove non-existence)
NDEA	N-nitrosodiethylamine		Side reaction products	N/A	no (No longer controlled in finished finerenone after multiple batch statistics prove non-existence)
NDIPA	N-nitrosodiisopropylamine		Side reaction products	N/A	no (No longer controlled in finished finerenone after multiple batch statistics prove non-existence)
NDBA	N-nitrosodibutylamine		Side reaction products	N/A	no (No longer controlled in finished finerenone after multiple batch statistics prove non-existence)
NIEPA	N-nitrosoethylisopropylamine		Side reaction products	N/A	no (No longer controlled in finished finerenone after multiple batch statistics prove non-existence)
NMBA	N-nitroso-methyl-4-aminobutyric acid		Side reaction products	N/A	no (No longer controlled in finished finerenone after multiple batch statistics prove non-existence)

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NMPA	N-nitrosotoluidine		Side reaction products	N/A	no (No longer controlled in finished finerenone after multiple batch statistics prove non-existence)
NDPA	N-nitrosodipropyl amine		Side reaction products	N/A	no (No longer controlled in finished finerenone after multiple batch statistics prove non-existence)

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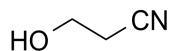
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### (1) YA2304-39

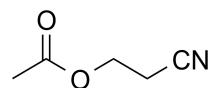
YA2304-39 is the incomplete raw material in the synthesis of the starting material YA2304-2. Its molecular structure formula is as follows:



The Applicant intends to control YA2304-39 as a specific impurity by no more than 5.0% in the starting material YA2304-2 and calculate the impurity content by self-comparison method with correction factor. Refer to the validation report of YA2304-2 Related Substance Methodology under item 3.2.2.3.6.1 that YA2304-39 has a correction factor of 0.9 relative to YA2304-2. At the same time, the reasonability of the impurity limit in the standard of the applicant's design of the impurity experiment is investigated. The investigation results show that the removal capacity of the registered process for impurity YA2304-39 is NLT 5.0%. The experimental process is detailed in section 3.2.S.2.3.5.2. Therefore, impurity YA2304-39 is no longer controlled in the finished Finerenone.

### (2) YA2304-57

YA2304-57 is a by-product in the synthesis of the starting material YA2304-2. Its molecular structure formula is as follows:



The applicant intends to control YA2304-57 as a specific impurity in the starting material YA2304-2 by no more than 2.0% and calculate the impurity content by self-comparison method with correction factor. Refer to the validation report of YA2304-2 Related Substance Methodology under item 3.2.2.3.6.1 that YA2304-57 has a correction factor of 0.9 relative to YA2304-2. At the same time, the applicant designed an experiment to

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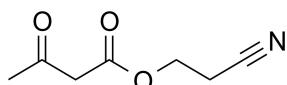
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investigate the reasonability of the impurity limit in the standard for adding impurities, and the investigation results show that the removal capacity of the registered process for impurity YA2304-57 is NLT 2.0%. The experimental process is detailed in section 3.2.S.2.3.5.2. Therefore, the impurity YA2304-57 is no longer controlled in the finished Finerenone.

### **(3) YA2304-2**

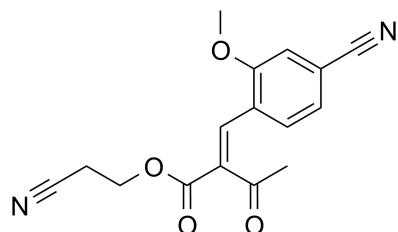
YA2304-2 is the incomplete starting material in Finerenone synthesis. Its molecular structure formula is as follows:



The applicant intends to use YA2304-2 as a specific impurity in the intermediate YA2304-5 to control no more than 0.5%, and calculate the impurity content by external standard method. At the same time, the applicant designed an experiment to investigate the rationality of the impurity limit in the standard for adding impurities, and the investigation results show that the removal capacity of the registration process for impurity YA2304-2 is NLT 0.5%. The experimental process is detailed in section 3.2.S.2.4.3.1.1. Therefore, impurity YA2304-2 is no longer controlled in the finished Finerenone.

### **(4) YA2304-3**

YA2304-3 is an intermediate product in the synthesis of the intermediate YA2304-5. Its molecular structure formula is as follows:



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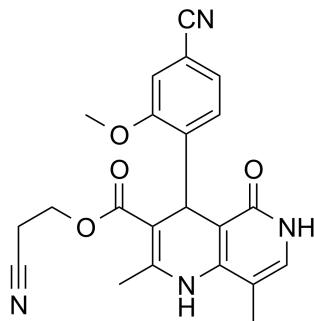
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The applicant intends to control YA2304-3 as a specific impurity not exceeding 1.0% in the intermediate YA2304-5, and calculate the impurity content by self-comparison method with correction factor. Refer to the validation report of YA2304-5 on Substance Methodology under item 3.2.S.2.4.3.1.3.1 that YA2304-3 has a correction factor of 2.4 relative to YA2304-5. At the same time, the reasonableness of the impurity limit in the test standard for impurity addition designed by the applicant shows that the removal ability of the registration process for impurity YA2304-3 is NLT 1.0%. The experimental process is detailed in section 3.2.S.2.4.3.1.1. Therefore, impurity YA2304-3 is no longer controlled in the finished finalizone.

### **(5) YA2304-5**

YA2304-5 is an intermediate in Finerenone synthesis. Its molecular structure formula is as follows:



The applicant intends to control YA2304-5 as a specific impurity not exceeding 0.3% in the intermediate YA2304-8, and calculate the impurity content by self-comparison method with correction factor. Refer to the validation report of YA2304-8 on Substance Methodology under item 3.2.S.2.4.3.2.3. The correction factor of YA2304-5 relative to YA2304-8 is 1.2. At the same time, the reasonability of the impurity limit in the test standard for impurity addition designed by the applicant shows that the removal capacity of the registration process for impurity YA2304-5 is NLT 0.3%, and

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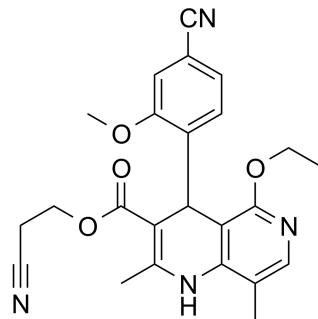
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the experimental process is detailed in section 3.2.S.2.4.3.2.1. Therefore, impurity YA2304-5 is no longer controlled in the finished Finerenone.

### **(6) YA2304-6**

YA2304-6 is an intermediate product in the synthesis of the intermediate YA2304-8. Its molecular structure formula is as follows:



The applicant intends to control YA2304-6 as a specific impurity not exceeding 0.3% in the intermediate YA2304-8, and calculate the impurity content by self-comparison method with correction factor. Refer to the validation report of YA2304-8 Related Substance Methodology under item 3.2.S.2.4.3.2.3, YA2304-6 has a correction factor of 1.1 relative to YA2304-8. At the same time, the reasonability of the impurity limit in the test standard for impurity addition designed by the applicant shows that the removal capacity of the registration process for impurity YA2304-6 is NLT 0.3%, and the experimental process is detailed in section 3.2.S.2.4.3.2.1. Therefore, impurity YA2304-6 is no longer controlled in the finished Finerenone.

### **(7) YA2304-7**

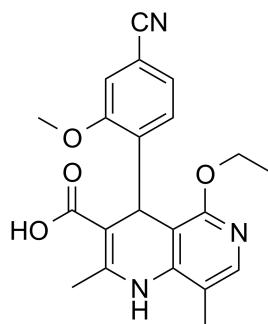
YA2304-7 is an intermediate product in the synthesis of the intermediate YA2304-8. Its molecular structure formula is as follows:

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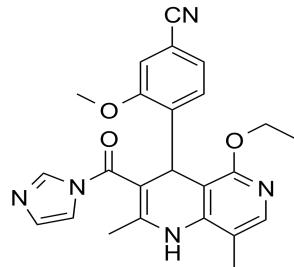
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The applicant intends to control YA2304-7 as a specific impurity not exceeding 0.15% in the intermediate YA2304-8, and calculate the impurity content by self-comparison method with correction factor. Refer to the validation report of YA2304-8 Related Substance Methodology under item 3.2.S.2.4.3.2.3. It can be seen that YA2304-7 has a correction factor of 1.0 relative to YA2304-8. At the same time, the applicant designed an impurity addition experiment to investigate the reasonability of the impurity limit in the standard. The experimental process is detailed in section 3.2.S.2.4.3.2.1. Therefore, impurity YA2304-7 is no longer controlled in the finished Finerenone.

### **(8) YA2304-55**

YA2304-55 is an intermediate product in the synthesis of the intermediate YA2304-8. Its molecular structure formula is as follows:



The applicant intends to control YA2304-55 as a specific impurity not exceeding 0.20% in the intermediate YA2304-8, and calculate the impurity content by self-comparison method with correction factor. Refer to the

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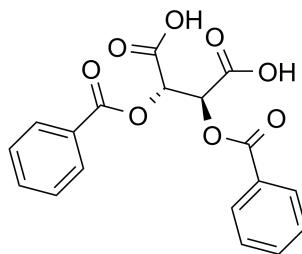
Version: 001

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validation report of YA2304-8 on Substance Methodology under item 3.2.S.2.4.3.2.3 that YA2304-55 has a correction factor of 1.2 relative to YA2304-8. At the same time, the reasonability of the impurity limit in the test standard designed by the applicant for adding impurities shows that the removal capacity of the registered process for impurity YA2304-55 is NLT 0.20%, and the impurity limit meets the process requirements. The experimental process is detailed in section 3.2.S.2.4.3.2.1. Therefore, impurity YA2304-55 is no longer controlled in the finished Finerenone.

### **(9) YA2304-10**

YA2304-10 is the resolution reagent in the synthesis of intermediate YA2304-CP. Its molecular structure formula is as follows:



The applicant intends to control the impurity YA2304-10 as a specific impurity in the finished Finerenonee, with the limit of not exceeding the identification threshold of 0.10%, and calculate the peak area according to the external standard method.

### **(10) YA2304-12**

YA2304-12 is a possible by-product in the synthesis of intermediate YA2304-CP, and the impurity may be produced by hydrolysis of Finerenone under acidic or alkaline conditions. According to the degradation experimental data in 3.2.S.4.3.1 Methodology-verification of related substances of the finished product, Finerenone did not degrade impurity YA2304-12 under weak acid (destruction by 2mL of 0.1mol/l hydrochloric

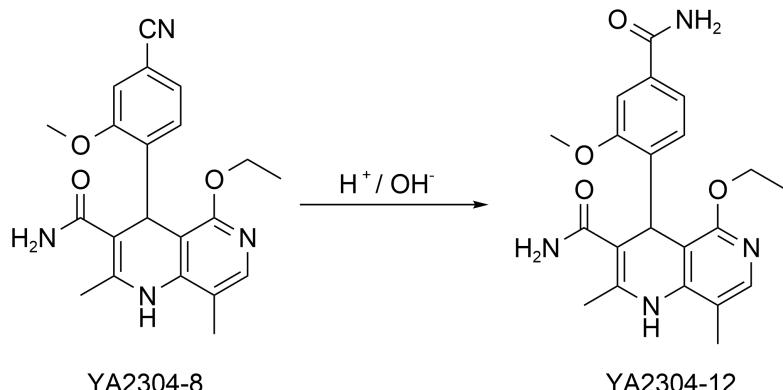
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acid for 24h). The known impurity YA2304-12 (content 0.07%) was mainly produced by the degradation of Finerenone under the condition of weak base (destruction of 2mL of 0.1mol/l sodium hydroxide for 24h). Its molecular structure formula and chemical reaction process are as follows:



The Applicant initially controlled impurity YA2304-12 as a specific impurity in the finished Finerenonee to a limit of 0.15% above the defined threshold. Refer to the Validation report of the Methodology for the Analysis of Substances in 3.2.S.4.3.1 that YA2304-12 has a correction factor of 1.2 relative to Finerenone. The applicant tested three commercial-scale batches of Finerenone (batch No. 231201, 240101 and 240102) by the method of substance related, and no impurity YA2304-12 was detected in each sample. For specific test data, please refer to Table 3.2.S.3.3.2.1 -2. In addition, the impurity was also investigated by the applicant in the stability study. See 3.2.S.7.3 stability data for the above three commercial-scale batch Finerenone apis under accelerated stability test conditions (temperature  $40^\circ\text{C} \pm 2^\circ\text{C}$ ; Humidity  $75\% \pm 5\%$ ) and long-term stability test conditions (temperature  $30^\circ\text{C} \pm 2^\circ\text{C}$ , relative humidity RH $65\% \pm 5\%$ ) for 6 months, the impurity YA2304-12 was not detected, indicating that the Finerenone raw material will not produce impurity YA2304-12 during stable placement. Therefore, the applicant finally controlled the impurity YA2304-12 in the

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finished product as a non-specific impurity to not exceed the identification threshold of 0.10%.

### **(11) YA2304-14, YA2304-15 and YA2304-16, YA2304-18**

Impurity formation path and mutual conversion relationship of YA2304-14, YA2304-15 and YA2304-16 and YA2304-18 are as follows:

- ① Triethyl orthoacetate may contain a small amount of methyl and isopropyl substituted impurities, and YA2304-50 and YA2304-51 can be formed by alkylation reaction with YA2304-5, and YA2304-16 and YA2304-18 can be formed by hydrolysis reaction in the following steps. And then the corresponding derivatives YA2304-14 and YA2304-15 are generated by ammonolysis.
- ② In the presence of methanol and isopropyl alcohol, Finerenone may also generate impurities YA2304-14 and YA2304-15 by ether exchange, respectively.
- ③ YA2304-14 and YA2304-15 may be hydrolyzed under acidic conditions to produce impurities YA2304-16 and YA2304-18, respectively.

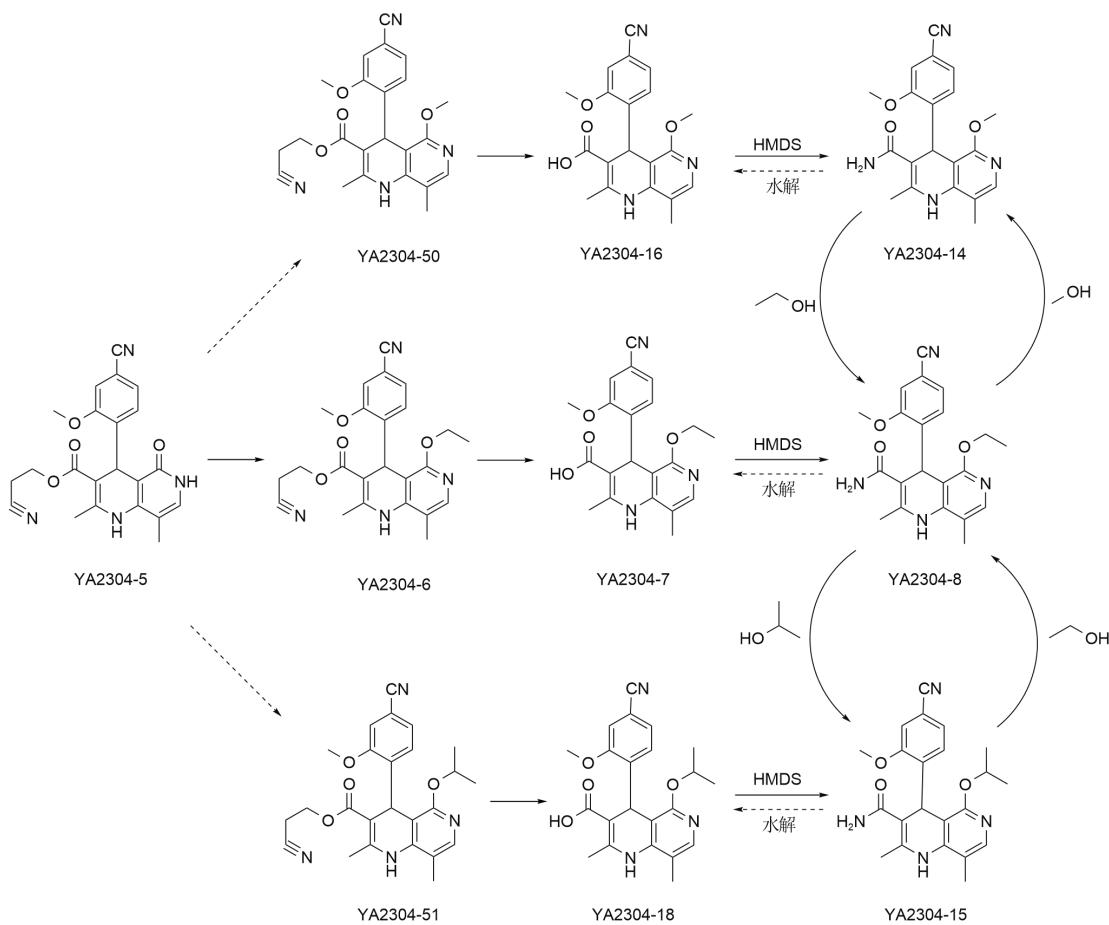
The molecular structure formula and chemical reaction process of each impurity are as follows:

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Impurities YA2304-14 and YA2304-15 are process impurities that are finally converted in the finished product. The original research import registration preparation standard JX20220072 controls these two impurities (the code of the impurity in the import registration standard is BAY1814286 and BAY1814288, respectively) as non-specific impurities shall not exceed 0.2%. The applicant intends to more strictly control the impurities YA2304-14 and YA2304-15 as specific impurities in the finished Finerenone, which shall not exceed the defined threshold of 0.15%.

The Applicant originally controlled the impurities YA2304-16 and YA2304-18 as specific impurities in the finished finnaldone to the extent that they both did not exceed the defined threshold of 0.15%. Refer to the 3.2.S.4.3.1 Validation Report on the Methodology of Substance Analysis that YA2304-16 has a correction factor of 1.0 with respect to Finerenone and YA2304-18 has

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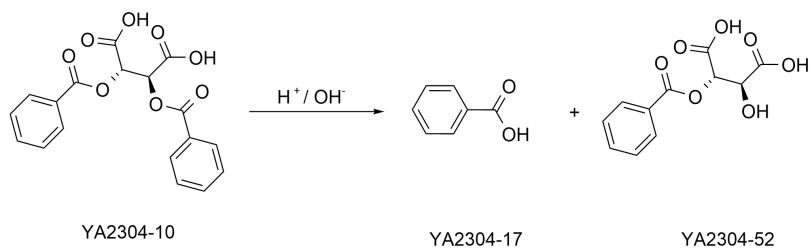
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a correction factor of 1.1 with respect to Finerenone. The applicant tested three commercial-scale batches of Finerenone (batch No. 231201, 240101 and 240102) by using the substance related method. Impurities YA2304-16 and YA2304-18 in each sample were not detected. For specific test data, please refer to Table 3.2.S. In addition, the impurity was also investigated by the applicant in the stability study. See 3.2.S.7.3 stability data for the above three commercial-scale batch Finerenone apis under accelerated stability test conditions (temperature  $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ; Humidity  $75\% \pm 5\%$ ) and long-term stability test conditions (temperature  $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , relative humidity RH $65\% \pm 5\%$ ) for 6 months, impurities YA2304-16 and YA2304-18 were not detected. It indicated that the impurities YA2304-16 and YA2304-18 would not be produced during the stable placement of Finerenone. As a result, the applicant ultimately controls the impurities YA2304-16 and YA2304-18 as non-specific impurities in the finished product without exceeding the identification threshold of 0.10%.

### (12) YA2304-17 and YA2304-52

Impurities YA2304-17 and YA2304-52 are hydrolyzed products of the resolution reagent YA2304-10 (D-(+)-dibenzoyltartaric acid), and their molecular structure formula and chemical reaction process are as follows:



The applicant initially controlled the impurity YA2304-17 as a specific impurity in the finished Finerenone, with the limit not exceeding the defined threshold of 0.15%, and calculated according to the external standard method. The Applicant tested three commercial-scale batches of Finerenone

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No. 231201, 240101 and 240102 by the method of substance related, and no impurity YA2304-17 was detected in each sample. For specific test data, please refer to Table 3.2.S. In addition, the impurity was also investigated by the applicant in the stability study. Refer to the stability data of 3.2.S.7.3, it can be seen that the above three batches of commercial-scale mass produced Finerenone apis were placed under accelerated stability test conditions (temperature  $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , humidity  $75\% \pm 5\%$ ) and long-term stability test conditions (temperature  $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , relative humidity RH $65\% \pm 5\%$ ) for 6 months. Impurity YA2304-17 was also not detected, indicating that the impurity YA2304-17 would not be produced during the stable placement of the Finerenone bulk drug. In summary, the registered process has a good ability to remove impurity YA2304-17 and does not degrade during the stability period to produce impurity YA2304-17. The applicant intends to eventually use impurity YA2304-17 as a specific impurity in the crude product, which shall not exceed the defined threshold of 0.15% and shall not control impurity YA2304-17 in the finished product.

The applicant tested three commercial-scale batches of Finerenone No. 231201, 240101 and 240102 by using the Substance II method. No impurity YA2304-52 was detected in each sample. For specific test data, please refer to Table 3.2.S. The test results showed that the registered process had a good ability to remove impurity YA2304-52. Therefore, the applicant does not control the impurity YA2304-52 in the finished product.

### **(13) YA2304-19**

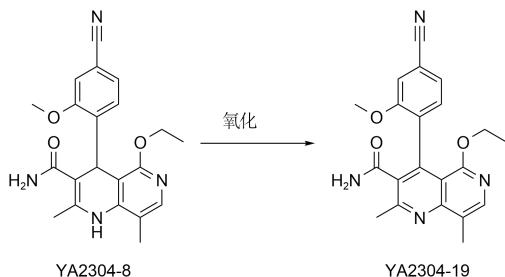
YA2304-19 is the oxidative degradation product in the process of preparing YA2304-CP. Its molecular structure formula and chemical reaction process are as follows:

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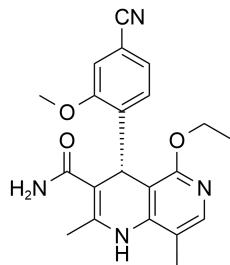
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The impurity (the code of the impurity in the import registration standard is BAY1040818) shall not be controlled more than 0.5% as a specific impurity in the original research import registration preparation standard JX20220072, and the applicant intends to use the impurity as a specific impurity in the finished Finerenone to more strictly control that the impurity shall not exceed the defined threshold of 0.15%. Refer to the validation report of 3.2.S.4.3.1 Related Substance analysis Methodology, YA2304-19 has a correction factor of 0.7 relative to Finerenone.

### (14) YA2304-20

YA2304-20 is the enantiomer impurity of the finished product. The chemical structure formula is as follows:



The impurity was not controlled in JX20220072, the standard for imported registered preparations of the original research. The applicant intends to control the impurity as a specific impurity not exceeding the defined threshold value of 0.15% in the quality standard of Finerenone, and calculate the content of the impurity according to the external standard method.

### (15) Imidazole

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Imidazole is the by-product of the reaction and the hydrolyzed product of reagent CDI. The applicant tested three commercially-produced batches of Finerenone No. 231201, 240101 and 240102 by the method of Substance II. No imidazole was detected in each sample. For specific test data, please refer to Table 3.2.S. The test results showed that the registered process had a good scavenging ability for imidazole. Therefore, the applicant does not control the imidazole in the finished product.

### **(16) Unknown degradation impurities**

Refer to the degradation experimental data in 3.2.S.4.3.1 Methodological verification of finished product related substances. Under the condition of destruction by weak acid (destruction by 2mL of 0.1mol/l hydrochloric acid for 24h), the raw material of Finerenone mainly degraded to produce known impurities YA2304-16 (content < 0.05%) and unknown impurities of RRT≈0.59 (content 2.02%). The known impurity YA2304-12 (content 0.07%) was mainly degraded under the condition of weak base (destruction of 2mL of 0.1mol/l sodium hydroxide for 24h). The degradation of Finerenone under oxidation destruction (2mL destruction of 3% hydrogen peroxide for 24h) mainly produced unknown impurities YA2304-19 (content 0.12%) and RRT≈0.64 (content 0.43%). The solid samples of Finerenone were destroyed by light (4500±500Lux, 90±5μWcm<sup>2</sup> for 30d) degradation mainly produced 5 known impurities YA2304-14, YA2304-15, YA2304-16, YA2304-17, YA2304-19, and unknown impurities with RRT≈1.53 (content of 0.05%). The 5 known impurities produced by degradation except the impurity YA2304-19 content of 0.17%, the other known impurities produced by degradation content of < 0.05%; Two known impurities, YA2144-16 and YA2144-19, and three unknown impurities, RRT≈0.64, RRT≈0.87 and RRT≈0.96, were mainly produced by the degradation of fenelidone bulk drug solution by light

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(4500±500Lux, 90±5μWcm<sup>2</sup> destroying the test product solution for 24h).

The contents of YA2304-16 and YA2304-19 produced by degradation were < 0.05% and 0.54%, and the contents of RRT≈0.64, RRT≈0.87 and RRT≈0.96 produced by degradation were 0.06%, 0.06% and 0.08%, respectively. These unknown impurities can be effectively separated by the method of related substances. Under other conditions, the unknown impurities were not degraded in the samples.

3.S.7.3 Accelerated Stability Test and long-term stability test data showed that three commercial-scale batches of Finerenone 231201, 240101 and 240102 were subjected to accelerated stability test at 6 months (temperature 40°C±2°C, All impurities during the relative humidity RH75%±5% and the 6-month long-term stability test (temperature 30°C±2°C, relative humidity RH65%±5%) were in line with the internal control standards, and no new unknown degradation impurities were produced.

In accordance with the provisions of ICHQ1A(R2) section 2.1.2: If some degradation products of the degradation test have been confirmed to be not generated under accelerated or long-term stability test conditions, it is not necessary to control the impurity. Therefore, the Applicant shall not control the said unknown degradation impurity as a specific impurity in the finished product and shall not exceed the identification threshold of 0.10% as a non-specific impurity.

### **Detection of potential organic impurities in commercial-scale mass production of Finerenone**

The detection of potential organic impurities in three commercial-scale batches of Finerenone with batch NO.s 231201, 240101 and 240102 are shown in Table 3.2.S. 4.1-2.

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Table 3.2.S.3.2.1-2 Detection of each potential organic impurity in Finer enone

Impurity name \ Batch No.	231201	240101	240102
Related substance	YA2304-10	Not detected	Not detected
	YA2304-12	Not detected	Not detected
	YA2304-14	Not detected	Not detected
	YA2304-15	Not detected	Not detected
	YA2304-16	Not detected	Not detected
	YA2304-17	Not detected	Not detected
	YA2304-18	Not detected	Not detected
	YA2304-19	< 0.05%	< 0.05%
	Other single i mpurities	< 0.05%	< 0.05%
	Total Miscella neous	< 0.05%	< 0.05%
Related Sub stance II	YA2304-52	Not detected	Not detected
	Imidazole	Not detected	Not detected
Enantiomer	YA2304-20	0.03%	0.03%
			0.02%

### 3.2.S.3.2.2 Inorganic Impurities

Inorganic impurities in Finerenone may originate from reaction reagents, catalysts used in the production process, and inorganic salts used in the post-treatment process. For inorganic impurities that may be introduced into the starting material, the applicant intends to set up a incandescent residue item in each starting material quality standard to control inorganic impurities. Therefore, the applicant combined with 3.2.S.2.2.3 process description to analyze and study the inorganic impurities in the process of Finerenone preparation, and formulated the corresponding control strategy, as detailed in Table 3.2.S.3.2.2-1.

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**Table 3.2.S.3.2.2-1 Analysis and Control Strategies of Inorganic Impurities in Finerenone**

Names of Impurities	Impurity source and removal analysis	Control Limits	Is the negatio n of entry criteria
Sodium acetate	Sodium acetate is the reagent used in step 2. This impurity is soluble in water and can be removed by subsequent washing and crystallization processes. The applicant intends to control sodium acetate by burning residue.	Incandescent residue ≤0.1%	is
Sulfuric acid	Sulfuric acid is the reagent used in step 2 and is converted to sodium sulfate. Sodium sulfate dissolves easily in water and can be removed by subsequent washing and crystallization processes. The applicant intends to control sodium sulfate by burning residue.	Incandescent residue ≤0.1%	is
Sodium hydroxide	Sodium hydroxide is the reagent used in step 2, which can be converted into sodium chloride. Sodium chloride is soluble in water and can be removed by subsequent washing and crystallization processes. The applicant intends to control sodium chloride by burning residue.	Incandescent residue ≤0.1%	is
Hydrochloric acid	Hydrochloric acid is the reagent used in step 2 and is converted to sodium chloride. Sodium chloride is soluble in water and can be removed by subsequent washing and crystallization processes. The applicant intends to control sodium chloride by burning residue.	Incandescent residue ≤0.1%	is
Sodium phosphate	Sodium phosphate is the reagent used in Step 3 that is partially converted to convert disodium hydrogen phosphate after the base is modulated in step 3. The impurities of	Burning residue ≤0.1%	is

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	disodium hydrogen phosphate and sodium phosphate are soluble in water and can be removed by subsequent washing and crystallization processes. The applicant intends to control disodium hydrogen phosphate and sodium phosphate through incandescent residue.		
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### **Chlorides and sulfates**

Finerenone can remove trace amounts of chloride and sulfate that may remain after multiple washing, filtration, and recrystallization processes. The applicant has tested the chlorides and sulfates of multiple batches of commercial-scale mass-produced Finerenone in accordance with General Rules 0801 and 0802 of Part 4 of the 2020 Edition of the Chinese Pharmacopoeia. The test results of chloride and sulfate are not more than 0.02%, and the test results of sulfate are not more than 0.1%, as detailed in Table 3.2.S.3.2.2-2. Since the incandescent residue can also control chloride and sulfate, the applicant intends not to set up a test item for chloride and sulfate in the finished product standard.

### **The detection of various inorganic impurities in commercial-scale batch production of Finerenone**

The applicant has tested the burning residue, chloride and sulfate of three batches of commercial-scale mass produced Finerenone (batch No. 231201, 240101 and 240102 respectively), and the test results all meet the requirements of the standard. The test results are shown in Table 3.2.S.3.2.2-2.

Table 3.2.S.3.2.2-2 Statistics of detection data of inorganic impurities in

#### Finerenone

Test items \ Batch No.	231201	240101	240102
Finerenone			

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Incandescent residue ≤0.1%	conforms	conforms	conforms
Chloride ≤0.02%	conforms	conforms	conforms
Sulfate ≤0.1%	conforms	conforms	conforms

### **3.2.S.3.2.3 Residual solvents**

Solvent Control Strategies". It intends to control triethylamine in starting material YA2304-2 with reference to ICHQ3C(R9) and the four General Rules. The residual solvents that may be introduced into starting material YA2304-1 are acetone, N, N-dimethylformamide, dichloromethane, ethanol, methanol and methyl tert-butyl ether, the residual solvents that may be introduced into starting material YA2304-2 are ethyl acetate and triethylamine, and the residual solvents that may be introduced into starting material YA2304-2 are methanol and ethanol. In the preparation of Finerenonee, the applicant used eight dissolution reagents, including piperidine, acetic acid, isopropyl alcohol, secondary butanol, n-methylpyrrolidone, toluene, tetrahydrofuran and ethanol. The reagent was triethyl acetate reaction and hydrolysis to produce ethanol and ethyl acetate, and a trace amount of benzene may be introduced into the solvent acetone, toluene and ethanol.

In summary, 16 residual solvents such as acetone, N, N-dimethylformamide, dichloromethane, ethanol, methyl tert-butyl ether, ethyl acetate, triethylamine, methanol, acetic acid, piperidine, isopropyl alcohol, secondary butanol, n-methylpyrrolidone, toluene, tetrahydrofuran and benzene may be present in the finished finnalidone. The applicant intends to control acetone, N, N-dimethylformamide, dichloromethane, methanol and methyl tert-butyl ether in starting material YA2304-1 with reference to ICHQ3C(R9) and the Four General Rules 0861 of the 2020 edition of the Chinese

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Pharmacopoeia. The relevant control strategies are detailed in section 3.2.S.2.3.3.2 "Residual Solvent Control Strategy"; It is proposed to control methanol in starting material YA2304-4 with reference to ICHQ3C(R9) and General Rules 0861 of the fourth part of the 2020 edition of Chinese Pharmacopoeia. The relevant control strategies are detailed in section 3.2.S.2.3.5.2 "Residual Solvent Control Strategy"; Acetic acid is the reagent used in step 1, which can be easily removed by dissolving in aqueous solution with alkali-forming inorganic salts in subsequent steps, and the applicant intends not to conduct research control on it; For the remaining residual solvents, the applicant classifies them with reference to ICHQ3C(R9) and the fourth General Rule 0861 of the 2020 edition of the Chinese Pharmacopoeia, and formulates the corresponding control limits, as detailed in Table 3.2.S.3.2.3-1.

Table 3.2.S.3.2.3-1 Classification and control limits of residual solvents

Name of solvent	Steps to use	Solvent classification	Limits
Ethyl acetate	Starting material YA2304-2 is introduced, reagent triethyl orthoacetate reaction and hydrolysis	Class 3	≤0.5%
Isopropyl alcohol	Steps 1	Class 3	≤0.5%
sec-butanol	Step 1	Class 3	≤0.5%
N-methylpyrrolidone	Step 2	Class 2	≤0.053%
Toluene	Step 2	Class 2	≤0.089%
tetrahydrofuran	Step 2	Class 2	≤0.072%
Ethanol	Steps 1,3,4	Class 3	≤0.5%
Piperidine	Steps 1	Class 4	≤0.1%

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benzene	Toluene and ethanol introduced	Class 1	≤0.0002%
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The applicant has developed two sets of GC methods and one set of HPLC methods to detect the residual solvent of the finished product, one set of GC methods to detect ethyl acetate, ethanol, isopropyl alcohol, secondary butanol, tetrahydrofuran, toluene, N-methylpyrrolidone, the other set of GC methods to detect benzene, and one set of HPLC methods to detect piperidine. At the same time, the applicant has carried out systematic methodological verification on the above three sets of residual solvent methods, and the verification results show that the method is specific, linear and has a good recovery rate. The quantification limit of ethanol is 0.01%, the quantification limit of isopropanol is 0.01%, the quantification limit of ethyl acetate is 0.01% and the quantification limit of secondary butanol is 0.008%. The limit of quantification of tetrahydrofuran is 0.007%, the limit of quantification of toluene is 0.004%, the limit of quantification of N-methylpyrrolidone is 0.006%, the limit of quantification of benzene is 0.000053% and the limit of quantification of piperidine is 0.01%, all of which are far below the standard control limit. See 3.2.S.4.3.4, 3.2.S.4.3.5 and 3.2.S.4.3.6 for the relevant methodological validation.

### **Detection of residual solvents in commercial-scale batch production of Finerenone**

The applicant has tested the residual solvent of three batches of commercial-scale mass produced Finerenone (batch No. 231201, 240101 and 240102 respectively), and the test results all meet the standard requirements. The detection information is shown in Table 3.2.S.3.2.3-2.

Table 3.2.S.3.2.3-2 Test data of residual solvent in Finerenone

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Batch	Limit s	10% of limits	231201	240101	240102	LOD	LOQ
Ethanol	≤0. 5%	≤0.05%	0.1%	0.2%	0.1%	0.003%	0.01%
Isopropyl alcohol	≤0. 5%	≤0.05%	Not detected	Not detected	Not detected	0.003%	0.01%
Ethyl acetate	≤0. 5%	≤0.05%	Not detected	Not detected	Not detected	0.003%	0.01%
sec-butanol	≤0. 5%	≤0.05%	Not detected	Not detected	Not detected	0.002%	0.008%
tetrahydrafuran	≤0.07 2%	≤0.0072%	Not detected	Not detected	Not detected	0.002%	0.007%
Toluene	≤0.08 9%	≤0.0089%	Not detected	Not detected	Not detected	0.001%	0.004%
N-methylpyrrolidone	≤0.05 3%	≤0.0053%	Not detected	Not detected	Not detected	0.002%	0.006%
piperidine	≤0. 1%	≤0.01%	Not detected	Not detected	Not detected	0.003%	0.01%
benzene	≤0.00 02%	≤0.00002 %	Not detected	Not detected	Not detected	0.000016 %	0.000053 %

According to the test results of three commercial-scale batches of self-made samples for residual solvents, except for ethanol used in the last step of the process, the remaining eight residual solvents were not detected (LOD is detailed in Table 3.2.S.3.2.3-2), all below 10% of their acceptable limits. Therefore, the applicant in the final draft quality standards, the solvent used in the last step of the process of ethanol and the second class of solvents

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N-methylpyrrolidone, toluene and tetrahydrofuran set into the quality standards for control, the limit of ethanol shall not be 0.5%; N-methylpyrrolidone shall not exceed 0.053%; Toluene must not exceed 0.089%; Tetrahydrofuran shall not exceed 0.072%, and the remaining solvents shall not be controlled.

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### **3.2.S.3.2.4 Impurities of Metal Elements**

With reference to ICH-Q3D (R2), the applicant conducts risk assessment of potential metal element impurities in the preparation of finalizone from three aspects, including process introduction, material introduction and equipment introduction, and includes palladium and highly toxic Class 1 and 2A metal element impurities as follows:

#### **palladium**

The catalyst palladium acetate was used in the preparation of Finerenone starting material YA2304-1, and trace amounts of palladium may remain in the finished product. The oral acceptable limit of palladium referenced to ICHQ3D(R2) is 10 $\mu$ g/g (i.e. 10 PPM). The Applicant tested three commercial-scale batches of Finerenone (lots 231201, 240101 and 240102), each containing significantly less than 1ppm of palladium (1 0% of the acceptable limit), as detailed in Table 3.2.S.3.2.4-1.

Table.2.S.3.2.4-1 Statistics of palladium content in Finerenone

Batch No. Elemental impurities	231201	240101	240102	10% of the acceptable limit
Palladium content	0.053 ppm	0.084 ppm	0.009 ppm	1ppm

In summary, the palladium content of Finerenone produced by registered process was less than 10% (i.e. 1ppm) of the oral acceptable limit of palladium in ICH-Q3D (R2). With reference to the "Implementation of policy on elemental impurities in the Certification Procedure" published by EDQM, the applicant can see that if the metal element is introduced before the final synthetic reaction, And if the corresponding metal element test results in 3 batches of commercial-scale finished products are all lower than 30% of the acceptable limit, the finished product standard may not include the impurity in the routine test project, as detailed

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in 3.2.S.4.5. Based on this analysis, the risk of Finerenone introducing palladium element is low, and the registered process can control the impurity below 10% of the acceptable limit. Therefore, the applicant does not control it.

### **lead**

See ICH-Q3D (R2) for an oral PDE of 5 $\mu$ g/ day for lead. The maximum daily dose (MDD) of Finerenone is 20mg (much less than 1g), and the applicant calculated an oral acceptable limit of 5 $\mu$ g/g (i.e. 5 PPM) for lead based on a daily intake of 1g. In order to exclude the possibility of the introduction of more toxic Group 1 (lead) metal elements into various manufacturing materials and equipment, the Applicant tested three commercial-scale batches of Finerenone (Batch No. 231201, 240101 and 240102). The levels of lead detected in these three batches were all below 30% of the acceptable oral limits (see Table 3.2.S.3.2.4-2 for details). Therefore, the Applicant intends not to control the elemental impurity lead in the finished product.

Table 3.2.S.3.2.4-2 Statistics of lead detection data in Finerenone

Elemental impurities Batch No.	231201	240101	240102	30% of the acceptable limit
Lead content	0.380 ppm	0.386 ppm	0.093 ppm	1.5 ppm

### **Other Class 1 and Class 2A elemental impurities**

See ICH-Q3D (R2) for oral Pdes of 15 $\mu$ g/ day, 5 $\mu$ g/ day, 30 $\mu$ g/ day, 50 $\mu$ g/ day, 200 $\mu$ g/ day, and 100 $\mu$ g/ day for arsenic, cadmium, mercury, cobalt, nickel, and vanadium, respectively. The maximum daily dose (MDD) of Finerenone is 20mg (much less than 10g), The applicant calculated the oral acceptable limits of arsenic, cadmium, mercury, lead, cobalt, nickel and

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vanadium with a daily intake of 10g as 1.5 $\mu$ g/g (i.e. 1.5ppm), 0.5 $\mu$ g/g (i.e. 0.5ppm), 3 $\mu$ g/g (i.e. 3 PPM), 5 $\mu$ g/g (i.e. 5 PPM), 20 $\mu$ g/g (i.e. 20 PPM), 10 $\mu$ g/g (i.e. 20 PPM), respectively. I.e. 10ppm). In order to exclude the possibility of the introduction of highly toxic Class 1 (arsenic, cadmium and mercury) and Class 2A (cobalt, nickel and vanadium) metal elements into each production material and production equipment, the applicant tested three commercial-scale batch produced batches of Finerenone (Batch No. 231201, 240101 and 240102). The levels of lead detected in these three batches were all below 30% of the acceptable oral limits (see Table 3.2.4-3 for details). Therefore, the Applicant intends not to control the element impurities of Class 1 (arsenic, cadmium and mercury) and Class 2A (cobalt, nickel and vanadium) in the finished product.

Table 3.2.S.3.2.4-3 Finerenone Category 1 and 2A element impurity

inspection status

Elemental impurities \ Batch No.	231201	240101	240102	LODs	30% of acceptable limits
Arsenic levels	Undetected	Not detected	Not detected	0.004 ppm	0.45 ppm
Cadmium content	Undetected	Not detected	Not detected	0.001 ppm	0.15 ppm
Mercury content	Undetected	Not detected	Not detected	0.020 ppm	0.9 ppm
Cobalt content	0.005 ppm	0.028 ppm	Undetected	0.001 ppm	1.5 ppm
Nickel content	0.174 ppm	0.351 ppm	0.088 ppm	0.011 ppm	6ppm
Vanadium content	Undetected	0.004 ppm	Undetected	0.002 ppm	3ppm

### **Heavy metal**

Metal reaction reagents are not used in the preparation process of Finer

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enone, considering that the materials used and the equipment may introduce trace heavy metals. Therefore, the applicant proposed a heavy metal test item in Finerenone, and controlled its limit to not exceed 10 ppm. At the same time, the applicant tested three commercial-scale batches of Finerenone No. 231201, 240101 and 240102 for heavy metals, and the test results did not exceed 10ppm, as detailed in 3.2.S.3.2.4-4.

Table 3.2.S.3.2.4-4 Statistics of heavy metal detection data in Finerenone

Batch No.	231201	240101	240102
≤10ppm	conforms	conforms	conforms

### **3.2.S.3.2.5 Potential genotoxic impurities**

The applicant combined the preparation process of the starting material and the preparation process of Finerenone to conduct genotoxic impurity analysis. Among them, the starting material YA2304-1 may introduce YA2304-23, YA2304-24, YA2304-33, YA2304-43, dimethyl sulfate and other five potential genotoxic impurities. The starting material YA2304-2 may introduce one potential genotoxic impurity such as YA2304-38; Two potential genotoxic impurities (YA2304-40, YA2304-41) may be introduced into starting material YA2304-4. Potential genotoxic impurities such as YA2304-1, YA2304-4, YA2304-47, diethyl sulfate, diisopropyl sulfate, di-sec-butylsulfate, 4-dimethylaminopyridine and nitrosamines may be introduced in the preparation of Finerenone. Specific studies are as follows:

#### **(1) YA2304-23**

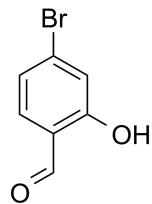
YA2304-23 is an incomplete raw material in the synthesis of starting material YA2304-1. This impurity has a potential genotoxic warning structure and is a potential genotoxic impurity. Its molecular structure formula is as follows:

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The applicant intends to control the impurity YA2304-23 under the relevant substances in the starting material YA2304-1, and design the impurity removal experiment to investigate the rationality of the impurity limit. The results show that when the impurity YA2304-23 content in the starting material YA2304-1 is 0.5%, The registration process can effectively ensure that the impurity YA2304-23 content in the subsequent production of intermediate YA2304-8 is far less than 30% of the maximum acceptable limit of genotoxic impurities in the finished product (i.e. 22.5ppm). The experimental process is detailed in section 3.2.S.2.3.3.2. At the same time, the applicant tested three commercial-scale batches of Finerenone (Batch No. 231201, 240101 and 240102), and no impurity YA2304-23 was detected in any of the batches (LOD: 6.02ppm), i.e., well below 30% of the acceptable limit (i.e. 22.5ppm), as detailed in Table 3.2.S.3.2.5-5.

According to Method 3 under item 8.1 of ICH-M7, when an impurity standard higher than the acceptable limit of the API is proposed in the starting material, the appropriate analytical method combined with the knowledge of the impurity destination and removal as well as relevant process control is adopted to ensure that the impurity level in the API is less than 30% of the acceptable limit, and the data of the commercial scale of multiple batches are collected. If it is proved that the impurity level is below 30% of the acceptable limit, the impurity can no longer be controlled in the subsequent process.

Therefore, the Applicant intends to control YA2304-23 as a specific impurity

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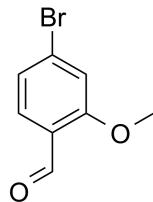
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by no more than 0.5% in the starting material YA2304-1, and this impurity will no longer be controlled in the finished product.

### **(2) YA2304-24**

YA2304-24 is an intermediate in the synthesis of the starting material YA2304-1. This impurity has a potential genotoxic warning structure and is a potential genotoxic impurity. Its molecular structure formula is as follows:



The applicant intends to control the impurity YA2304-24 under the relevant substances in the starting material YA2304-1, and design the impurity removal experiment to investigate the rationality of the impurity limit. The results show that when the impurity YA2304-24 content in the starting material YA2304-1 is 0.5%, The registration process can effectively ensure that the impurity YA2304-24 content in the subsequent production of intermediate YA2304-8 is far less than 30% of the maximum acceptable limit of genotoxic impurity in the finished product (i.e. 22.5ppm). The experimental process is detailed in section 3.2.S.2.3.3.2. At the same time, the applicant tested three commercial-scale batches of Finerenone (Batch No. 231201, 240101 and 240102), and no impurity YA2304-24 was detected in any of the batches (LOD: 6.09ppm), i.e., well below 30% of the acceptable limit (i.e. 22.5ppm), as detailed in Table 3.2.S.3.2.5-5.

Referring to Method 3 under item 8.1 of ICH-M7, the Applicant intends to control YA2304-24 as a specific impurity by no more than 0.5% in the starting material YA2304-1, which will no longer be controlled in the finished product.

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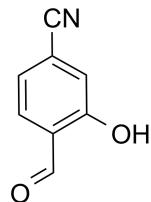
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### (3) YA2304-33

YA2304-33 is a by-product in the synthesis of the starting material YA2304-1.

This impurity has a potential genotoxic warning structure and is a potential genotoxic impurity. Its molecular structure formula is as follows:



The applicant intends to control the impurity YA2304-33 under the relevant substances in the starting material YA2304-1, and design the impurity removal experiment to investigate the rationality of the impurity limit. The results show that when the impurity YA2304-33 content in the starting material YA2304-1 is 0.5%, The registration process can effectively ensure that the impurity YA2304-33 content in the subsequent production of intermediate YA2304-8 is far less than 30% of the maximum acceptable limit of genotoxic impurities in the finished product (i.e. 22.5ppm). The experimental process is detailed in section 3.2.S.2.3.3.2. At the same time, the applicant tested three commercial-scale batches of Finerenone (Batch No. 231201, 240101 and 240102), and no impurity YA2304-33 was detected in any of the batches (LOD: 5.91ppm), i.e., well below 30% of the acceptable limit (i.e. 22.5ppm), as detailed in Table 3.2.S.3.2.5-5.

Referring to Method 3 under item 8.1 of ICH-M7, the Applicant intends to control YA2304-33 as a specific impurity by no more than 0.5% in the starting material YA2304-1, which will no longer be controlled in the finished product.

### (4) YA2304-43

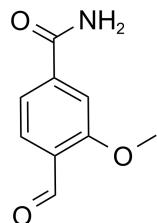
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YA2304-43 is the degradation product of starting material YA2304-1. This impurity has a potential genotoxic warning structure and is a potential genotoxic impurity. Its molecular structure formula is as follows:



The applicant intends to control the impurity YA2304-43 under the relevant substances in the starting material YA2304-1, and design the impurity removal experiment to investigate the rationality of the impurity limit. The results show that when the impurity YA2304-43 content in the starting material YA2304-1 is 0.5%, The registration process can effectively ensure that the impurity YA2304-43 content in the subsequent production of intermediate YA2304-8 is far less than 30% of the maximum acceptable limit of genotoxic impurity in the finished product (i.e. 22.5ppm). The experimental process is detailed in section 3.2.S.2.3.3.2. At the same time, the applicant tested three commercial-scale batches of Finerenone (Batch No. 231201, 240101 and 240102), and no impurity YA2304-43 was detected in any of the batches (LOD: 7.33ppm), i.e., well below 30% of the acceptable limit (i.e. 22.5ppm), as detailed in Table 3.2.S.3.2.5-5.

Referring to Method 3 under item 8.1 of ICH-M7, the Applicant intends to control YA2304-43 as a specific impurity by no more than 0.5% in the starting material YA2304-1, and this impurity will no longer be controlled in the finished product.

### **(5) YA2304-38**

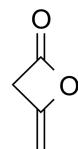
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YA2304-38 is an incomplete raw material in the synthesis of starting material YA2304-2. This impurity has a potential genotoxic warning structure and is a potential genotoxic impurity. Its molecular structure formula is as follows:



The applicant queried the microbial mutation test (Ames test) data of the compound from the Ministry of Health, Labor and Welfare of Japan (Occupational safety website), and it can be seen that the Ames test result of 4-methylene-2-oxacyclobutanone (YA2304-38) was Negative. The screenshot of the query result is as follows:

Diketene (ジケン)	
Chemical Name:	Diketene
Synonym	4-Methylene-2-oxetanone Acetyl ketene Oxetanone, 4-methylene-
Molecular weight:	84.08
Melting point:	-6.5°C
Boiling point:	127.4°C, 69~70°C (100mmHg)
Flashing point:	34°C
Chemical Structure	$\begin{array}{c} \text{CH}_2=\text{C}-\text{CH}_2 \\   \quad   \\ \text{O}-\text{C}=\text{O} \end{array}$
CAS No :	674-82-8
MITI No:	(5)-13
Source of Substance:	Wako Pure Chem. Ind., Ltd.
Lot. No.:	SAN5723
Purity:	99 %
Vehicle:	Acetone
Mutagenicity in Bacterial Test:	Negative
IARC Evaluation:	not yet cited

According to the requirements of ICH-M7, the impurity can be classified into 5 categories of potential genotoxic impurities and can be controlled as

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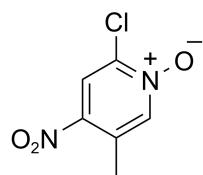
non-mutagenic impurities. The applicant intends to limit impurity YA2304-38 to 1.0% under residual solvent in starting material YA2304-2, and to design an impurity removal experiment to investigate the rationality of the impurity limit. The results show that when the impurity YA2304-38 content in starting material YA2304-2 is 1.0%, The registration process can effectively ensure the removal of impurity YA2304-38 in the subsequent production of Finerenonee. The experimental process is detailed in section 3.2.S.2.3.5.2. At the same time, the applicant tested three commercial-scale batches of Finerenone No. 231201, 240101 and 240102, and no impurity YA2304-38 (LOD: 0.027%) was detected in each batch of samples. For details, see Table 3.2.5-1. Therefore, the applicant intends to control YA2304-38 as a specific impurity by no more than 1.0% in the starting material YA2304-2, and this impurity will no longer be controlled in the finished product.

Table 3.2.5-1 Test data for YA2304-38 in Finerenone

Batch No.	231201	240101	240102	LOD
YA2304-38	Undetected	Not detected	Not detected	0.027%

### (6) YA2304-40

YA2304-40 is an incomplete raw material in the synthesis of starting material YA2304-4. This impurity has a potential genotoxic warning structure and is a potential genotoxic impurity. Its molecular structure formula is as follows:



The applicant intends to control the impurity YA2304-40 under the relevant substances in the starting material YA2304-4, and design the impurity removal experiment to investigate the rationality of the impurity limit. The

## **Drug Master File of Finerenone**

Document: YA2304-Quality

Version: 001

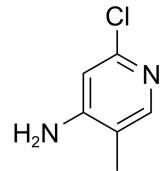
Date: Sep. 2024

results show that when the impurity YA2304-4 content is 0.5%, the impurity YA2304-40 should not exceed 0.5%. The registration process can effectively ensure that the impurity YA2304-40 content in the subsequent production of Finerenonee is far less than 30% of the maximum acceptable limit of genotoxic impurity in the finished product (i.e. 22.5ppm). The experimental process is detailed in section 3.2.S.2.3.7.2. At the same time, the applicant tested three commercial-scale batches of Finerenone (Batch No. 231201, 240101 and 240102), and no impurity YA2304-40 was detected in any of the batches (LOD: 12.59ppm), i.e., well below 30% of the acceptable limit (i.e. 22.5ppm), as detailed in Table 3.2.S.3.2.5-5.

Referring to Method 3 under item 8.1 of ICH-M7, the Applicant intends to control YA2304-40 as a specific impurity by no more than 0.5% in the starting material YA2304-4, which will no longer be controlled in the finished product.

### **(7) YA2304-41**

YA2304-41 is an intermediate in the synthesis of the starting material YA2304-4. This impurity has a potential genotoxic warning structure and is a potential genotoxic impurity. Its molecular structure formula is as follows:



The applicant intends to control the impurity YA2304-41 under the relevant substances in the starting material YA2304-4, and design the impurity removal experiment to investigate the reasonableness of the impurity limit. The results show that when the impurity YA2304-41 content in the starting material YA2304-4 is 0.5%, The registration process can effectively ensure

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Version: 001

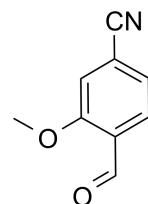
Date: Sep. 2024

that the impurity YA2304-41 content in the subsequent production of Finerenonee is far less than 30% of the maximum acceptable limit of genotoxic impurity in the finished product (i.e. 22.5ppm). The experimental process is detailed in section 3.2.S.2.3.7.2. At the same time, the applicant tested three commercial-scale batches of Finerenone (Batch No. 231201, 240101 and 240102), and no impurity YA2304-41 was detected in any of the batches (LOD: 7.51ppm), i.e., well below 30% of the acceptable limit (i.e. 22.5ppm), as detailed in Table 3.2.S.3.2.5-5.

Referring to Method 3 under Item 8.1 of ICH-M7, the Applicant intends to control YA2304-41 as a specific impurity by no more than 0.5% in the starting material YA2304-4, which will no longer be controlled in the finished product.

### **(8) YA2304-1**

YA2304-1 is an incomplete starting material in the synthesis of Finerenonee. This impurity has a potential genotoxic warning structure and is a potential genotoxic impurity. Its molecular structure formula is as follows:



The applicant intends to control the impurity YA2304-1 in the intermediate YA2304-5 under the relevant substances by 0.5%, and design the impurity removal experiment to investigate the rationality of the impurity limit. The results show that when the impurity YA2304-1 content in the intermediate YA2304-5 is 0.5%, The registration process can effectively ensure that the impurity YA2304-1 content in the subsequent production of Finerenonee is far less than 30% of the maximum acceptable limit of genotoxic impurity in

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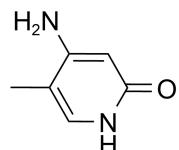
Date: Sep. 2024

the finished product (i.e. 22.5ppm). The experimental process is detailed in section 3.2.S.2.4.3.1.1. At the same time, the applicant tested three commercial-scale batches of Finerenone (Batch No. 231201, 240101 and 240102), and no impurity YA2304-1 was detected in any of the batches (LOD: 12.86ppm), i.e., well below 30% of the acceptable limit (i.e. 22.5ppm), as detailed in Table 3.2.S.3.2.5-5.

Referring to Method 3 under item 8.1 of ICH-M7, the Applicant intends to control YA2304-1 as a specific impurity by no more than 0.5% in the intermediate YA2304-5, which will no longer be controlled in the finished product.

### **(9) YA2304-4**

YA2304-4 is an incomplete starting material in Finerenone synthesis. This impurity has a potential genotoxic warning structure and is a potential genotoxic impurity. Its molecular structure formula is as follows:



The applicant intends to control the impurity YA2304-5 in the intermediate YA2304-5 under the relevant substances under 1.5%, and design the impurity removal experiment to investigate the rationality of the impurity limit. The results show that when the impurity YA2304-5 content in the intermediate YA2304-5 is 1.5%, The registration process can effectively ensure that the content of impurity YA2304-4 in the subsequent production of Finerenonee is far less than 30% of the maximum acceptable limit of genotoxic impurity in the finished product (i.e. 22.5ppm). The experimental process is detailed in section 3.2.S.2.4.3.1.1. At the same time, the applicant tested three commercial-scale batches of Finerenone (Batch No. 231201,

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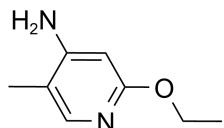
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240101 and 240102), and no impurity YA2304-4 was detected in any of the batches (LOD: 12.41ppm), i.e., well below 30% of the acceptable limit (i.e. 22.5ppm), as detailed in Table 3.2.S.3.2.5-5.

Referring to Method 3 under item 8.1 of ICH-M7, the Applicant intends to control YA2304-4 as a specific impurity by no more than 1.5% in the intermediate YA2304-5, which will no longer be controlled in the finished product.

### **(10) YA2304-47**

YA2304-47 is a derivative impurity of the starting material YA2304-4 involved in the subsequent reaction. This impurity has a potential genotoxic warning structure and is a potential genotoxic impurity. Its molecular structure formula is as follows:



The applicant intends to limit impurity YA2304-4 to 1.5% under related substances in the intermediate YA2304-5, and design an impurity removal experiment to investigate the reasonableness of the impurity limit. The results show that when the impurity YA2304-4 content in the intermediate YA2304-5 is 1.5%, The registration process can effectively ensure that the impurity YA2304-47 content in the subsequent production of Finerenonee is far less than 30% of the maximum acceptable limit of genotoxic impurity in the finished product (i.e. 22.5ppm). The experimental process is detailed in section 3.2.S.2.4.3.1.1. At the same time, the applicant tested three commercial-scale batches of Finerenone (Batch No. 231201, 240101 and 240102), and no impurity YA2304-47 was detected in any of the batches

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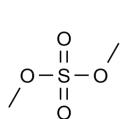
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(LOD: 6.37ppm), i.e., well below 30% of the acceptable limit (i.e. 22.5ppm), as detailed in Table 3.2.S.3.2.5-5.

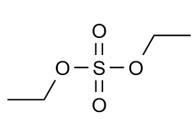
Referring to Method 3 under Item 8.1 of ICH-M7, the Applicant intends to control YA2304-4 as a specific impurity by no more than 1.5% in the intermediate YA2304-5, and its derived impurity YA2304-47 will no longer be controlled in the finished product.

### **(11) Sulfate impurities**

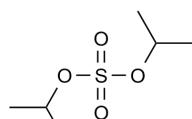
Dimethyl sulfate is an incomplete reaction reagent in the synthesis of starting material YA2304-1. Diethyl sulfate, diisopropyl sulfate and disec-butyl sulfate are the by-products that may be formed by the reaction of concentrated sulfuric acid with ethanol, isopropyl alcohol and 2-butanol in the synthesis of finalizone. Four potential genotoxic impurities are dimethyl sulfate, diethyl sulfate, diisopropyl sulfate, and disec-butyl sulfate. Their molecular structure formula is as follows:



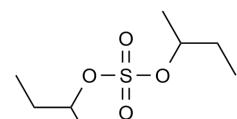
硫酸二甲酯



硫酸二乙酯



硫酸二异丙酯



硫酸二仲丁酯

With reference to ICH-M7, the acceptable limit of a single genotoxic impurity in Finerenone was calculated based on TTC as 75ppm, that is, the maximum acceptable limit of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and di-sec-butylsulfate in Finerenone was 75ppm.

Since NLT 3 impurities containing genotoxic warning structures are controlled in the finished fineridone, the applicant calculated the control limits of multiple genotoxic impurities in the finished fineridone with reference to ICH-M7 (R1) : The maximum daily dose (MDD) of Finerenone is 20mg, and the maximum daily tolerable dose (TTC) of the sum of the

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impurities containing the genotoxic warning structure is 5 $\mu$ g according to the following formula:

$$\text{Genotoxic impurity limit (ppm)} = \text{TTC/MDD}$$

TTC: Maximum tolerable daily dose,  $\mu$ g/ day,

MDD: maximum daily dose, g/ day;

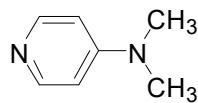
The calculated acceptable limit of the sum of impurities containing genotoxic warning structure in finalizone was 250ppm, that is, the maximum acceptable limit of the sum of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and di-sec-butyl sulfate in finalizone was 250ppm.

The applicant tested the contents of the above four sulfate genotoxic impurities in three commercial-scale batches of Finerenone No. 231201, 240101 and 240101. Dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and di-sec-butylsulfate were not detected. The specific test results are shown in Table 3.2.S.3.2.5-4, and the relevant methodology verification report is shown in 3.2.S.4.3.6.

Referring to Method 1 under ICH-M7 8.1, periodic confirmatory testing may be performed if the level of mutagenic impurities in the API is below 30% of the acceptable limit in at least 6 consecutive pilot batches or 3 consecutive production batches. Therefore, the applicant intends to schedule the genotoxic impurities of sulfate as part of the release criteria for finished Finerenone with a testing cycle of one batch for every 10 batches.

### **(12) 4-dimethylaminopyridine (abbreviated: DMAP)**

4-dimethylaminopyridine contains a genotoxicity warning structure, CAS number 1122-58-3, the molecular structure formula is as follows:



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The applicant queried Ames test data of the compound through the European Chemicals Agency (ECHA) database and found that 4-dimethylaminopyridine has been detected in *Salmonella typhimurium* TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia coli* WP2 uvr A reverse mutation test (Ames test) did not show mutagenic activity, as shown in the screenshot below:

### Test results 1

Species / strain:	<u>S. typhimurium TA 1535, TA 1537, TA 98 and TA 100</u>
Metabolic activation:	with and without
Genotoxicity:	<u>negative</u>
Cytotoxicity / choice of top concentrations:	no cytotoxicity nor precipitates, but tested up to recommended limit concentrations

### Test results 3

Species / strain:	<u>E. coli WP2 uvr A</u>
Metabolic activation:	with and without
Genotoxicity:	<u>negative</u>
Cytotoxicity / choice of top concentrations:	no cytotoxicity nor precipitates, but tested up to recommended limit concentrations

### Applicant's summary and conclusion

Conclusions:	<u>Interpretation of results (migrated information):</u> <u>negative</u>
<u>4-Dimethylaminopyridine is not mutagenic, as tested in several strains of <i>Salmonella typhimurium</i> and <i>E. coli</i>, both with and without rat and hamster liver metabolic activation.</u>	

According to the requirements of ICH-M7, the impurity can be classified into 5 potentially genotoxic impurities and can be controlled as non-mutagenic impurities, i.e. the acceptable limit of the impurity is 0.10%. 4-dimethylaminopyridine is a catalyst added in the ammoniation reaction of step 3 of the preparation process of non-nellidone, and the amount of feed is very small; The compound is soluble in water and ethanol, and is an organic base, after the subsequent two alcohol water system heating, filtration and a recrystallization of ethanol is easy to be removed, and D- (+) -dibenzoyl tartar conversion into water-soluble organic salt is also easy to be removed. The applicant tested three commercial-scale

Finerenone

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batches of Finerenone (batch No. 231201, 240101, 240102), and found that 4-dimethylaminopyridine was not detected in each batch of samples, as detailed in Table 3.2.S.3.2.5-2.

Table 3.2.S.3.2.5-2 Detection data of 4-dimethylaminopyridine in Finerenone

Names of impurities	231201	240101	240102	LOD
4-dimethylaminopyridine	Not detected	Not detected	Not detected	0.0005%

See 3.2.S.4.3.2 for the LOD of 4-dimethylaminopyridine (DMAP) in the finished product related Substance II analysis method is 0.0005%, which is far below the acceptable limit for this impurity. According to this analysis, although 4-dimethylaminopyridine (DMAP) was added in the preparation process of Finerenonee, the impurity can be effectively removed by the registration process. Therefore, the applicant intends not to control 4-dimethylaminopyridine (DMAP) in the finished product.

### **(13) nitrosamine impurities**

The applicant intends to conduct the following evaluation and research on 8 possible nitrosamine impurities such as NDMA, NDEA, NDIPA, NDBA, NIEPA, NMBA, NMPA, NDPA:

The AI values of NDMA, NDEA, NDIPA, NIEPA, NMBA and NMPA are announced in the "Control of Nitrosamine Impurities in Human Drugs" issued by FDA as follows:

Nitrosamine	AI Limit (ng/day) <sup>1,2</sup>
NDMA	96
NDEA	26.5
NMBA	96
NMPA	26.5
NIEPA	26.5
NDIPA	26.5

The AI values of the NDBA published in the Control of Nitrosamine Impurities in Human Drugs by the CHMP are as follows:

NDBA**(924-16-3)	26.5
------------------	------

The TD<sub>50</sub> value of the oral pathway of NDPA (CAS: 621-64-7) rats was 0.186mg/kg/day published in the CPDB, and the query screenshot is as

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follows:

### **Rats and Mice: Cancer Test Summary**

Rat Target Sites		Mouse Target Sites		TD <sub>50</sub> (mg/kg/day)	
Male	Female	Male	Female	Rat	Mouse
no test	eso liv nas	no test	no test	0.186	no test

Based on the above query results, the applicant calculated the AI of NDPA with reference to ICH-M7 (R2) according to the following formula, in which the AI of NDPA under oral route is 186ng/ day, the calculation formula is as follows:

$$\text{Maximum daily intake AI (ng/ day)} = \text{TD}_{50} \times 50\text{kg} / 50000$$

The applicant intends to refer to the "Control of Nitrosamine Impurities in Human Drugs" issued by CHMP to calculate the impurity limit according to TTC method, in which the AI of impurity NDPA is calculated according to 18ng/ day. The relevant references are described as follows:

In cases where robust TD<sub>50</sub> values as point of departure for excess cancer risk calculations are not available, the SWP recommends using a class specific threshold of theoretical concern (TTC) of 18 ng/d as default option with the possibility to justify a higher limit based on the structure-activity-relationship (SAR) approach described in the ICH M7(R1). Of note, the class specific AI of 18 ng/d for nitrosamines recommended by SWP as outlined below was determined using a novel methodology not widely used at this stage.

The maximum daily dose (MDD) of Finerenone is 20mg, and the applicant calculates the above impurity limit with reference to ICH-M7 (R2) according to the following formula, the calculation results are detailed in Table 3.2.5-3.

$$\text{Impurity limit (ppm)} = \text{AI/MDD}$$

AI: Maximum daily intake, ng/ day; MDD: maximum daily dose, mg/ day;

The maximum acceptable limits of NDMA, NDEA, NDIPA, NEIPA, NMBA, NMPA, NDBA and NDPA in finalizone were calculated to be 4.8ppm, 1.32ppm, 1.32ppm, 1.32ppm, 1.32ppm, 4.8ppm, 1.32ppm, 1.32ppm and 0.9ppm, respectively . The applicant intends to strictly control the above eight nitrosamine impurities in the finished product to not exceed 0.3ppm during

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the research process.

The applicant intends to develop LC-MS method to detect the above eight nitrosamine impurities in combination with the above impurity limits, and conducts corresponding methodological research with reference to the General Rule 9101 of the 2020 edition of Chinese Pharmacopoeia. The verification results show that the method is specific, linear and has a good recovery rate, and the LOD of each impurity is about 10% of the impurity control limit. For details, see Table 3.2.5-3. At the same time, NDMA, NDEA, NDIPA, NDBA, NEIPA, NMBA, NMPA and NDPA were not detected in the three commercial-scale batches of Finerenone No. 231201, 240101 and 240102. In other words, the detected amounts were all less than 10% of the impurity control limit, as detailed in Table 3.2.S.3.2.5-4.

Table 3.2.5-3 Acceptable limits of nitrosamine impurities in finalizone

Names of Impurities	Impurity AI (ng/day)	Acceptable limits based on TTC calculations (ppm)	More stringent acceptable limits (ppm) proposed by the applicant	LOD (ppm)
NDMA	96	4.8	0.3	0.0310
NDEA	26.5	1.32	0.3	0.0336
NDIPA	26.5	1.32	0.3	0.0319
NEIPA	26.5	1.32	0.3	0.0303
NMBA	96	4.8	0.3	0.0298
NMPA	26.5	1.32	0.3	0.0327
NDBA	26.5	1.32	0.3	0.0341
NDPA	18	0.9	0.3	0.0354

February 26, 2021, Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of the EMA As described in Q&A No. 15 of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products, When the amount of nitrosamine impurities detected is less than 10% of the acceptable limit in

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the finished product calculated by AI, the test may not be carried out, as follows:

### **15. When should a test for nitrosamines be included in the MA dossier?**

When a nitrosamine is identified after Step 2 confirmatory testing, a limit will usually need to be included in the specifications of the finished product and the product must comply if tested. If the root cause has been identified in the finished product manufacturing process or nitrosamines have been detected in the finished product but the actual source of contamination remains unclear, routine testing of the finished product is required by default.

The control point (finished product, API or an intermediate) for nitrosamines should be selected in such a way that it will give assurance of presence of the impurity below the acceptable limit based on acceptable intake (AI) in the finished product. Testing is usually expected to be carried out in the finished product, however if the source of a nitrosamine impurity is identified in the active substance manufacturing process, control options 1 to 3 as stated in ICH M7(R1) guideline could be used to demonstrate that the nitrosamine will not be present above the acceptable limit based on AI in the finished product. Even if the control point for the nitrosamine is not at finished product level, a limit has to be included in the finished product specification and batches should comply if tested. Testing of raw materials (e.g. excipients) should also be considered if these are potential sources of nitrosamine impurities.

Exceptions from routine testing may be possible, if the root cause of contamination is demonstrated to be well-understood:

- Only if the amount of nitrosamine present is consistently below 10% of the acceptable limit based on AI in the finished product, then testing could be omitted.
- Only if nitrosamine levels are consistently below 30% of the acceptable limit based on AI in the API or the finished product, skip-testing according to the ICH Q6A definition could be acceptable.

The translation of the underlined part in red is as follows:

Routine testing can only be avoided if the level of nitrosamine is consistently below 10% of the acceptable limit for AI-based calculations in the finished product.

In summary, in the three batches of commercial-scale mass production of finnalidone, the quality inspection quantity of eight nitrosamine impurities, such as NDMA, NDEA, NDIPA, NDBA, NEIPA, NMBA, NMPA and NDPA, is far less than 10% of the acceptable limit. The applicant intends not to control the above eight nitrosamine impurities in the finished product.

### **The detection situation of potential genotoxic impurities in commercial-scale mass production of Finerenone**

Table 3.2.S.3.2.5-4 Detection results of nitrosamine impurities in Finerenone

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Batch No.				LOD	Acceptable limits based on TTC calculations	More stringent acceptable limits drawn up by the applicant
Impurities	231201	240101	240102			
NDMA	Not detected	Not detected	Not detected	0.0310 ppm	4.8 ppm	0.3 ppm
NDEA	Not detected	Not detected	Not detected	0.0336 ppm	1.32 ppm	0.3 ppm
NDIPA	Not detected	Not detected	Not detected	0.0319 ppm	1.32 ppm	0.3 ppm
NEIPA	Not detected	Not detected	Not detected	0.0303 ppm	1.32 ppm	0.3 ppm
NMBA	Not detected	Not detected	Not detected	0.0298 ppm	4.8 ppm	0.3 ppm
NMPA	Not detected	Not detected	Not detected	0.0327 ppm	1.32 ppm	0.3 ppm
NDBA	Not detected	Not detected	Not detected	0.0341 ppm	1.32 ppm	0.3 ppm
NDPA	Not detected	Not detected	Not detected	0.0354 ppm	0.9 ppm	0.3 ppm

Table 3.2.S.3.2.5-5 Summary of genotoxic impurity detection results in commercial-scale batch production of Finerenone

Batch No.				LOD	30% of acceptable limits for genotoxic impurities
Impurities	231201	240101	240102		
YA2304-23	Not detected	Not detected	Not detected	6.02 ppm	22.5 ppm
YA2304-24	Not detected	Not detected	Not detected	6.09 ppm	22.5 ppm
YA2304-33	Not detected	Not detected	Not detected	5.91 ppm	22.5 ppm
YA2304-43	Not detected	Not detected	Not detected	12.22 ppm	22.5 ppm
YA2304-40	Not	Not	Not	12.59 ppm	22.5 ppm

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	detected	detected	detected		
YA2304-41	Not detected	Not detected	Not detected	7.51 ppm	22.5 ppm
YA2304-1	Not detected	Not detected	Not detected	12.86 ppm	22.5 ppm
YA2304-4	Not detected	Not detected	Not detected	12.41 ppm	22.5 ppm
YA2304-47	Not detected	Not detected	Not detected	6.37 ppm	22.5 ppm
Dimethyl sulfate	Not detected	Not detected	Not detected	2.2 ppm	22.5 ppm
Diethyl sulfate	Not detected	Not detected	Not detected	2.3 ppm	22.5 ppm
Diisopropyl sulfate	Not detected	Not detected	Not detected	2.3 ppm	22.5 ppm
Di-sec-butyl sulfate	Not detected	Not detected	Not detected	2.3 ppm	22.5 ppm

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### 3.2.S.4 Control of Drug Substance

#### 3.2.S.4.1 Specification

Specifications of Finerenone are summarized in the following table.

Table 3.2.S.4.1-1 Specifications of Finerenone

Test Items		Acceptance Criteria	Method
Characters	Appearance	white to yellow powder	Visual inspection
	Solubility	This product should be dissolved in methanol, slightly soluble in ethanol, acetonitrile and acetone, slightly soluble in isopropyl alcohol, and almost insoluble in water	In-house method
Identification	IR	The infrared light absorption pattern of this product should be consistent with that of the control product	Operating procedures of infrared absorption spectrophotometry (in-house method)
	HPLC	In the chromatogram recorded under the enantiomer determination item, the retention time of the main peak of the test solution should be consistent with the retention time of the main peak of the system suitability solution	Operating procedures of HPLC (in-house method)
Tests	Related substances	YA2304-10 NMT 0.10%; YA2304-14 NMT 0.15%; YA2304-15 NMT 0.15%; YA2304-19 NMT 0.15%; Other single impurities NMT 0.10%; Total impurities NMT 1.0%	Operating procedures of HPLC (in-house method)
	Enantiomers	YA2304-20 NMT 0.15%	Operating procedures of HPLC

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<b>Test Items</b>	<b>Acceptance Criteria</b>	<b>Method</b>
		(in-house method)
Genotoxic impurities	Dimethyl sulfate NMT 75ppm; Diethyl sulfate NMT 75ppm; Diisopropyl sulfate NMT 75ppm; Disec-butyl sulfate NMT 75ppm; The sum of genotoxic impurities NMT 250ppm	Operating procedures of GC-MS (in-house method)
Residual solvents	Ethanol NMT 0.5 % Tetrahydrofuran NMT 0.072% Toluene NMT 0.089% N-methylpyrrolidone NMT 0.053%	Operating procedures of GC (in-house method)
Water	NMT 0.5%	Operating procedures of Loss on drying determination (in-house method)
Residue on ignition	NMT 0.1%	Operating procedures of residue on ignition determination (in-house method)
Heavy metals	NMT 10 ppm	Operating procedures of heavy metals determination (in-house method)
Microbial limits	TAMC: NMT $10^3$ CFU/g TYMC: NMT $10^2$ CFU/g Absence of Escherichia coli (1g)	Operating procedures of microbial limits determination (in-house method)
Assay	98.0% to 102.0% (anhydrous substance)	Operating procedures of HPLC (in-house method)

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### **3.2.S.4.2 Analytical Procedures**

#### **CHARACTERS**

##### **Appearance**

white to yellow powder by visual inspection.

##### **Solubility**

It is dissolved in methanol, slightly soluble in ethanol, acetonitrile and acetone, slightly soluble in isopropyl alcohol and almost insoluble in water.

#### **IDENTIFICATION**

##### **Infrared Absorption Spectrophotometry**

Examine by *Operating procedures of infrared absorption spectrophotometry* (in-house method).

Comparison: Finerenone reference standard.

The IR spectra of the substance to be examined should comply with that of the Finerenone reference standard.

##### **High Performance Liquid Chromatography**

In the chromatogram recorded under the enantiomer determination, the retention time of the main peak of the test solution should be consistent with the retention time of the main peak of the system suitability solution. (*refers to enantiomer*).

#### **TESTS**

##### **Related Substances**

Examine by *Operating procedures of high performance liquid chromatography*

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(in-house method).

Solvent acetonitrile-water (30:70, v/v)

*Test solution:* Take an appropriate amount of this product, weigh it accurately, dissolve it in a brown volumetric flask with a suitable amount of solvent and sonicate it, and dilute it quantitatively to make a solution containing about 0.4mg per 1ml. Shake well to obtain the solution.

*Reference solution:* Accurately measure an appropriate amount of the test sample solution, dilute it quantitatively with solvent to prepare a solution containing approximately 0.4  $\mu$  g per 1ml, shake well, and obtain.

*Reference stock solution ①:* Take appropriate amounts of YA2304-10 and YA2304-17 reference standards, weigh them accurately, dissolve them in a solvent, and dilute them quantitatively to prepare a mixed solution containing approximately 200  $\mu$  g of YA2304-10 and YA2304-17 per 1ml.

*Reference solution:* Precisely measure an appropriate amount of reference stock solution ①, dissolve it in a solvent and dilute it quantitatively to prepare a mixed solution containing approximately 0.4  $\mu$  g of YA2304-10 and 0.4  $\mu$  g of YA2304-17 per 1ml.

*Reference stock solution ②:* Take appropriate amounts of YA2304-14, YA2304-15, YA2304-16, and YA2304-19 reference samples, weigh them accurately, dissolve and dilute them in acetonitrile to prepare a mixed solution containing approximately 200  $\mu$  g of YA2304-14, YA2304-15, YA2304-16, and YA2304-19 per 1ml.

*System suitability solution:* Accurately weigh about 10mg of YA2304 reference substance and place it in a 25ml volumetric flask. Add an appropriate amount of solvent and sonicate to dissolve it. Accurately add 100

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μl each of reference stock solution ① and reference stock solution ②, dilute to the mark with solvent, shake well, and obtain the solution.

### *Chromatographic system*

—stationary phase: Octadecylsilane bonded silica gel (YMC Trairt C18 , 4.6mm×150mm, 3μm, or other similar column).

—temperature: 20°C.

*Mobile phase:* see the gradient table blow.

- *mobile phase A:* phosphoric acid solution (take 300μl phosphoric acid, add 1000ml water, mix well);

- *mobile phase B:* acetonitrile;

Time(min)	Mobile phase A(%)	Mobile phase B(%)
0	85	15
2	80	20
5	80	20
8	69	31
13	55	45
14	55	45
18	50	50
22	20	80

*Flow rate:* 1.0 mL/min.

*Detection:* at 251nm or 230 nm .

*Injection volume:* 5 μL.

*System suitability:* System suitability solution:

- Reference solution (230nm): The peak order is YA2304-17, YA2304-10;
- System suitability solution (251nm): The peak order is YA2304-14 YA2304、YA2304-15、YA2304-17、YA2304-16、YA2304-10、YA2304-19; The separation

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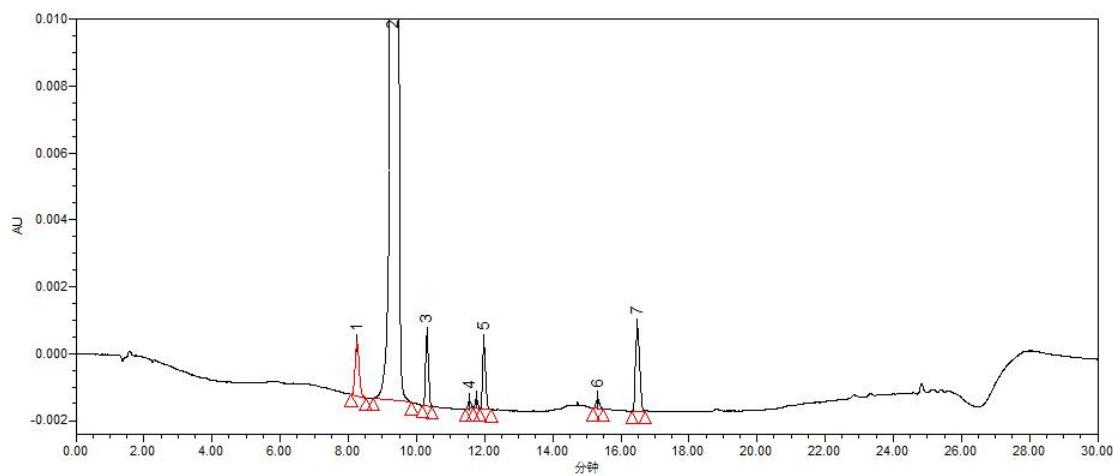
degree between YA2304, YA2304-14, and YA2304-15 NLT 1.5; The separation degree between YA2304-17 and YA2304-16 and adjacent peaks NLT 1.0;

- Reference solution (251nm): The signal-to-noise ratio (S/N) of the main peak NLT 20.

**Impurity Table 1**

Name	Correction factor	Acceptance criteria, NMT(%)
YA2304-14	1.0	0.10 (at 230nm)
YA2304	1.0	0.15 (at 251m)
YA2304-15	1.2	0.15 (at 251m)
YA2304-19	0.7	0.15 (at 251m)
Other individual impurity	1.0	0.10
Total impurities	-	1.0

Typical chromatogram of system suitability solution



注：

1号峰为杂质YA2304-14；2号峰为非奈利酮（YA2304）；3号峰为杂质YA2304-15；4号峰为杂质YA2304-17；  
5号峰为杂质YA2304-16；6号峰为杂质YA2304-10；7号峰为杂质YA2304-19

## Enantiomers

Examine by *Operating procedures of high performance liquid chromatography* (in-house method).

Solvent acetonitrile-water (30:70, v/v)

*Test solution:* Take an appropriate amount of this product, weigh it

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accurately, dissolve it in a solvent and dilute it to make a solution containing approximately 1mg per 1ml.

*Reference solution:* Take an appropriate amount of YA2304-20 reference substance, weigh it accurately, dissolve it in a solvent and dilute it to prepare a solution containing approximately 1.5  $\mu$  g per 1ml.

*System suitability solution:* Accurately weigh about 20mg of YA2304 reference substance, place it in a 20ml volumetric flask, dissolve and dilute to the mark with the reference substance solution, shake well, and obtain.

### *Chromatographic system*

—*stationary phase:* Covalent bonding of cellulose tri - (3,5-dichlorophenylcarbamate) on the surface of silicone gel (Da Sai Lu CHIRALPAK IC, 250  $\times$  4.6mm, 5  $\mu$  m, or other similar column).

—*temperature:* 40°C.

*Mobile phase:* see the gradient table blow.

- 20mmol/L ammonium acetate buffer (pH adjusted to 3.1±0.05 with phosphoric acid) -acetonitrile (70:30, v/v);

*Flow rate:* 1.0 mL/min.

*Detection:* at 251nm.

*Injection volume:* 5  $\mu$ L.

*Run time:* 40 min

*System suitability:* System suitability solution:

- System suitability solution: The peak order is YA230420, YA2304; The separation degree between YA2304, YA2304-14 NLT 1.5;

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- Reference solution : The signal-to-noise ratio (S/N) of the main peak NLT 30.

*Limits* (calculate by external standard method):

- YA2304-20 NMT 0.15%

### **Residual solvents**

#### **Acetonitrile , Tetrahydrofuran , and n-butanol**

Examine by *Operating procedures of gas chromatography* (in-house method).

*Solvent:* N, N-dimethylformamide (DMF)

*Test solution:* Take an appropriate amount of this product, weigh it accurately, dissolve it in a solvent and dilute it to prepare a solution containing approximately 50mg of non nalide per 1ml.

*Reference solution:* Take appropriate amounts of ethanol, tetrahydrofuran, toluene, and N-methylpyrrolidone, accurately weigh them, and dilute with solvent to prepare a mixed solution containing approximately 250 µ g of ethanol, 36 µ g of tetrahydrofuran, 44.5 µ g of toluene, and 26.5 µ g of N-methylpyrrolidone per 1ml.

Operating conditions:

Column	6% cyanopropyl phenyl-94% dimethylpolysiloxane, DB-624, 60m×0.53mm, 3.00µm, or other column with similar polarity.		
Column temperature	Initial keep at 40 °C for 5min, raise to 60 °C at rate of 10 °C/min, keep at 60 °C for 5min. raise to 120 °C at rate of 10 °C/min, keep at 120 °C for 5min. raise to 240 °C at rate of 10 °C/min, keep at 240 °C for 5min.		
Injection port temperature	240 °C	Split ratio	10:1 (recommended)
Detector	FID	Detector temperature	240 °C
Carrier gas	N <sub>2</sub>	Flow rate	1.5 mL/min
Injection volume		1.0 uL	

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*System suitability:* reference solution

- elution order: ethanol, tetrahydrofuran, toluene, N-methylpyrrolidone.
- *resolution:* minimum 1.5 between the adjacent peaks.

*Limits* (calculate by external standard method):

- ethanol NMT 0.5%;
- tetrahydrofuran NMT 0.072%;
- toluene NMT 0.089%;
- N-methylpyrrolidone NMT 0.053%

### **Water**

Examine in accordance with Standard operating procedure of Water determination (in-house method)

NMT 0.5%, determined on 0.2 g.

### **Residue on ignition**

Examine by *Operating procedures of residue on ignition determination* (in-house method).

NMT 0.1%, determined on 1.0 g.

### **Heavy metals**

Examine by *Operating procedures of heavy metals determination* (in-house method).

NMT 10 ppm, determined on the residue obtained in the test for *Residue on ignition*.

### **Microbial limits**

Examine by *Operating procedures of microbial limits determination* (in-house method)

Limits:

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Total aerobic microbial counts (TAMC) is NMT  $10^3$  CFU/g;

mould and yeast count (TYMC) is NMT  $10^2$  CFU/g; and

*E.coli* is absent in each 1 g of the substance to be examined.

### **Assay**

Examine by Operating procedures of high performance liquid chromatography (in-house method).

Solvent mixture: acetonitrile, water (30:70 V/V);

Test solution: Take an appropriate amount of this product, weigh it accurately, dissolve it in a solvent with ultrasound, and dilute it to make a solution containing approximately 0.02mg per 1ml.

Reference solution: Take an appropriate amount of non maleketone reference substance, accurately weigh it, dissolve it in solvent ultrasound and dilute it to prepare a solution containing approximately 0.02mg of non maleketone per 1ml.

### *Chromatographic system*

Except for the detection wavelength of 251nm, all others are Refer to the relevant material items.

### *System suitability*

-Reference solution: The theoretical number of trays NLT 3000 calculated based on the Finerenone peak, and the tailing factor NMT 1.5;

-The reference solution should be injected 6 times, and the relative standard deviation of the main peak area NMT 2%.

Calculate the percentage content of Finerenone ( $C_{21}H_{22}N_4O_3$ ) by external standard method.

It contains 98.0% to 102.0% of  $C_{21}H_{22}N_4O_3$ , calculated on the anhydrous basis.

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### **3.2.S.4.3 Validation of Analytical Procedure**

#### **3.2.S.4.3.1 Method Validation of Related Substances in Finerenone**

##### **I. Analytical Procedures**

Please refer to the analytical procedures of related substances in Finerenone in section 3.2.S.4.2.

##### **II. Validation Results and Discussion**

###### **a. Selection of Detection Wavelength**

###### **➤ Acceptance Criteria**

The selection of the detection wavelength is carried out by measuring Finerenone (YA2304), YA2304-10, YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-17, YA2304-18 and YA2304-19 at 200nm ~ 400nm wavelengths Uv scanning, YA2304-10 and YA2304-17 should have large absorption at 230nm; Finerenone (YA2304), YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18 and ya2304-19 should have greater absorption at 251nm.

###### **➤ Solution Preparation**

Blank solution: solvent.

YA2304-12 Reference product reserve liquid: Take YA2304-12 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with solvent and dilute to scale, shake well, and then get. (About 200 $\mu$ g/ml)

YA2304-14 reference product reserve liquid: Take YA2304-14 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with acetonitrile and dilute to scale, shake well, ready. (Approx. 200 $\mu$ g/ml)

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YA2304 reference product reserve liquid: Take YA2304 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with acetonitrile and dilute to scale, shake well, ready. (Approx. 200 $\mu$ g/ml)

YA2304-15 reference product reserve liquid: Take YA2304-15 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with acetonitrile and dilute to scale, shake well, ready. (Approx. 200 $\mu$ g/ml)

YA2304-16 reference product reserve liquid: Take YA2304-16 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with acetonitrile and dilute to scale, shake well, ready. (Approx. 200 $\mu$ g/ml)

YA2304-18 reference product reserve liquid: Take YA2304-18 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with acetonitrile and dilute to scale, shake well, ready. (Approx. 200 $\mu$ g/ml)

YA2304-19 reference product reserve liquid: Take YA2304-19 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with acetonitrile and dilute to scale, shake well, ready. (Approx. 200 $\mu$ g/ml)

YA2304-10 reference product reserve liquid: Take YA2304-10 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with solvent and dilute to scale, shake well, and then obtain. (About 200 $\mu$ g/ml)

YA2304-17 reference product reserve liquid: Take YA2304-17 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with solvent and dilute to scale, shake well, and then obtain. (About 200 $\mu$ g/ml)

Mixed control solution ① : Take 0.6ml of each control product reserve solution of YA2304-12, YA2304-14, YA2304, YA2304-15, YA2304-16, YA2304-18, YA2304-19, YA2304-10 and YA2304-17 and put them into a 20ml measuring bottle. Dilute it with solvent to scale and shake well; Then accurately measure 1ml of the above solution, place it in a 10ml measuring

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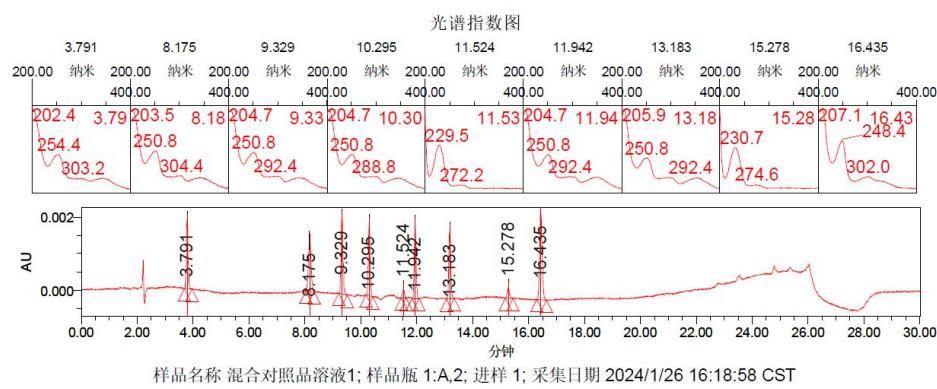
bottle, dilute it with solvent to the scale, shake well, and then get. (Both about 0.6μg/ml)

### ➤ Injection Sequence

After the blank solution, inject 1 needle mixed with reference solution ① and record the chromatogram.

### ➤ Results and Discussion

Wavelength Scanning Spectrum of Reference solution:



Results of Detection Wavelength Selection:

Ingredient name	Retention time (min)	Whether there is a large absorption at 230nm or 251nm
YA2304-10	15.278	Yes, at 230nm
YA2304-17	11.524	Yes, at 230nm
YA2304-12	3.791	Yes, at 251nm
YA2304-14	8.175	Yes, at 251nm
YA2304	9.329	Yes, at 251nm
YA2304-15	10.295	Yes, at 251nm
YA2304-16	11.942	Yes, at 251nm
YA2304-18	13.183	Yes, at 251nm
YA2304-19	16.435	Yes, at 251nm

Conclusion: Impurities YA2304-10 and YA2304-17 have large absorption at 230nm wavelength; The principal component and impurities YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18 and YA2304-19 all have large absorption at 251nm.

### b. System Suitability Determination

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### ➤ **Acceptance Criteria**

The blank solution should be free of interference; Reference solution chromatogram (230nm), the peak order is YA2304-17, YA2304-10; In the chromatogram of system suitability solution (251nm), the peak order is YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19. The separation degree between YA2304 and YA2304-14 and YA2304-15 should be NLT 1.5, and the separation degree between YA2304-17 and YA2304-16 and adjacent peaks should be NLT 1.0; In the chromatogram obtained by contrast solution (251nm) for 6 consecutive days, the RSD of the main peak NMT 1.0%, the RSD of the peak area NMT 5%, and the signal-to-noise ratio (S/N) should NLT 20.

### ➤ **Solution preparation**

Blank solution: solvent.

YA2304-12 Reference storage solution: Share "YA2304-12 Reference Storage solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-14 Reference storage solution: Share "YA2304-14 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304 Reference stock: Share "YA2304 Reference Stock" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-15 Reference storage solution: Share "YA2304-15 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-16 Reference storage solution: Share "YA2304-16 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

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YA2304-18 Reference storage solution: Share "YA2304-18 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-19 Reference storage solution: Share "YA2304-19 Reference storage solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-10 Reference storage Solution: Share "YA2304-10 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-17 Reference storage solution: Share "YA2304-17 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

Test product solution: Take about 10mg of this product, weigh it accurately, put it in a 25ml measuring bottle, dissolve it with solvent and dilute it to the scale, shake well, then get. (About 0.4mg/ml)

Control solution: accurately measure 1ml of the test product solution, place it in a 20ml measuring bottle, dilute it with solvent to the scale, and shake well; Precision measure 1ml of the above solution, place it in a 50ml measuring bottle, dilute it with solvent to the scale, shake well, and then get. (About 0.4 $\mu$ g/ml)

Reference solution: accurately measure 1ml each of YA2304-10 and YA2304-17 reference solution, place them in a 50ml measuring bottle, dilute them with solvent to scale, and shake well; Accurately measure 1ml of the above solution, place it in a 10ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (YA2304-10 and YA2304-17 contain about 0.4 $\mu$ g/ml)

System suitability solution: Accurately weigh YA2304 reference product about 10mg, place it in 25ml bottle, add appropriate amount of solvent to dissolve by ultrasound, then accurately add 100 $\mu$ l each of YA2304-14,

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YA2304-15, YA2304-16, YA2304-19, YA2304-10 and YA2304-17 reference product reserve solution. Dilute with solvent to scale, shake well, ready. (YA2304-14, YA2304-15, YA2304-16, YA2304-19, YA2304-10 and YA2304-17 all about 0.8 $\mu$ g/ml)

### ➤ **Injection Sequence**

After the blank solution, inject 1 needle of system suitability solution, 1 needle of reference solution, 6 needles of reference solution, and record the chromatogram.

### ➤ **Results and Discussion**

Results of System Suitability:

Name of spectrum	Substance name	YA2304-1 4	YA2304	YA2304-1 5	YA2304-1 7	YA2304-1 6
System suitability solution (251nm)	RT (min)	8.255	9.307	10.306	11.563	11.979
	Minimum separation between YA2304 and YA2304-14 and YA2304-15		4.9	Minimum separation between YA2304-17 and YA2304-16 and adjacent peaks		1.2
Map name	Number of samples injected	Main peak retention time (min)		Main peak area	Principal peak signal-to-noise ratio (S/N)	
Control solution (251nm)	1	9.378		6845	54.4	
	2	9.381		6900	44.5	
	3	9.381		6907	71.7	
	4	9.389		6991	73.6	
	5	9.387		6976	97.1	
	6	9.382		6726	78.4	

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	RSD(n=6)	0.05%	1.5%	/
Conclusion: There was no interference in the blank solution, the chromatogram of the control solution (230nm), the peak sequence was YA2304-17, YA2304-10; In the chromatogram of system suitable solution (251nm), the peak order was YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19. The minimum separation degree between YA2304 and YA2304-14 and YA2304-15 is 4.9 (NLT 1.5), and the minimum separation degree between YA2304-17 and YA2304-16 and adjacent peaks is 1.2 (NLT 1.0); In the chromatogram obtained by contrast solution (251nm) for 6 consecutive days, the RSD of main peak retention time was 0.05% (not more than 1.0%), the RSD of main peak area was 1.5% (not more than 5%), and the signal-to-noise ratio (S/N) was NLT 20. All the above meet the verification requirements, indicating that the method has good applicability in system.				

### **c. Specificity**

#### **i. Peak Specificity**

##### **➤ Acceptance Criteria**

The blank solution should be free from interference; In the chromatogram of system suitability solution (251nm), the peak order is YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19, The separation degree between YA2304 and YA2304-14 and YA2304-15 should be NLT 1.5, and the separation degree between YA2304-17 and YA2304-16 and adjacent peaks should be NLT 1.0; In the chromatogram of test product solution (251nm) and specific mixed solution (251nm), the separation degree between YA2304-17 and YA2304-16 and adjacent peaks should NLT 1.0, and the separation degree between other adjacent peaks should NLT 1.5; The retention time of each known impurity peak in the reference solution, system suitability solution, test product solution and specific mixed solution should be consistent with the corresponding main peak in the impurity positioning solution, and the

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relative retention time of each known impurity peak in the specific mixed solution should be basically consistent with the quality standard.

### **➤ Solution preparation**

Blank solution: solvent

YA2304-12 Reference storage solution: Share "YA2304-12 Reference Storage solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-14 Reference storage solution: Share "YA2304-14 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304 Reference stock: Share "YA2304 Reference Stock" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-15 Reference storage solution: Share "YA2304-15 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-16 Reference storage solution: Share "YA2304-16 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-18 Reference storage solution: Share "YA2304-18 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-19 Reference storage solution: Share "YA2304-19 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-10 Reference storage Solution: Share "YA2304-10 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-17 Reference storage solution: Share "YA2304-17 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

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YA2304-12 Impurity location solution: accurately measure 0.6ml of YA2304-12 reference product reserve, place it in a 20ml measuring bottle, dilute it with solvent to the scale, and shake well; Then precisely measure 1ml, place it in a 10ml measuring bottle, dilute it with solvent to the scale, and shake well, then it is ready. (About 0.6 $\mu$ g/ml)

YA2304-14 Impurity location solution: accurately measure 0.6ml of YA2304-14 reference product reserve, place it in a 20ml measuring bottle, dilute it with solvent to the scale, and shake well; Then precisely measure 1ml, place it in a 10ml measuring bottle, dilute it with solvent to the scale, and shake well, then it is ready. (About 0.6 $\mu$ g/ml)

YA2304-15 Impurity location solution: accurately measure 0.6ml of YA2304-15 reference product reserve, place it in a 20ml measuring bottle, dilute it with solvent to the scale, and shake well; Then precisely measure 1ml, place it in a 10ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 0.6 $\mu$ g/ml)

YA2304-16 Impurity location solution: accurately measure 0.6ml of YA2304-16 reference product reserve, place it in a 20ml measuring bottle, dilute it with solvent to the scale, and shake well; Then precisely measure 1ml, place it in a 10ml measuring bottle, dilute it with solvent to the scale, and shake well, then it is ready. (About 0.6 $\mu$ g/ml)

YA2304-18 Impurity location solution: Accurately measure 0.6ml of YA2304-18 reference product reserve, place it in a 20ml measuring bottle, dilute it with solvent to the scale, and shake well; Then precisely measure 1ml, place it in a 10ml measuring bottle, dilute it with solvent to the scale, and shake well, then it is ready. (About 0.6 $\mu$ g/ml)

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YA2304-19 Impurity location solution: accurately measure 0.6ml of YA2304-19 reference product reserve, place it in a 20ml measuring bottle, dilute it with solvent to the scale, and shake well; Then precisely measure 1ml, place it in a 10ml measuring bottle, dilute it with solvent to the scale, and shake well, then it is ready. (About 0.6 $\mu$ g/ml)

YA2304-17 Impurity location solution: accurately measure 0.6ml of YA2304-17 reference product reserve, place it in a 20ml measuring bottle, dilute it with solvent to the scale, and shake well; Then precisely measure 1ml, place it in a 10ml measuring bottle, dilute it with solvent to the scale, and shake well, then it is ready. (About 0.6 $\mu$ g/ml)

YA2304-10 Impurity location solution: accurately measure 0.6ml of YA2304-10 reference product reserve, place it in a 20ml measuring bottle, dilute it with solvent to the scale, and shake well; Then precisely measure 1ml, place it in a 10ml measuring bottle, dilute it with solvent to the scale, and shake well, then it is ready. (About 0.6 $\mu$ g/ml)

Reference solution: Share "Reference solution" under "4.2 System Suitability".  
(YA2304-10, YA2304-17 containing approximately 0.4 $\mu$ g/ml)

Test solution: Share "test solution" under "4.2 System Suitability". (Approx.  
0.4mg/ml)

Control solution: Share "Control solution" under "4.2 System Suitability".  
(Approx. 0.4 $\mu$ g/ml)

System Suitability Solution: Share "System Suitability Solution" under "4.2 System Suitability". (YA2304-14, YA2304-15, YA2304-16, YA2304-19, YA2304-10 and YA2304-17 all approx. 0.8 $\mu$ g/ml)

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Specific reserve liquid: Take 1.5ml of each control product reserve liquid of YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18, YA2304-19, YA2304-17 and YA2304-10 respectively and place them in the same 20ml bottle and dilute them to the scale with solvent. Shake well, then it is ready. (Contains YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18, YA2304-19, YA2304-17 and YA2304-10 about 15 $\mu$ g/ml each)

Specific mixed solution: accurately weigh 10mg of this product, place it in a 25ml measuring bottle, add an appropriate amount of solvent and ultrasonic to dissolve it, then precisely add 1.0ml of specific reserve solution, dilute it with solvent to the scale, shake well, and get. (Including YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18, YA2304-19, YA2304-17 and YA2304-10 are about 0.6 $\mu$ g/ml)

### ➤ **Injection Sequence**

After the blank solution, Sample system suitability solution, test product solution, reference product solution, reference solution, specific mixed solution, YA2304-12 impurity location solution, YA2304-14 impurity location solution, YA2304-15 impurity location solution, YA2304-16 impurity location solution, YA2304-18 impurity location solution, YA2304-19 Impurity location solution, YA2304-10 impurity location solution and YA2304-17 impurity location solution were each injected, and the chromatogram was recorded.

### ➤ **Results and Discussion**

Results of Peak Specificity:

Solution name	Detection wavelength	Component name	Component concentration ( $\mu$ g/ml)	Rt (min)	RRT	Minimum separation from
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							adjacent peaks
YA2304-12 Impurity locating solution	251nm	YA2304-12	0.6005	3.826	/	/	
YA2304-14 Impurity locating solution	251nm	YA2304-14	0.6032	8.268	/	/	
Contrast solution	251nm	YA2304	0.4113	9.378	/	/	
YA2304-15 Impurity locating solution	251nm	YA2304-15	0.6055	10.314	/	/	
YA2304-17 Impurity locating solution	251nm	YA2304-17	0.6040	11.563	/	/	
	230nm			11.556	/	/	
YA2304-16 Impurity locating solution	251nm	YA2304-16	0.5797	11.976	/	/	
YA2304-18 Impurity locating solution	251nm	YA2304-18	0.6014	13.221	/	/	
YA2304-10 Impurity locating solution	251nm	YA2304-10	0.6092	15.316	/	/	
	230nm			15.312	/	/	
YA2304-19 Impurity locating	251nm	YA2304-19	0.6008	16.496	/	/	

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solution						
Reference solution	230nm	YA2304-17	0.6040	11.558	/	/
		YA2304-10	0.6092	15.319	/	/
System Suitability solution	251nm	YA2304-14	0.8043	8.255	0.89	4.9
		YA2304	402.4291	9.307	1.00	4.9
		YA2304-15	0.8073	10.306	1.11	5.5
		YA2304-17	0.8054	11.563	1.24	1.2
		YA2304-16	0.7730	11.979	1.29	1.4
		YA2304-10	0.8122	15.316	1.65	5.5
		YA2304-19	0.8011	16.486	1.77	5.5
		YA2304-12	/	3.815	0.41	4.5
Sample solution	251nm	YA2304	411.2800	9.292	1.00	6.8
		YA2304-19	/	16.476	1.77	24.4
		YA2304-17	/	/	/	/
	230nm	YA2304-10	/	/	/	/
Specific mixed solution	251nm	YA2304-12	0.6005	3.810	0.41	3.1
		YA2304-14	0.6032	8.233	0.89	4.7
		YA2304	410.0400	9.289	1.00	5.0
		YA2304-15	0.6055	10.295	1.11	5.6
		YA2304-17	0.6040	11.549	1.24	1.2
		YA2304-16	0.5797	11.972	1.29	1.4
		YA2304-18	0.6014	13.207	1.42	7.4
		YA2304-10	0.6092	15.305	1.65	5.7
	230nm	YA2304-19	0.6008	16.474	1.77	5.7
		YA2304-12	0.6005	3.806	0.41	9.5
		YA2304-14	0.6032	8.229	0.89	5.0

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YA2304	410.0400	9.285	1.00	5.0
YA2304-15	0.6055	10.291	1.11	5.6
YA2304-17	0.6040	11.547	1.24	1.2
YA2304-16	0.5797	11.967	1.29	1.5
YA2304-18	0.6014	13.203	1.42	7.4
YA2304-10	0.6092	15.304	1.65	5.6
YA2304-19	0.6008	16.470	1.77	5.6

Conclusion: Blank solution has no interference; In the chromatogram of system suitability solution (251nm), the peak order was YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19. The minimum separation degree between YA2304 and YA2304-14 and YA2304-15 is required to be 4.9 (NLT 1.5), and the minimum separation degree between YA2304-17 and YA2304-16 and adjacent peaks is 1.2 (NLT 1.0); In the chromatogram of test product solution (251nm) and specific mixed solution (251nm), the minimum separation degree between YA2304-17 and YA2304-16 and adjacent peaks is 1.2 (NLT 1.0), and the minimum separation degree between other adjacent peaks is 3.1 (NLT 1.5). In the chromatogram of specific mixed solution (230nm), the minimum separation degree between YA2304-17 and YA2304-16 and adjacent peaks was 1.2 (NLT 1.0), and the minimum separation degree between other adjacent peaks was 5.0 (NLT 1.5). The retention time of each known impurity peak in the reference solution, the system suitability solution, the test product solution and the specific mixed solution was consistent with the corresponding main peak in the impurity localization solution, and the relative retention time of each known impurity peak in the specific mixed solution was basically consistent with the quality standard; The above are in line with the verification requirements.

### **ii. Forced Degradation Studies**

#### **➤ Acceptance Criteria**

The forced degradation test is to examine the stability of the sample under acid, alkali, oxidation, high temperature, light and other conditions. By examining the separation of impurities from the sample and the material balance rate, the feasibility of the method is verified. It is required that the

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degradation degree of the principal component NMT 20% under each destruction condition, and the separation degree between the peaks of impurities greater than 0.05% in the samples after degradation should NLT 1.0, the purity Angle of the main peak should be less than the purity threshold, and the material balance rate should be between 90% and 110%.

### **➤ Solution preparation**

Blank solution: solvent.

YA2304-14 Reference storage solution: Share "YA2304-14 Reference Storage solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304 Reference stock: Share "YA2304 Reference Stock" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-15 Reference storage solution: Share "YA2304-15 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-16 Reference storage solution: Share "YA2304-16 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-19 Reference storage solution: Share "YA2304-19 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-10 Reference storage Solution: Share "YA2304-10 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-17 Reference storage solution: Share "YA2304-17 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

System suitability solution: Prepare by the same method as "System Suitability solution" under "4.2 System Suitability". (YA2304-14, YA2304-15, YA2304-16, YA2304-19, YA2304-10 and YA2304-17 are about 0.8 $\mu$ g/ml)

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Control product solution ① : Accurately measure 1ml of YA2304 control product reserve solution, place it in 20ml measuring bottle, dilute it with solvent to scale, and shake well; Accurately measure 1ml of the above solution, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 0.4 $\mu$ g/ml)

Control product solution ② : accurately measure 1ml of YA2304-10 and YA2304-17 control product reserve solution respectively, place them in 50ml measuring bottle, dilute them with solvent to scale, and shake well; Accurately measure 1ml of the above solution, place it in a 10ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (YA2304-10 and YA2304-17 contain about 0.4 $\mu$ g/ml)

Undamaged test product solution: Take about 10mg of this product, weigh it accurately, place it in a 25ml measuring bottle, dissolve it with solvent and dilute it to the scale, shake well, and then get. (About 0.4mg/ml)

High temperature destruction of test product solution: take appropriate amount of this product, 105°C destruction for 5 days, precision weigh about 10mg, put in 25ml measuring bottle, dissolve with solvent and dilute to the scale, shake well, then get.

Light damage test product solution: Take appropriate amount of this product, light damage ( $25\pm2^{\circ}\text{C}$ ,  $60\pm10\%\text{RH}$ ,  $4500\pm500\text{LUX}$ ,  $90\pm5\mu\text{Wcm}^2$ ) 30 days after damage, precision weigh about 10mg, put in 25ml measuring bottle, dissolve with solvent and dilute to the scale, shake well, filter, ready.

The blank solution of acid and base destruction: take 2ml of 0.1mol/L hydrochloric acid solution, place it in 25ml bottle, add 2ml of 0.1mol/L sodium hydroxide solution to neutralize it, add 1ml acetonitrile, dilute it with

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solvent to the scale, shake well, and get.

Acid destruction of test product solution: Take about 10mg of this product, put it in 25ml bottle, add 2ml of 0.1mol/L hydrochloric acid solution to destroy it for 24h, add 2ml of 0.1mol/L sodium hydroxide solution to neutralize it, add 1ml of acetonitrile, dissolve it with solvent and dilute it to the scale, shake well, and obtain.

Alkali destruction test solution: Take about 10mg of this product, place it in 25ml bottle, add 2ml of 0.1mol/L of sodium hydroxide solution to destroy it for 24h, add 2ml of 0.1mol/L of hydrochloric acid solution to neutralize it, add 1ml of acetonitrile, dissolve it with solvent and dilute it to the scale, shake well, and obtain.

Oxidizing and destroying blank solution: take 2ml of 3% hydrogen peroxide solution, place it in 25ml measuring bottle, add 0.5ml acetonitrile, dilute it with solvent to scale, shake well, and get.

Oxidizing destruction of test product solution: Take about 10mg of this product, weigh it accurately, put it in 25ml bottle, add 2ml of 3% hydrogen peroxide solution and destroy it at room temperature for 24h, then add 0.5ml of acetonitrile, dilute it with solvent to the scale, shake well, and obtain.

Sample of light destruction solution: Take about 10mg of this product, accurately weigh it, put it in 25ml transparent measuring bottle, dissolve it with solvent and dilute it to the scale, shake well, and destroy it under (25±2°C, 60±10%RH, 4500±500LUX, 90±5μWcm<sup>2</sup>) under light condition for 24h, ready to obtain.

### ➤ **Injection Sequence**

After the blank solution, the system suitable solution was injected with 1

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needle, the control solution (1) and the control solution (2) were injected with 2 needles each. The blank solution of oxidation destruction, the blank solution of acid and alkali destruction, the undamaged test product solution, the acid destruction test product solution, the alkali destruction test product solution, the oxidation destruction test product solution, the high temperature destruction test product solution, the light destruction test product solution and the light destruction solution sample were each 1 needle, and the chromatogram was recorded.

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### ➤ Results and Discussion

Results of Forced Degradation Studies:

Name of solution	Undamaged test product solution	Acid destroys test solution	Alkali destroys the test solution	Oxidation destroys the test solution	High temperature destroys the test solution	Light destroys the test solution	Light destroys the solution sample
Failure conditions	/	0.1mol/l hydrochloric acid 2mL destruction for 24h	Destroy 0.1mol/l sodium hydroxide 2mL for 24h	3% hydrogen peroxide 2mL destruction for 24h	Damage at 105°C for 5d	4500±500Lux, 90±5µWcm <sup>2</sup> destroy solid samples for 30d	4500±500Lux, 90±5µWcm <sup>2</sup> destroy the test solution for 24h
Main peak purity Angle	0.313	0.259	0.276	0.300	0.266	0.286	0.261
Main peak purity threshold	1.170	1.104	1.207	1.207	1.231	1.110	1.167
Main peak purity (%)	99.93%	97.87%	99.89%	99.32%	99.95%	99.41%	99.02%
I m	YA2304-12	< 0.05%	< 0.05%	0.07%	< 0.05%	< 0.05%	< 0.05%
	YA2304-14	Not detected	Not detected	Not detected	Not detected	< 0.05%	Not detected

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p u ri ty c o nt e nt	YA2304-15	Not detected	< 0.05%	Not detected				
	YA2304-16	Not detected	< 0.05%	Not detected	Not detected	Not detected	< 0.05%	< 0.05%
	YA2304-18	Not detected						
	YA2304-19	< 0.05%	< 0.05%	< 0.05%	0.12%	< 0.05%	0.17%	0.54%
	YA2304-17	Not detected	< 0.05%	< 0.05%				
	YA2304-10	Not detected						
	RRT≈0.49 impurity	Not detected	Not detected	Not detected	< 0.05%	Not detected	Not detected	Not detected
	RRT≈0.53 impurity	Not detected	Not detected	Not detected	< 0.05%	Not detected	Not detected	Not detected
	RRT≈0.58 impurity	Not detected	Not detected	Not detected	< 0.05%	Not detected	Not detected	Not detected
	RRT≈0.59 impurity	Not detected	2.02%	Not detected	Not detected	Not detected	Not detected	< 0.05%
	RRT≈0.64 impurity	< 0.05%	Not detected	Not detected	0.43%	Not detected	< 0.05%	0.06%
	RRT≈0.67 impurity	Not detected	< 0.05%					

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	RRT≈0.71 impurity	Not detected	< 0.05%					
	RRT≈0.73 impurities	Not detected	< 0.05%	Not detected				
	RRT≈0.79 impurity	Not detected	Not detected	Not detected	< 0.05%	Not detected	< 0.05%	Not detected
	RRT≈0.87 impurity	Not detected	0.06%					
	RRT≈0.96 impurity	Not detected	< 0.05%	0.08%				
	RRT≈1.14 impurities	Not detected	< 0.05%	Not detected				
	RRT≈1.16 impurity	Not detected	< 0.05%	Not detected				
	RRT≈1.27 impurity	< 0.05%	< 0.05%	< 0.05%	< 0.05%	< 0.05%	< 0.05%	< 0.05%
	RRT≈1.33 impurities	Not detected	< 0.05%					
	RRT≈1.38	Not detected	< 0.05%	Not detected				

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	impurities							
RRT≈1.45 impurity	Not detected	Not detected	Not detected	< 0.05%	Not detected	Not detected	Not detected	Not detected
RRT≈1.52 impurity	Not detected	< 0.05%						
RRT≈1.53 impurities	Not detected	0.05%	Not detected					
RRT≈1.67 impurity	Not detected	< 0.05%	Not detected					
RRT≈1.74 impurity	Not detected	< 0.05%	Not detected					
Total Miscellaneous	< 0.05%	2.02%	0.07%	0.56%	< 0.05%	0.22%	0.74%	
Material balance ratio	/	99.02%	100.01%	99.91%	99.96%	98.15%	99.59%	

Conclusion: In the chromatogram of system applicability solution (251nm), the minimum separation degree between YA2304 and YA2304-14 and YA2304-15 is 5.8 (NLT 1.5), and the minimum separation degree between YA2304-17 and YA2304-16 and adjacent peaks is 1.7 (NLT 1.0). The signal-to-noise ratio (SNR) of the two luminous solutions ① were 37.3 and 40.4 (both greater than 20), respectively, indicating that the system applicability met the requirements. There was no significant change in the impurity quality of Finerenone raw material under the condition of high temperature (105°C for 5d). Under the condition of destruction by weak acid (destruction by 2mL of 0.1mol/l hydrochloric acid

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for 24h), Finerenone was mainly degraded to produce known impurities YA2144-16 (content < 0.05%) and unknown impurities of RRT≈0.59 (content 2.02%). The known impurity YA2304-12 (content 0.07%) was mainly degraded under the condition of weak base (destruction of 2mL of 0.1mol/l sodium hydroxide for 24h). The degradation of Finerenone under oxidation destruction (2mL destruction of 3% hydrogen peroxide for 24h) mainly produced unknown impurities YA2304-19 (content 0.12%) and RRT≈0.64 (content 0.43%). The solid samples of Finerenone were destroyed by light (4500±500Lux, 90±5μWcm<sup>2</sup> for 30d) degradation mainly produced 5 known impurities YA2304-14, YA2304-15, YA2304-16, YA2304-17, YA2304-19, and unknown impurities with RRT≈1.53 (content of 0.05%). The 5 known impurities produced by degradation except the impurity YA2304-19 content of 0.17%, the other known impurities produced by degradation content of < 0.05%; Two known impurities, YA2144-16 and YA2144-19, and three unknown impurities, RRT≈0.64, RRT≈0.87 and RRT≈0.96, were mainly produced by the degradation of fenelidone bulk drug solution by light (4500±500Lux, 90±5μWcm<sup>2</sup> destroying the test product solution for 24h). The contents of YA2304-16 and YA2304-19 produced by degradation were < 0.05% and 0.54%, and the contents of RRT≈0.64, RRT≈0.87 and RRT≈0.96 produced by degradation were 0.06%, 0.06% and 0.08%, respectively. Under each failure condition, the degradation degree of principal component was less than 20%, and the purity Angle of main peak was less than the purity threshold. The material balance rate under each destruction condition is in the range of 90%~110%, and the peaking separation degree of impurities greater than 0.05% in the degraded samples is NLT 1.0, which can be effectively separated, indicating that the method has good specificity. Meet the verification requirements.

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### **d. Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

#### **➤ Acceptance Criteria**

The limit of quantitation (LOQ) and the limit of detection (LOD) are achieved by testing and diluting the signal-to-noise ratio of each impurity reference solution with a certain concentration. The signal-to-noise ratio of each impurity peak in 6 LOQ solutions is NLT 10, the RSD of each impurity peak retention time should NMT 2.0%, and the RSD of the peak area should NMT 10%. The signal-to-noise ratio of each impurity peak in 3-needle LOD solution is NLT 3.

#### **➤ Solution preparation**

Blank solution: solvent

YA2304-12 Reference storage solution: Share "YA2304-12 Reference Storage solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-14 Reference storage solution: Share "YA2304-14 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304 Reference stock: Share "YA2304 Reference Stock" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-15 Reference storage solution: Share "YA2304-15 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-16 Reference storage solution: Share "YA2304-16 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

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YA2304-18 Reference storage solution: Share "YA2304-18 Reference Storage Solution" under "3.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-19 Reference storage solution: Share "YA2304-19 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-10 Reference storage Solution: Share "YA2304-10 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-17 Reference storage solution: Share "YA2304-17 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

Quantitative limited stock solution: Accurately measure 1ml of each reference liquid for YA2304-12, YA2304-14, YA2304, YA2304-15, YA2304-16, YA2304-18, YA2304-19, YA2304-10 and YA2304-17 and place them in the same 20ml bottle. Dilute to the scale with solvent, shake well, and then get.

Limited quantitation solution: Accurately measure 0.3ml of limited quantitation reserve liquid, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and get. (Parallel preparation of 6 parts)

Detection limit solution: accurately measure the first dose limit solution 5ml, place it in a 10ml measuring bottle, dilute it with solvent to the scale, shake well, and then get.

### **➤ Injection Sequence**

After the blank solution, 6 parts of the limited quantitative solution were injected with 1 needle each, and the detection limited solution was injected with 3 needles repeatedly, and the chromatogram was recorded.

### **➤ Results and Discussion**

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### Results of LOQ Determination:

Impurity Designation	Solution name	Concentration /( $\mu\text{g}/\text{ml}$ )	Equivalent to test product solution concentration /%	Retention time /min	Peak area	S/N	
YA2304-12	Limited quantitative solution ①	0.1201	0.0300	3.796	162 9	14. 3	
	Limited quantification solution ②			3.799	164 2	15. 1	
	Limited quantification solution ③			3.804	163 4	15. 1	
	Limited quantification solution ④			3.798	168 3	14. 1	
	Limited quantitation solution ⑤			3.801	155 6	14. 6	
	Limited quantification solution ⑥			3.803	173 5	15. 4	
	RSD			0.09%	4%	/	
Verdict: The limiting solution concentration of impurity YA2304-12 was 0.1201 $\mu\text{g}/\text{ml}$ (relative to 0.0300% of the test product concentration), the RSD of the retention time of 6 parts of the limiting solution was 0.09% (no more than 2.0%), the RSD of the peak area was 4% (no more than 10%), and the S/N ranged from 14.1 to 15.4. All of them were greater than 10. Meet verification requirements.							
YA2304-14	Limited quantitative	0.1206	0.0302	8.167	204 4	11. 1	

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	solution ①						
	Limited quantification solution ②			8.173	200 4	11. 7	
	Limited quantification solution ③			8.180	202 2	11. 8	
	Limited quantification solution ④			8.174	187 0	10. 4	
	Limited quantification solution ⑤			8.178	200 7	11. 2	
	Limited quantification solution ⑥			8.174	199 7	11. 7	
	RSD			0.06%	4%	/	
Verdict: The limiting solution concentration of impurity YA2304-14 was 0.1206µg/ml (relative to 0.0302% of the test product), the RSD of the retention time of 6 parts of the limiting solution was 0.06% (no more than 2.0%), the RSD of the peak area was 4% (no more than 10%), and the S/N ranged from 10.4 to 11.8. Were all greater than 10. Meet verification requirements.							
YA2304 (in place of unknown impurities)	Limited quantification solution ①	0.1229	0.0307	9.319	209 5	15. 2	
	Limited quantification solution ②			9.322	199 9	16. 1	
	Limited quantitation solution ③			9.329	202 4	16. 0	
	Limited			9.324	210	15.	

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	quantification solution ④				6	1
	Limited quantification solution ⑤			9.328	208 3	16. 1
	Limited quantification solution ⑥			9.327	209 7	16. 8
	RSD			0.05%	2.2 %	/
<p>Verdict: The RSD of principal component YA2304 (in place of unknown impurity) was 0.1229µg/ml (relative to 0.0307% of test substance), the RSD of retention time and peak area of 6 parts of RSD were 0.05% (no more than 2.0%) and 2.2% (no more than 10%), respectively. S/N ranged from 15.1 to 16.8, all of which were greater than 10. Meet the verification requirements.</p>						
YA2304-15	Limited quantitation solution ①	0.1211 0.0303		10.266	173 7	14. 4
	Limited quantification solution ②			10.269	178 7	15. 4
	Limited quantification solution ③			10.276	169 5	15. 3
	Limited quantification solution ④			10.269	168 9	14. 1
	Limited quantification solution ⑤			10.273	182 5	15. 3
	Limited quantification solution ⑥			10.276	169 4	15. 3

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		RSD	0.05%	4%	/
Verdict: The limiting solution concentration of impurity YA2304-15 was 0.1211 $\mu$ g/ml (relative to 0.0303% of the test product), the RSD of the retention time of 6 parts of the limiting solution was 0.05% (no more than 2.0%), the RSD of the peak area was 4% (no more than 10%), and the S/N ranged from 14.1 to 15.4. All of them were greater than 10. Meet verification requirements.					
YA2304-17 (230nm)	Limited quantitation solution ①	0.1208	0.0302	11.493	314 3      15. 4
	Limited quantification solution ②			11.496	325 8      16. 5
	Limited quantification solution ③			11.503	323 3      14. 7
	Limited quantification solution ④			11.496	318 1      14. 3
	Limited quantification solution ⑤			11.501	317 0      14. 1
	Limited quantification solution ⑥			11.504	322 0      15. 0
	RSD		0.04%	1.4 %      /	
Verdict: The limited quantification solution concentration of impurity YA2304-17 was 0.1208 $\mu$ g/ml (relative to 0.0302% of the test product), the RSD of the retention time of 6 parts of the limited quantification solution was 0.04% (no more than 2.0%), the RSD of the peak area was 1.4% (no more than 10%), and the S/N ranged from 14.1 to 16.5. Were all greater than 10. Meet verification requirements.					

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YA2304-16	Limited quantitative solution ①	0.1159	0.0290	11.919	195 3	14. 8	
	Limited quantification solution ②			11.924	190 7	15. 6	
	Limited quantification solution ③			11.931	189 8	15. 3	
	Limited quantification solution ④			11.922	186 2	14. 2	
	Limited quantification solution ⑤			11.928	192 8	15. 2	
	Limited quantification solution ⑥			11.932	184 8	15. 5	
	RSD			0.05%	2.1 %	/	
Conclusion: The limiting solution concentration of impurity YA2304-16 was 0.1159µg/ml (relative to 0.0290% of the test product), the RSD of the retention time of 6 parts of the limiting solution was 0.05% (no more than 2.0%), the RSD of the peak area was 2.1% (no more than 10%), and the S/N ranged from 14.2 to 15.6. Were all greater than 10. Meet verification requirements.							
YA2304-18	Limited quantitative solution ①	0.1203	0.0301	13.167	187 2	13. 9	
	Limited quantification solution ②			13.169	170 6	14. 6	
	Limited			13.177	186	14.	

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	quantification solution ③				7	8
	Limited quantification solution ④			13.170	185 0	13. 7
	Limited quantification solution ⑤			13.171	188 3	14. 6
	Limited quantification solution ⑥			13.178	175 1	14. 8
	RSD			0.04%	5%	/
	Verdict: The limiting solution concentration of impurity YA2304-18 was 0.1203µg/ml (relative to 0.0301% of the test product), the RSD of the retention time of 6 parts of the limiting solution was 0.04% (no more than 2.0%), the RSD of the peak area was 5% (no more than 10%), and the S/N was between 13.7 and 14.8. Were all greater than 10. Meet verification requirements.					
YA2304-10 (230nm)	Limited quantification solution ①	0.1218	0.0305	15.271	276 2	12. 1
	Limited quantification solution ②			15.274	273 5	12. 6
	Limited quantification solution ③			15.282	259 7	11. 1
	Limited quantification solution ④			15.275	274 2	11. 0
	Limited quantification solution ⑤			15.277	266 0	10. 6

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	Limited quantitation solution ⑥			15.281	258 6	11. 2
		RSD		0.028%	2.9 %	/
Verdict: The limiting solution concentration of impurity YA2304-10 was 0.1218µg/ml (relative to 0.0305% of the test product), the RSD of the retention time of 6 parts of the limiting solution was 0.028% (no more than 2.0%), the RSD of the peak area was 2.9% (no more than 10%), and the S/N was between 10.6 and 12.6. Were all greater than 10. Meet verification requirements.						
YA2304-19	Limited quantitative solution ①	0.1202	0.0300	16.431	299 4	17. 5
	Limited quantification solution ②			16.434	318 8	19. 1
	Limited quantification solution ③			16.442	304 6	19. 0
	Limited quantification solution ④			16.437	312 2	17. 6
	Limited quantification solution ⑤			16.438	300 5	18. 5
	Limited quantification solution ⑥			16.438	313 9	19. 5
				RSD	0.023%	2.6 %
Verdict: The limiting solution concentration of impurity YA2304-19 was 0.1202µg/ml (relative to 0.0300% of the test product), the RSD of the						

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	retention time of 6 parts of the limiting solution was 0.023% (no more than 2.0%), the RSD of the peak area was 2.6% (no more than 10%), and the S/N was between 17.5 and 19.5. Were all greater than 10. Meet verification requirements.
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Results of LOD Determination:

Names of impurities	Detection limit solution Sample injection	Concentration /( $\mu\text{g}/\text{ml}$ )	Equivalent to test product concentration /%	Retention time /min	Peak area	S/N
YA2304-12	1	0.0601	0.0150	3.799	832	7.3
	2			3.799	831	7.3
	3			3.802	804	6.8
	Conclusion: The detection limit solution concentration of impurity YA2304-12 is $0.0601 \mu\text{g}/\text{ml}$ (relative to 0.0150% of the test product concentration), and the S/N of the 3-needle detection limit solution is between 6.8 and 7.3, all of which are greater than 3. In line with the verification requirements.					
YA2304-14	1	0.0603	0.0151	8.175	1000	5.8
	2			8.167	936	5.5
	3			8.176	964	5.2
	Conclusion: The limit solution concentration of impurity YA2304-14 is $0.0603 \mu\text{g}/\text{ml}$ (relative to 0.0151% of the test product), and the S/N of the 3-needle limit solution is between 5.2 and 5.8, all of which are greater than 3. In line with the verification requirements.					
YA2304	1	0.0615	0.0154	9.322	1234	9.3
	2			9.321	1138	8.9
	3			9.327	1166	8.5
	Conclusion: The limited solution concentration of principal component YA2304 (in place of unknown impurity) was $0.0615 \mu\text{g}/\text{ml}$ (relative to 0.0154% of the test substance), and the S/N of the 3-needle limited					

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	solution was between 8.5 and 9.3, all of which were greater than 3. It met the verification requirements.					
YA2304-15	1	0.0605	0.0151	10.270	909	7.7
	2			10.271	911	8.1
	3			10.274	851	6.9
Conclusion: The limit solution concentration of impurity YA2304-15 is 0.0605 $\mu$ g/ml (relative to 0.0151% of the test product), and the S/N of the 3-needle limit solution is between 6.9 and 8.1, all of which are greater than 3. In line with the verification requirements.						
YA2304-17 (230nm)	1	0.0604	0.0151	11.498	1613	7.6
	2			11.497	1637	8.0
	3			11.503	1624	7.8
Conclusion: The limit solution concentration of impurity YA2304-17 is 0.0604 $\mu$ g/ml (relative to 0.0151% of the test product), and the S/N of the 3-needle limit solution ranges from 7.6 to 8.0, all of which are greater than 3. In line with the verification requirements.						
YA2304-16	1	0.0580	0.0145	11.926	961	7.6
	2			11.921	984	7.6
	3			11.930	1006	7.3
Conclusion: The limit solution concentration of impurity YA2304-16 is 0.0580 $\mu$ g/ml (relative to 0.0145% of the test product), and the S/N of the 3-needle limit solution ranges from 7.3 to 7.6, all of which are greater than 3. In line with the verification requirements.						
YA2304-18	1	0.0601	0.0150	13.174	976	7.5
	2			13.171	965	7.3
	3			13.176	913	6.6
Conclusion: The limit solution concentration of impurity YA2304-18 is 0.0601 $\mu$ g/ml (relative to 0.0150% of the test product), and the S/N of the 3-needle limit solution is between 6.6 and 7.5, all of which are greater than 3. In line with the verification requirements.						

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YA2304-10 (230nm)	1	0.0609	0.0152	15.274	1373	5.8
	2			15.274	1443	5.9
	3			15.278	1364	5.9
	Conclusion: The limit solution concentration of impurity YA2304-10 is 0.0609 $\mu$ g/ml (relative to 0.0152% of the test product), and the S/N of the 3-needle limit solution is between 5.8 and 5.9, all of which are greater than 3. In line with the verification requirements.					
YA2304-19	1	0.0601	0.0150	16.435	1486	9.1
	2			16.437	1557	9.6
	3			16.440	1557	8.6
	Conclusion: The detection limit solution concentration of impurity YA2304-19 is 0.0601 $\mu$ g/ml (relative to 0.0150% of the test product), and the S/N of the 3-needle detection limit solution is between 8.6 and 9.6, all of which are greater than 3. In line with the verification requirements.					

### **e. Linearity and Range**

#### **➤ Acceptance Criteria**

Known impurities at 251nm wavelength (YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18 and YA2304-19) and 230nm wavelength (YA2304-10, YA2304-17) : Within the range of limited quantitation concentration ~ equivalent to 0.3% of the test product solution concentration, 6 concentration points were selected fairly evenly, and the concentration of each impurity was taken as the horizontal coordinate and the peak area was taken as the vertical coordinate as the linear regression curve. The regression coefficient R of the obtained linear graph should NLT 0.990, and the RSD of the response factor should NMT 10%. Y-axis intercepts are within 25% of 100% response value.

Unknown impurities at 251nm wavelength (substituted by YA2304) : Within

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the range of limited quantitation concentration ~ equivalent to 0.3% of the test product solution concentration, 6 concentration points were selected fairly uniformly, and the concentration of each impurity was taken as the horizontal coordinate and the peak area was taken as the vertical coordinate as the linear regression curve. The regression coefficient (R) of the regression curve was NLT 0.990, and the RSD of the response factor was not more than 10%. Y-axis intercept accounted for 100% of the response value of the percentage within 25%.

### ➤ **Solution Preparation**

Blank solution: solvent

YA2304-12 Reference storage solution: Share "YA2304-12 Reference Storage solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-14 Reference storage solution: Share "YA2304-14 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304 Reference stock: Share "YA2304 Reference Stock" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-15 Reference storage solution: Share "YA2304-15 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-16 Reference storage solution: Share "YA2304-16 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-18 Reference storage solution: Share "YA2304-18 Reference storage solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-19 Reference storage solution: Share "YA2304-19 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

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YA2304-10 Reference storage Solution: Share "YA2304-10 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-17 Reference storage solution: Share "YA2304-17 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

Linear reserve solution: Take 1.0ml of each control product reserve liquid of YA2304-12, YA2304-14, YA2304, YA2304-15, YA2304-16, YA2304-18, YA2304-19, YA2304-10 and YA2304-17 and place them in a 20ml measuring bottle. Dilute to the scale with solvent, shake well, and then get. (Both about 10 $\mu$ g/ml)

Linear solution ① (LOQ solution) : prepared by the same method as "Limit of quantification solution" under "Limits of quantification and Detection Limits 4.4".

Linear solution ② (equivalent to 0.05% of the test product solution) : accurately measure 0.5ml of linear reserve solution, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and obtain. (About 0.2 $\mu$ g/ml)

Linear solution ③ (equivalent to 0.10% of the test product solution) : accurately measure 1.0ml of linear reserve solution, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and obtain. (About 0.4 $\mu$ g/ml)

Linear solution ④ (equivalent to 0.15% of the test solution) : accurately measure 1.5ml of linear reserve liquid, place it in 25ml measuring bottle, dilute it with solvent to the scale, shake well, and get. (About 0.6 $\mu$ g/ml)

Linear solution ⑤ (equivalent to 0.20% of the test product solution) :

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accurately measure 2.0ml of linear reserve liquid, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and get.  
(About 0.8 $\mu$ g/ml)

Linear solution ⑥ (equivalent to 0.30% of the test product solution) : accurately measure 3.0ml of linear reserve liquid, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and get.  
(About 1.2 $\mu$ g/ml)

### ➤ **Injection Sequence**

After the blank solution, the linear solution ①~⑥ was injected with 1 needle each from low concentration to high concentration, and the chromatogram was recorded.

### ➤ **Results and Discussion**

Results of Linearity and Range Study:

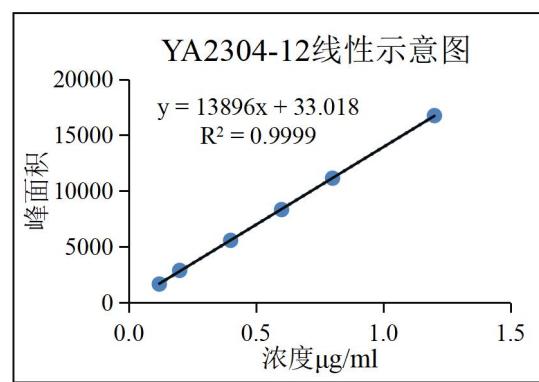
Names of impurities	Solution name	Equivalent to test product solution concentration percentage /%	Concentration /( $\mu$ g/ml)	Peak area	Response factor
YA2304-12	Linear solution ①	0.0300	0.1201	1669	13896
	Linear solution ②	0.0500	0.2002	2892	14447
	Linear solution ③	0.1001	0.4004	5582	13943
	Linear solution ④	0.1501	0.6005	8337	13883
	Linear solution ⑤	0.2002	0.8007	11146	13920
	Linear solution ⑥	0.3003	1.2011	16748	13944

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	Response factor RSD/%	1.6			
	Linear graph	 <p>YA2304-12线性示意图</p> <p>Y-axis: 峰面积 (Peak Area) ranging from 0 to 20000. X-axis: 浓度 μg/ml (Concentration) ranging from 0.0 to 1.5.</p> <p>The plot shows six data points forming a straight line. The equation of the line is <math>y = 13896x + 33.018</math> and the correlation coefficient is <math>R^2 = 0.9999</math>.</p>			
	Linear equations	$y = 13896x + 33.018$			
	Correlation coefficient R	0.99997			
	Absolute value of Y-axis intercept as % /% of 100% response value	0.39			
	<p>Conclusion: At 251nm wavelength, the RSD of YA2304-12 in the concentration range of 0.1201μg/ml~1.2011μg/ml is 1.6%, less than 10%. The linear equation is <math>y = 13896x + 33.018</math>, and the correlation coefficient R is 0.99997, greater than 0.990. The absolute value of Y-axis intercept accounts for 0.39% of 100% response value, which is less than 25%; It indicates that impurity YA2304-12 has a good linear relationship in the concentration range of 0.1201μg/ml~1.2011μg/ml.</p>				
YA2304-14	Linear solution ①	0.0302	0.1206	1957	16221
	Linear solution ②	0.0503	0.2011	3275	16288
	Linear solution ③	0.1005	0.4021	6460	16064
	Linear solution ④	0.1508	0.6032	9837	16308
	Linear solution ⑤	0.2011	0.8043	13285	16518
	Linear solution ⑥	0.3016	1.2064	19868	16468
	Response factor RSD/%	1.1			

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	Linear graph				
	Linear equations			$y = 16536x - 85.568$	
	Correlation coefficient R			0.99995	
	Absolute value of Y-axis intercept as % /% of 100% response value			0.87	
	<p>Conclusion: At 251nm wavelength, the RSD of YA2304-14 in the concentration range of 0.1206μg/ml~1.2064μg/ml is 1.1%, less than 10%. The linear equation is <math>y = 16536x - 85.568</math>, and the correlation coefficient R is 0.99995, greater than 0.990. The absolute value of Y-axis intercept accounts for 0.87% of 100% response value, which is less than 25%; It indicates that impurity YA2304-14 has a good linear relationship in the concentration range of 0.1206μg/ml~1.2064μg/ml.</p>				
YA2304 (principal component instead of unknown impurity)	Linear solution ①	0.0307	0.1229	2058	16740
	Linear solution ②	0.0512	0.2049	3438	16779
	Linear solution ③	0.1025	0.4098	6805	16605
	Linear solution ④	0.1537	0.6147	10130	16479
	Linear solution ⑤	0.2049	0.8196	13463	16426
	Linear solution ⑥	0.3074	1.2294	20246	16468
	Response factor RSD/%		1.0		

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	Linear graph		<p>YA2304(代替未知杂质)线性示意图  <math>y = 16404x + 57.399</math>  <math>R^2 = 1</math></p>		
	Linear equations		$y = 16404x + 57.399$		
	Correlation coefficient R		1.000		
	Absolute value of Y-axis intercept as % /% of 100% response value		0.87		
	<p>Conclusion: At 251nm wavelength, the RSD of YA2304 (principal component instead of unknown impurity) is 1.0%, less than 10%, in the concentration range of 0.1229μg/ml~1.2294μg/ml. The linear equation is <math>y = 16404x + 57.399</math>, and the correlation coefficient R is 1.000, greater than 0.990. The percentage of Y-axis intercept in 100% response value is 0.87%, which is within 25%; This indicates that YA2304 (principal component instead of unknown impurity) has a good linear relationship in the concentration range of 0.1229μg/ml~1.2294μg/ml.</p>				
YA2304-15	Linear solution ①	0.0303	0.1211	1801	14873
	Linear solution ②	0.0505	0.2018	2828	14013
	Linear solution ③	0.1009	0.4036	5854	14503
	Linear solution ④	0.1514	0.6055	8660	14303
	Linear solution ⑤	0.2018	0.8073	11573	14336
	Linear solution ⑥	0.3027	1.2109	17425	14390
	Response factor RSD/%		2.0		

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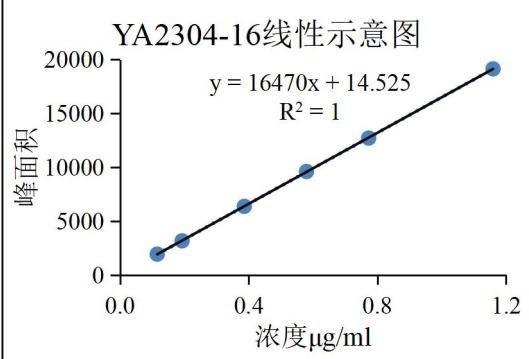
	Linear graph		 $y = 14367x + 1.2724$ $R^2 = 0.9999$		
	Linear equations		$y = 14367x + 1.2724$		
	Correlation coefficient R		0.99996		
	Absolute value of Y-axis intercept as % /% of 100% response value		0.015		
	<p>Conclusion: At 251nm wavelength, the RSD of YA2304-15 in the concentration range of 0.1211μg/ml~1.2109μg/ml is 2.0%, less than 10%. The linear equation is <math>y = 14367x + 1.2724</math>, and the correlation coefficient R is 0.99996, greater than 0.990. The absolute value of Y-axis intercept as a percentage of 100% response value is 0.015%, within 25%; It indicates that impurity YA2304-15 has a good linear relationship in the concentration range of 0.1211μg/ml~1.2109μg/ml.</p>				
YA2304-16	Linear solution	0.0290	0.1159	1942	16749
	Linear solution ②	0.0483	0.1932	3175	16430
	Linear solution ③	0.0966	0.3865	6371	16484
	Linear solution ④	0.1449	0.5797	9607	16571
	Linear solution ⑤	0.1932	0.7730	12706	16438
	Linear solution ⑥	0.2899	1.1595	19121	16491
	Response factor RSD/%		0.8		

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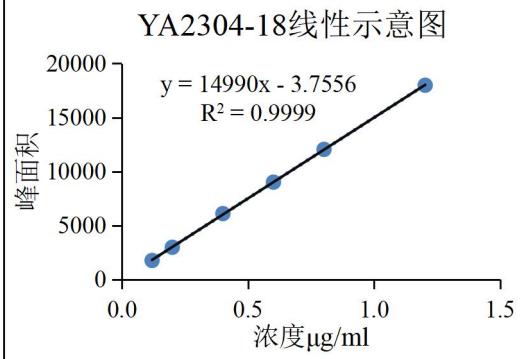
	Linear graph				
	Linear equations	$y = 16470x + 14.525$			
	Correlation coefficient R	1.000			
	Absolute value of Y-axis intercept as % /% of 100% response value	0.15			
Conclusion: At 251nm wavelength, the RSD of YA2304-16 in the concentration range of 0.1159μg/ml~1.1595μg/ml is 0.8%, less than 10%. The linear equation is $y = 16470x + 14.525$ , and the correlation coefficient R is 1.000, greater than 0.990. The absolute value of Y-axis intercept as a percentage of 100% response value is 0.15%, within 25%; It indicates that impurity YA2304-16 has a good linear relationship in the concentration range of 0.1159μg/ml~1.1595μg/ml.					
YA2304-18	Linear solution ①	0.0301	0.1203	1751	14559
	Linear solution ②	0.0501	0.2005	2973	14832
	Linear solution ③	0.1002	0.4009	6094	15201
	Linear solution ④	0.1503	0.6014	9008	14980
	Linear solution ⑤	0.2005	0.8018	12043	15020
	Linear solution ⑥	0.3007	1.2027	17988	14956
	Response factor RSD/%		1.5		

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	Linear graph				
	Linear equations	$y = 14990x - 3.7556$			
	Correlation coefficient R	0.99996			
	Absolute value of Y-axis intercept as % /% of 100% response value	0.042			
	Conclusion: At 251nm wavelength, the RSD of YA2304-18 in the concentration range of 0.1203μg/ml~1.2027μg/ml is 1.5%, less than 10%. The linear equation is $y = 14990x - 3.7556$ , and the correlation coefficient R is 0.99996, greater than 0.990. The absolute value of Y-axis intercept as a percentage of 100% response value is 0.042%, which is within 25%; It indicates that impurity YA2304-18 has a good linear relationship in the concentration range of 0.1203μg/ml~1.2027μg/ml.				
YA2304-17	Linear solution ①	0.0302	0.1208	3143	26017
	Linear solution ②	0.0503	0.2013	5288	26264
	Linear solution ③	0.1007	0.4027	10626	26388
	Linear solution ④	0.1510	0.6040	16044	26562
	Linear solution ⑤	0.2013	0.8054	21376	26542
	Linear solution ⑥	0.3020	1.2080	32097	26569
	Response factor RSD/%		0.9		

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		<p style="text-align: center;">YA2304-17线性示意图</p> <p style="text-align: center;"><math>y = 26638x - 75.991</math> <math>R^2 = 1</math></p>			
	Linear graph				
	Linear equations	$y = 26638x - 75.991$			
	Correlation coefficient R	1.000			
	Absolute value of Y-axis intercept as % /% of 100% response value	0.48			
<p>Conclusion: Under the condition of 230nm wavelength, the RSD of YA2304-17 is 0.9%, less than 10%, in the concentration range of 0.1208μg/ml~1.2080μg/ml. The linear equation is <math>y = 26638x-75.991</math>, and the correlation coefficient R is 1.000, greater than 0.990. The absolute value of Y-axis intercept as a percentage of 100% response value is 0.48%, within 25%; It indicates that impurity YA2304-17 has a good linear relationship in the concentration range of 0.1208μg/ml~1.2080μg/ml.</p>					
YA2304-10	Linear solution ①	0.0305	0.1218	2603	21366
	Linear solution ②	0.0508	0.2031	4267	21014
	Linear solution ③	0.1015	0.4061	8180	20143
	Linear solution ④	0.1523	0.6092	12086	19841
	Linear solution ⑤	0.2031	0.8122	16286	20052
	Linear solution ⑥	0.3046	1.2183	24255	19909
	Response factor RSD/%		4		

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	Linear graph		<p>YA2304-10线性示意图  <math>y = 19735x + 192.83</math>  <math>R^2 = 0.9999</math></p>		
	Linear equations			$y = 19735x + 192.83$	
	Correlation coefficient R			0.99996	
	Absolute value of Y-axis intercept as % /% of 100% response value			2.4	
	<p>Conclusion: Under the condition of 230nm wavelength, the RSD of YA2304-10 is 4%, less than 10%, in the concentration range of 0.1218μg/ml~1.2183μg/ml. The linear equation is <math>y = 19735x + 192.83</math>, and the correlation coefficient R is 0.99996, greater than 0.990. The absolute value of Y-axis intercept accounts for 2.4% of 100% response value, which is within 25%; It indicates that impurity YA2304-10 has a good linear relationship in the concentration range of 0.1218μg/ml~1.2183μg/ml.</p>				
YA2304-19	Linear solution ①	0.0300	0.1202	3087	25690
	Linear solution ②	0.0501	0.2003	5054	25236
	Linear solution ③	0.1001	0.4005	10300	25715
	Linear solution ④	0.1502	0.6008	15189	25281
	Linear solution ⑤	0.2003	0.8011	20363	25419
	Linear solution ⑥	0.3004	1.2016	30691	25541
	Response factor RSD/%		0.8		

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	Linear graph	
	Linear equation	$y = 25507x - 18.855$
	Correlation coefficient R	0.99997
	Absolute value of Y-axis intercept as % /% of 100% response value	0.12
Conclusion: At 251nm wavelength, the RSD of YA2304-19 in the concentration range of 0.1202μg/ml~1.2016μg/ml is 0.8%, less than 10%. The linear equation is $y = 25507x - 18.855$ , and the correlation coefficient R is 0.99997, greater than 0.990. The absolute value of Y-axis intercept as a percentage of 100% response value is 0.12%, within 25%; It indicates that impurity YA2304-19 has a good linear relationship in the concentration range of 0.1202μg/ml~1.2016μg/ml.		

### Validation Results of Correction Factor (f):

Names of impurities	Linear equation slope	Correction factor	
		Standard regulations	Determination results
YA2304-12	13896	1.2	1.2
YA2304-14	16536	1.0	1.0
YA2304	16404	1.0	1.0
YA2304-15	14367	1.2	1.1
YA2304-16	16470	1.0	1.0
YA2304-18	14990	1.1	1.1
YA2304-19	25507	0.7	0.6

Verdict: The correction factors of YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18, YA2304-18 and YA2304-19 relative to the principal component YA2304 are 1.18, 0.99, 1.14, 1.00 and 1.0, respectively, based on the impurities and the slope of the principal component linear equation 9, 0.64, revised to approximately 1.2, 1.0,

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1.1, 1.0, 1.1, 0.6. Compared with the correction factors specified in the quality standard, except for impurity YA2304-15 and impurity YA2304-19, the correction factors are 0.1 different from the standard comparison, and the other impurities are consistent within the deviation range. Therefore, the correction factor in the quality standard is not revised.

### **f. Accuracy**

#### ➤ **Acceptance Criteria**

The accuracy of unknown impurities is achieved by determining the recovery rate between the theoretical addition and the actual detected amount of YA2304 reference in the solvent at different concentrations (LOQ concentration, 100% limit concentration and 150% limit concentration). It is required that the recovery rate of unknown impurity LOQ concentration solution ~150% limit concentration solution should be 85%~110%, and the RSD of recovery NMT 10%.

The recovery rate of known impurities is by adding each impurity in the test product solution within the range of 30% limit concentration ~ 150% limit concentration of each impurity reference products (note: Impurity YA2304-10 is added to 0.045% test product solution concentration ~ 0.225% test product solution concentration impurity reference), and the recovery rate between the theoretical added amount and the actual detected amount is achieved. It is required that the recovery rate of known impurity 30% limit concentration sampling solution ~150% limit concentration solution is 85%~110%, and the RSD of each impurity recovery rate NMT 10%.

#### ➤ **Solution Preparation**

### **i. Identified Impurities**

Blank solution: solvent

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YA2304-12 Reference storage solution: Share "YA2304-12 Reference Storage solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-14 Reference storage solution: Share "YA2304-14 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-15 Reference storage solution: Share "YA2304-15 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-16 Reference storage solution: Share "YA2304-16 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-18 Reference storage solution: Share "YA2304-18 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-19 Reference storage solution: Share "YA2304-19 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-10 Reference storage Solution: Share "YA2304-10 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-17 Reference storage solution: Share "YA2304-17 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

Test product solution: Take YA2304 sample about 10mg, weigh it accurately, put it in 25ml measuring bottle, dissolve it with solvent and dilute it to scale, shake well, and then get it. Prepare 2 parts in parallel. (About 0.4mg/ml)

Mixed reference solution: accurately measure 1ml each of YA2304-10 reference solution, YA2304-17 reference solution and YA2304 reference solution, place them in the same 20ml bottle, dilute to scale with solvent, and shake well; Precisely measure 1ml of the above solution, place it in a 25ml

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measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (YA2304-10, YA2304-17 and YA2304 contain about 0.4 $\mu$ g/ml)

System suitability solution: Accurately weigh YA2304 reference product about 10mg, place it in 25ml bottle, add appropriate amount of solvent to dissolve by ultrasound, then accurately add 100 $\mu$ l each of YA2304-14, YA2304-15, YA2304-16, YA2304-19, YA2304-10 and YA2304-17 reference product reserve solution. Dilute with solvent to scale, shake well, ready. (YA2304-14, YA2304-15, YA2304-16, YA2304-19, YA2304-10 and YA2304-17 all about 0.8 $\mu$ g/ml)

Sample recovery reserve liquid: Take 1.5ml of each control product reserve liquid of YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18, YA2304-19, YA2304-17 and YA2304-10 into the same 20ml measuring bottle and dilute it to the scale with solvent. Shake well, then it is ready. (About 15 $\mu$ g/ml)

30% sample recovery solution (Note: YA2304-10 is 0.045% test solution concentration) : Take YA2304 sample about 10mg, accurately weigh, put into 25ml measuring bottle, add appropriate amount of solvent to dissolve by ultrasound, accurately add 0.3ml of sample recovery liquid, dilute with solvent to scale, shake well, and then obtain. Prepare 3 parts in parallel.

100% sample recovery solution (Note: YA2304-10 is 0.15% test product solution concentration) : Take about 10mg of YA2304 sample, accurately weigh it, place it in a 25ml measuring bottle, add an appropriate amount of solvent to dissolve it by ultrasound, accurately add 1.0ml of the reserve liquid with sample recovery rate, dilute it with solvent to the scale, shake well, and obtain. Prepare 3 parts in parallel.

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150% sample recovery solution (Note: YA2304-10 is 0.225% test product solution concentration) : Take about 10mg of YA2304 sample, accurately weigh it, place it in 25ml measuring bottle, add an appropriate amount of solvent to dissolve by ultrasound, accurately add 1.5ml of the reserve liquid with sample recovery rate, dilute it with solvent to the scale, shake well, and obtain. Prepare 3 parts in parallel.

### **ii. Unidentified Impurities**

Blank solution: solvent

YA2304 Reference storage solution: Share "YA2304 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

Recovery rate of reserve liquid: accurately measure YA2304 reference product reserve liquid 1ml, place it in 20ml measuring bottle, dilute it with solvent to scale, shake well, then obtain. (About 10 $\mu$ g/ml)

Control product solution: accurately measure the recovery rate of 1ml of reserve liquid, put it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then get. (About 0.4 $\mu$ g/ml)

LOQ recovery solution: accurately measure the recovery rate of 0.3ml of reserve liquid, put it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then get. 3 parts in parallel (about 0.12 $\mu$ g/ml)

100% recovery solution: accurately measure the recovery rate of the reserve liquid 1.0ml, put it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. 3 parts in parallel (about 0.4 $\mu$ g/ml)

150% recovery solution: accurately measure the recovery rate of 1.5ml of the

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reserve liquid, put it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. 3 parts in parallel (about 0.6 $\mu$ g/ml)

### ➤ **Injection Sequence**

After the blank solution, 1 needle was injected into the system suitability solution, 2 needles into the mixed control solution, 1 needle into each of 2 sample solutions and 9 sample recovery solutions; 2 needles were injected into the control solution and 1 needle into each of the 9 recovery solutions, and the chromatogram was recorded.

### ➤ **Results and Discussion**

Results of Accuracy Study:

Impurities Name	Solution name	Quantity of test product detected ( $\mu$ g/ml)	Amount added ( $\mu$ g/ml)	Measured amount ( $\mu$ g/ml)	Recovery rate (%)
YA2304-12	30% sample recovery solution ①	0.0248	0.1802	0.1992	96.8
	30% sample recovery solution ②	0.0249	0.1802	0.2054	100.1
	30% recovery solution ③	0.0249	0.1802	0.2048	99.9
	100% recovery solution ①	0.0251	0.6005	0.6142	98.1
	100% recovery solution ②	0.0252	0.6005	0.6143	98.1
	100% recovery solution ③	0.0251	0.6005	0.6266	100.2
	150% recovery solution ①	0.0251	0.9008	0.9121	98.5
	150% recovery solution ②	0.0251	0.9008	0.9046	97.6
	150% recovery solution ③	0.0252	0.9008	0.9083	98.0

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	Average recovery rate (%)			98.6
	RSD (%)			1.3
	Verdict: The impurity YA2304-12 in the test product was 0.006%. The control product YA2304-12 was added to the test product in the limit concentration range of 30%~150% (that is, 0.1802 $\mu$ g/ml~0.9008 $\mu$ g/ml). The recovery rate of each concentration point was 96.8%~100.2%. In the range of 85%~110%; The average recovery rate was 98.6%. The RSD of recovery was 1.3%, less than 10%; Therefore, the accuracy of impurity YA2304-12 is good in the range of 0.1802 $\mu$ g/ml~0.9008 $\mu$ g/ml.			
Impurities Name	Solution name	Test quantity ( $\mu$ g/ml)	Amount added ( $\mu$ g/ml)	Measured amount ( $\mu$ g/ml)
YA2304-14	30% recovery solution ①	Not detected	0.1810	0.1830
	30% sample recovery solution ②		0.1810	0.1810
	30% recovery solution ③		0.1810	0.1849
	100% recovery solution ①		0.6032	0.5880
	100% recovery solution ②		0.6032	0.5879
	100% recovery solution ③		0.6032	0.5846
	150% recovery solution ①		0.9048	0.8827
	150% recovery solution ②		0.9048	0.8781
	150% recovery solution ③		0.9048	0.8760
	Average recovery rate (%)		98.5	
	RSD (%)		2.1	
Conclusions: The impurity YA2304-14 was not detected in the test product, and the control product YA2304-14 was added to the test product in the limit concentration range of 30% to 150% (that is, in the range of 0.1810 $\mu$ g/ml to 0.9048 $\mu$ g/ml). The recoveries of each concentration point were 96.8% to 102.2%, and the recoveries were 85% to 110%. The average recoveries were 98.5%; The RSD of recovery was 2.1%, less than 10%; Therefore, the accuracy of impurity YA2304-14 was				

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good in the range of 0.1810µg/ml~0.9048µg/ml.						
Impurities Name	Solution name	Quantity of test product detected (µg/ml)	Amount added (µg/ml)	Measured amount (µg/ml)	Recovery rate (%)	
YA2304-15	30% recovery solution ①	Not detected	0.1816	0.1867	102.8	
	30% sample recovery solution ②		0.1816	0.1858	102.3	
	30% recovery solution ③		0.1816	0.1857	102.2	
	100% recovery solution ①		0.6055	0.5950	98.3	
	100% recovery solution ②		0.6055	0.6005	99.2	
	100% sample recovery solution ③		0.6055	0.5982	98.8	
	150% recovery solution ①		0.9082	0.8881	97.8	
	150% recovery solution ②		0.9082	0.8878	97.8	
	150% recovery solution ③		0.9082	0.8951	98.6	
	Average recovery rate (%)				99.7	
	RSD (%)				2.1	
Conclusions: The impurity YA2304-15 was not detected in the test product. The control product YA2304-15 was added in the limit concentration range of 30%~150% (0.1816µg/ml~0.9082µg/ml). The recoveries of each concentration point were 97.8%~102.8%, and the recoveries were 85%~110%. The average recoveries were 99.7%; The RSD of recovery was 2.1%, less than 10%; Therefore, the accuracy of impurity YA2304-15 was good in the range of 0.1816µg/ml~0.9082µg/ml.						
Impurities Name	Solution name	Quantity of test product detected (µg/ml)	Amount added (µg/ml)	Measured amount (µg/ml)	Recovery rate (%)	
YA2304-16	30% recovery solution ①	Not detected	0.1739	0.1606	92.4	

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	30% sample recovery solution ②		0.1739	0.1588	91.3	
	30% recovery solution ③		0.1739	0.1625	93.4	
	100% recovery solution ①		0.5797	0.5519	95.2	
	100% recovery solution ②		0.5797	0.5515	95.1	
	100% recovery solution ③		0.5797	0.5516	95.1	
	150% recovery solution ①		0.8696	0.8297	95.4	
	150% recovery solution ②		0.8696	0.8282	95.2	
	150% recovery solution ③		0.8696	0.8240	94.7	
	Average recovery rate (%)			94.2		
	RSD (%)			1.6		
Conclusions: The impurity YA2304-16 was not detected in the test product, and the control product YA2304-16 was added in the limit concentration range of 30%~150% (0.1739μg/ml~0.8696μg/ml). The recoveries of each concentration point were 91.3%~95.4%, and the recoveries were 85%~110%. The average recoveries were 94.2%; The RSD of recovery was 1.6%, less than 10%. Therefore, the accuracy of impurity YA2304-16 was good in the range of 0.1739μg/ml~0.8696μg/ml.						
Impurities Name	Solution name	Quantity of test product detected (μg/ml)	Dosage (μg/ml)	Measured amount (μg/ml)	Recovery rate (%)	
YA2304-18	30% recovery solution ①	Not detected	0.1804	0.1762	97.7	
	30% sample recovery solution ②		0.1804	0.1777	98.5	
	30% recovery solution ③		0.1804	0.1760	97.5	
	100% recovery solution ①		0.6014	0.5856	97.4	
	100% recovery solution ②		0.6014	0.5921	98.5	

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	100% recovery solution ③		0.6014	0.5892	98.0	
	150% recovery solution ①		0.9020	0.8734	96.8	
	150% recovery solution ②		0.9020	0.8742	96.9	
	150% recovery solution ③		0.9020	0.8775	97.3	
	Average recovery rate (%)				97.6	
	RSD (%)				0.7	
	Conclusions: The impurity YA2304-18 was not detected in the test product, and the control product YA2304-18 was added to the test product in the limit concentration range of 30% to 150% (that is, 0.1804μg/ml to 0.9020μg/ml). The recoveries of each concentration point were 96.8% to 98.5%, and all were 85% to 110%. The average recoveries were 97.6%; The RSD of recovery was 0.7%, less than 10%; Therefore, the accuracy of impurity YA2304-18 was good in the range of 0.1804μg/ml~0.9020μg/ml.					
Impurities Name	Solution name	Quantity of test product detected (μg/ml)	Amount added (μg/ml)	Measured amount (μg/ml)	Recovery rate (%)	
YA2304-19	30% recovery solution ①	0.0359	0.1802	0.2094	96.3	
	30% sample recovery solution ②	0.0360	0.1802	0.2271	106.0	
	30% recovery solution ③	0.0359	0.1802	0.2021	92.2	
	100% recovery solution ①	0.0362	0.6008	0.6206	97.3	
	100% recovery solution ②	0.0363	0.6008	0.6123	95.9	
	100% recovery solution ③	0.0362	0.6008	0.6123	95.9	
	150% recovery solution ①	0.0363	0.9012	0.9011	96.0	
	150% recovery solution ②	0.0363	0.9012	0.9003	95.9	

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	150% recovery solution ③	0.0363	0.9012	0.9039	96.3	
	Average recovery rate (%)				96.8	
	RSD (%)				4	
	Verdict: The impurity YA2304-19 in the test product was 0.009%. The control product YA2304-19 in the limit concentration range of 30%~150% was added to the test product (that is, 0.1802μg/ml~0.9012μg/ml). The recovery rate of each concentration point was 92.2%~106.0%. In the range of 85%~110%; The average recovery rate was 96.8%. The RSD of recovery was 4%, less than 10%; Therefore, the accuracy of impurity YA2304-19 is good in the range of 0.1802μg/ml~0.9012μg/ml.					
Impurities Name	Solution name	Quantity of test product detected (μg/ml)	Amount added (μg/ml)	Measured amount (μg/ml)	Recovery rate (%)	
YA2304-10 (230nm)	45% sample recovery solution ①	Not detected	0.1827	0.1848	101.1	
	45% sample recovery solution ②		0.1827	0.1848	101.1	
	45% sample recovery solution ③		0.1827	0.1815	99.3	
	150% recovery solution ①		0.6092	0.5928	97.3	
	150% recovery solution ②		0.6092	0.5990	98.3	
	150% recovery solution ③		0.6092	0.5948	97.6	
	225% recovery solution ①		0.9137	0.8880	97.2	
	225% recovery solution ②		0.9137	0.8936	97.8	
	225% recovery solution ③		0.9137	0.8880	97.2	
	Average recovery (%)				98.6	
	RSD (%)				1.7	
Verdict: The impurity YA2304-10 was not detected in the test product, and the control product YA2304-10 was added in the concentration range of 0.045%~0.225% of the test product (that is, 0.1827μg/ml~0.9137μg/ml). The recovery rate of each concentration point was 97.2%~101.1%. In the						

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	range of 85%~110%; The average recovery rate was 98.6%. The RSD of recovery was 1.7%, less than 10%; The results indicated that impurity YA2304-10 was in the range of 0.1827 $\mu$ g/ml~0.9137 $\mu$ g/ml, and the accuracy was good.					
Impurities Name	Solution name	Quantity of test product detected ( $\mu$ g/ml)	Amount added ( $\mu$ g/ml)	Measured amount ( $\mu$ g/ml)	Recovery rate (%)	
YA2304-17 (230nm)	30% sample recovery solution ①	Not detected	0.1812	0.1757	96.9	
	30% sample recovery solution ②		0.1812	0.1779	98.2	
	30% recovery solution ③		0.1812	0.1779	98.2	
	100% recovery solution ①		0.6040	0.5915	97.9	
	100% recovery solution ②		0.6040	0.5944	98.4	
	100% recovery solution ③		0.6040	0.5925	98.1	
	150% recovery solution ①		0.9060	0.8922	98.5	
	150% recovery solution ②		0.9060	0.8903	98.3	
	150% recovery solution ③		0.9060	0.8929	98.5	
	Average recovery rate (%)				98.1	
	RSD (%)				0.5	
	Conclusions: The impurity YA2304-17 was not detected in the test product, and the control product YA2304-17 was added to the test product in the limit concentration range of 30% to 150% (that is, 0.1812 $\mu$ g/ml to 0.9060 $\mu$ g/ml). The recoveries of each concentration point were 96.9% to 98.5%, and all were 85% to 110%. The average recoveries were 98.1%; The RSD of recovery was 0.5%, less than 10%; Therefore, the accuracy of impurity YA2304-17 was good in the range of 0.1812 $\mu$ g/ml~0.9060 $\mu$ g/ml.					
Impurities Name	Solution name	Quantity of test product detected	Amount added ( $\mu$ g/ml)	Measured quantity ( $\mu$ g/ml)	Recovery rate (%)	

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		( $\mu\text{g}/\text{ml}$ )			
YA2304 (principal component instead of unknown impurity)	LOQ recovery solution ①	/	0.1229	0.1270	103.3
	LOQ recovery solution ②		0.1229	0.1232	100.2
	LOQ recovery solution ③		0.1229	0.1295	105.3
	100% recovery solution ①		0.4098	0.4078	99.5
	100% recovery solution ②		0.4098	0.4114	100.4
	100% recovery solution ③		0.4098	0.4139	101.0
	150% recovery solution ①		0.6147	0.6227	101.3
	150% recovery solution ②		0.6147	0.6139	99.9
	150% recovery solution ③		0.6147	0.6176	100.5
	Average recovery rate (%)		101.3		
		RSD (%)			1.9
Conclusion: YA2304 (principal component instead of unknown impurity) was added to the solvent in the limit concentration range of LOQ~150% (that is, $0.1229\mu\text{g}/\text{ml} \sim 0.6147\mu\text{g}/\text{ml}$ ), and the recoveries of each concentration point were 99.5%-105.3%, and all were between 85% and 110%. The average recovery rate was 101.3%. The RSD of recovery was 1.9%, less than 10%; Therefore, the impurity YA2304 (principal component instead of unknown impurity) was in the range of $0.1229\mu\text{g}/\text{ml} \sim 0.6147\mu\text{g}/\text{ml}$ , and the accuracy was good.					

### **g. Precision**

Precision refers to the proximity between the results obtained by multiple sampling of the same uniform test product under specified test conditions, and its precision is judged by examining repeatability and intermediate precision.

#### **i. Repeatability**

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### **➤ Acceptance Criteria**

6 repeatable sample solutions were prepared by Analyst A on date A and determined by instrument A. The blank solution should be free of interference; Reference solution chromatogram (230nm), the peak order is YA2304-17, YA2304-10; In the chromatogram of system suitability solution (251nm), the peak order is YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19. The separation degree between YA2304 and YA2304-14 and YA2304-15 should be NLT 1.5, and the separation degree between YA2304-17 and YA2304-16 and adjacent peaks should be NLT 1.0; In the contrast solution chromatogram (251nm), the signal-to-noise ratio (S/N) of the main peak should NLT 20. In 6 samples of repetitive test solution with limited concentration, the impurity peak less than 0.5 times of the main peak area of the control solution should be ignored, and the RSD of other impurities should NMT 5%, and the RSD of total impurity should NMT 5%.

### **➤ Solution Preparation**

Blank solution: solvent

YA2304-12 Reference storage solution: Share "YA2304-12 Reference Storage solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-14 Reference storage solution: Share "YA2304-14 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304 Reference stock: Share "YA2304 Reference Stock" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

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YA2304-15 Reference storage solution: Share "YA2304-15 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-16 Reference storage solution: Share "YA2304-16 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-18 Reference storage solution: Share "YA2304-18 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-19 Reference storage solution: Share "YA2304-19 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-10 Reference storage Solution: Share "YA2304-10 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-17 Reference storage solution: Share "YA2304-17 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

System suitability solution: Share "System Suitability Solution" under "4.3.2 Forced degradation Test".

Reference solution: share "reference solution ②" under "4.3.2 Forced degradation Test". (YA2304-10, YA2304-17 containing about 0.4 $\mu$ g/ml)

Repeatable reference stock solution: Accurately measure 0.75ml of each of YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18, YA2304-19, YA2304-17 and YA2304-10 of the reference product reserves, place them in a 10ml measuring bottle, and dilute them to the scale with solvent. Shake well, then it is ready.

Repeatable test product solution: accurately weigh the YA2304 sample about 10mg, place it in 25ml measuring bottle, add an appropriate amount of

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solvent to dissolve by ultrasound, accurately add 1ml repeatable reference product reserve solution, dilute it with solvent to scale, shake well, and obtain. (6 copies were prepared in parallel, named as repeatable test product solution ①~⑥)

Control solution: accurately measure 1ml of the test product solution, place it in a 20ml measuring bottle, dilute it with solvent to the scale, and shake well; Precision measure 1ml of the above solution, place it in a 50ml measuring bottle, dilute it with solvent to the scale, shake well, and then get. (named as control solution ①~⑥)

### ➤ **Injection Sequence**

After the blank solution, 1 needle was injected into the system suitability solution, 2 needles into the control solution, 1 needle into each of 6 control solutions and 6 repetitive test solutions, and the chromatogram was recorded.

### ➤ **Results and Discussion**

Results of Repeatability Study:

Name of solution		Repeatable test product ①	Repeate t test produ ct ②	Repea t sampl e ③	Repea t sampl e ④	Repetiti ve test product ⑤	Repetiti ve test product ⑥	RS D (%) (n= 6)
Impurity content /%	Impurity YA2304-12	0.156	0.154	0.157	0.155	0.155	0.156	0.7
	Impurity YA2304-14	0.149	0.148	0.148	0.150	0.149	0.149	0.5
	Impurity	0.158	0.155	0.157	0.156	0.156	0.156	0.7

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	YA2304-15							
	Impurity YA2304-16	0.141	0.138	0.139	0.139	0.140	0.139	0.9
	Impurity YA2304-18	0.148	0.146	0.148	0.147	0.147	0.146	0.5
	Impurity YA2304-19	0.166	0.165	0.167	0.172	0.164	0.164	1.7
	Impurity YA2304-17	0.147	0.147	0.148	0.148	0.148	0.146	0.6
	Impurity YA2304-10	0.148	0.147	0.148	0.148	0.148	0.147	0.4
	Unknown n impurity (RRT≈0.64)	<0.05%	<0.05 %	<0.05 %	<0.05 %	<0.05%	<0.05%	/
	Unknown n impurity (RRT≈1.27)	<0.05%	<0.05 %	<0.05 %	<0.05 %	<0.05%	<0.05%	/
	Total impurity	1.213	1.201	1.212	1.215	1.206	1.204	0.5
Verdict: Impurities YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18 and YA2304-19 were calculated by the principal component self-control method with correction factor under the wavelength of 251nm. Impurities YA2304-10 and YA2304-17 were calculated at 230nm wavelength by external standard method. The impurity peak less than 0.5 times of the main peak area of the control solution was ignored in the 6 samples with limited concentration repeatable test solution. The RSD of other impurities was less than 5%, and the RSD of total impurities was less than 5%, which met the requirements. The method has good repeatability.								

### ii. Intermediate Precision

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### **➤ Acceptance Criteria**

6 parts of intermediate precision test product solution were prepared by Analyst B on date B and determined by instrument B. The blank solution should be free of interference; Reference solution chromatogram (230nm), the peak order is YA2304-17, YA2304-10; In the chromatogram of system suitability solution (251nm), the peak order is YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19. The separation degree between YA2304 and YA2304-14 and YA2304-15 should be NLT 1.5, and the separation degree between YA2304-17 and YA2304-16 and adjacent peaks should be NLT 1.0; In the chromatogram obtained by contrast solution (251nm) for 6 consecutive days, the RSD of the main peak NMT 1.0%, the RSD of the peak area NMT 5%, and the signal-to-noise ratio (S/N) should NLT 20. In 6 intermediate precision test solutions, the impurity peak less than 0.5 times of the main peak area of the control solution should be ignored, the RSD of other impurities should NMT 5%, and the RSD of total impurity should NMT 5%.

In the results of 12 tests of repeatability and intermediate precision, the RSD of other impurities NMT 10% and the RSD of total impurities NMT 10% if the impurity peak is less than 0.5 times the main peak area of the control solution.

### **➤ Solution Preparation**

Blank solution: solvent

YA2304-12 Reference storage solution: Share "YA2304-12 Reference Storage solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

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YA2304-14 Reference storage solution: Share "YA2304-14 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304 Reference stock: Share "YA2304 Reference Stock" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-15 Reference storage solution: Share "YA2304-15 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-16 Reference storage solution: Share "YA2304-16 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-18 Reference storage solution: Share "YA2304-18 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-19 Reference storage solution: Share "YA2304-19 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-10 Reference storage Solution: Share "YA2304-10 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-17 Reference storage solution: Share "YA2304-17 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

System suitability solution: Prepare by the same method as "System Suitability solution" under "4.2 System Suitability". (YA2304-14, YA2304-15, YA2304-16, YA2304-19, YA2304-10 and YA2304-17 are about 0.8 $\mu$ g/ml)

Reference solution: Prepare it by the same method as "reference solution" under "4.2 System Suitability". (YA2304-10 and YA2304-17 both contain about 0.4 $\mu$ g/ml)

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Intermediate precision control product reserve liquid: Take 0.75ml of each control product reserve liquid of YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18, YA2304-19, YA2304-17 and YA2304-10 and place them in a 10ml measuring bottle and dilute them to the scale with solvent. Shake well, then it is ready.

Intermediate precision test product solution: accurately weigh the YA2304 sample about 10mg, place it in a 25ml measuring bottle, add an appropriate amount of solvent and ultrasonic to dissolve it, then precisely add 1ml of the intermediate precision control product reserve, dilute it with solvent to the scale, shake well, and obtain. (6 parts were prepared in parallel, named as intermediate precision test product solution ①~⑥)

Control solution: accurately measure 1ml of the test product solution, place it in a 20ml measuring bottle, dilute it with solvent to the scale, and shake well; Precision measure 1ml of the above solution, place it in a 50ml measuring bottle, dilute it with solvent to the scale, shake well, and then get. (named as control solution ①~⑥)

### **➤ Injection Sequence**

After the blank solution, 1 needle was injected into the system suitable solution, 2 needles were injected into the control solution, 6 needles were repeated into the control solution, 1 needle was injected into the control solution ②~⑥ and 1 needle was injected into the intermediate precision test solution ①~⑥, and the chromatogram was recorded.

### **➤ Results and Discussion**

System Suitability of Intermediate Precision Study:

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Map Name	Substance name	YA2304-1 4	YA2304	YA2304-1 5	YA2304-1 7	YA2304-1 6
System suitability solution (251nm)	RT (min)	8.335	9.348	10.352	11.561	12.027
	Minimum separation between YA2304 and YA2304-14 and YA2304-15		5.7	Minimum separation between YA2304-17 and YA2304-16 and adjacent peaks		1.6
Map name	Number of samples injected	Main peak retention time (min)		Main peak area	Principal peak signal-to-noise ratio (S/N)	
Control solution (251nm)	1	9.394	7013	93.2		
	2	9.380	6926	99.1		
	3	9.384	6843	107.0		
	4	9.404	6972	107.7		
	5	9.414	7098	139.5		
	6	9.409	7247	83.1		
	RSD(n=6)	0.15	2.1	/		
Conclusion: There was no interference in the blank solution, the chromatogram of the control solution (230nm), the peak sequence was YA2304-17, YA2304-10; In the chromatogram of system suitable solution (251nm), the peak order was YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19. The minimum separation degree between YA2304 and YA2304-14 and YA2304-15 is 5.7 (NLT 1.5), and the minimum separation degree between YA2304-17 and YA2304-16 and adjacent peaks is 1.6 (NLT 1.0); In the chromatogram obtained by contrast solution (251nm) for 6 consecutive days, the RSD of the main peak retention time was 0.15% (not more than 1.0%), the RSD of the main peak area was 2.1% (not more than 5%), and the signal-to-noise ratio (S/N) was NLT 20. In accordance with the verification requirements.						

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Results of Precision Study:

Name of solution		Repeatable test product ①	Repeat test product ②	Repeat sample ③	Repeat sample ④	Repetitive test product ⑤	Repetitive test product ⑥	RSD (%) (n=6)
Impurity content / %	Impurity YA2304-12	0.156	0.154	0.157	0.155	0.155	0.156	0.7
	Impurity YA2304-14	0.149	0.148	0.148	0.150	0.149	0.149	0.5
	Impurity YA2304-15	0.158	0.155	0.157	0.156	0.156	0.156	0.7
	Impurity YA2304-16	0.141	0.138	0.139	0.139	0.140	0.139	0.9
	Impurity YA2304-18	0.148	0.146	0.148	0.147	0.147	0.146	0.5
	Impurity YA2304-19	0.166	0.165	0.167	0.172	0.164	0.164	1.7
	Impurity YA2304-17	0.147	0.147	0.148	0.148	0.148	0.146	0.6
	Impurity YA2304-10	0.148	0.147	0.148	0.148	0.148	0.147	0.4
	Unknown impurity (RRT≈0.64)	<0.05%	<0.05%	<0.05%	<0.05%	<0.05%	<0.05%	/
	Unknown impurity (RRT≈1.27)	<0.05%	<0.05%	<0.05%	<0.05%	<0.05%	<0.05%	/
Total impurity		1.213	1.201	1.212	1.215	1.206	1.204	0.5

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Verdict: Impurities YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18 and YA2304-19 were calculated by the principal component self-control method with correction factor under the wavelength of 251nm. Impurities YA2304-10 and YA2304-17 were calculated at 230nm wavelength by external standard method. The impurity peak less than 0.5 times of the main peak area of the control solution was ignored in the 6 samples with limited concentration repeatable test solution. The RSD of other impurities was less than 5%, and the RSD of total impurities was less than 5%, which met the requirements. The method has good repeatability.

Solution name		Intermediate precision test product ①	Intermediate precision test product ②	Intermediate precision sample ③	Intermediate precision test product ④	Intermediate precision test product ⑤	Intermediate precision test product ⑥	RSD (%) (n=6)	RSD (%) (n=12)
Impurity content /%	Impurity YA2304-12	0.145	0.148	0.150	0.150	0.151	0.149	1.5	2.6
	Impurity YA2304-14	0.142	0.142	0.147	0.144	0.146	0.143	1.4	2.0
	Impurity YA2304-15	0.151	0.151	0.154	0.153	0.154	0.153	1.0	1.5
	Impurity YA2304-16	0.131	0.131	0.135	0.135	0.135	0.133	1.6	2.6
	Impurity YA2304-18	0.140	0.140	0.145	0.144	0.143	0.141	1.4	2.0
	Impurity YA2304-19	0.164	0.163	0.171	0.171	0.167	0.165	2.1	1.9
	Impurity YA2304-17	0.142	0.142	0.142	0.142	0.142	0.142	0.22	2.1
	Impurity YA2304-10	0.148	0.146	0.144	0.146	0.146	0.148	1.1	0.9
	Unknown impurity (RRT≈0.64)	<0.05%	<0.05%	<0.05%	<0.05%	<0.05%	<0.05%	/	<0.05%

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	Unknown impurity (RRT≈1.27)	<0.05%	<0.05%	<0.05%	<0.05%	<0.05%	<0.05%	/	<0.05%
	Total impurity	1.163	1.162	1.188	1.185	1.185	1.174	1.0	1.6

Conclusion: Impurities YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18 and YA2304-19 were calculated by the principal component self-control method with correction factor under the wavelength of 251nm. Impurities YA2304-10 and YA2304-17 were calculated at 230nm wavelength by external standard method. The impurity peak less than 0.5 times of the main peak area of the control solution was ignored in the 6 parts of intermediate precision test solution with limit concentration. The RSD of other impurities was less than 5%, and the RSD of total impurities was less than 5%, which met the requirements. The intermediate precision of this method is good. In the 12 tests of repeatability and intermediate precision, the impurity peak less than 0.5 times of the main peak area of the control solution was ignored, the RSD of other impurities was less than 10%, and the RSD of total impurities was less than 10%, which met the requirements.

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### **h. Solution Stability Studies**

#### ➤ **Acceptance Criteria**

Each solution is placed under 5°C (refrigerated) condition, and the control solution, system suitability solution and test product solution are injected at different times. The chromatogram of system suitability solution is required to be (251nm). The peak order is YA2304-14, YA2304, YA2304-15, YA2304-16, YA2304-17, YA2304-10, YA2304-19, and the separation degree between YA2304 and YA2304-14 and YA2304-15 should NLT 1.5. The separation degree between YA2304-17 and YA2304-16 and adjacent peaks should NLT 1.0; In the chromatogram of the control product solution (230nm), the peak sequence was YA2304-17, YA2304-10; The RSD of the peak-peak area of each impurity in the chromatogram of the reference solution NMT 10%. In the chromatogram of the control solution (251nm), the signal-to-noise ratio (S/N) of the main peak should NLT 20, the RSD of the peak area NMT 10%, the impurity peak less than 0.5 times the main peak area of the control solution should not be ignored in the chromatogram of the test solution and the solution with the maximum concentration and miscellaneous test product, and the RSD of the peak area of other single impurities NMT 10%. And there should be no new impurities that interfere with the detection of related substances.

#### ➤ **Solution Preparation**

Blank solution: solvent

YA2304-12 Reference storage solution: Share "YA2304-12 Reference Storage solution" under "4.1 Wavelength Selection". (Approx. 200µg/ml)

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YA2304-14 Reference storage solution: Share "YA2304-14 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-15 Reference storage solution: Share "YA2304-15 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-16 Reference storage solution: Share "YA2304-16 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-18 Reference storage solution: Share "YA2304-18 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-19 Reference storage solution: Share "YA2304-19 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-10 Reference storage Solution: Share "YA2304-10 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-17 Reference storage solution: Share "YA2304-17 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

Reference solution: Prepare it by the same method as "reference solution" under "4.2 System Suitability". (YA2304-10, YA2304-17 containing about 0.4 $\mu$ g/ml)

Test solution: Prepare the test solution by the same method as "test solution" under "4.2 System Suitability". (Approx. 0.4mg/ml)

Reference solution: Prepare it by the same method as "reference solution" under "4.2 System Applicability". (Approx. 0.4 $\mu$ g/ml)

System Suitability Solution: Share "System Suitability Solution" from "4.6 Accuracy" known impurity. (YA2304-14, YA2304-15, YA2304-16, YA2304-19, YA2304-10 and YA2304-17 all approx. 0.8 $\mu$ g/ml)

Impurity reserve liquid: Accurately measure 0.75ml of each control product reserve liquid of YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18, YA2304-19, YA2304-17 and YA2304-10 into the same 10ml bottle and dilute it to the scale with solvent. Shake well, then it is ready. (Contains YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18, YA2304-19, YA2304-17 and YA2304-10 about 15 $\mu$ g/ml each)

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Add mixed test product solution: accurately weigh 10mg of this product, place it in a 25ml measuring bottle, add an appropriate amount of solvent and ultrasonic to dissolve it, then accurately add 1.0ml of impurity reserve liquid, dilute it with solvent to the scale, shake well, and get. (Including YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18, YA2304-19, YA2304-17 and YA2304-10 are about 0.6 $\mu$ g/ml)

### ➤ **Injection Sequence**

The suitable solution of the system was placed at 5°C, 1 needle was injected at 0h, 8h, 19h, 26.5h, 46h, 59h and 100h respectively, and the chromatogram was recorded.

The mixed test solution was placed at 5°C, and 1 needle was injected at 0h, 8h, 19h, 25h, 46h, 59h and 100h, respectively, and the chromatogram was recorded.

The test solution was placed at 5°C, and 1 needle was injected at 0h, 8h, 19h, 38.5h, 46h, 59h and 100h, respectively, and the chromatogram was recorded.

The control solution was placed at 5°C and injected with 1 needle each at 0h, 8h, 19h, 39h, 46h, 59h and 100h, respectively, and the chromatogram was recorded.

The control solution was placed at 5°C, and 1 needle was injected at 0h, 8h, 19h, 38h, 46h, 59h and 100h, respectively, and the chromatogram was recorded.

### ➤ **Results and Discussion**

System suitability solution stability test results:

Investigation items	Control solution							RSD %
	0h	8h	19h	38h	46h	59h	100h	

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Main peak area	6709	6535	6563	6771	6836	6606	6732	1.7
Principal peak signal-to-noise ratio	75.4	65.3	92.2	89.8	54.0	103.0	92.8	/
Conclusion: When the control solution was placed at 5°C for 100h, the chromatogram of the control solution (251nm) showed that the main peak signal-to-noise ratio (S/N) was NLT 54.0 (required to be NLT 20), and the RSD of the peak area was 1.7% (required to be no more than 10%). All of the above meet the verification requirements, indicating that the control solution is stable within 100h under 5°C.								

Stability test results of control solution:

Investigation Items	Impurity names	Reference solution							RSD %
		0h	8h	19h	39h	46h	59h	100h	
Peak area	YA2304-17	1103 7	1099 0	1104 2	1109 6	1100 6	1123 5	1103 1	0.8
	YA2304-10	8322	8233	8327	8411	8336	8361	8219	

Conclusion: When the control solution was placed at 5°C for 100h, the chromatogram of the control solution (230nm) was followed by YA2304-17 and YA2304-10, and the RSD of the peak-peak area of each impurity in the chromatogram was no more than 0.9% (required to be no more than 10%). The verification requirements were met, indicating that the control solution was stable within 100h under 5°C.

Stability test results of system suitability solution:

Investigation Items	Time of visit						
	0h	8h	19h	26.5 h.	46h	59h	100h
Degree of separation between YA2304 and YA2304-14	5.9	6.0	5.9	5.9	5.9	5.8	5.8
Degree of separation between YA2304 and YA2304-15	6.8	6.9	6.8	6.8	6.8	6.8	6.7
Minimum separation between YA2304-17 and adjacent peaks	1.6	1.6	1.6	1.6	1.6	2.2	2.2
Minimum separation degree between YA2304-16 and adjacent peaks	1.6	1.6	1.6	1.6	1.6	1.6	1.6

Verdict: The system suitable solution was placed at 5°C for 100h, and the

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chromatogram of the system suitable solution was 251nm. The peak order was YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19. The minimum separation degree between YA2304 and YA2304-14 and YA2304-15 is NLT 5.8 (NLT 1.5 required), and the minimum separation degree between YA2304-17 and YA2304-16 and adjacent peaks is NLT 1.6 (NLT 1.0 required); All of the above meet the verification requirements, indicating that the system suitable solution is stable within 100h under 5°C.

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Stability test results of test solutions:

Time Point		Detection wavelength	0h	8h	19h	38.5 h.	46h	59h	100h	RSD/%
Impurity peak area	Impurity YA2304-12	251nm	382	333	343	386	467	337	356	Content < 0.05%, RSD does not do statistics
	Unknown impurity (RRT≈0.64)		1182	1090	1360	1327	1308	1166	458	Content < 0.05%, RSD did not do statistics
	Impurity YA2304-14		Not detected	Content < 0.05%, RSD does not do statistics						
	Impurity YA2304-15		Not detected	Content < 0.05%, RSD does not do statistics						
	Unknown impurity (RRT≈1.27)		1220	1198	1161	1174	1266	1201	1181	Content < 0.05%, RSD does not do statistics
	Impurity YA2304-16		Not detected	Content < 0.05%, RSD does not do statistics						
	Impurity YA2304-18		Not detected	Content < 0.05%, RSD does not do statistics						
	Impurity YA2304-19		560	481	575	611	588	767	409	Content < 0.05%, RSD does not do statistics

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	Impurity YA2304-17	230nm	Not detecte d	Content < 0.05%, RSD does not do statistics					
	Impurity YA2304-10		Not detecte d	Content < 0.05%, RSD does not do statistics					
Conclusion: When the test solution was placed at 5°C for 100h, the impurity peak smaller than 0.5 times of the main peak area of the control solution was ignored in the chromatogram of the test solution. The known impurities were detected except YA2304-12 and YA2304-19, and both were less than 0.05%, and the rest were not detected. Other single unknown impurities were all less than 0.05%, the RSD of each impurity peak area was not counted (not more than 10%), and no new impurities were added to interfere with the detection of related substances. The above are in line with the verification requirements, indicating that the test solution is stable within 100h under 5°C.									

### Stability test results of solution with miscellaneous test products

Time Point		Detection wavelength	0h	8h	19h	25h	46h	59h	100h	RSD/%
Im pur ity pea k are a	Impurity YA2304-12	251nm	8738	8628	8837	8744	8810	8825	8731	0.9
	Unknown impurity (RRT≈0.64)		/	945	1233	1061	1491	1223	1666	Content < 0.05%, RSD does not do statistics
	Impurity YA2304-14		10267	9809	9895	10078	10015	10050	9889	1.6
	Impurity YA2304-15		8793	8747	8744	8807	8888	8899	8669	1.0
	Unknown impurity (RRT≈1.27)		1082	1049	1104	1109	1096	1102	1107	Content < 0.05%, RSD did not do statistics
	Impurity YA2304-16		9394	9292	9227	9074	8798	8843	8449	4

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	Impurity YA2304-18	230nm	9107	9027	8791	8755	8574	8452	8111	4
	Impurity YA2304-19		15639	15602	15665	15644	15942	15998	15712	1.1
	Impurity YA2304-17		16023	15969	15952	15986	16017	16400	16199	1.1
	Impurity YA2304-10		12233	12086	12173	12076	12218	12178	11975	0.8

Conclusions: The impurity peak less than 0.5 times the main peak area of the control solution was ignored in the chromatogram of the impurity solution added at 5°C for 100h. At 230nm wavelength, the RSD of the known impurity YA2304-17 and YA2304-10 was not greater than 1.1% (10% is not required). At 251nm wavelength, the RSD of known impurities YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18 and YA2304-19 are not greater than 4% (10% is not required); The RSD of other single unknown impurity is less than 0.05%, the RSD of the single unknown impurity is not counted (not more than 10%), and no new impurity interferes with the detection of related substances; The above are in line with the verification requirements, indicating that the solution of added impurity is stable within 100h at 5°C.

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### **i. Robustness**

#### **➤ Acceptance Criteria**

Durability is achieved by examining the content of various impurities in the test solution under normal chromatographic conditions (changing the column temperature, the initial proportion of mobile phase, the flow rate, the column lot number) and testing the limit concentration under normal chromatographic conditions. It is required that in the spectrum obtained by the system applicable solution under each condition (251nm), the peak order is YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19. The separation degree between YA2304 and YA2304-14 and YA2304-15 should be NLT 1.5, and the separation degree between YA2304-17 and YA2304-16 and adjacent peaks should be NLT 1.0; In the chromatogram of the reference product solution (230nm), the order of the peaks is YA2304-17, YA2304-10; In the control solution chromatogram (251nm), the signal-to-noise ratio (S/N) of the main peak should NLT 20.

Under different conditions of the same chromatographic parameters, the RSDS of each impurity content greater than 0.05% in the test product NMT 10%, and the RSDS of total impurity content NMT 10%; Or the absolute deviation of the impurity content NMT 20% of the limit value, the absolute deviation of the total impurity content NMT 40% of the limit value.

Variations of Method Parameters in Robustness Study

Chromato graphic Parameter s	Standard conditions	Variable Values
Column temperatu	20 °C	18 ° C, 22 ° C

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Parameter	Description	Specification
Initial ratio of mobile phase	Mobile phase A- Mobile phase B (85:15)	Mobile phase A- Mobile phase B (87:13), Mobile phase A- Mobile phase B (83:17)
Flow rate	1.0 ml/min	0.95ml/min, 1.05ml/min
Chromatographic column	Column 1 (YMC Trairt C18 4.6×150mm, 3μm, SN: 117EB10004, Serial No. : CXC18-05)	Column 2 (YMC Trairt C18 4.6×150mm, 3μm, SN: 129ZB00034, No. : ZJC18-35)

### ➤ **Solution Preparation**

Blank solution: solvent

YA2304-12 Reference storage solution: Share "YA2304-12 Reference Storage solution" under "4.1 Wavelength Selection". (Approx. 200μg/ml)

YA2304-14 Reference storage solution: Share "YA2304-14 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200μg/ml)

YA2304 Reference stock: Share "YA2304 Reference Stock" under "4.1 Wavelength Selection". (Approx. 200μg/ml)

YA2304-15 Reference storage solution: Share "YA2304-15 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200μg/ml)

YA2304-16 Reference storage solution: Share "YA2304-16 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200μg/ml)

YA2304-18 Reference storage solution: Share "YA2304-18 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200μg/ml)

YA2304-19 Reference storage solution: Share "YA2304-19 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200μg/ml)

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YA2304-10 Reference storage Solution: Share "YA2304-10 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

YA2304-17 Reference storage solution: Share "YA2304-17 Reference Storage Solution" under "4.1 Wavelength Selection". (Approx. 200 $\mu$ g/ml)

Reference solution: share "Reference solution ②" under "4.3.2 Forced degradation Test". (YA2304-10, YA2304-17 containing about 0.4 $\mu$ g/ml)

System suitability solution: Share "System Suitability solution" under "4.3.2 Forced degradation Test".

Impurity reserve solution: Prepare by the same method as "impurity reserve solution" under "4.8 Solution stability". (Each about 15 $\mu$ g/ml)

Add impurity test product solution: accurately weigh YA2304 sample about 10mg, put it in 25ml measuring bottle, add appropriate amount of solvent to dissolve by ultrasound, accurately add 1ml of impurity reserve liquid, dilute it with solvent to scale, shake well, and then get. (Prepared 2 parts in parallel, named as adding miscellaneous test product solution ①~②)

Control solution: accurately measure 1ml of mixed test product solution, place it in a 20ml measuring bottle, dilute it with solvent to the scale, and shake well; Precision measure 1ml of the above solution, place it in a 50ml measuring bottle, dilute it with solvent to the scale, shake well, and then get. (named as control solution ①~②)

### **➤ Injection Sequence**

After the blank solution, under different durability conditions (normal condition, column temperature, flow rate, initial proportion of mobile phase, column), the system suitable solution was injected with 1 needle, the control

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solution was injected with 2 needles, 2 control solution and 2 mixed test solution with 1 needle each, and the chromatogram was recorded.

### ➤ **Results and Discussion**

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Table 3.2.S.4.3.1-19 Durability test results - Test of system suitability

Chromato graphic Condition s	Parameter values	Separation degree between YA2304 and YA2304-14	Degree of separation between YA2304 and YA2304-15	Minimum separation degree between YA2304-17 and adjacent peaks	Minimum separation degree between YA2304-16 and adjacent peaks	Main peak signal-to-noise ratio of control solution	
						Sample Injection 1	Sample Injection 2
Column temperatu re (C)	20	5.8	6.8	2.2	1.6	99.1	107.5
	18	5.7	6.6	1.2	1.9	104.4	75.9
	22	5.8	6.9	3.5	1.4	86.0	91.1
Flow rate (ml/min)	1.0	5.8	6.8	2.2	1.6	99.1	107.5
	0.95	5.7	6.7	1.7	1.7	109.8	106.6
	1.05	5.9	6.9	2.8	1.6	96.8	106.5
Mobile phase A- Mobile phase B	85:15	5.8	6.8	2.2	1.6	99.1	107.5
	83:17 (ratio 1)	6.0	6.9	2.5	1.6	78.8	81.5
	87:13 (ratio 2)	5.6	6.7	2.0	1.7	96.5	90.9
Column	Column 1	5.8	6.8	2.2	1.6	99.1	107.5
	Column 2	5.6	6.4	1.7	1.6	72.5	67.2
Conclusion: Under single factor change of column temperature, flow rate, initial ratio of fluidity, chromatographic column and normal chromatographic conditions, the peak order of contrast solution (230nm) is YA2304-17, YA2304-10; In the chromatogram of system suitability solution (251nm), the peak order was YA2304-14, YA2304, YA2304-15, YA2304-17, unknown impurities, YA2304-16, YA2304-10, YA2304-19. The minimum separation degree between YA2304 and YA2304-14 and YA2304-15 is NLT 5.6 (NLT 1.5 required), and the minimum separation degree between YA2304-17 and YA2304-16 and adjacent peaks is 1.2 (NLT 1.0 required); In the contrast solution chromatogram (251nm), the signal-to-noise ratio of the main peak was NLT 67.2 (NLT 20), and the system applicability met the verification requirements.							

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### Durability test results

Chromato graphy Condition s	Paramet er Values	Impurity content (%)										
		Impurity YA2304-12	Impurity YA2304-14	Impurity YA2304-15	Impurity YA2304-16	Impurity YA2304-18	Impurity YA2304-19	Impurity YA2304-17	Impurity YA2304-10	Unknown impurity (RRT≈0.64)	Unknown impurity (RRT≈1.26)	Total impurity
Column temperature (C)	20	0.152	0.146	0.153	0.130	0.137	0.163	0.143	0.145	< 0.05%	< 0.05%	1.169
		0.152	0.145	0.155	0.130	0.137	0.165	0.147	0.149	< 0.05%	< 0.05%	1.180
	18	0.150	0.142	0.151	0.128	0.136	0.161	0.144	0.144	< 0.05%	< 0.05%	1.155
		0.157	0.150	0.157	0.135	0.141	0.169	0.147	0.147	< 0.05%	< 0.05%	1.202
	22	0.149	0.146	0.151	0.128	0.134	0.161	0.143	0.145	< 0.05%	< 0.05%	1.155
		0.156	0.150	0.159	0.133	0.138	0.169	0.146	0.149	< 0.05%	< 0.05%	1.200
RSD/%		2.2	2.3	2.1	2.2	1.7	2.3	1.4	1.5	Content < 0.05%, do not do statistic s	Content < 0.05%, no statistic s	1.8
Flow rate (ml/min)	1.0	0.152	0.146	0.153	0.130	0.137	0.163	0.143	0.145	< 0.05%	< 0.05%	1.169
		0.152	0.145	0.155	0.130	0.137	0.165	0.147	0.149	< 0.05%	< 0.05%	1.180
	0.95	0.150	0.142	0.150	0.125	0.131	0.161	0.142	0.144	< 0.05%	< 0.05%	1.145
		0.156	0.147	0.156	0.130	0.136	0.168	0.145	0.146	< 0.05%	< 0.05%	1.184
	1.05	0.150	0.147	0.154	0.127	0.132	0.163	0.143	0.144	< 0.05%	< 0.05%	1.161
		0.156	0.151	0.158	0.129	0.138	0.168	0.148	0.148	< 0.05%	< 0.05%	1.196

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RSD/%		1.9	2.1	1.7	1.7	2.1	1.7	1.6	1.6	Content < 0.05%, do not do statistic s	Content < 0.05%, no statistic s	1.6
Mobile phase A- Mobile phase B	85:15	0.152	0.146	0.153	0.130	0.137	0.163	0.143	0.145	< 0.05%	< 0.05%	1.169
		0.152	0.145	0.155	0.130	0.137	0.165	0.147	0.149	< 0.05%	< 0.05%	1.180
	83:17 (ratio 1)	0.152	0.149	0.152	0.126	0.131	0.163	0.143	0.142	< 0.05%	< 0.05%	1.158
		0.159	0.150	0.158	0.129	0.137	0.170	0.148	0.147	< 0.05%	< 0.05%	1.198
	87:13 (ratio 2)	0.148	0.143	0.149	0.123	0.130	0.162	0.143	0.142	< 0.05%	< 0.05%	1.139
		0.154	0.148	0.156	0.126	0.134	0.169	0.146	0.147	< 0.05%	< 0.05%	1.179
RSD/%		2.4	1.8	2.2	2.6	2.6	2.0	1.6	1.9	Content < 0.05%, do not do statistic s	Content < 0.05%, no statistic s	1.8
Column	Column 1	0.152	0.146	0.153	0.130	0.137	0.163	0.143	0.145	< 0.05%	< 0.05%	1.169
		0.152	0.145	0.155	0.130	0.137	0.165	0.147	0.149	< 0.05%	< 0.05%	1.180
	Column 2	0.153	0.144	0.150	0.119	0.125	0.162	0.141	0.142	< 0.05%	< 0.05%	1.136
		0.158	0.149	0.156	0.120	0.127	0.169	0.144	0.145	< 0.05%	< 0.05%	1.169
RSD/%		1.8	1.7	1.7	6%	5%	1.9	1.9	2.1	Content < 0.05%, do not do	Content < 0.05%, no statistic	1.7

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									statistic s	s	
Conclusion: RSD of impurity YA2304-12 is less than 2.4% (less than 10% is required) under column temperature, flow rate, initial ratio of mobile phase and normal chromatography conditions. RSDS of YA2304-14 were no more than 2.3% (no more than 10% required). RSD of YA2304-15 content shall NMT 2.2% (NMT 10%); RSD of YA2304-16 content shall NMT 6% (NMT 10%); RSD of YA2304-18 content shall NMT 5% (NMT 10%); RSD of YA2304-19 content shall NMT 2.3% (NMT 10%); RSD of YA2304-17 content should NMT 1.9% (NMT 10%); RSD of YA2304-10 content should NMT 2.1% (NMT 10%); The contents of unknown impurity RRT≈0.64 and unknown impurity RRT≈1.26 are less than 0.05, and RSD is not used for statistics. All of them met the verification conditions.											

### III. Summary of Method Validation

#### Summaries of Method Validation of Related Substances in Finerenone

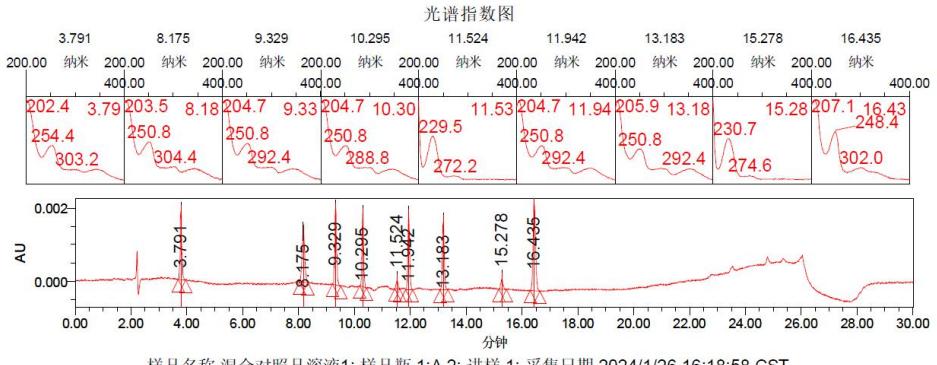
Item	Acceptable criteria	Verification of results
Wavelength selection	Impurities YA2304-10 and YA2304-17 have large absorption at 230nm wavelength; The principal component and impurities YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18 and YA2304-19 all have large absorption at 251nm wavelength.	Impurities YA2304-10 and YA2304-17 have greater absorption at 230nm wavelength; The principal component and impurities YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18 and YA2304-19 all have large absorption at 251nm. In the mixed control product solution ①, The retention times of YA2304-10, ya2304-17, ya2304-12, ya2304-14, YA2304, ya2304-15, YA2304-16, ya2304-18 and ya2304-19 were 15.278min, 11.524min and 3.791m, respectively in, 8.175min, 9.329min, 10.295min, 11.942min, 13.183min, 16.435min, mixed control solution ① spectral diagram is as follows:

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		 <p>光谱指数图</p> <p>样品名称 混合对照品溶液1; 样品瓶 1A,2; 进样 1; 采集日期 2024/1/26 16:18:58 CST</p>
System suitability	<p>The blank solution should have no interference, and the chromatogram of the reference solution (230nm), the peak order is YA2304-17, YA2304-10; In the chromatogram of system suitability solution (251nm), the peak order is YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19. The separation degree between YA2304 and YA2304-14 and YA2304-15 should be NLT 1.5, and the separation degree between YA2304-17 and YA2304-16 and adjacent peaks should be NLT 1.0; Compared with the chromatogram of solution (251nm) for 6 consecutive days, the RSD of the main</p>	<p>There was no interference in the blank solution, and in the chromatogram of the control solution (230nm), the peak exit sequence was YA2304-17 and YA2304-10; In the chromatogram of system suitability solution (251nm), the peak order is YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19. The minimum separation degree between YA2304 and YA2304-14 and YA2304-15 is 4.9 (NLT 1.5), and the minimum separation degree between YA2304-17 and YA2304-16 and adjacent peaks is 1.2 (NLT 1.0); In the chromatogram obtained by contrast solution (251nm) for 6 consecutive days, the RSD of main peak retention time was 0.05% (not more than 1.0%), the RSD of main peak area was 1.5% (not more than 5%), and the signal-to-noise ratio (S/N) was NLT 20. All the above meet the verification requirements, indicating that the method has good applicability in system.</p>

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	peak retention time NMT 1.0%, the RSD of the peak area NMT 5%, and the signal-to-noise ratio (S/N) should NLT 20.									
Specificity	<p>Blank solution should be free of interference; In the chromatogram of system suitability solution (251nm), the peak order is YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19, The separation degree between YA2304 and YA2304-14 and YA2304-15 should be NLT 1.5, and the separation degree between YA2304-17 and YA2304-16 and adjacent peaks should be NLT 1.0; In the chromatogram of test product solution (251nm) and specific mixed solution (251nm), the separation degree between YA2304-17 and YA2304-16 and adjacent peaks should NLT 1.0, and the separation degree between other adjacent peaks should NLT 1.5; The retention time of each known impurity peak in the reference solution, system suitability solution, test product solution and specific mixed solution should be</p> <p>The blank solution has no interference; In the chromatogram of system suitability solution (251nm), the peak order is YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19, The minimum separation degree between YA2304 and YA2304-14 and YA2304-15 is required to be 4.9 (NLT 1.5), and the minimum separation degree between YA2304-17 and YA2304-16 and adjacent peaks is 1.2 (NLT 1.0); In the chromatogram of test product solution (251nm) and specific mixed solution (251nm), the minimum separation degree between YA2304-17 and YA2304-16 and adjacent peaks is 1.2 (NLT 1.0), and the minimum separation degree between other adjacent peaks is 3.1 (NLT 1.5). In the chromatogram of specific mixed solution (230nm), the minimum separation degree between YA2304-17 and YA2304-16 and adjacent peaks was 1.2 (NLT 1.0), and the minimum separation degree between other adjacent peaks was 5.0 (NLT 1.5). The retention time of each known impurity peak in the reference solution, the system suitability solution, the test product solution and the specific mixed solution was consistent with the corresponding main peak in the impurity localization solution, and the relative retention time of each known impurity peak in the specific mixed solution was basically consistent with the quality standard. The concentration of YA2304-10, YA2304-17, YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18 and YA2304-19 in the specific mixed solution was 0.15% of the concentration of principal components in the test solution.</p>	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Name of the</th> <th>Testing Wavel</th> <th>Component name</th> <th>Component concentr</th> <th>Rt (min)</th> <th>RRT</th> <th>Minimum separati</th> <th>The percentage equivalent</th> </tr> </thead> </table>	Name of the	Testing Wavel	Component name	Component concentr	Rt (min)	RRT	Minimum separati	The percentage equivalent
Name of the	Testing Wavel	Component name	Component concentr	Rt (min)	RRT	Minimum separati	The percentage equivalent			

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		solut ion	ength		ation ( $\mu$ g/ml)			on from adjacen t peaks	to the concentratio n of the principal component in the test solution
consistent with the corresponding main peak in the impurity positioning solution, and the relative retention time of each known impurity peak in the specific mixed solution should be basically consistent with the quality standard.	251n m	Speci fic mixe d soluti on	YA2304-12	0.6005	3.810	0.41	3.1	0.15%	
			YA2304-14	0.6032	8.233	0.89	4.7	0.15%	
			YA2304	410.0400	9.289	1.00	5.0	100%	
			YA2304-15	0.6055	10.295	1.11	5.6	0.15%	
			YA2304-17	0.6040	11.549	1.24	1.2	0.15%	
			YA2304-16	0.5797	11.972	1.29	1.4	0.15%	
			YA2304-18	0.6014	13.207	1.42	7.4	0.15%	
			YA2304-10	0.6092	15.305	1.65	5.7	0.15%	
			YA2304-19	0.6008	16.474	1.77	5.7	0.15%	
	230n m		YA2304-12	0.6005	3.806	0.41	9.5	0.15%	
			YA2304-14	0.6032	8.229	0.89	5.0	0.15%	
			YA2304	410.0400	9.285	1.00	5.0	100%	
			YA2304-15	0.6055	10.291	1.11	5.6	0.15%	
			YA2304-17	0.6040	11.547	1.24	1.2	0.15%	
			YA2304-16	0.5797	11.967	1.29	1.5	0.15%	
			YA2304-18	0.6014	13.203	1.42	7.4	0.15%	
			YA2304-10	0.6092	15.304	1.65	5.6	0.15%	
			YA2304-19	0.6008	16.470	1.77	5.6	0.15%	

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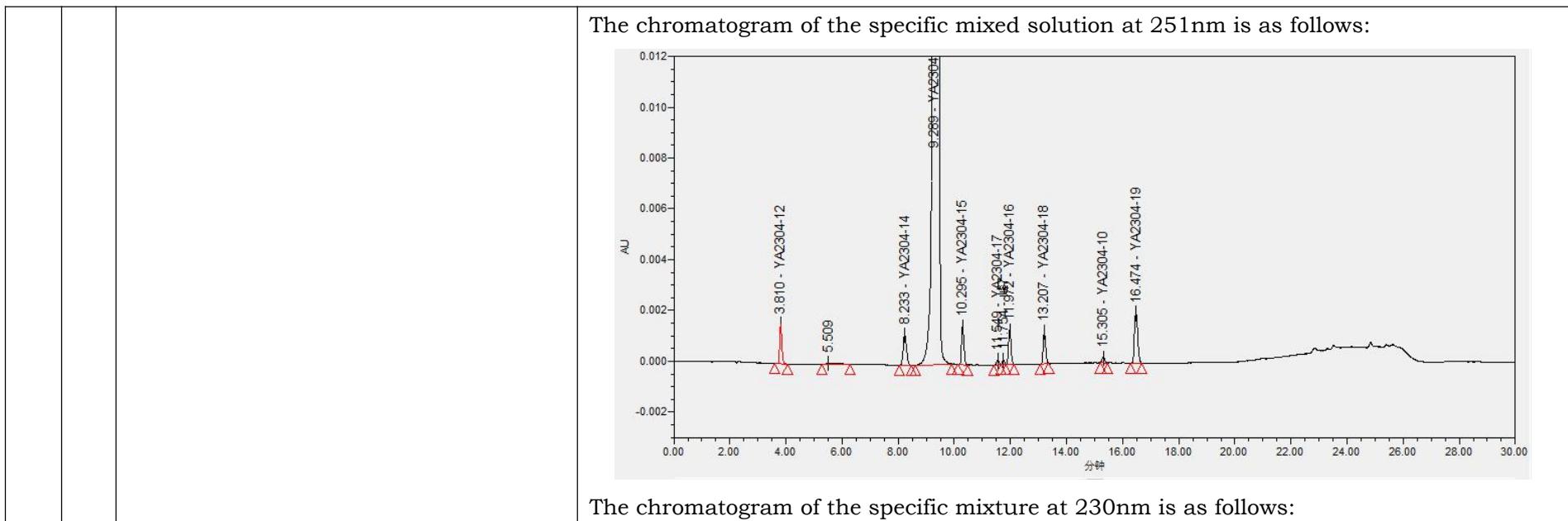
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Limits of quantification and detection	The S/N of non-nellidone and all impurities in the 6 RMS should be NLT 10, the RSD of the peak retention time of each component should be no more than 2.0%, and the RSD of the peak area should be NLT 10%. The S/N of fineridone and each impurity in the 3-needle detection limited solution atlas was NLT 3.	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th rowspan="2" style="text-align: left; vertical-align: bottom;"><b>Impurity /principal component</b></th><th colspan="2" style="text-align: center; border-bottom: 1px solid black;"><b>Detection limits</b></th><th style="text-align: center; border-bottom: 1px solid black;"><b>Limit of quantitation</b></th></tr> <tr> <th style="text-align: center; border-bottom: 1px solid black;"><b>Concentration (equivalent to the percentage of solution concentration of the test product)</b></th><th style="text-align: center; border-bottom: 1px solid black;"><b>Concentration (equivalent to the test product solution concentration percentage)</b></th><th style="text-align: center; border-bottom: 1px solid black;"></th></tr> </thead> <tbody> <tr> <td>YA2304-12</td><td style="text-align: center;">0.0601<math>\mu</math>g/ml (0.0150%)</td><td style="text-align: center;">0.1201<math>\mu</math>g/ml (0.0300%)</td><td></td></tr> <tr> <td>YA2304-14</td><td style="text-align: center;">0.0603<math>\mu</math>g/ml (0.0151%)</td><td style="text-align: center;">0.1206<math>\mu</math>g/ml (0.0302%)</td><td></td></tr> <tr> <td>YA2304</td><td style="text-align: center;">0.0615<math>\mu</math>g/ml (0.0154%)</td><td style="text-align: center;">0.1229<math>\mu</math>g/ml (0.0307%)</td><td></td></tr> <tr> <td>YA2304-15</td><td style="text-align: center;">0.0605<math>\mu</math>g/ml (0.0151%)</td><td style="text-align: center;">0.1211<math>\mu</math>g/ml (0.0303%)</td><td></td></tr> <tr> <td>YA2304-10</td><td style="text-align: center;">0.0604<math>\mu</math>g/ml (0.0151%)</td><td style="text-align: center;">0.1208<math>\mu</math>g/ml (0.0302%)</td><td></td></tr> </tbody> </table>	<b>Impurity /principal component</b>	<b>Detection limits</b>		<b>Limit of quantitation</b>	<b>Concentration (equivalent to the percentage of solution concentration of the test product)</b>	<b>Concentration (equivalent to the test product solution concentration percentage)</b>		YA2304-12	0.0601 $\mu$ g/ml (0.0150%)	0.1201 $\mu$ g/ml (0.0300%)		YA2304-14	0.0603 $\mu$ g/ml (0.0151%)	0.1206 $\mu$ g/ml (0.0302%)		YA2304	0.0615 $\mu$ g/ml (0.0154%)	0.1229 $\mu$ g/ml (0.0307%)		YA2304-15	0.0605 $\mu$ g/ml (0.0151%)	0.1211 $\mu$ g/ml (0.0303%)		YA2304-10	0.0604 $\mu$ g/ml (0.0151%)	0.1208 $\mu$ g/ml (0.0302%)	
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Finerenone

Confidential

Hinye Pharmaceutical Co., Ltd.

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		7			
		YA2304-1 6	0.0580µg/ml (0.0145%)	0.1159µg/ml (0.0290%)	
		YA2304-1 8	0.0601µg/ml (0.0150%)	0.1203µg/ml (0.0301%)	
		YA2304-1 0	0.0609µg/ml (0.0152%)	0.1218µg/ml (0.0305%)	
		YA2304-1 9	0.0601µg/ml (0.0150%)	0.1202µg/ml (0.0300%)	
Linearity, range and correction factors	<p>Known impurities at 251nm wavelength (YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18 and YA2304-19) and 230nm wavelength (YA2304-10, YA2304-17) : Within the range of limited quantitation concentration ~ equivalent to 0.3% of the test product solution concentration, 6 concentration points were selected fairly evenly, and the concentration of each impurity was taken as the horizontal coordinate and the peak area was taken as the vertical coordinate as the linear regression curve. The regression coefficient R of the obtained linear graph should NLT 0.990, and the RSD of the response factor should NMT 10%. Y-axis</p>	<b>Impurities / Principal component</b>	<b>Concentration (equivalent to percentage of solution concentration of test product)</b>	<b>Linear equation</b>	<b>Correction factor</b>
		YA2304-1 2	0.1201µg/ml to 1.2011µg/ml (0.0300% to 0.3003%)	$y = 13896x + 33.018$	1.2
		YA2304-1 4	0.1206µg/ml to 1.2064µg/ml (0.0302% to 0.3016%)	$y = 16536x - 85.568$	1.0
		YA2304	0.1229µg/ml to 1.2294µg/ml (0.0307% to 0.3074%)	$y = 16404x + 57.399$	1.0
		YA2304-1 5	0.1211µg/ml to 1.2109µg/ml (0.0303% to 0.3027%)	$y = 14367x + 1.2724$	1.1
		YA2304-1 6	0.1159µg/ml to 1.1595µg/ml (0.0290% to 0.2899%)	$y = 16470x + 14.525$	1.0
		YA2304-1 8	0.1203µg/ml to 1.2027µg/ml (0.0301% to 0.3007%)	$y = 14990x - 3.7556$	1.1

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	<p>intercepts are within 25% of 100% response value.</p> <p>Unknown impurities at 251m wavelength (substituted by YA2304) : Within the range of limited quantitation concentration ~ equivalent to 0.3% of the test product solution concentration, 6 concentration points were selected fairly uniformly, and the concentration of each impurity was taken as the horizontal coordinate and the peak area was taken as the vertical coordinate as the linear regression curve. The regression coefficient (R) of the regression curve was NLT 0.990, and the RSD of the response factor was not more than 10%. Y-axis intercept accounted for 100% of the response value of the percentage within 25%.</p>	<table border="1"> <tr> <td>YA2304-1 7</td><td>0.1208µg/ml to 1.2080µg/ml (0.0302% to 0.3020%)</td><td>y = 26638x - 75.991</td><td>/</td></tr> <tr> <td>YA2304-1 0</td><td>0.1218µg/ml to 1.2183µg/ml (0.0305% to 0.3046%)</td><td>y = 19735x + 192.83</td><td>/</td></tr> <tr> <td>YA2304-1 9</td><td>0.1202µg/ml~1.2016µg/ml (0.0300%~0.3004%)</td><td>y = 25507x - 18.855</td><td>0.6</td></tr> </table>	YA2304-1 7	0.1208µg/ml to 1.2080µg/ml (0.0302% to 0.3020%)	y = 26638x - 75.991	/	YA2304-1 0	0.1218µg/ml to 1.2183µg/ml (0.0305% to 0.3046%)	y = 19735x + 192.83	/	YA2304-1 9	0.1202µg/ml~1.2016µg/ml (0.0300%~0.3004%)	y = 25507x - 18.855	0.6
YA2304-1 7	0.1208µg/ml to 1.2080µg/ml (0.0302% to 0.3020%)	y = 26638x - 75.991	/											
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YA2304-1 9	0.1202µg/ml~1.2016µg/ml (0.0300%~0.3004%)	y = 25507x - 18.855	0.6											
Accuracy	<p>Unknown impurities: The recovery rate of unknown impurity LOQ concentration sampling solution ~150% limit concentration solution is 85%~110%, and the RSD of recovery rate NMT 10%.</p> <p>Known impurities: The recovery rate</p>	<table border="1"> <thead> <tr> <th>Impurity/principal component</th><th>Conditions of labeling</th><th>Average recovery rate</th><th>RSD</th></tr> </thead> <tbody> <tr> <td>YA2304-12</td><td>Added 0.1802µg/ml~0.9008µg/ml</td><td>98.6%, n=9</td><td>1.3%, n=9</td></tr> <tr> <td>YA2304-14</td><td>Added 0.1810µg/ml~0.9048µg/ml</td><td>98.5%, n=9</td><td>2.1%, n=9</td></tr> </tbody> </table>	Impurity/principal component	Conditions of labeling	Average recovery rate	RSD	YA2304-12	Added 0.1802µg/ml~0.9008µg/ml	98.6%, n=9	1.3%, n=9	YA2304-14	Added 0.1810µg/ml~0.9048µg/ml	98.5%, n=9	2.1%, n=9
Impurity/principal component	Conditions of labeling	Average recovery rate	RSD											
YA2304-12	Added 0.1802µg/ml~0.9008µg/ml	98.6%, n=9	1.3%, n=9											
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<p>of known impurity 30% limit concentration solution ~150% limit concentration solution is 85%~110%, and the RSD of each impurity recovery rate NMT 10%.</p>	YA2304-15	Added 0.1816µg/ml~0.9082µg/ml	99.7%, n=9	2.1%, n=9																																								
	YA2304-16	Added 0.1739µg/ml~0.8696µg/ml	94.2%, n=9	1.6%, n=9																																								
	YA2304-18	Added 0.1804µg/ml~0.9020µg/ml	97.6%, n=9	0.7%, n=9																																								
	YA2304-19	Added 0.1802µg/ml~0.9012µg/ml	96.8%, n=9	4%, n=9																																								
	YA2304-10	Added 0.1827µg/ml~0.9137µg/ml	98.6%, n=9	1.7%, n=9																																								
	YA2304-17	Added 0.1812µg/ml~0.9060µg/ml	98.1%, n=9	0.5%, n=9																																								
	YA2304	Add 0.1229µg/ml~0.6147µg/ml	101.3%, n=9	1.9%, n=9																																								
Precision	Repeatability and intermediate precision: The impurity peak less than 0.5 times the main peak area of the control solution was ignored in the repetitive test solution of 6 parts plus limit concentration. The RSD of other impurities should NMT 5%, and the RSD of total impurity should NMT 5%.  In the results of 12 tests of repeatability and intermediate precision, the RSDS of other impurities that are less than 0.5 times of the main peak area	<table border="1"> <thead> <tr> <th>Impurities</th> <th>repeatability</th> <th>Intermediate precision</th> <th>Precision</th> </tr> </thead> <tbody> <tr> <td>YA2304-12</td> <td>0.7%, n=6</td> <td>1.5%, n=6</td> <td>2.6%, n=12</td> </tr> <tr> <td>YA2304-14</td> <td>0.5%, n=6</td> <td>1.4%, n=6</td> <td>2.0%, n=12</td> </tr> <tr> <td>YA2304-15</td> <td>0.7%, n=6</td> <td>1.0%, n=6</td> <td>1.5%, n=12</td> </tr> <tr> <td>YA2304-16</td> <td>0.9%, n=6</td> <td>1.6%, n=6</td> <td>2.6%, n=12</td> </tr> <tr> <td>YA2304-18</td> <td>0.5%, n=6</td> <td>1.4%, n=6</td> <td>2.0%, n=12</td> </tr> <tr> <td>YA2304-19</td> <td>1.7%, n=6</td> <td>2.1%, n=6</td> <td>1.9%, n=12</td> </tr> <tr> <td>YA2304-10</td> <td>0.6%, n=6</td> <td>0.22%, n=6</td> <td>2.1%, n=12</td> </tr> <tr> <td>YA2304-17</td> <td>0.4%, n=6</td> <td>1.1%, n=6</td> <td>0.9%, n=12</td> </tr> <tr> <td>Other</td> <td>Contents all &lt; 0.05%</td> <td>Content &lt; 0.05%, not</td> <td>Content &lt; 0.05%, not</td> </tr> </tbody> </table>	Impurities	repeatability	Intermediate precision	Precision	YA2304-12	0.7%, n=6	1.5%, n=6	2.6%, n=12	YA2304-14	0.5%, n=6	1.4%, n=6	2.0%, n=12	YA2304-15	0.7%, n=6	1.0%, n=6	1.5%, n=12	YA2304-16	0.9%, n=6	1.6%, n=6	2.6%, n=12	YA2304-18	0.5%, n=6	1.4%, n=6	2.0%, n=12	YA2304-19	1.7%, n=6	2.1%, n=6	1.9%, n=12	YA2304-10	0.6%, n=6	0.22%, n=6	2.1%, n=12	YA2304-17	0.4%, n=6	1.1%, n=6	0.9%, n=12	Other	Contents all < 0.05%	Content < 0.05%, not	Content < 0.05%, not		
Impurities	repeatability	Intermediate precision	Precision																																									
YA2304-12	0.7%, n=6	1.5%, n=6	2.6%, n=12																																									
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YA2304-15	0.7%, n=6	1.0%, n=6	1.5%, n=12																																									
YA2304-16	0.9%, n=6	1.6%, n=6	2.6%, n=12																																									
YA2304-18	0.5%, n=6	1.4%, n=6	2.0%, n=12																																									
YA2304-19	1.7%, n=6	2.1%, n=6	1.9%, n=12																																									
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YA2304-17	0.4%, n=6	1.1%, n=6	0.9%, n=12																																									
Other	Contents all < 0.05%	Content < 0.05%, not	Content < 0.05%, not																																									

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	<p>of the control solution should not be more than 10%, and the RSDS of total impurities should not be more than 10%.</p>	<table border="1"> <thead> <tr> <th>individual impurities</th><th>not applicable</th><th>applicable</th><th>applicable</th></tr> </thead> <tbody> <tr> <td>Total Clutter</td><td>0.5%, n=6</td><td>1.0%, n=6</td><td>1.6%, n=12</td></tr> </tbody> </table>	individual impurities	not applicable	applicable	applicable	Total Clutter	0.5%, n=6	1.0%, n=6	1.6%, n=12
individual impurities	not applicable	applicable	applicable							
Total Clutter	0.5%, n=6	1.0%, n=6	1.6%, n=12							
Solution stability	<p>Each solution was placed at 5°C (refrigerated) for a period of time. During the investigation, the signal-to-noise ratio (S/N) of the main peak (251nm) in the chromatogram of the control solution should NLT 20, and the RSD of the peak area NMT 10%; The peak area RSD of each impurity in the chromatogram of the control solution (230nm) NMT 10%; In the chromatogram of system suitability solution (251nm), the peak exit sequence is YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19. The separation degree between YA2304 and YA2304-14 and YA2304-15 should be NLT 1.5, and the separation degree between YA2304-17 and YA2304-16 and adjacent peaks should be NLT 1.0; If the impurity peak less than 0.5 times the main peak area of the control solution is ignored in the chromatogram of the test product solution and the limited concentration of</p>	<p>Control solution: under the condition of 5°C for 100h, the chromatogram of the control solution (251nm), the main peak signal-to-noise ratio (S/N) is NLT 54.0 (NLT 20), and the peak area RSD is 1.7% (NMT 10%); The above are in line with the verification requirements, indicating that the control solution is stable within 100h under 5°C.</p> <p>Reference solution: placed at 5°C for 100h, the chromatogram of the reference solution (230nm), the peak order is YA2304-17, YA2304-10, the RSD of the peak-peak area of each impurity in the chromatogram of the reference solution is not more than 0.9% (the requirement is not more than 10%); The verification requirements were met, indicating that the control solution was stable within 100h under 5°C.</p> <p>System suitability solution: Placed at 5°C for 100h, the chromatogram of system suitability solution (251nm), the peak order is YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19, The minimum separation degree between YA2304 and YA2304-14 and YA2304-15 is NLT 5.8 (NLT 1.5 required), and the minimum separation degree between YA2304-17 and YA2304-16 and adjacent peaks is NLT 1.6 (NLT 1.0 required); All of the above meet the verification requirements, indicating that the system suitable solution is stable within 100h under 5°C.</p> <p>Test solution: After being placed at 5°C for 100h, impurity peaks smaller than 0.5 times the main peak-peak area of control solution were ignored in the chromatogram of test solution. Known impurities were detected except YA2304-12 and YA2304-19, both of which were less than 0.05%, and other impurities were not detected. Other single unknown impurities were all less than 0.05%, the RSD of each impurity peak area was not counted (not more than 10%), and no new impurities were added to interfere with the detection of related substances. The above are in line with the verification requirements,</p>								

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	impurities, the RSD of the other impurity peak area NMT 10%, and there shall not be any new impurities that interfere with the detection of related substances.	indicating that the test solution is stable within 100h under 5°C.  The sample solution with limited concentration of impurities: under the condition of 5°C for 100h, the impurity peak less than 0.5 times the main peak area of the control solution was ignored in the chromatogram of the sample solution with mixed impurities. At 230m wavelength, the RSD of the peak area of the known impurities YA2304-17 and YA2304-10 was not more than 1.1% (the requirement NMT 10%). At 251nm wavelength: the RSD of known impurities YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18 and YA2304-19 are not greater than 4% (required not to exceed 10%); The RSD of other single unknown impurity is less than 0.05%, the RSD of the single unknown impurity is not counted (not more than 10%), and no new impurity interferes with the detection of related substances; The above are in line with the verification requirements, indicating that the solution of added impurity is stable within 100h at 5°C.  The above solutions do not need to be used in the new preparation.
Durability	Under the condition of single factor change column temperature, flow rate, initial gradient of mobile phase, column lot number and normal chromatographic conditions, the chromatogram obtained by system suitability solution under each condition (251nm) is required. The peak order is YA2304-14, YA2304, YA2304-15, YA2304-17, YA2304-16, YA2304-10, YA2304-19, and the separation degree between YA2304 and YA2304-14 and YA2304-15 should NLT 1.5. The separation degree between	Column temperature 20°C±2°C, mobile phase ratio (85:15) ±2, flow rate 1.0ml/min±0.05ml/min, different batches of chromatographic columns were investigated as follows:  System applicability: Reference solution chromatogram (230nm), the peak sequence was YA2304-17, YA2304-10; In the chromatogram of system suitability solution (251nm), the peak exit sequence was YA2304-14, YA2304, YA2304-15, YA2304-17, unknown impurities, YA2304-16, YA2304-10, YA2304-19, The minimum separation degree between YA2304 and YA2304-14 and YA2304-15 is NLT 5.6 (NLT 1.5 required), and the minimum separation degree between YA2304-17 and YA2304-16 and adjacent peaks is 1.2 (NLT 1.0 required); In the contrast solution chromatogram (251nm), the signal-to-noise ratio of the main peak was NLT 67.2 (NLT 20), and the system applicability met the verification requirements.  Sample solution: the RSD of impurity YA2304-12 is no more than 2.4% (the

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<p>YA2304-17 and YA2304-16 and adjacent peaks should NLT 1.0; In the chromatogram of the control product solution (230nm), the peak sequence was YA2304-17, YA2304-10; In the control solution chromatogram (251nm), the signal-to-noise ratio (S/N) of the main peak should NLT 20.</p> <p>Under different conditions of the same chromatographic parameters, the RSDS of each impurity content greater than 0.05% in the test product NMT 10%, and the RSDS of total impurity content NMT 10%; Or the absolute deviation of the impurity content NMT 20% of the limit value, the absolute deviation of the total impurity content NMT 40% of the limit value.</p>	<p>requirement is no more than 10%); RSD of YA2304-14 shall NMT 2.3% (NMT 10%); RSD of YA2304-15 content shall NMT 2.2% (NMT 10%); RSD of YA2304-16 content shall NMT 6% (NMT 10%); RSD of YA2304-18 content shall NMT 5% (NMT 10%); RSD of YA2304-19 content shall NMT 2.3% (NMT 10%); RSD of YA2304-17 content should NMT 1.9% (NMT 10%); RSD of YA2304-10 content should NMT 2.1% (NMT 10%); The contents of unknown impurity RRT≈0.64 and unknown impurity RRT≈1.26 are less than 0.05, and RSD is not used for statistics. All of them met the verification conditions.</p>
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### **3.2.S.4.3.2 Methodological verification of relevant substances II of finished products**

#### **I. Analytical Procedures**

Please refer to the analytical procedures of related substances in Finerenone in section 3.2.S.4.2.

#### **II. Validation Results and Discussion**

##### **a. Wavelength selection**

###### **➤ Acceptance Criteria**

The detection wavelength is selected by ultraviolet scanning of the principal component at 200nm ~ 400nm wavelength, requiring all impurities (YA2304-52, imidazole and 4-dimethylaminopyridine) to have greater absorption at 210nm wavelength.

###### **➤ Solution preparation**

Solvent: acetonitrile: water (50:50)

Blank solution: solvent.

YA2304-52 reference product reserve liquid (about 100 $\mu$ g/ml) : accurately weigh YA2304-52 reference product about 5mg, place it in a 50ml bottle, add an appropriate amount of solvent to dissolve by ultrasound, dilute it with solvent to the scale, shake well, and obtain.

Imidazole reference liquid (about 100 $\mu$ g/ml) : accurately weigh imidazole reference liquid (about 5mg), place it in a 50ml measuring bottle, add appropriate amount of solvent and ultrasonic to dissolve it, dilute it with solvent to the scale, shake well, and then obtain.

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4-dimethylaminopyridine (DMAP) reference product reserve liquid (about 100 $\mu$ g/ml) : precision weigh 4-dimethylaminopyridine reference product about 5mg, put in a 50ml bottle, add the appropriate amount of solvent ultrasonic to dissolve, dilute with solvent to the scale, shake well, ready.

Reference product solution: precisely measure YA2304-52 reference product reserve liquid, imidazole reference product reserve liquid and 4-dimethylaminopyridine reference product reserve liquid 1ml, put in the same 20ml bottle, dilute with solvent to the scale, shake well, ready. (About 5 $\mu$ g/ml for each)

### ➤ Injection procedure

After the chromatographic system balance, take the blank solution and the reference solution each injection 1 needle, record the chromatogram.

### ➤ Measurement results

test results of detection wavelength selection

Name of solution	Test substance	Retention time (min)	Whether there is a large absorption at 210nm
Reference solution	YA2304-52	6.392	is
	Imidazole	7.362	is
	DMAP	16.667	is
Reference solution wavelength scan	<p>光谱指数图</p> <p>样品名称: 对照品溶液; 样品瓶 1:C,2; 进样 1; 采集日期 2024/4/18 15:28:06 CST</p>		

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Conclusion: All impurities (YA2304-52, imidazole and 4-dimethylaminopyridine) have great absorption at 210nm wavelength.

### **(4.2) System applicability**

#### **➤ Acceptance Criteria**

The blank solution should be free of interference; The control solution should be injected for 6 consecutive times, and the peak order should be YA2304-52, imidazole and 4-dimethylaminopyridine. The separation degree between imidazole peak and YA2304-52 peak should be NLT 1.5, the retention time RSD of each impurity peak should be no more than 1.0%, and the RSD of the peak area of each impurity peak should be no more than 5%.

#### **➤ Solution preparation**

Blank solution: solvent.

YA2304-52 Reference storage solution (approx. 100 $\mu$ g/ml) : Share YA2304-52 reference storage solution under "4.1 wavelength selection".

Imidazole reference product reserve (approx. 100 $\mu$ g/ml) : Imidazole reference product reserve under "4.1 wavelength selection".

4-dimethylaminopyridine (DMAP) reference storage solution (about 100 $\mu$ g/ml) : 4-dimethylaminopyridine (DMAP) storage solution under "4.1 wavelength selection".

Reference product solution: according to "4.1 wavelength selection" under the reference product solution prepared by the same method. (Each about 5 $\mu$ g/ml)

#### **➤ Injection procedure**

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After the chromatographic system was balanced, the blank solution was injected with 1 needle, the control solution was injected with 6 needles again, and the chromatogram was recorded.

### ➤ Measurement results

Results of system suitability test

Investigation parameters		Control solution was injected						RSD
		1	2	3	4	5	6	
YA230 4-52	RT (min)	6.348	6.354	6.361	6.357	6.347	6.347	0.10%
	Peak area	24168	24307	23941	24129	23890	24107	0.7%
Imidazole	RT (min)	7.389	7.388	7.390	7.390	7.386	7.384	0.04%
	Peak area	41795 3	41800 4	41776 4	41971 1	41871 1	41875 6	0.18%
DMAP	RT (min)	16.764	16.764	16.771	16.774	16.771	16.767	0.025 %
	Peak area	45883 2	45868 8	45820 8	45761 9	46089 2	46086 8	0.4%
Degree of separation between imidazole peak and YA2304-52 peak		1.9	1.9	1.9	1.9	1.9	1.9	/
Conclusion: The blank solution has no interference; The control solution was injected for 6 consecutive times, and the peak order was YA2304-52, imidazole and 4-dimethylaminopyridine. The separation degree between imidazole peak and YA2304-52 peak was 1.9 (NLT 1.5), and the retention time RSD of each impurity peak was no more than 0.10% (NMT 1.0%). The RSD of the peak area of each impurity peak was no more than 0.7% (NMT 5%); All the above meet the verification requirements, indicating that the method has good system applicability.								

### (4.3) Specificity

#### ➤ Acceptance Criteria

The blank solution should be free of interference; In the chromatogram obtained from the control solution, the peak order is YA2304-52, imidazole, 4-dimethylaminopyridine, and the separation degree between the imidazole

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peak and YA2304-52 peak should NLT 1.5. The retention time of each impurity peak in the control solution, the test solution and the specific mixed solution should be consistent with the retention time of the main peak in the impurity localization solution. The separation degree between the imidazole peak and YA2304-52 peak in the specific mixed solution should be NLT 1.5. All impurities studied under the related substances of the finished product (including YA2304-10, YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-17, YA2304-18 and YA2304-19) shall not interfere with YA2304-52, imidazole and imidazole in the tested product 4-dimethylaminopyridine test.

### **➤ Solution preparation**

Blank solution: solvent.

YA2304-52 Reference storage solution (approx. 100 $\mu$ g/ml) : Share YA2304-52 reference storage solution under "4.1 wavelength selection".

Imidazole reference product reserve (approx. 100 $\mu$ g/ml) : Imidazole reference product reserve under "4.1 wavelength selection".

4-dimethylaminopyridine (DMAP) reference reserve liquid (about 100 $\mu$ g/ml) : Share 4-dimethylaminopyridine (DMAP) reserve liquid under "4.1 wavelength selection".

YA2304-10 reference product reserve liquid (about 200 $\mu$ g/ml) : accurately weigh YA2304-10 reference product about 5mg, put it in 25ml measuring bottle, add appropriate amount of solvent to dissolve by ultrasound, dilute it with solvent to the scale, shake well, and then obtain.

YA2304-12 reference product reserve (about 200 $\mu$ g/ml) : precision weigh YA2304-12 reference product about 5mg, place in 25ml measuring bottle,

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add appropriate amount of solvent and ultrasonic to dissolve, dilute with solvent to the scale, shake well, ready.

YA2304-14 reference product reserve liquid (about 200 $\mu$ g/ml) : precisely weigh YA2304-14 reference product about 5mg, place it in 25ml measuring bottle, add acetonitrile to dissolve by ultrasound, dilute it with acetonitrile to the scale, shake well, and then obtain.

YA2304-15 reference product reserve liquid (about 200 $\mu$ g/ml) : precisely weigh YA2304-15 reference product about 5mg, place it in 25ml measuring bottle, add appropriate amount of acetonitrile to dissolve, dilute it with acetonitrile to the scale, shake well, and then obtain.

YA2304-16 reference product reserve liquid (about 200 $\mu$ g/ml) : Accurately weigh about 5mg of YA2304-16 reference product, place it in a 25ml bottle, add acetonitrile to dissolve by ultrasound, dilute it with acetonitrile to the scale, shake well, and obtain.

YA2304-17 reference product reserve liquid (about 200 $\mu$ g/ml) : precisely weigh YA2304-17 reference product about 5mg, place it in 25ml measuring bottle, add appropriate amount of solvent to dissolve by ultrasound, dilute it with solvent to the scale, shake well, and then obtain.

YA2304-18 reference product reserve liquid (about 200 $\mu$ g/ml) : precisely weigh YA2304-18 reference product about 5mg, place it in 25ml measuring bottle, add acetonitrile to dissolve by ultrasound, dilute it with acetonitrile to the scale, shake well, and then obtain.

YA2304-19 reference product reserve liquid (about 200 $\mu$ g/ml) : precisely weigh YA2304-19 reference product about 5mg, place it in 25ml measuring

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bottle, add appropriate amount of acetonitrile to dissolve, dilute it with acetonitrile to the scale, shake well, and then obtain.

Reference solution: Share the reference solution under "4.2 System Suitability". (Approx. 5 $\mu$ g/ml each)

YA2304-52 positioning solution: Accurately measure 1ml of YA2304-52 reference product reserve, place it in a 20ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 5 $\mu$ g/ml)

Imidazole positioning solution: accurately measure 1ml of imidazole reference product reserve liquid, place it in 20ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 5 $\mu$ g/ml)

4-dimethylaminopyridine (DMAP) positioning solution: accurately measure 4-dimethylaminopyridine (DMAP) reference product reserve liquid 1ml, placed in 20ml measuring bottle, diluted with solvent to scale, shake well, ready. (About 5 $\mu$ g/ml)

YA2304-10 positioning solution: Accurately measure 1ml of YA2304-10 reference product reserve, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 8 $\mu$ g/ml)

YA2304-12 positioning solution: accurately measure 1ml of YA2304-12 reference product reserve, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 8 $\mu$ g/ml)

YA2304-14 positioning solution: accurately measure 1ml of YA2304-14 reference product reserve, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 8 $\mu$ g/ml)

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YA2304-15 positioning solution: accurately measure 1ml of YA2304-15 reference product reserve liquid, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 8 $\mu$ g/ml)

YA2304-16 positioning solution: accurately measure 1ml of YA2304-16 reference product reserve, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 8 $\mu$ g/ml)

YA2304-17 Positioning solution: Accurately measure 1ml of YA2304-17 reference product reserve, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 8 $\mu$ g/ml)

YA2304-18 positioning solution: Accurately measure 1ml of YA2304-18 reference product reserve, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 8 $\mu$ g/ml)

YA2304-19 positioning solution: Accurately measure 1ml of YA2304-19 reference product reserve, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 8 $\mu$ g/ml)

Test product solution: Take YA2304 about 100mg, accurately weigh it, place it in a 20ml measuring bottle, add an appropriate amount of solvent and ultrasonic to dissolve it, dilute it with solvent to the scale, shake well, and get it. (About 5mg/ml)

Specific mixed solution: Take YA2304 test product about 100mg, weigh it accurately, place it in a 20ml bottle, add an appropriate amount of solvent to dissolve by ultrasound, then add YA2304-52 reference product reserve, imidazole reference product reserve and 4-dimethylaminopyridine reference

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product reserve 1ml each, dilute it with solvent to the scale, shake well, and obtain. (Finerenone about 5mg/ml, each impurity about 5 $\mu$ g/ml)

### ➤ **Injection procedure**

After the chromatographic system balance, take blank solution, reference solution, test solution, specific mixed solution and each impurity location solution into 1 needle, record the chromatogram. (The chromatogram was obtained by sharing the test solution under the item "4.8 solution stability" for 2h; The control product solution shared the atlas obtained from the test product solution under "4.8 solution stability" for 16h)

### ➤ **Measurement results**

Name of solution	Substance name	Concentration ( $\mu$ g/ml)	RT (min)	Interference with YA2304-52, imidazole and DMAP detection
Blank solution	/	/	/	no
YA2304-10 Positioning solution	YA2304-10	8.9522	19.424	no
YA2304-12 Positioning solution	YA2304-12	8.4174	19.649	no
YA2304-14 Positioning solution	YA2304-14	8.1736	21.933	no
YA2304-15 Positioning solution	YA2304-15	7.9170	24.256	no
YA2304-16 Positioning solution	YA2304-16	7.5335	23.470	no
YA2304-17 Positioning solution	YA2304-17	8.6177	11.377	no
YA2304-18 Positioning solution	YA2304-18	8.1525	25.279	no
YA2304-19 Positioning solution	YA2304-19	7.8582	20.263	no
YA2304-52 Positioning solution	YA2304-52	5.2991	6.354	/

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Solution name	Substance name	Concentration ( $\mu\text{g}/\text{ml}$ )	RT (min)	Degree of separation from the previous peak
Reference solution	YA2304-52	5.2991	6.332	/
	Imidazole	5.1637	7.380	1.9
	DMAP	5.1695	16.750	14.9
Solution for the test product	YA2304-52	/	/	/
	Imidazole	/	/	/
	DMAP	/	/	/
	YA2304	5014.6000	Peak overload, uncredited	/
Specific mixed solution	YA2304-52	5.2991	6.354	/
	Imidazole	5.1637	7.386	1.9
	DMAP	5.1695	16.756	15.0
	YA2304	5017.3500	Peak overload, uncredited	/
Conclusion: the blank solution has no interference; In the chromatogram obtained from the control solution, the sequence of peaks was YA2304-52, imidazole, 4-dimethylaminopyridine, and the separation degree between imidazole peaks and YA2304-52 peaks was 1.9 (required to be NLT 1.5). The retention time of each impurity peak in the control solution, the test solution and the specific mixed solution was consistent with that of the main peak in the impurity positioning solution. The separation degree between the imidazole peak and YA2304-52 peak in the specific mixed solution was 1.9 (NLT 1.5); The impurities studied under the related substances of finished products (including YA2304-10, YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-17, YA2304-18 and YA2304-19) did not interfere with YA2304-52, imidazole and 4- in the test product Detection of dimethylaminopyridine.				

### (4.4) Limits of quantification and detection

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### **➤ Acceptance Criteria**

The S/N of each impurity in the 6 limited quantification solution maps should NLT 10, the RSD of the peak retention time of each impurity should not be more than 2.0%, and the RSD of the peak area should not be more than 10%. The S/N of each impurity in the 3-needle detection limited solution atlas should NLT 3.

### **➤ Solution preparation**

Blank solution: solvent.

YA2304-52 Reference storage solution (approx. 100 $\mu$ g/ml) : Share YA2304-52 reference storage solution under "4.1 wavelength selection".

Imidazole reference product reserve (approx. 100 $\mu$ g/ml) : Imidazole reference product reserve under "4.1 wavelength selection".

4-dimethylaminopyridine (DMAP) reference reserve liquid (about 100 $\mu$ g/ml) : Share 4-dimethylaminopyridine (DMAP) reserve liquid under "4.1 wavelength selection".

Quantitative limited reserve liquid: accurately measure imidazole reference product reserve liquid 400 $\mu$ l and 4-dimethylaminopyridine (DMAP) reference product reserve liquid 300 $\mu$ l, put in the same 20ml bottle, dilute with solvent to the scale, shake well, it is obtained.

Limited quantitative solution: precisely measure YA2304-52 reference product reserve liquid 250 $\mu$ l and limit quantitative reserve liquid 1ml, place in the same 20ml measuring bottle, dilute with solvent to scale, shake well, ready. Prepare 6 parts in parallel.

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Detection limit solution: accurately remove the first limit of quantification solution 3ml, place it in a 10ml measuring bottle, dilute it with solvent to the scale, shake well, and then get.

### ➤ **Injection procedure**

After the chromatographic system was balanced, the blank solution and the parallel preparation of 6 parts of the limited quantitative solution were injected with 1 needle each, the detection of the limited solution was repeated with 3 needles, and the chromatogram was recorded.

### ➤ **Measurement results**

Results of limit of quantitation test

Impurities Name	Solution name	Concentration /( $\mu$ g/ml)	Equivalent to test product concentration percentage	Retention time /min	Peak area	S/N
YA2304 -52	Limited quantitative solution ①	1.3248	0.03%	6.337	5472	24.0
	Limited quantification solution ②			6.348	5344	23.5
	Limited quantification solution ③			6.352	5352	34.9
	Limited quantification solution ④			6.340	5391	26.9
	Limited quantification solution ⑤			6.355	5307	27.5
	Limited quantification solution ⑥			6.337	5087	27.8

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	RSD	0.13%	2.5%	/
Conclusion: The limited quantification concentration of YA2304-52 was 1.3248µg/ml, which was equivalent to 0.03% of the solution concentration of the tested product. The signal to noise ratio (S/N) of YA2304-52 peaks in the 6 limited quantification solutions were all in the range of 23.5~34.9 (the requirement should be greater than 10). The RSD of retention time was 0.13% (no more than 2.0% required), and the RSD of peak area was 2.5% (no more than 10% required); All of the above meet the verification requirements.				
Imidazo le	Limited quantitative solution ①	0.1033	0.002%	7.384
	Limited quantification solution ②			8362
	Limited quantification solution ③			39.4
	Limited quantification solution ④			7.383
	Limited quantification solution ⑤			8180
	Limited quantification solution ⑥			39.3
	RSD		7.385	8001
Verdict: The limit of quantization of imidazole was 0.1033µg/ml, which was equivalent to 0.002% of the concentration of the tested solution. The signal to noise ratio (S/N) of the imidazole peak in the 6 limited quantification solutions were all in the range of 39.3~56.5 (the requirement should be greater than 10), and the RSD of the retention time was 0.06% (the requirement should NMT 2.0%). The RSD of the peak area was 4% (NMT 10%); All of the above meet the verification requirements.				
DMAP	Limited quantification solution ①	0.0775	0.002%	16.749
	Limited			6368
				22.3
				16.822
				7206
				22.4

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	quantification solution ②					
	Limited quantification solution ③			16.765	6718	32.8
	Limited quantification solution ④			16.789	7661	27.4
	Limited quantification solution ⑤			16.784	7547	28.0
	Limited quantitation solution ⑥			16.767	6117	23.6
	RSD			0.16%	10%	/
Verdict: The limit of quantitation concentration of 4-dimethylaminopyridine (DMAP) was 0.0775µg/ml, which was equivalent to 0.002% of the solution concentration. The signal to noise ratio (S/N) of DMAP peaks in 6 limited quantitation solutions were all in the range of 22.3~32.8 (the requirement should be greater than 10). The RSD of retention time was 0.16% (NMT 2.0%), and the RSD of peak area was 10% (NMT 10%); All of the above meet the verification requirements.						

### Test results of detection limits

Impurities Name	Detection limit solution injection	Concentration / (µg/ml)	Equivalent to test product concentration percentage /%	Retention time /min	Peak area	S/N
YA2304 -52	1	0.3974	0.008%	6.340	1263	6.5
	2			6.362	946	5.2
	3			6.346	1129	6.3
	Conclusion: The detection limit concentration of YA2304-52 is 0.3974µg/ml, which is equivalent to 0.008% of the concentration of the test solution. The signal-to-noise ratio (S/N) of YA2304-52 peak after repeated injection of the detection limit solution is in the range of 5.2~6.5 (the requirement should be greater than 3), which meets the verification requirements.					
3.	1	0.0310	0.0006%	7.396	2597	11.9

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Imidazo le	2			7.389	2537	11.8
	3			7.387	2584	13.6
Conclusion: The detection limit concentration of imidazole is 0.0310 $\mu$ g/ml, which is equivalent to 0.0006% of the concentration of the test solution. The signal-to-noise ratio (S/N) of the 3-needle imidazole peak after repeated injection of the detection limit solution is in the range of 11.8~13.6 (the requirement should be greater than 3), which meets the verification requirements.						
DMAP	1	0.0233	0.0005%	16.879	1814	5.0
	2			16.804	1708	4.6
	3			16.844	1164	4.7
	Conclusion: The detection limit concentration of 4-dimethylaminopyridine (DMAP) is 0.0233 $\mu$ g/ml, which is equivalent to 0.0005% of the concentration of the test solution. The signal-to-noise ratio (S/N) of the DMAP peak of the detection limit solution is in the range of 4.6~5.0 (the requirement should be greater than 3), which meets the verification requirements.					

### **(4.5) Linearity and range**

#### **➤ Acceptance Criteria**

In the range equivalent to the LOQ concentration of each impurity ~ 200% limit concentration, take 6 concentration points more evenly, take the concentration of each impurity as the horizontal coordinate, the peak area as the vertical coordinate as the linear regression curve, the regression coefficient R of the linear graph should NLT 0.990, the RSD of the response factor should NMT 10%. Y-axis intercepts are within 25% of 100% response value.

#### **➤ Solution Preparation**

Blank solution: solvent.

YA2304-52 Reference storage solution (approx. 100 $\mu$ g/ml) : Share YA2304-52 reference storage solution under "4.1 wavelength selection".

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Imidazole reference product reserve (approx. 100 $\mu$ g/ml) : Imidazole reference product reserve under "4.1 wavelength selection".

4-dimethylaminopyridine (DMAP) reference reserve liquid (about 100 $\mu$ g/ml) : Share 4-dimethylaminopyridine (DMAP) reserve liquid under "4.1 wavelength selection".

Linear solution ① : share the 6th dose limit solution prepared in parallel under the item "4.4 dose limit and detection limit".

Linear solution ② : precisely measure 0.5ml of YA2304-52 reference product reserve, imidazole reference product reserve and 4-dimethylaminopyridine reference product reserve in the same 20ml bottle, dilute to the scale with solvent, shake well, and obtain. (Each about 2.5 $\mu$ g/ml)

Linear solution ③ : Precision measure YA2304-52 reference product reserve liquid, imidazole reference product reserve liquid and 4-dimethylaminopyridine reference product reserve liquid of 0.75ml, place in the same 20ml bottle, dilute with solvent to scale, shake well, then obtain. (Each about 3.75 $\mu$ g/ml)

Linear solution ④ : Accurately measure YA2304-52 reference product reserve, imidazole reference product reserve and 4-dimethylaminopyridine reference product reserve of 1ml each, place in the same 20ml bottle, dilute with solvent to scale, shake well, and obtain. (Each about 5 $\mu$ g/ml)

Linear solution ⑤ : precisely measure YA2304-52 reference product reserve liquid, imidazole reference product reserve liquid and 4-dimethylaminopyridine reference product reserve liquid of 1.5ml, place in

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the same 20ml bottle, dilute to the scale with solvent, shake well, then obtain.

(Each about 7.5 $\mu$ g/ml)

Linear solution ⑥ : Accurately measure YA2304-52 reference product reserve liquid, imidazole reference product reserve liquid and 4-dimethylaminopyridine reference product reserve liquid 2ml, place in the same 20ml bottle, dilute with solvent to scale, shake well, and obtain. (Each about 10 $\mu$ g/ml)

### ➤ **Injection procedure**

After the chromatographic system is balanced, take a blank solution into 1 needle, linear solution ①~ linear solution ⑥ from low concentration to high concentration into 1 needle, record the chromatogram.

### ➤ **Measurement results**

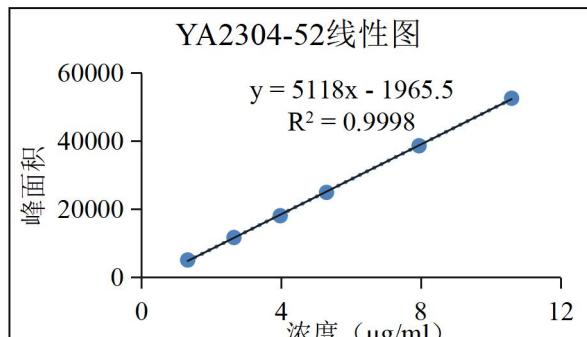
Substances Name	Solution name	Equivalent to percentage of reference solution concentration	Concentration ( $\mu$ g/ml)	Peak area	Response factor
YA230 4-52	Linear solution ①	0.03%	1.3248	5103	3852
	Linear solution ②	0.05%	2.6495	11692	4413
	Linear solution ③	0.08%	3.9743	18083	4550
	Linear solution ④	0.11%	5.2991	24926	4704
	Linear solution ⑤	0.16%	7.9486	38602	4856
	Linear solution ⑥	0.21%	10.5982	52524	4956
	Response factor RSD			9%	

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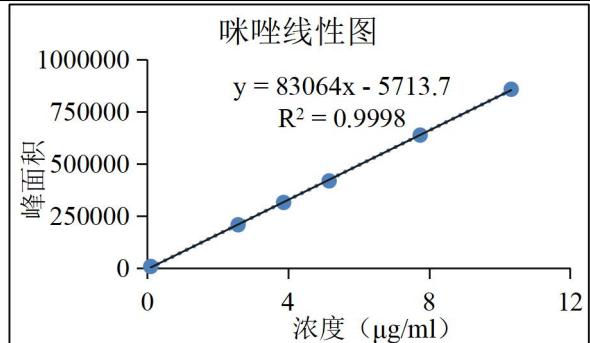
	Linear graph				
	Linear equations	$Y = 5118 - 1965.5 x$			
	Slope	5118			
	Correlation coefficient $r$	0.9999			
	Absolute value of Y-axis intercept as a percentage of 100% response value	8.32%			
	Conclusion: When YA2304-52 is in the concentration range of 1.3248 $\mu$ g/ml~10.5982 $\mu$ g/ml, the linear equation is $y=5118x-1965.5$ , the correlation coefficient $r$ is 0.9999 (NLT 0.990), and the RSD of the response factor is 9% (NMT 10%). The absolute value of Y-axis intercept as a percentage of 100% response value is 8.32% (not more than 25% required). All the above meet the requirements, indicating that under this method, when YA2304-52 is in the concentration range of 1.3248 $\mu$ g/ml~10.5982 $\mu$ g/ml, the linear relationship between peak area and concentration is good.				
Imidazole	Linear solution ①	0.002%	0.1033	7966	77135
	Linear solution ②	0.05%	2.5818	207402	80332
	Linear solution ③	0.08%	3.8727	314281	81152
	Linear solution ④	0.10%	5.1637	417642	80881
	Linear solution ⑤	0.15%	7.7455	636837	82220
	Linear solution ⑥	0.21%	10.3273	856435	82929
	Response factor RSD		2.5%		

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	Linear graph				
	Linear equations	$Y = 83064 - 5713.7 x$			
	Slope	83064			
	Correlation coefficient <i>r</i>	0.9999			
	Absolute value of Y-axis intercept as a percentage of 100% response value	1.39%			
	Conclusion: When imidazole is in the concentration range of 0.1033μg/ml~10.3273μg/ml, the linear equation is $y=83064x-5713.7$ , the correlation coefficient <i>r</i> is 0.9999 (NLT 0.990), and the RSD of the response factor is 2.5% (NMT 10%). The absolute value of Y-axis intercept as a percentage of 100% response value is 1.39% (required not to be greater than 25%). All the above meet the requirements, indicating that under this method, when imidazole is in the concentration range of 0.1033μg/ml~10.3273μg/ml, the linear relationship between peak area and concentration is good.				
DMAP	Linear solution ①	0.002%	0.0775	6879	88713
	Linear solution ②	0.05%	2.5848	230453	89159
	Linear solution ③	0.08%	3.8771	346096	89266
	Linear solution ④	0.10%	5.1695	465309	90010
	Linear solution ⑤	0.16%	7.7543	710454	91621
	Linear solution ⑥	0.21%	10.3390	969730	93793
	Response factor RSD			2.2%	

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	Linear graph	
	Linear equations	y=93774x-10958
	Slope	93774
	Correlation coefficient r	0.9997
	Absolute value of Y-axis intercept as a percentage of 100% response value	2.39%
Conclusion: When 4-dimethylaminopyridine (DMAP) was in the concentration range of 0.0775μg/ml~10.3390μg/ml, the linear equation was y=93774x-10958, the correlation coefficient r was 0.9997 (NLT 0.990), and the RSD of the response factor was 2.2% (NMT 10%). The absolute value of Y-axis intercept as a percentage of 100% response value is 2.39% (not more than 25% required). All of the above meet the requirements, indicating that under this method, when 4-dimethylaminopyridine (DMAP) is in the concentration range of 0.0775μg/ml to 10.3390μg/ml, the peak area has a good linear relationship with the concentration.		

### (4.6) Accuracy

#### ➤ Acceptance Criteria

The recovery rate of known impurities is achieved by adding each impurity reference in the test product solution within the range of 50% concentration to 150% limit concentration, and testing the recovery rate between the theoretical addition amount and the actual detection amount. The required recovery rate is 80%~115%, and the RSD of the recovery rate of each impurity is not more than 10%.

#### ➤ Solution preparation

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## **Drug Master File of Finerenone**

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

Blank solution: solvent.

YA2304-52 Reference storage solution (approx. 100 $\mu$ g/ml) : Share YA2304-52 reference storage solution under "4.1 wavelength selection".

Imidazole reference product reserve (approx. 100 $\mu$ g/ml) : Imidazole reference product reserve under "4.1 wavelength selection".

4-dimethylaminopyridine (DMAP) reference storage solution (about 100 $\mu$ g/ml) : Share 4-dimethylaminopyridine (DMAP) storage solution under "4.1 wavelength selection".

Reference product solution: according to "4.1 wavelength selection" under the reference product solution prepared by the same method. (Each about 5 $\mu$ g/ml)

Test product solution: Prepare the test product solution under "4.3 specific properties" by the same method. Prepare 2 parts in parallel.

50% recovery solution: Take YA2304 test product about 100mg, weigh it accurately, place it in 20ml measuring bottle, add appropriate amount of solvent to dissolve by ultrasound, then add YA2304-52 reference product reserve, imidazole reference product reserve and 4-dimethylaminopyridine reference product reserve 0.5ml each, dilute with solvent to scale, shake well, and obtain. 3 parts were prepared in parallel. (Finerenone about 5mg/ml, each impurity about 2.5 $\mu$ g/ml)

100% recovery solution: Take YA2304 test product about 100mg, accurately weigh, put into 20ml bottle, add appropriate amount of solvent to dissolve by ultrasound, add YA2304-52 reference product reserve liquid, imidazole reference product reserve liquid and 4-dimethylaminopyridine reference

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product reserve liquid 1.0ml each, dilute with solvent to scale, shake well, then obtain. 3 parts were prepared in parallel. (Finerenone about 5mg/ml, each impurity about 5 $\mu$ g/ml)

150% recovery solution: Take YA2304 test product about 100mg, accurately weigh, put into 20ml bottle, add appropriate amount of solvent to dissolve by ultrasound, add YA2304-52 reference product reserve, imidazole reference product reserve and 4-dimethylaminopyridine reference product reserve 1.5ml each, dilute with solvent to scale, shake well, then obtain. 3 parts were prepared in parallel. (Finerenone about 5mg/ml, each impurity about 7.5 $\mu$ g/ml)

### ➤ **Injection procedure**

After the chromatographic system was balanced, the blank solution was injected with 1 needle, the control solution was injected with 2 needles repeatedly, 2 samples of test solution and 9 recovery solutions were injected with 1 needle each, and the chromatogram was recorded.

### ➤ **Measurement results**

Substance Name	Solution name	Amount to add ( $\mu$ g/ml)	Measured amount ( $\mu$ g/ml)	Recovery rate	Average recovery rate	RSD
YA2304-5 2	50% recovery solution -1	2.6495	2.4303	91.7%	99.3%	5%
	50% recovery solution -2	2.6495	2.5275	95.4%		
	50% recovery solution -3	2.6495	2.4833	93.7%		
	100% recovery solution -1	5.2991	5.3386	100.7%		
	100% recovery	5.2991	5.4065	102.0%		

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	solution -2							
	100% recovery solution -3	5.2991	5.4013	101.9%				
	150% recovery solution -1	7.9486	8.0957	101.8%				
	150% recovery solution -2	7.9486	8.1642	102.7%				
	150% recovery solution -3	7.9486	8.2538	103.8%				
	Conclusions: YA2304-52 was added into the test samples at concentrations ranging from 2.6495µg/ml to 7.9486µg/ml. The recoveries were all in the range of 91.7% to 103.8% (85% to 110%), and the RSD of the recoveries was 5% (less than 10%). All the above met the requirements, indicating that the method had good accuracy in detecting YA2304-52 content.							
	50% recovery solution -1	2.5818	2.5271	97.9%				
	50% recovery solution -2	2.5818	2.5098	97.2%				
	50% recovery solution -3	2.5818	2.5329	98.1%				
	100% recovery solution -1	5.1637	5.1654	100.0%				
Imidazole	100% recovery solution -2	5.1637	5.2085	100.9%	99.5%	1.5%		
	100% recovery solution -3	5.1637	5.1694	100.1%				
	150% recovery solution -1	7.7455	7.8567	101.4%				
	150% recovery solution -2	7.7455	7.7757	100.4%				
	150% recovery solution -3	7.7455	7.7179	99.6%				
	Conclusions: The recoveries of imidazole control products in the concentration range of 2.5818µg/ml to 7.7455µg/ml were in the range of 97.2% to 101.4% (85% to 110%), and the RSD of recovery was 1.5% (no more than 10%). All of the above met the requirements, indicating that the method was accurate in detecting imidazole content.							

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DMAP	50% recovery solution -1	2.5848	2.5840	100.0%	102.1 %	1.8%
	50% recovery solution -2	2.5848	2.6040	100.7%		
	50% recovery solution -3	2.5848	2.6423	102.2%		
	100% recovery solution-1	5.1695	5.2211	101.0%		
	100% recovery solution -2	5.1695	5.2575	101.7%		
	100% recovery solution -3	5.1695	5.1986	100.6%		
	150% recovery solution -1	7.7543	8.0727	104.1%		
	150% recovery solution -2	7.7543	8.1400	105.0%		
	150% recovery solution -3	7.7543	8.0275	103.5%		
	Conclusions: The recoveries of 4-dimethylaminopyridine (DMAP) in the concentration range of 2.5848 $\mu$ g/ml to 7.7543 $\mu$ g/ml were all in the range of 100.0% to 105.0% (85% to 110%), and the RSD of recovery was 1.8% (less than 10%). All the above met the requirements, indicating that the method was accurate in detecting the content of 4-dimethylaminopyridine (DMAP).					

### **(4.7) Precision**

Precision refers to the proximity between the results obtained by multiple sampling of the same uniform test product under specified test conditions, and its precision is judged by examining repeatability and intermediate precision.

#### **(4.7.1) Repeatability**

##### **➤ Acceptance Criteria**

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6 copies of repeatable sample solution were prepared by analyst A on date A and measured by instrument A. The blank solution was required to be free of interference; The separation degree between the imidazole peak and YA2304-52 peak in the control solution should be NLT 1.5; The RSD of all impurities in the solution of 6 repeatable test products NMT 10%.

### **➤ Solution preparation**

Blank solution: solvent.

YA2304-52 Reference storage solution (approx. 100 $\mu$ g/ml) : Share YA2304-52 reference storage solution under "4.1 wavelength selection".

Imidazole reference product reserve (approx. 100 $\mu$ g/ml) : Imidazole reference product reserve under "4.1 wavelength selection".

4-dimethylaminopyridine (DMAP) reference solution (approx. 100 $\mu$ g/ml) : Share 4-dimethylaminopyridine (DMAP) under "43.1 wavelength selection".

Reference solution: Prepare it by the same method as the reference solution under "4.1 wavelength selection". (Each about 5 $\mu$ g/ml)

Repetitive test product solution: Take YA2304 test product about 100mg, accurately weigh it, place it in a 20ml measuring bottle, add appropriate amount of solvent to dissolve by ultrasound, then add YA2304-52 reference product reserve, imidazole reference product reserve and 4-dimethylaminopyridine reference product reserve 1.0ml each, dilute with solvent to scale, shake well, and obtain. 6 parts were prepared in parallel. (YA2304 about 5mg/ml, each impurity about 5 $\mu$ g/ml)

### **➤ Injection procedure**

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After the chromatographic system was balanced, 1 needle was injected into the blank solution, 2 needles into the control solution, 1 needle into each of the 6 repetitive test product solutions, and the chromatogram was recorded.

### ➤ Determination result

Name of solution	Inspection items		Sample number				RESU LTS	
Reference solution	Degree of separation between imidazole peaks and YA2304-52 peaks		1			2.0		
			2			2.0		
Investigation Items	Solution name						RSD	
	Repeate d test product solutio n 1	Repetiti ve test product solutio n 2	Repetiti ve test product solutio n 3	Repetiti ve test product solutio n 4	Repetiti ve test product solutio n 5	Repetiti ve test product solutio n 6		
	YA2304-52 content	0.11%	0.10%	0.10%	0.10%	0.10%	0.11%	1.2%
	Imidazole content	0.10%	0.10%	0.10%	0.10%	0.10%	0.10%	0.9%
	DMAP content	0.10%	0.10%	0.10%	0.10%	0.10%	0.10%	1.6%
	Conclusion: The blank solution has no interference; The separation degree between imidazole peaks and YA2304-52 peaks in control solution was 2.0 (NLT 1.5 required). The RSDS of all impurities in the 6 repeatable samples were no more than 1.6% (no more than 10% required); All the above met the verification requirements, indicating that the method has good repeatability.							

### (4.7.2) Intermediate precision

#### ➤ Brief Description

6 intermediate precision sample solutions were prepared by Analyst B on date B. It is required that the blank solution should be interference free; The control solution was injected for 6 consecutive times, and the peak order was YA2304-52, imidazole and 4-dimethylaminopyridine. The separation degree between imidazole peak and YA2304-52 peak should be NLT 1.5, the

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retention time RSD of each impurity peak should be no more than 1.0%, and the RSD of the peak area of each impurity peak should be no more than 5%. RSD of all impurities in 6 intermediate precision test solutions NMT 10%.

The RSD of each impurity content NMT 10% for the results of 12 tests of repeatability and intermediate precision.

### **➤ Solution preparation**

Blank solution: solvent.

YA2304-52 Reference storage solution (about 100 $\mu$ g/ml) : Share YA2304-52 reference storage solution under "4.1 Wavelength selection".

Imidazole reference product reserve (approx. 100 $\mu$ g/ml) : Imidazole reference product reserve under "4.1 wavelength selection".

4-dimethylaminopyridine (DMAP) reference reserve liquid (about 100 $\mu$ g/ml) : Share 4-dimethylaminopyridine (DMAP) reserve liquid under "4.1 wavelength selection".

Reference product solution: according to "4.1 wavelength selection" under the reference product solution prepared by the same method. (Each about 5 $\mu$ g/ml)

Intermediate precision test solution: Take YA2304 test product about 100mg, weigh it accurately, place it in a 20ml measuring bottle, add appropriate amount of solvent to dissolve by ultrasound, then add YA2304-52 reference product reserve liquid, imidazole reference product reserve liquid and 4-dimethylaminopyridine reference product reserve liquid 1.0ml each, dilute

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it with solvent to the scale, shake well, and obtain. 6 parts were prepared in parallel. (YA2304 about 5mg/ml, each impurity about 5µg/ml)

### ➤ **Injection procedure**

Intermediate precision - System applicability: After the chromatographic system balance, take blank solution into 1 needle, control solution into 6 needles, record the chromatogram.

Intermediate precision - Sample detection: After the chromatographic system was balanced, 1 needle was injected into the blank solution, 2 needles were injected into the control solution, and 1 needle was injected into each of the 6 intermediate precision test solutions, and the chromatogram was recorded.

### ➤ **Determination result**

Results of intermediate precision - system suitability test

Investigation parameters		Control solution was injected						RSD
		1	2	3	4	5	6	
YA230 4-52	RT (min)	6.348	6.348	6.351	6.351	6.354	6.351	0.04%
	Peak area	25275	25258	25184	25462	25289	25551	0.6%
imidazole	RT (min)	7.348	7.348	7.350	7.352	7.353	7.352	0.030 %
	Peak area	41650 3	41639 8	41707 9	41785 9	41758 3	41799 7	0.17%
DMAP	RT (min)	16.646	16.647	16.652	16.657	16.659	16.657	0.04%
	Peak area	47114 1	47143 3	47226 3	47320 7	46753 7	47310 5	0.5%
Degree of separation between imidazole peak and YA2304-52 peak		1.9	1.9	1.9	1.9	1.9	1.9	/
Conclusion: The blank solution has no interference; The control solution was injected for 6 consecutive times, and the peak order was YA2304-52, imidazole and 4-dimethylaminopyridine. The separation degree between imidazole peak and								

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YA2304-52 peak was 1.9 (NLT 1.5), and the retention time RSD of each impurity peak was no more than 0.04% (NMT 1.0%). The RSD of the peak area of each impurity peak was no more than 0.6% (NMT 5%); All the above meet the verification requirements, indicating that the method has good system applicability.

### Results of repeatability test

Investigation items	Solution name						RSD (n=6)
	RSD (n = 6) 1 Repeatability test solution n	Repetitive product solution n 2	Repetitive product solution n 3	Repetitive product solution n 4	Repetitive product solution n 5	Repetitive product solution n 6	
YA2304-52 content	0.11%	0.10%	0.10%	0.10%	0.10%	0.11%	1.2%
Imidazole content	0.10%	0.10%	0.10%	0.10%	0.10%	0.10%	0.9%
DMAP content	0.10%	0.10%	0.10%	0.10%	0.10%	0.10%	1.6%
Survey items	Solution name						RSD (n=6) RSD (n=12)
	Intermediate precision test sample solution n 1	Intermediate precision sample solution n 2	Intermediate precision sample solution n 3	Intermediate precision sample solution n 4	Intermediate precision sample solution n 5	Intermediate precision sample solution n 6	
YA2304-52 content	0.10%	0.11%	0.11%	0.11%	0.11%	0.10%	1.1 %
Imidazole content	0.10%	0.10%	0.10%	0.10%	0.10%	0.10%	1.1 %
DMAP content	0.10%	0.10%	0.11%	0.11%	0.11%	0.10%	2.2 %
Conclusion: RSDS of all impurities in 6 intermediate precision test solutions are not more than 1.3% (the requirement is not more than 10%). All the above meet the verification requirements, indicating that the intermediate precision of the method is good; RSDS of all impurities in 12 repetitive test products and intermediate precision test products were not more than 2.2% (the requirement was not more than 10%); The above all meet the verification requirements, indicating that the method has good							

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precision.

### **(4.8) Solution stability**

#### **➤ Brief Description**

Each solution is placed in a 5°C injector, and the control solution and test solution are injected at different times. The separation degree between imidazole peak and YA2304-52 peak in the control solution should be NLT 1.5, and the RSD of the peak area of each impurity peak should be no more than 5%; The RSDS of all impurities in the test solution should NMT 10%, and no new impurities to be measured (YA2304-52, imidazole, 4-dimethylaminopyridine) should be detected.

#### **➤ Solution preparation**

Blank solution: solvent.

Reference solution: Share the reference solution under "4.2 System Suitability". (Approx. 5 $\mu$ g/ml)

Test solution: Share the test solution under "4.3 specific Properties".

#### **➤ Injection Sequence**

After the chromatographic system was balanced, the control solution placed in the 5°C injector was injected with 1 needle at 0h, 2h, 4h, 16h, 24h, 40h and 48h respectively, and the test solution placed in the 5°C injector was injected with 1 needle at 0h, 2h, 4h, 12h, 20h, 36h and 44h respectively to record the chromatogram. (The chromatogram of reference solution 0h and 2h shared the chromatogram of reference solution 1 and 4 needles of repeated injection of 6 needles under "4.2 system applicability", and the

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chromatogram of reference solution 16h shared the chromatogram of reference solution under "4.3 specificity"; The test product solution 2h atlas was shared with the test product solution under "4.3 specificity")

### ➤ Measurement results

#### Stability test results of reference solution

Investigation		Reference solution							RSD
		Items	0h	2h	4h	16h	24h	40h	
Peak area	YA230 4-52	24168	24129	23898	24389	24371	24282	24439	0.8 %
	Imidazole	41795 3	41971 1	41872 0	42153 2	42406 2	41994 6	42191 7	0.5 %
	DMAP	45883 2	45761 9	46219 1	46923 3	47399 0	47453 2	47218 2	1.6 %
Degree of separation between imidazole peaks and YA2304-52 peaks		1.9	1.9	1.9	1.9	1.9	2.0	2.0	/
Conclusion: When the control solution was placed in the 5°C injector for 48 h, the separation degree between imidazole peak and YA2304-52 peak was NLT 1.9 (required to be NLT 1.5), and the RSD of the peak-peak area of each impurity was not more than 1.6% (required to be no more than 5%). All the above met the verification requirements, indicating that the control product solution was stable within 48h after being placed in the 5°C injector.									

#### Stability test results of sample solution

Investigation		Test solution							RSD
		Items	0h	2h	4h	12h	20h	36h	
Content	YA2304 -52	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	/
	Imidazole	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	/

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	DMAP	Not detected	/						
Conclusion: YA2304-52, imidazole and 4-dimethylaminopyridine (DMAP) were not detected when the sample solution was placed in the sample injector at 5°C for 44h, and no other impurity peaks affecting the detection of the above three impurities were detected. The results indicated that the tested product solution was stable for 44h after being placed in the 5°C injector.									

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### III. Summary of Method Validation

#### Methodological Verification Results of Finished Product Related Substances II

Item	Acceptable criteria	Verification of results
Wavelength selection	All impurities (YA2304-52, imidazole and 4-dimethylaminopyridine) have greater absorption at 210nm wavelength	<p>All impurities (YA2304-52, imidazole and 4-dimethylaminopyridine) have greater absorption at 210nm wavelength. In the control solution, the retention time of YA2304-52, imidazole and 4-dimethylaminopyridine was 6.392min, 7.362min and 16.667min, respectively. The spectral diagram of the control solution is as follows:</p> <p style="text-align: center;">光谱指数组图</p> <p style="text-align: center;">样品名称 对照品溶液; 样品瓶 1:C,2; 进样 1; 采集日期 2024/4/18 15:28:06 CST</p>
System suitability	The blank solution should be free of interference; The control solution should be injected for 6 consecutive times, and the peak order should be YA2304-52, imidazole and 4-dimethylaminopyridine. The separation degree between imidazole	There was no interference in the blank solution; The control solution was injected for 6 consecutive times, the peak order was YA2304-52, imidazole, 4-dimethylaminopyridine, the separation degree between imidazole peak and YA2304-52 peak was 1.9 (NLT 1.5), and the retention time RSD of each impurity peak was no more than 0.10% (NMT 1.0%). The RSD of the peak area of each impurity peak was no more than 0.7% (NMT 5%); All the above meet the verification requirements, indicating that the method has good system

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	peak and YA2304-52 peak should be NLT 1.5, the retention time RSD of each impurity peak should be no more than 1.0%, and the RSD of the peak area of each impurity peak NMT 5%.	applicability.																								
Specificity	<p>The blank solution should be free of interference; In the chromatogram obtained from the control solution, the peak order is YA2304-52, imidazole, 4-dimethylaminopyridine, and the separation degree between the imidazole peak and YA2304-52 peak was 1.9 (required to be NLT 1.5); The retention time of each impurity peak in the control solution, the test solution and the specific mixed solution was consistent with that of the main peak in the impurity positioning solution. The separation degree between the imidazole peak and YA2304-52 peak in the specific mixed solution was 1.9 (NLT 1.5); The impurities studied under the related substances of finished products (including YA2304-10, YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-17, YA2304-18 and YA2304-19) did not interfere with YA2304-52, imidazole and 4- in the test product Detection of dimethylaminopyridine. The concentration of YA2304-52, imidazole and 4-dimethylaminopyridine in the specific mixed solution was 0.10% of the principal component concentration in the test solution.</p>	<p>Blank solution without interference; In the chromatogram obtained from the control solution, the peak order was YA2304-52, imidazole, 4-dimethylaminopyridine, and the separation degree between the imidazole peak and YA2304-52 peak was 1.9 (required to be NLT 1.5); The retention time of each impurity peak in the control solution, the test solution and the specific mixed solution was consistent with that of the main peak in the impurity positioning solution. The separation degree between the imidazole peak and YA2304-52 peak in the specific mixed solution was 1.9 (NLT 1.5); The impurities studied under the related substances of finished products (including YA2304-10, YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-17, YA2304-18 and YA2304-19) did not interfere with YA2304-52, imidazole and 4- in the test product Detection of dimethylaminopyridine. The concentration of YA2304-52, imidazole and 4-dimethylaminopyridine in the specific mixed solution was 0.10% of the principal component concentration in the test solution.</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Name of solution</th> <th>Component name</th> <th>Component concentration (<math>\mu\text{g/ml}</math>)</th> <th>Rt (min)</th> <th>Degree of separation from the previous peak</th> <th>The percentage equivalent to the concentration of the principal component in the test solution</th> </tr> </thead> <tbody> <tr> <td>Specifi c</td> <td>YA2304-52</td> <td>5.2991</td> <td>6.354</td> <td>/</td> <td>0.10%</td> </tr> <tr> <td>mixed</td> <td>Imidazole</td> <td>5.1637</td> <td>7.386</td> <td>1.9</td> <td>0.10%</td> </tr> <tr> <td></td> <td>DMAP</td> <td>5.1695</td> <td>16.756</td> <td>15.0</td> <td>0.10%</td> </tr> </tbody> </table>	Name of solution	Component name	Component concentration ( $\mu\text{g/ml}$ )	Rt (min)	Degree of separation from the previous peak	The percentage equivalent to the concentration of the principal component in the test solution	Specifi c	YA2304-52	5.2991	6.354	/	0.10%	mixed	Imidazole	5.1637	7.386	1.9	0.10%		DMAP	5.1695	16.756	15.0	0.10%
Name of solution	Component name	Component concentration ( $\mu\text{g/ml}$ )	Rt (min)	Degree of separation from the previous peak	The percentage equivalent to the concentration of the principal component in the test solution																					
Specifi c	YA2304-52	5.2991	6.354	/	0.10%																					
mixed	Imidazole	5.1637	7.386	1.9	0.10%																					
	DMAP	5.1695	16.756	15.0	0.10%																					

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<p>YA2304-14, YA2304-15, YA2304-16, YA2304-17, YA2304-18 and YA2304-19) shall not interfere with YA2304-52, imidazole and imidazole in the tested product 4-dimethylaminopyridine test.</p>	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="padding: 2px;">solution</td><td style="padding: 2px;">YA2304</td><td style="padding: 2px;">5017.350</td><td style="padding: 2px;">Peak overload, uncredited</td><td style="padding: 2px; text-align: center;">/</td><td style="padding: 2px;">100%</td></tr> </table> <p>The chromatogram of the specific mixed solution is as follows:</p>	solution	YA2304	5017.350	Peak overload, uncredited	/	100%									
solution	YA2304	5017.350	Peak overload, uncredited	/	100%											
<p>Limits of quantification and detection</p>	<p>The S/N of each impurity in the 6 limited quantitation solution maps should NLT 10, the RSDS of the peak retention time of each impurity should not be more than 2.0%, and the RSDS of the peak area should not be more than 10%. The S/N of each impurity in the 3-needle detection limited solution atlas</p>	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th rowspan="2" style="text-align: center; width: 25%;">Impurities</th> <th style="text-align: center;">Detection Limits</th> <th style="text-align: center;">Limit of quantitation</th> </tr> <tr> <th style="text-align: center;">Concentration (equivalent to the concentration percentage of the test product solution)</th> <th style="text-align: center;">Concentration (equivalent to the test product solution concentration percentage)</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;">YA2304-52</td><td style="text-align: center;">0.3974µg/ml (0.008%)</td><td style="text-align: center;">1.3248µg/ml (0.03%)</td></tr> <tr> <td style="text-align: center;">Imidazole</td><td style="text-align: center;">0.0310µg/ml (0.0006%)</td><td style="text-align: center;">0.1033µg/ml (0.002%)</td></tr> <tr> <td style="text-align: center;">4-dimethylaminopyridine</td><td style="text-align: center;">0.0233µg/ml (0.0005%)</td><td style="text-align: center;">0.0775µg/ml (0.002%)</td></tr> </tbody> </table>	Impurities	Detection Limits	Limit of quantitation	Concentration (equivalent to the concentration percentage of the test product solution)	Concentration (equivalent to the test product solution concentration percentage)	YA2304-52	0.3974µg/ml (0.008%)	1.3248µg/ml (0.03%)	Imidazole	0.0310µg/ml (0.0006%)	0.1033µg/ml (0.002%)	4-dimethylaminopyridine	0.0233µg/ml (0.0005%)	0.0775µg/ml (0.002%)
Impurities	Detection Limits	Limit of quantitation														
	Concentration (equivalent to the concentration percentage of the test product solution)	Concentration (equivalent to the test product solution concentration percentage)														
YA2304-52	0.3974µg/ml (0.008%)	1.3248µg/ml (0.03%)														
Imidazole	0.0310µg/ml (0.0006%)	0.1033µg/ml (0.002%)														
4-dimethylaminopyridine	0.0233µg/ml (0.0005%)	0.0775µg/ml (0.002%)														

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	NLT 3.																			
Linearity vs. Range	In the range equivalent to the LOQ concentration of each impurity ~ 200% limit concentration, take 6 concentration points more evenly, take the concentration of each impurity as the horizontal coordinate and the peak area as the vertical coordinate as the linear regression curve, the regression coefficient R of the linear graph should NLT 0.990, and the RSD of the response factor should not be more than 10%. Y-axis intercepts are within 25% of 100% response value.	<table border="1"> <thead> <tr> <th>Impurities</th><th>Concentration (equivalent to the percentage of solution concentration of the test product)</th><th>Linear equation</th></tr> </thead> <tbody> <tr> <td>YA2304-52</td><td>1.3248µg/ml to 10.5982µg/ml (0.03% to 0.21%)</td><td><math>Y = 5118 - 1965.5 x</math></td></tr> <tr> <td>Imidazole</td><td>0.1033µg/ml~10.3273µg/ml (0.002%~0.21%)</td><td><math>Y = 83064 - 5713.7 x</math></td></tr> <tr> <td>4-dimethylamino pyridine</td><td>0.0775µg/ml to 10.3390µg/ml (0.002% to 0.21%)</td><td><math>y=93774x-10958</math></td></tr> </tbody> </table>			Impurities	Concentration (equivalent to the percentage of solution concentration of the test product)	Linear equation	YA2304-52	1.3248µg/ml to 10.5982µg/ml (0.03% to 0.21%)	$Y = 5118 - 1965.5 x$	Imidazole	0.1033µg/ml~10.3273µg/ml (0.002%~0.21%)	$Y = 83064 - 5713.7 x$	4-dimethylamino pyridine	0.0775µg/ml to 10.3390µg/ml (0.002% to 0.21%)	$y=93774x-10958$				
Impurities	Concentration (equivalent to the percentage of solution concentration of the test product)	Linear equation																		
YA2304-52	1.3248µg/ml to 10.5982µg/ml (0.03% to 0.21%)	$Y = 5118 - 1965.5 x$																		
Imidazole	0.1033µg/ml~10.3273µg/ml (0.002%~0.21%)	$Y = 83064 - 5713.7 x$																		
4-dimethylamino pyridine	0.0775µg/ml to 10.3390µg/ml (0.002% to 0.21%)	$y=93774x-10958$																		
Accuracy	The recovery rate of known impurities is achieved by adding each impurity reference in the range of 50% concentration to 150% limit concentration in the test product solution, and testing the recovery rate between the theoretical addition amount and the actual detection amount. The required recovery rate is 80%~115%, and the RSD of the recovery rate of each	<table border="1"> <thead> <tr> <th>Impurities</th><th>Marking condition</th><th>Average recovery rate</th><th>RSD</th></tr> </thead> <tbody> <tr> <td>YA2304-52</td><td>Added 2.6495µg/ml to 7.9486µg/ml</td><td>99.3%, n=9</td><td>5%, n=9</td></tr> <tr> <td>Imidazole</td><td>Added 2.5818µg/ml to 7.7455µg/ml</td><td>99.5%, n=9</td><td>1.5%, n=9</td></tr> <tr> <td>4-dimethylamino pyridine</td><td>Added 2.5848µg/ml~7.7543µg/ml</td><td>102.1%, n=9</td><td>1.8%, n=9</td></tr> </tbody> </table>			Impurities	Marking condition	Average recovery rate	RSD	YA2304-52	Added 2.6495µg/ml to 7.9486µg/ml	99.3%, n=9	5%, n=9	Imidazole	Added 2.5818µg/ml to 7.7455µg/ml	99.5%, n=9	1.5%, n=9	4-dimethylamino pyridine	Added 2.5848µg/ml~7.7543µg/ml	102.1%, n=9	1.8%, n=9
Impurities	Marking condition	Average recovery rate	RSD																	
YA2304-52	Added 2.6495µg/ml to 7.9486µg/ml	99.3%, n=9	5%, n=9																	
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4-dimethylamino pyridine	Added 2.5848µg/ml~7.7543µg/ml	102.1%, n=9	1.8%, n=9																	

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	impurity is NMT 10%.				
Precision	Repeatability and intermediate precision: the RSD of each impurity in 6 repeatability (or intermediate precision test product) solution NMT 10%. Precision: Repeatability and intermediate precision 12 test results, each impurity content RSD NMT 10%.	<b>Impurities</b>	<b>Repeatability</b>	<b>Intermediate precision</b>	<b>Precision</b>
	YA2304-52	1.2%, n=6	1.1%, n=6	1.1%, n=12	
	Imidazole	0.9%, n=6	1.1%, n=6	1.8%, n=12	
	4-dimethylaminopyridine	1.6%, n=6	1.3%, n=6	2.2%, n=12	
Solution stability	Each solution was placed in a 5°C injector, and the control solution and the test solution were injected at different times. The separation degree between imidazole peak and YA2304-52 peak in the control solution NLT 1.5, and the RSD of the peak area of each impurity peak should be no more than 5%; The RSDS of all impurities in the test solution NMT 10%, and no new impurities to be measured (YA2304-52, imidazole, 4-dimethylaminopyridine) should be detected.	<p>Control solution: Within 48 h of being placed in a 5°C injector, the separation degree between imidazole peak and YA2304-52 peak was NLT 1.9 (required to be NLT 1.5), and the RSD of each impurity peak area was not more than 1.6% (required to be no more than 5%); The above all met the verification requirements, indicating that the control solution was stable within 48h after being placed in the 5°C injector.</p> <p>Test product solution: YA2304-52, imidazole and 4-dimethylaminopyridine (DMAP) were not detected within 44h after being placed in the sample injector at 5°C, and no other impurity peaks affecting the detection of the above three impurities were detected; The results indicated that the test solution was stable within 44h after being placed in the 5°C injector.</p> <p>The above solutions do not need new preparation for temporary use.</p>			

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### **3.2.S.4.3.3 Methodological verification of Enantiomers of finished products**

#### **I. Analytical Procedures**

Please refer to the analytical procedures of Enantiomers in Finerenone in section 3.2.S.4.2.

#### **II. Validation Results and Discussion**

##### **a. Wavelength selection**

###### **➤ Acceptance Criteria**

The selection of detection wavelength requires UV scanning of impurity YA2304-20 at 200nm ~ 400nm wavelength, impurity YA2304-20 should have greater absorption at 251nm.

###### **➤ Solution preparation**

Solvent: acetonitrile: water (30:70, v/v)

Blank solution: solvent.

Reserve liquid of reference product: Take YA2304-20 about 7.5mg of reference product, accurately weigh it, place it in a 50ml measuring bottle, add appropriate amount of solvent to dissolve by ultrasound, cool it, dilute it with solvent to scale, shake well, and then get. (Approx. 150 $\mu$ g/ml)

Control product solution: accurately measure 1ml of the control product reserve solution, place it in a 100ml measuring bottle, dilute it with solvent to the scale, shake well, and get.

###### **➤ Injection procedure**

After the blank solution, 1 needle of the mixed control solution was injected and the chromatogram was recorded.

###### **➤ Measurement results**

Name of	Test	Retention time	Maximum absorption wavelength (nm)
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solution	substance	(min)	
Reference solution	YA2304-20	25.626	205.9, 250.8, 292.4, 340.3
Spectrogram of reference solution	<p style="text-align: center;">光谱指数组 25.626 纳米</p> <p style="text-align: center;">AU</p>		
Conclusion	Impurity YA2304-20 has a large absorption at 251nm, which meets the verification requirements		

### **(4.2) System suitability**

#### ➤ **Acceptance Criteria**

The blank solution should be free of interference; The separation degree between the main peak and YA2304-20 peak in the chromatogram of system suitability solution should be greater than 1.5; In the chromatogram obtained for 6 consecutive days, the signal-to-noise ratio (S/N) of YA2304-20 peak should NLT 30, the RSD of the retention time NMT 1.0%, and the RSD of the peak area NMT 5%.

#### ➤ **Solution Preparation**

Blank solution: solvent.

Reference product reserve solution: Prepare the reference product reserve solution according to "4.1 wavelength selection" under the same method.

Reference solution: prepared in the same way as the reference solution under "4.1 wavelength selection".

System suitability solution: Take YA2304 reference product about 20mg, accurately weigh it, place it in a 20ml measuring bottle, dissolve it with the reference product solution and dilute it to the scale, shake well, and then get it.

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### ➤ **Injection procedure**

After the instrument is balanced, 1 needle of blank solution is injected, 1 needle of system suitability solution is injected, and 6 needles of control solution are injected continuously.

### ➤ **Measurement results**

Name of solution	Sample number	Separation degree of main peak from YA2304-20 peak		
System suitability solution	1	3.0		
Reference solution	Sample number	Retention time (min)	Peak area	Signal-to-noise ratio
	1	24.926	23887	80.8
	2	24.933	23768	72.6
	3	24.922	23570	76.0
	4	24.926	23907	72.3
	5	24.882	23670	71.5
	6	24.912	23708	83.4
	RSD%(n=6)	0.08	0.6	/

Conclusion: The blank solution has no interference; The separation degree between YA2304-20 peak and main peak in the system applicability solution is 3.0 (the requirement should NLT 1.5). In the chromatogram obtained from the control product solution, the signal-to-noise ratio of the main peak was between 71.5 and 83.4 (the requirement should be NLT 30); The RSD of peak retention time of YA2304-20 in the photoproduct solution for 6 consecutive days was 0.08% (the requirement should NMT 1.0%); The RSD of peak area is 0.6% (the requirement should NMT 5%); All meet the verification requirements. It shows that this method has good system applicability.

### **(4.3) Specificity**

#### **(4.3.1) Peak identification test**

### ➤ **Acceptance Criteria**

The blank solution should be free of interference; The separation degree between the main peak and YA2304-20 peak in the system suitability

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solution should be greater than 1.5; The peak signal-to-noise ratio (S/N) of YA2304-20 in the chromatogram of reference solution should NLT 30; The separation degree between YA2304-20 peaks and adjacent peaks in the chromatogram of test solution and specific mixed solution should be greater than 1.5, and the retention time should be consistent with the peak retention time of YA2304-20 in the chromatogram of control solution. Other single impurity localization solutions do not interfere with YA2304-20 peak production.

### ➤ **Solution preparation**

Blank solution: solvent.

Reference stock solution: Share reference stock solution under "4.2 System Suitability".

Reference solution: Prepare the reference solution in the same way as the reference solution under "4.1 wavelength selection".

System suitability solution: Share system suitability solution under "4.2 System Suitability".

YA2304 reference product reserve liquid: Take about 7.5mg of this product, accurately weigh it, place it in a 50ml measuring bottle, add an appropriate amount of solvent to dissolve with ultrasound, cool it, dilute it with solvent to the scale, shake well, and then get.

YA2304 positioning solution: precisely measure 1ml of YA2304 reference product reserve solution, place it in 100ml measuring bottle, dilute it with solvent to scale, shake well, ready.

Test product solution: Take about 20mg of this product, weigh it accurately, put it in a 20ml measuring bottle, dissolve it with solvent and dilute it to the scale, shake well, ready.

Specific mixed solution: Take about 20mg of this product, accurately weigh it,

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put it in a 20ml measuring bottle, dissolve it with the control product solution and dilute it to the scale, shake well, ready.

YA2304-10 Reference product reserve liquid: Take YA2304-10 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with solvent and dilute to the scale, shake well, ready. (Approx. 200 $\mu$ g/ml)

YA2304-12 reference product reserve liquid: Take YA2304-12 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with solvent and dilute to scale, shake well, and then obtain. (Approx. 200 $\mu$ g/ml)

YA2304-14 reference product reserve liquid: Take YA2304-14 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with acetonitrile and dilute to scale, shake well, and then obtain. (Approx. 200 $\mu$ g/ml)

YA2304-15 reference product reserve liquid: Take YA2304-15 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with acetonitrile and dilute to scale, shake well, and then obtain. (Approx. 200 $\mu$ g/ml)

YA2304-16 reference product reserve liquid: Take YA2304-16 reference product about 5mg, accurately weigh, place in 25ml bottle, dissolve with acetonitrile and dilute to scale, shake well, ready. (Approx. 200 $\mu$ g/ml)

YA2304-17 reference product reserve liquid: Take YA2304-17 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with solvent and dilute to scale, shake well, and then obtain. (Approx. 200 $\mu$ g/ml)

YA2304-18 reference product reserve liquid: Take YA2304-18 reference product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with acetonitrile and dilute to scale, shake well, and then obtain. (Approx. 200 $\mu$ g/ml)

YA2304-19 reference product reserve liquid: Take YA2304-19 reference

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product about 5mg, accurately weigh, put into 25ml measuring bottle, dissolve with acetonitrile and dilute to scale, shake well, then obtain. (Approx. 200 $\mu$ g/ml)

YA2304-10 positioning solution: Accurately measure 0.75ml of YA2304-10 reference product reserve into 100ml measuring bottle, dilute with solvent to scale, shake well, and then obtain.

YA2304-12 positioning solution: precisely measure 0.75ml of YA2304-12 reference storage solution into 100ml measuring bottle, dilute it with solvent to scale, shake well, ready.

YA2304-14 positioning solution: precisely measure 0.75ml of YA2304-14 reference storage solution into 100ml measuring bottle, dilute with solvent to scale, shake well, ready.

YA2304-15 positioning solution: precisely measure 0.75ml of YA2304-15 reference product storage solution into 100ml measuring bottle, dilute with solvent to scale, shake well, ready.

YA2304-16 positioning solution: precisely measure 0.75ml of YA2304-16 reference storage solution into 100ml measuring bottle, dilute with solvent to scale, shake well, ready.

YA2304-17 positioning solution: precisely measure 0.75ml of YA2304-17 reference storage solution into 100ml measuring bottle, dilute with solvent to scale, shake well, ready.

YA2304-18 positioning solution: precisely measure 0.75ml of YA2304-18 reference storage solution into 100ml measuring bottle, dilute with solvent to scale, shake well, ready.

YA2304-19 positioning solution: precisely measure 0.75ml of YA2304-19 reference product storage solution into 100ml measuring bottle, dilute with solvent to scale, shake well, ready.

### **➤ Injection Sequence**

After the instrument is balanced, 1 needle is injected into blank solution, 1 needle is injected into system suitability solution, 1 needle is injected into

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reference solution, 1 needle is injected into test solution, 1 needle is injected into specific mixed solution, and the other positioning solutions are injected into 1 needle respectively.

### ➤ Measurement results

Solution name	Component name	Concentration ( $\mu\text{g}/\text{ml}$ )	Rt (min)	Separation	S/N
System suitability solution	YA2304	1021.2271	20.180	3.0	/
	YA2304-20	1.4983	24.852		
Reference solution	YA2304-20	1.4983	24.858	/	62.2
YA2304 Locate solution	YA2304	1.5559	20.240	/	/
YA2304-10 Locates the solution	YA2304-10	1.5534	3.906	/	/
YA2304-12 Locate the solution	YA2304-12	1.4942	5.627	/	/
			5.935	/	/
YA2304-14 Locate the solution	YA2304-14	1.5137	15.696	/	/
			18.853	/	/
YA2304-15 Locate the solution	YA2304-15	1.5366	22.827	/	/
			27.493	/	/
YA2304-16 Locate the solution	YA2304-16	1.4226	11.400	/	/
			12.070	/	/
YA2304-17 Locate the solution	YA2304-17	1.5798	4.505	/	/
YA2304-18 Locate the solution	YA2304-18	1.5268	15.456	/	/
			15.956	/	/
YA2304-19 Locate the solution	YA2304-19	1.5790	18.768	/	/
			21.475	/	/
Sample solution	YA2304	1003.6000	20.182	3.0	/
	YA2304-20	/	24.794		/
Specific mixed solution	YA2304	1033.1000	20.183	3.0	/
	YA2304-20	1.4983	24.872		/

Conclusion: Blank solution has no interference; The separation degree between YA2304-20 peak and main peak in the system applicability solution is 3.0 (the requirement should NLT 1.5). In the chromatogram obtained from the control solution, S/N was 62.2 (the requirement should NLT 30); The separation degree

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between YA2304-20 peaks and adjacent peaks in the specific mixed solution and the test solution was greater than 1.5, and the retention time was consistent with that of YA2304-20 peaks in the chromatogram of the control solution. Impurities YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18 and YA2304-19 all have a chiral center, so the above impurities all have two peaks under the current chromatographic conditions. Other single impurities (YA2304-10, YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-17, YA2304-18 and YA2304-19) do not interfere with the peak of YA2304-20; Meet the requirements of verification.

### **(4.3.2) Forced degradation test**

#### **➤ Acceptance Criteria**

The blank solution should be free of interference; The separation degree between the main peak and YA2304-20 peak in the system suitability solution should be greater than 1.5; The peak signal-to-noise ratio (S/N) of YA2304-20 in the chromatogram of reference solution should NLT 30; The degradation degree of principal component NMT 20% under each failure condition; After degradation, the separation degree of all impurities in the sample and YA2304-20 should NLT 1.0.

#### **➤ Solution preparation**

Blank solution: solvent.

Reference product reserve solution: Prepare the reference product reserve solution according to "4.1 wavelength selection" under the same method.

Reference solution: prepared in the same way as the reference solution under "4.1 wavelength selection".

System suitability solution: prepared in the same way as system suitability solution under "4.1 wavelength selection".

Undamaged test product solution: take about 20mg of this product,

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accurately weigh it, place it in a 20ml measuring bottle, add an appropriate amount of solvent to dissolve by ultrasound, cool it, dilute it with solvent to the scale, shake well, and get.

Acid destruction blank solution: take 2ml of 0.1mol/L hydrochloric acid solution, place it in 20ml measuring bottle, add 2ml of 0.1mol/L sodium hydroxide solution to neutralize it, dilute it with solvent to the scale, shake well, and get it.

Acid destruction of test product solution: take about 20mg of this product, accurately weigh it, place it in 20ml bottle, add 2ml of 0.1mol/L hydrochloric acid solution at room temperature for destruction for 24h, add 2ml of 0.1mol/L sodium hydroxide solution for neutralization, add an appropriate amount of solvent to dissolve by ultrasound, dilute it with solvent to the scale, shake well, and obtain.

Alkali destruction blank solution: take 2ml of 0.1mol/L sodium hydroxide solution, place it in 20ml measuring bottle, add 2ml of 0.1mol/L hydrochloric acid solution to neutralize it, dilute it with solvent to the scale, shake well, and obtain.

Alkali destruction test product solution: take about 20mg of this product, weigh it accurately, put it in 20ml measuring bottle, add 2ml of 0.1mol/L sodium hydroxide solution at room temperature for destruction for 24h, add 2ml of 0.1mol/L hydrochloric acid solution for neutralization, add an appropriate amount of solvent to dissolve by ultrasound, dilute it with solvent to the scale, shake well, and obtain.

Oxidizing destruction of blank solution: take 2ml of 3% hydrogen peroxide

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solution, place it in 20ml measuring bottle, dilute it with solvent to the scale, shake well, and obtain.

Oxidizing destruction of test product solution: Take about 20mg of this product, weigh it accurately, put it in 20ml bottle, add 2ml of 3% hydrogen peroxide solution and destroy it at room temperature for 24h, add an appropriate amount of solvent and ultrasonic to dissolve it, dilute it with solvent to the scale, shake well, and obtain.

High temperature destruction of the test product solution: accurately weigh about 20mg of this product damaged for 5 days at 105°C, place it in a 20ml measuring bottle, add an appropriate amount of solvent and ultrasonic to dissolve it, dilute it with solvent to the scale, shake well, and obtain.

Light damage test product solution: accurately weigh about 20mg of this product after 20 days of damage under light (25±2°C, 4500±500Lux, 90±5 $\mu$ W/cm<sup>2</sup>), place it in a 20ml bottle, add an appropriate amount of solvent to dissolve by ultrasound, dilute it with solvent to the scale, shake well, and obtain.

### **➤ Injection Sequence**

After the instrument balance, blank solution, system suitability solution, reference solution, undamaged test product solution, acid damage blank solution, alkali damage blank solution, oxidation damage blank solution, acid damage test product solution, alkali damage test product solution, oxidation damage test product solution, high temperature damage test product solution, light damage test product solution into 1 needle each.

### **➤ Test result**

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Name of solution	Damage conditions	YA2304-20 degree of peak separation from adjacent	Degree of degradati on of principal compone nt	S/N
System suitability solution	/	2.6	/	/
Reference solution	/	/	/	33.7
Undamaged test product solution	/	/	/	/
Acid destroys test solution	Destroy 2ml of 0.1mol/L hydrochloric acid solution for 24h	/	1.25	/
Alkali destroys the test solution	Destroy 2ml of 0.1mol/L sodium hydroxide solution for 24h	/	/	/
Destroy the test product solution by oxidation	2ml 3% hydrogen peroxide solution was destroyed at room temperature for 24h	/	5.18	/
The test product solution was destroyed at high temperature	105°C damage for 5 days	/	/	/
Light destroyed the test solution	Light damage ( $25\pm2$ °C, $4500\pm500$ Lux, $90\pm5\mu\text{Wcm}^2$ ) for 20 days	/	0.18	/
Conclusion: The blank solution has no interference; The system applicability meets the requirements; The degradation degree of principal components was 1.25% under acid damage, 5.18% under oxidation damage, 0.18% under light damage, and alkali damage and high temperature damage did not degrade principal components. Impurity YA2304-20 was not degraded under the conditions of acid, alkali, oxidation, high temperature and light.				

### (4.4) Limits of quantification and detection

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### **➤ Acceptance Criteria**

The signal to noise ratio (S/N) of each impurity peak in 6 LOQ solutions should NLT 10, the RSD of retention time should not be more than 2.0%, and the RSD of peak area should not be more than 10%.

The SNR (S/N) of each impurity peak in the 3-pin detection limit (LOD) solution should NLT 3.

### **➤ Solution preparation**

Blank solution: solvent.

Reference stock solution: Share reference stock solution under "4.2 System Suitability".

Reference stock solution YA2304: Share reference stock solution YA2304 under "4.3.1 Peak Identification Test".

LOQ solution: Accurately measure 1ml of YA2304 reference product reserve solution and 1ml of reference product reserve solution, place in the same 100ml bottle, dilute to scale with solvent, and shake well; Accurately measure 5ml of the solution into 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then get. (Dispense 6 parts in parallel)

LOD solution: Accurately measure the first part of limited quantification solution 3ml, place it in a 10ml measuring bottle, dilute it with solvent to the scale, shake well, and then get.

### **➤ Injection Sequence**

After the balance of the instrument, 1 needle was injected into the blank solution, 1 needle was injected into the 6 parts of limited quantitation

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solution, and 3 needles were injected into the detection limited solution continuously.

### ➤ Measurement results

Results of limit of quantitation test

Impurities Name	Solution name	Concentration /( $\mu\text{g}/\text{ml}$ )	Equivalent to the percentage % of the concentration of the test product solution	Retention time /min	Peak area	S/N
YA2304	LOQ solution 1	0.3112	0.03	20.404	4914	18.6
	LOQ Solution 2			20.366	4939	18.9
	LOQ solution 3			20.410	5091	19.9
	LOQ Solution 4			20.408	5200	17.6
	LOQ Solution 5			20.355	4823	19.1
	LOQ solution 6			20.424	4925	15.8
	RSD%			0.14	2.8	/
Conclusion: The concentration of YA2304 in LOQ solution is 0.3112 $\mu\text{g}/\text{ml}$ , which is equivalent to 0.03% of the concentration of test product. The S/N of YA2304 peak in 6 LOQ solution spectra ranges from 15.8 to 19.9 (the requirement should be NLT 10), and the RSD of retention time is 0.14% (the requirement should be no more than 2.0%). The RSD of the peak area was 2.8% (the requirement should NMT 10%), which met the verification requirements.						
YA2304-20	LOQ Solution 1	0.2997	0.03	25.100	4859	14.2
	LOQ Solution 2			25.149	4747	14.1
	LOQ Solution 3			25.151	4791	15.1
	LOQ Solution 4			25.076	4581	12.8
	LOQ Solution 5			25.078	4797	14.5
	LOQ solution 6			25.020	4727	12.2
	RSD%			0.20	2.0	/
Verdict: The concentration of YA2304-20 in LOQ solution was 0.2997 $\mu\text{g}/\text{ml}$ , equivalent to 0.03% of the concentration of test product. The S/N of YA2304-20 peaks in 6 LOQ solution spectra ranged from 12.2 to 15.1 (the requirement should be NLT 10), and the RSD of retention time was 0.20% (the requirement should be no more than 2.0%). The RSD of the peak area was 2.0% (the requirement should NMT 10%), which met the verification requirements.						

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### Detection limit test results

Impurities Name	LOD solution injection	Concentration /( $\mu\text{g}/\text{ml}$ )	Equivalent to a percentage /% of the concentration of the test product	Retention time /min	Peak area	S/N
YA230 4	1	0.0934	0.01	20.443	1503	6.3
	2			20.379	1874	7.5
	3			20.469	1643	6.9
	Conclusion: The concentration of YA2304 in LOD solution is 0.0934 $\mu\text{g}/\text{ml}$ , which is equivalent to 0.01% of the concentration of the test product. The S/N of YA2304 peak in the 3-needle map is between 6.3 and 7.5 (the requirement should be NLT 3), which meets the verification requirements.					
YA230 4-20	1	0.0899	0.01	25.037	1564	5.2
	2			25.075	1529	5.4
	3			25.262	1335	4.7
	Conclusion: The concentration of YA2304-20 in LOD solution is 0.0899 $\mu\text{g}/\text{ml}$ , which is equivalent to 0.01% of the concentration of the test product. The S/N of YA2304-20 peak in the 3-needle atlas is between 4.7 and 5.4 (the requirement should be NLT 3), which meets the verification requirements.					

### (4.5) Linearity and range

#### ➤ Acceptance Criteria

Within the limit concentration range of YA2304-20 LOQ concentration ~ 200%, 6 concentration points were selected as linear regression curves with the concentration as the horizontal coordinate and YA2304-20 peak area as the vertical coordinate. The regression coefficient (R) of the regression curve was NLT 0.990, and the RSD of the response factor NMT 10%. Y-axis intercept accounts for less than 25% of 100% response value.

#### ➤ Solution preparation

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Blank solution: solvent.

Reference stock solution: Share reference stock solution under "4.2 System Suitability".

Reference stock solution YA2304: Share reference stock solution YA2304 under "4.3.1 Peak Identification Test".

Linear reserve liquid: precisely measure 1.0ml of the reference reserve liquid and 1.0ml of the YA2304 reference reserve liquid, place them in the same 10ml measuring bottle, dilute them with solvent to the scale, and shake well to obtain.

Linear solution 1 (LOQ solution) : prepared in the same way as the quantitative limit solution under "4.4 quantitative limit and detection limit".

Linear solution 2 (40% limit solution) : accurately measure 0.4ml linear reserve solution, place it in 10ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain.

Linear solution 3 (80% limit solution) : Accurately measure 0.8ml linear reserve solution, place it in 10ml measuring bottle, dilute it with solvent to the scale, shake well, and obtain.

Linear solution 4 (100% limit solution) : precisely measure 1.0ml linear reserve solution, place it in 10ml measuring bottle, dilute it with solvent to the scale, shake well, and get.

Linear solution 5 (150% limit solution) : precisely measure 1.5ml linear reserve solution, place it in 10ml measuring bottle, dilute it with solvent to the scale, shake well, and get.

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Linear solution 6 (200% limit solution) : accurately measure 2ml linear reserve solution, place it in 10ml measuring bottle, dilute it with solvent to the scale, shake well, and get.

### ➤ Injection Sequence

After instrument balance, blank solution 1 needle, each linear solution from low concentration to high concentration 1 needle injection.

### ➤ Measurement results

Results of linear and range tests

Impurities Name	Solution name	Equivalent to test product solution concentration percentage /%	Concentration /( $\mu\text{g}/\text{ml}$ )	Peak area	Response factor
YA230 4	Linear Solution 1	0.03	0.3112	5080	16324.77
	Linear solution 2	0.06	0.6224	9791	15731.87
	Linear Solution 3	0.12	1.2447	19328	15527.81
	Linear solution 4	0.16	1.5559	24393	15677.56
	Linear Solution 5	0.23	2.3339	36500	15639.21
	Linear solution 6	0.31	3.1118	49358	15861.37
	Response factor RSD/%			1.8	
Linear graph		<p>YA2304线性图</p> <p>峰面积</p> <p>浓度 (<math>\mu\text{g}/\text{ml}</math>)</p> <p><math>y = 15788x - 79.892</math></p> <p><math>R^2 = 0.9998</math></p>			

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YA230 4-20	Linear equations	$Y = 15788 - 79.892 x$		
	Correlation coefficient R	0.9999		
	Absolute Y-axis intercept percentage /% of 100% response value	0.34		
	Conclusion: YA2304 is in the range of 0.3112~3.1118 µg/ml, the linear equation is $y=15788x-79.892$ , $R=0.9999$ (should NLT 0.990), the response factor RSD is 1.8% (should not be more than 10%). The absolute value of Y-axis intercept accounts for 0.34% of 100% response value (NMT 25%), indicating that YA2304 detected by this method has a good linear relationship in the range of 0.3112~3.1118 µg/ml.			
	Linear Solution 1	0.03	0.2997	4602
	Linear Solution 2	0.06	0.5993	9081
	Linear Solution 3	0.12	1.1987	18112
	Linear solution 4	0.15	1.4983	22706
	Linear Solution 5	0.22	2.2475	33698
	Linear solution 6	0.30	2.9966	45656
Response factor RSD/%		0.9		
Linear graph				
Linear equations		$Y = 15161 - 28.894 x$		
Correlation coefficient R		0.9999		
Absolute Y-axis intercept percentage /% of 100% response value		0.13		
Conclusion: YA2304-20 in the range of 0.2997~2.9966 µg/ml, the linear equation is $y=15161x-28.894$ , $R=0.9999$ (should NLT 0.990), the response factor RSD is 0.9% (should not be more than 10%). The absolute value of				

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	Y-axis intercept accounts for 0.13% of 100% response value (NMT 25%), indicating that YA2304-20 detected by this method has a good linear relationship in the range of 0.2997~2.9966 µg/ml.
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### **(4.6) Accuracy**

#### ➤ **Brief Description**

Accuracy is achieved by determining the ratio (recovery rate) between the actual detected amount and the theoretical amount of YA2304-20 reference product added to the test product at different concentrations (30% to 150% limit concentration). It is required that the signal to noise ratio (S/N) of YA2304-20 peak in the chromatogram of the reference product should NLT 30; The 30% limit concentration recovery rate should be between 85% and 110%, the 100% to 150% limit concentration recovery rate should be between 90% and 108%, and the RSD of the recovery rate of 9 recovery samples NMT 10%.

#### ➤ **Solution preparation**

Blank solution: solvent.

Test product solution: Take about 20mg of this product, weigh it accurately, place it in a 20ml measuring bottle, add an appropriate amount of solvent to dissolve by ultrasound, cool it, dilute it with solvent to the scale, shake well, and then get. (Parallel preparation of 2 parts)

Reference product reserve liquid: The reference product reserve liquid under "4.1 wavelength selection" is prepared in the same way.

Reference solution: prepared in the same way as the reference solution under "4.1 wavelength selection".

System suitability solution: Share system suitability solution under "4.2

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System Suitability".

Reserve solution with sample recovery rate: accurately measure 2.5ml of the control product reserve solution, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain.

30% sample recovery solution: take about 20mg of this product, accurately weigh it, put it in a 20ml bottle, add an appropriate amount of solvent to dissolve by ultrasound, cool it, accurately add 0.6ml of sample recovery liquid, dilute it with solvent to the scale, shake well, and get. (Dispense 3 parts in parallel)

100% sample recovery solution: Take about 20mg of this product, weigh it accurately, place it in a 20ml measuring bottle, add an appropriate amount of solvent to dissolve by ultrasound, cool it, accurately add the reserve liquid with sample recovery rate of 2.0ml, dilute it with solvent to the scale, shake well, and get. (Dispense 3 parts in parallel)

150% sample recovery solution: Take about 20mg of this product, weigh it accurately, place it in a 20ml measuring bottle, add an appropriate amount of solvent to dissolve by ultrasound, cool it, accurately add the reserve liquid with sample recovery rate of 3.0ml, dilute it with solvent to the scale, shake well, and get. (Dispense 3 parts in parallel)

### ➤ **Injection procedure**

After the balance of the instrument, 1 needle was injected into the blank solution, 1 needle was injected into the system suitability solution, 2 needles were injected into the control solution, 1 needle was injected into the test solution, and 1 needle was injected into the solution with each recovery rate.

### ➤ **Measurement results**

Names of impurities	Solution name	Quantity of test product detected ( $\mu\text{g}/\text{ml}$ )	Amount added ( $\mu\text{g}/\text{ml}$ )	Measured amount ( $\mu\text{g}/\text{ml}$ )	Recovery rate (%)
YA2304-20	30% sample recovery solution 1	0.1465	0.4587	0.5893	96.5

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	30% addition recovery solution 2	0.1515	0.4587	0.5901	95.6
	30% addition recovery solution 3	0.1478	0.4587	0.5810	94.5
	100% sample recovery solution 1	0.1452	1.5289	1.6581	99.0
	100% addition recovery solution 2	0.1499	1.5289	1.6870	100.5
	100% addition recovery solution 3	0.1493	1.5289	1.6798	100.1
	150% addition recovery solution 1	0.1545	2.2933	2.4359	99.5
	150% addition recovery solution 2	0.1474	2.2933	2.4672	101.2
	150% addition recovery solution 3	0.1498	2.2933	2.4294	99.4
	Average recovery rate (%)				98.5
	RSD (%)				2.4
Verdict: YA2304-20 was added into the sample in the range of 0.4587~2.2933 ug/ml. The recovery rate of the sample was 94.5%~96.5% (the requirement was 85%~110%). 100%~150% recovery The sample recovery rate was 99.0%~101.2% (the requirement was 90%~108%), the average recovery rate of 9 recovery samples was 98.5%, and the recovery RSD was 2.4% (the requirement was not more than 10%), which met the verification requirements, indicating that the method was accurate.					

### **(4.7) Precision**

Precision refers to the proximity between the results obtained by multiple sampling of the same uniform test product under specified test conditions, and its precision is judged by examining repeatability and reproducibility.

#### **(4.7.1) Repeatability**

##### **➤ Acceptance Criteria**

6 repeatable sample solutions were prepared by Analyst A on date A and determined by instrument A. It is required that the separation degree

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between the main peak and YA2304-20 in the chromatogram of system suitability solution should be greater than 1.5; The signal to noise ratio (S/N) of YA2304-20 peak in the chromatogram of reference solution should NLT 30; The RSD of YA2304-20 in 6 repetitive test solutions should NMT 10%.

### ➤ **Solution Preparation**

Blank solution: solvent.

Reference stock solution: Share reference stock solution under "4.6 Accuracy".

Reference product solution: prepared in the same way as the reference product solution under "4.1 wavelength selection".

System suitability solution: Share system suitability solution under "4.2 System Suitability".

Repeatable reserve solution: accurately measure 2.5ml of the control product reserve solution, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain.

Repeatable test solution: take about 20mg of this product, accurately weigh it, place it in 20ml measuring bottle, add an appropriate amount of solvent to dissolve by ultrasound, cool it, add 2.0ml repeatable reserve liquid precisely, dilute it with solvent to the scale, shake well, ready. (Parallel preparation of 6 parts)

### ➤ **Injection procedure**

After the instrument is balanced, 1 needle is injected into blank solution, 1 needle is injected into system suitability solution, 1 needle is injected into control solution, and 1 needle is injected into repeated test solution.

### ➤ **Measurement results**

Name of solution	Number of injections	Degree of separation between main peak and YA2304-20 peak
System suitability solution	1	3.0

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Solution name		Number of injections		Signal-to-noise ratio			
Reference solution		1		91.4			
		2		68.2			
Repeat test product	1	2	3	4	5	6	RSD/%
YA2304-20 content /%	0.17	0.17	0.17	0.17	0.17	0.18	2.5

Conclusion: The system applicability meets the requirements; The RSD of YA2304-20 in 6 repetitive test products was 2.5% (the requirement NMT 10%). It met the verification requirements, indicating that the method had good repeatability.

### **(4.7.2) Reproducibility**

#### **➤ Brief Description**

6 reproducible sample solutions were prepared by QC personnel B on date B and determined by instrument B. It is required that the separation degree between the main peak and YA2304-20 peak in the chromatogram of system suitability solution should be greater than 1.5; The signal-to-noise ratio (S/N) of peak YA2304-20 in the chromatogram obtained for 6 consecutive days should NLT 30, the RSD of the retention time NMT 1.0%, and the RSD of the peak area NMT 5%. In 6 reproducible sample solutions, the RSD of YA2304-20 NMT 10%.

For the results of 12 repeatability and reproducibility tests, the RSD of YA2304-20 NMT 10%.

#### **➤ Solution preparation**

Blank solution: solvent

Reference stock solution: Share the reference stock solution under "4.6 Accuracy".

Reference product solution: prepared in the same way as the reference product solution under "4.1 wavelength selection".

System suitability solution: the system suitability solution under "4.2 System suitability" is prepared in the same way.

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Reproducible reserve solution: accurately measure 2.5ml of the control product reserve solution, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain

Reproducible test solution: Take about 20mg of this product, accurately weigh it, place it in a 20ml measuring bottle, add an appropriate amount of solvent to dissolve by ultrasound, cool it, add 2.0ml of reproducible reserve liquid precisely, dilute it with solvent to the scale, shake well, and obtain. (Parallel preparation of 6 parts)

### ➤ **Injection procedure**

After the balance of the instrument, 1 needle was injected into the blank solution, 1 needle was injected into the system suitability solution, 6 needles were injected into the control solution continuously, and 1 needle was injected into each of the reproducible test solution.

### ➤ **Measurement results**

Reproducibility - system suitability test results

Name of solution	Sample number	Separation degree of main peak from YA2304-20 peak		
System suitability solution	1	2.6		
Reference solution	Sample number	Retention time (min)	Peak area	Signal-to-noise ratio
	1	21.708	26122	49.3
	2	21.699	27518	45.7
	3	21.657	26997	41.9
	4	21.674	27146	43.6
	5	21.634	27328	41.4
	6	21.659	27296	43.5
	RSD%(n=6)	0.13	1.9	/

Conclusion: The blank solution has no interference; The separation degree between YA2304-20 peak and the main peak in the system applicability solution is 2.6 (the requirement should NLT 1.5). In the chromatogram obtained from the control product solution, the signal-to-noise ratio of the main peak was between 41.4 and 49.3 (the requirement should NLT 30); The RSD of peak retention time of YA2304-20 for 6 consecutive days was 0.13% (the requirement should be no more than 1.0%); The RSD% of peak area is 1.9% (the requirement should NMT 5%); All meet the verification requirements. It shows that this method has good system applicability.

Precision test results

Results of repeatability test	Reproducibility test results
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Solution name	YA2304-20 content /%	Solution name	YA2304-20 content /%
Repeatable test product solution 1	0.17	Reproducible test product solution 1	0.17
Reproducible sample solution 2	0.17	Reproducible test product solution 2	0.16
Repeatable sample solution 3	0.17	Reproducible test solution 3	0.16
Reproducible sample solution 4	0.17	Reproducible test product solution 4	0.18
Reproducible sample solution 5	0.17	Reproducible test product solution 5	0.17
Repeatable sample solution 6	0.18	Reproducible test product solution 6	0.18
RSD/% (n=6)	2.5	RSD/% (n=6)	5
Precision RSD/% (n=12)			4
Conclusion: The RSD of YA2304-20 in 6 reproducible samples was 5% (the requirement NMT 10%). The method met the requirements of verification, indicating good reproducibility of the method. The RSD of YA2304-20 measured for 12 times of repeatability and reproducibility was 4% (the requirement NMT 10%), which met the verification requirements, indicating that the method had good precision.			

### **(4.8) Solution stability**

#### **➤ Brief Description**

The system suitability solution, the control solution and the test product solution were placed at room temperature for a period of time, and the samples were injected at different time points. The separation degree between YA2304-20 peak and the main peak in the chromatogram of the system suitability solution should NLT 1.5; The peak signal-to-noise ratio (S/N) of YA2304-20 in the chromatogram of reference solution should NLT 30, and the RSD of peak area should not be more than 5%; In the chromatogram obtained by the test product solution, the RSD of YA2304-20 NMT 10% according to the peak area calculation by external standard method, and there shall be no new peak generation that interferes with the detection of YA2304-20.

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### ➤ **Solution preparation**

Reference solution: Share the reference solution under "4.2 System Suitability".

System Suitability solution: Share system suitability solution under "4.2 System Suitability".

Test product solution: Share test product solution under "4.3 Specificity - Peak identification".

### ➤ **Injection Sequence**

The system suitable solution was injected at 0h, 6h, 14.5h, 22h, 29h, 49h, 3d, 4d and 5d respectively. (System suitability solution 0h is system suitability solution under "System suitability", system suitability solution 6h is system suitability solution under "particularity-peak identification", system suitability solution 3d is system suitability solution under the second "accuracy", system suitability solution 4d is system suitability solution under the third "accuracy". System suitability solution 5d is system suitability solution under "repeatability",)

The control solution was injected at 0h, 6h, 14.5h, 22h, 29h and 49h, respectively. (Reference solution 0h was the reference solution under "system suitability", and reference solution 6h was the reference solution under "particularity-peak identification")

Sample solution was injected at 0h, 8.5h, 16h and 23h, respectively.

### ➤ **Measurement resultss**

Stability test results of solution

System suitability solution		Reference solution			Test product solution	
Time point	Degree of Separation	Point in Time	Peak area	Signal-to-noise ratio	Point in time	YA2304-20 content (%)
0h	3.0	0h	23887	80.8	0h	0.0111
6h	3.0	6h	23747	62.2	8.5 h.	0.0101
14.5 h.	3.0	14.5 h.	23929	77.2	16h	0.0112
22h	3.1	22h	23487	80.3	23h	0.0091
29h	3.1	29h	23527	62.7	/	/
49h	3.1	49h	23260	74.6	/	/

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3d	3.1	/	/	/	/	/
4d	3.0	/	/	/	/	/
5d	3.0	/	/	/	/	/
RSD/%	/	/	1.1	/	/	10

Conclusion: Within 5 days at room temperature, the separation degree between YA2304-20 peaks and main peaks in the system suitability solution is greater than 1.5 (the requirement should be NLT 1.5), which meets the verification requirements, indicating that the system suitability solution is stable within 5 days at room temperature.

Within 49h at room temperature, the RSD of YA2304-20 peaks in the control solution was 1.1% (not more than 10%). The peak-to-noise ratio of YA2304-20 was between 62.2 and 80.3 (the requirement should NLT 30), which met the verification requirements, indicating that the control solution was stable within 49h at room temperature.

Within 23h at room temperature, the RSD of YA2304-20 in the test solution is 10% (the requirement NMT 10%), which meets the verification requirements, indicating that the test solution is stable within 23h at room temperature.

### **(4.9) Durability**

#### **➤ Acceptance Criteria**

The separation degree between YA2304-20 peak and main peak should NLT 1.5 when column temperature, wavelength, flow rate, initial ratio of mobile phase and normal chromatographic conditions are changed by single factor. The peak signal-to-noise ratio of YA2304-20 in the chromatogram of reference solution should NLT 30; RSD of YA2304-20 in test product solution shall NMT 10%.

#### **➤ Solution Preparation**

Blank solution: solvent.

Reference stock solution: Share reference stock solution under "4.6 Accuracy".

Reference product solution: prepared in the same way as the reference product solution under "4.1 wavelength selection".

System suitability solution: The system suitability solution under "4.2 System Suitability" is prepared in the same way.

Durable reserve solution: accurately measure 2.5ml of the reference product reserve solution, place it in a 25ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain.

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Durability test solution: Take about 20mg of this product, accurately weigh it, put it in 20ml measuring bottle, add appropriate amount of solvent and ultrasonic to dissolve it, cool it, accurately measure 2.0ml of durable storage liquid, put it in the same 20ml measuring bottle, dilute it with solvent to the scale, shake well, and get it. (Parallel preparation of 2 parts)

The variation of the condition parameters is as follows:

Chromatographic parameters	Standard conditions	Variable Values
Column temperature	40 °C	38°C, 42°C
Initial ratio of mobile phase	Mobile phase A- Mobile phase B (70:30)	Mobile phase A- Mobile phase B (72:28), Mobile phase A- Mobile phase B (68:32)
Flow rate	1.0 ml/min	0.95ml/min, 1.05ml/min
Wavelength	251nm	249nm, 253nm

### ➤ **Injection procedure**

Under different chromatographic conditions, 1 needle of blank solution, 1 needle of system suitability solution, 2 needles of reference solution and 1 needle of durability test product solution were injected respectively.

### ➤ **Measurement results**

Results of durability - system suitability test

Chromatographic Conditions	Degree of separation between the main peak and YA2304-20 peaks in system suitability solution	The principal peak-to-noise ratio of the reference solution	
		1	2
Normal conditions	2.5	66.5	64.4
Column temperature 38 °C	2.5	60.0	65.4
Column temperature 42°C	2.5	70.4	77.1
The flow rate was 0.95ml/min	2.5	63.0	62.4

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The flow rate is 1.05ml/min	2.4	64.4	68.7
Mobile phase ratio (72:28)	1.9	46.2	48.9
Mobile phase ratio (68:32)	1.8	64.3	59.3
Wavelength 249nm	1.9	31.4	35.3
Wavelength 253nm	1.9	34.7	35.4
Conclusion: Under different chromatographic conditions, the separation degree between YA2304-20 peak and main peak in the system suitability solution is between 1.9 and 2.5 (the requirement should be NLT 1.5), which meets the verification requirements. The main peak signal-to-noise ratio (SNR) in the chromatogram of the control solution was between 31.4 and 77.1 (the requirement should be NLT 30), which met the verification requirements.			

### Durability test results

Chromatographic Conditions	Parameter values	Solution name	YA2304-20 content /%	RSD/%
Column temperature (° C)	38	Durability test product solution 1	0.17	2.6
		Durability test sample solution 2	0.16	
	40	Durability test sample solution 1	0.17	
		Durability test sample solution 2	0.17	
	42	Durability test product solution 1	0.17	
		Durability test sample solution 2	0.16	
Flow rate (ml/min)	0.95	Durability test solution 1	0.17	2.4
		Durability test sample solution 2	0.16	
	1.00	Durability test sample solution 1	0.17	
		Durability test sample solution 2	0.17	

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Wavelength (nm)	1.05	Durability test sample solution 1	0.17	
		Durability test sample solution 2	0.16	
Mobile phase ratio	249	Durability test solution 1	0.17	2.3
		Durability test sample solution 2	0.16	
	251	Durability test sample solution 1	0.17	
		Durability test sample solution 2	0.17	
	253	Durability test sample solution 1	0.17	
		Durability test sample solution 2	0.16	
Mobile phase ratio	72:28	Durability test product solution 1	0.16	2.4
		Durability test sample solution 2	0.16	
	70:30	Durability test sample solution 1	0.17	
		Durability test sample solution 2	0.17	
	68:32	Durability test sample solution 1	0.17	
		Durability test sample solution 2	0.16	
Conclusion: RSD of YA2304-20 was 2.6% (should NMT 10%) under different column temperature conditions, which met the verification requirements; At different flow rates, the RSD of YA2304-20 in test samples was 2.4% (should NMT 10%), which met the verification requirements; Under different wavelength conditions, the RSD of YA2304-20 in the test product was 2.3% (should NMT 10%), which met the verification requirements; Under different mobile phase ratios, the RSD of YA2304-20 in the test product was 2.4% (should NMT 10%), which met the verification requirements, indicating that the durability of the method was good.				

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### III. Summary of Method Validation

#### Methodological Verification Results of Finished Product Enantiomer

Project	Acceptable criteria	Verification of results
Wavelength selection	YA2304-20 should have a large absorption at 251nm.	<p>Impurity YA2304-20 has greater absorption at 251nm. In the control solution, the retention time of YA2304-20 is 25.626min. The spectrogram of the control solution is as follows:</p> <p style="text-align: center;">光谱指数组 25.626 纳米</p> <p style="text-align: center;">分钟</p>
System applicability	The blank solution should be free of interference; The separation degree between the main peak and YA2304-20 peak in the chromatogram of system suitability solution should be greater than 1.5; In the chromatogram obtained for 6 consecutive days, the signal-to-noise ratio (S/N) of YA2304-20 peak should NLT 30, the RSD of the retention time NMT 1.0%, and the RSD of the peak area NMT 5%.	<p>The blank solution has no interference; The separation degree between the main peak and YA2304-20 peak in the system suitability solution is 3.0 (the requirement should NLT 1.5); In the chromatogram obtained from the control product solution, the signal-to-noise ratio of the main peak was between 71.5 and 83.4 (the requirement should be NLT 30); The RSD of peak retention time of YA2304-20 in the photoproduct solution for 6 consecutive days was 0.08% (the requirement should NMT 1.0%); The RSD of peak area is 0.6% (the requirement should NMT 5%); All meet the verification requirements. It shows that this method has good system applicability.</p>
Speckle	Blank solution should be free of interference; The separation degree between the main peak and YA2304-20	The blank solution has no interference; The separation degree between the main peak and YA2304-20 peak in the system suitability solution is 3.0 (the requirement should NLT 1.5); In the chromatogram obtained from the control solution, S/N was 62.2 (the

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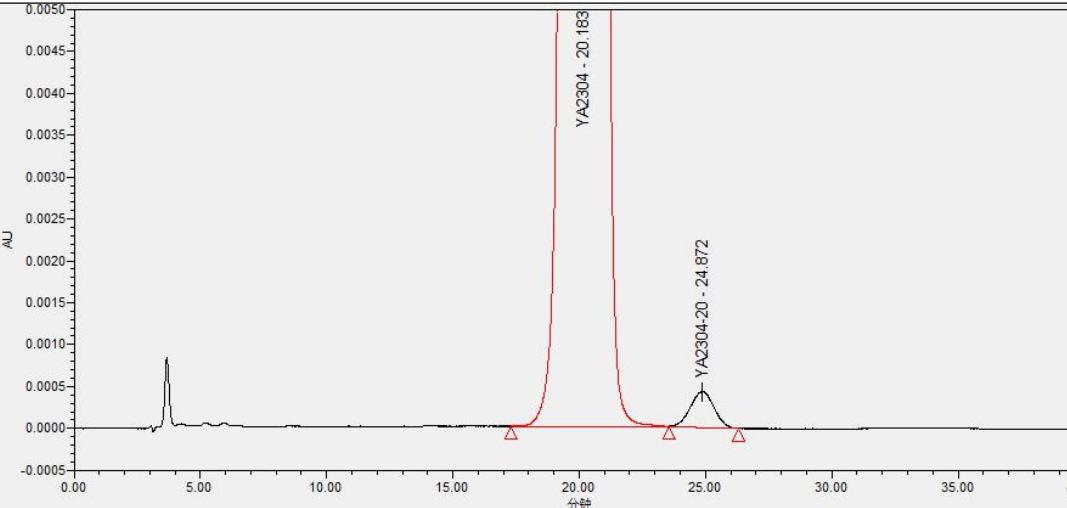
ci fi ci t y	ntifi cati on test	peak in the system suitability solution should be greater than 1.5; The peak signal-to-noise ratio (S/N) of YA2304-20 in the chromatogram of reference solution should NLT 30; The separation degree between YA2304-20 peaks and adjacent peaks in the chromatogram of test solution and specific mixed solution should be greater than 1.5, and the retention time should be consistent with the peak retention time of YA2304-20 in the chromatogram of control solution. Other single impurity localization solutions do not interfere with YA2304-20 peak production.	requirement should NLT 30); The separation degree between YA2304-20 peaks and adjacent peaks in the specific mixed solution and the test solution was greater than 1.5, and the retention time was consistent with that of YA2304-20 peaks in the chromatogram of the control solution. Impurities YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-18 and YA2304-19 all have a chiral center, so the above impurities all have two peaks under the current chromatographic conditions. Other single impurities (YA2304-10, YA2304-12, YA2304-14, YA2304-15, YA2304-16, YA2304-17, YA2304-18 and YA2304-19) do not interfere with the peak of YA2304-20; Meet the requirements of verification. The concentration of YA2304-20 in the specific mixed solution is equal to 0.15% of the principal component concentration in the test solution.					
			Name of solution	Component name	Component concentration ( $\mu\text{g/ml}$ )	Rt (min)	Separation	The percentage equivalent to the concentration of the principal component in the test product solution
			Specific mixed solution	YA2304	1033.1000	20.183	3.0	100%
				YA2304-20	1.4983	24.872		0.15%
The chromatogram of the specific mixed solution is as follows:								

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Limits of quantification and detection	The average signal-to-noise ratio (S/N) of each impurity peak in 6 LOQ solutions should NLT 10, the RSD of retention time should NMT 2.0%, and the RSD of peak area should NMT 10%. The SNR (S/N) of each impurity peak in the 3-pin detection limit (LOD) solution should NLT 3.	<b>Impurity/principal component</b>	<b>Detection limits</b> <b>Concentration (equivalent to the concentration percentage of the test product solution)</b>	<b>Limit of quantitation</b> <b>Concentration (equivalent to the test product solution concentration percentage)</b>
		YA2304-20	0.0899μg/ml (0.01%)	0.3112μg/ml (0.03%)
		YA2304	0.0934μg/ml (0.01%)	0.2997μg/ml (0.03%)
Linearity vs. range	With the concentration as the horizontal coordinate and the peak area as the vertical coordinate as the linear regression curve, the regression coefficient (R) of the regression curve is required to be NLT 0.990, the RSD of the response factor NMT 10%, and the Y-axis intercept is less than 25% of the percentage of 100% response value.	<b>Impurities / Principal component</b>	<b>Concentration (equivalent to percentage of solution concentration of test product)</b>	<b>Linear equation</b>
Accura	The required 30% limit	<b>Impurities</b>	<b>Marking condition</b>	<b>Average</b>
				<b>RSD</b>

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cy	concentration recovery rate should be 85%~110%, the required 100%~150% limit concentration recovery rate should be 90%~108%, and the RSD of the recovery rate NMT 10%.			<b>recovery rate</b>	
		YA2304-20	Added 0.4587 $\mu$ g/ml~2.2933 $\mu$ g/m	98.5%, n=9	2.4%, n=9
Precision	Repeatability and reproducibility: RSD of YA2304-20 in 6 repeatability (or reproducibility) test solution shall NMT 10%.  For the results of 12 repeatability and reproducibility tests, the RSD of YA2304-20 should NMT 10%.				
		<b>Impurities</b>	<b>repeatability</b>	<b>Reproducibility</b>	<b>Precision</b>
Solution stability	The system suitability solution, the control solution and the test product solution were placed at room temperature for a period of time, and the samples were injected at different time points. The separation degree between YA2304-20 peak and the main peak in the chromatogram of the system suitability solution should NLT 1.5; The peak signal-to-noise ratio (S/N) of YA2304-20 in the chromatogram of reference solution should NLT 30, and the RSD of peak area should not be more than 5%; In the chromatogram obtained by the test product solution, the RSD of YA2304-20 NMT 10% according to the peak area calculation by external standard method, and there shall be no new peak generation that interferes with the detection of YA2304-20.				
Durability	The separation degree between YA2304-20 peak and main peak should NLT 1.5 when column temperature,				

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wavelength, flow rate, mobile phase initiation ratio and normal chromatographic conditions are changed by single factor; The peak signal-to-noise ratio of YA2304-20 in the chromatogram of reference solution should NLT 30; RSD of YA2304-20 in test product solution shall NMT 10%.	System suitability: Under different chromatographic conditions, the separation degree between the main peak and YA2304-20 peak in the system suitability solution is between 1.9 and 2.5 (the requirement should be NLT 1.5), which meets the verification requirements. The main peak signal-to-noise ratio in the chromatogram of the reference product solution was between 31.4 and 77.1 (the requirement should be NLT 30), which met the verification requirements. Test product solution: under different chromatographic conditions, the RSD of YA2304-20 in the test product was no more than 10%, which met the verification requirements.
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### **3.2.S.4.3.4 Method Validation of Residual Solvents in Finerenone**

#### **I. Analytical Procedures**

Please refer to the analytical procedures of residual solvents in Finerenone in section 3.2.S.4.2.

#### **II. Validation Results and Discussion**

##### **a. System Suitability Determination**

###### **➤ Acceptance Criteria**

The blank solution should be free of interference; In the chromatogram obtained for 6 consecutive days, ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene, n-methylpyrrolidone peaks in turn, and the separation degree between each adjacent peak should be greater than 1.5. The RSD of the peak retention time of each solvent peak should NMT 1.0%, and the RSD of the peak area should NMT 10%.

###### **➤ Solution Preparation**

Solvent: N, n-dimethylformamide (DMF).

Ethanol reference product reserve liquid: precision weigh ethanol about 1000mg, put in 20ml bottle, dissolve with solvent and dilute to the scale, shake well, ready. (About 50000 $\mu$ g/ml)

Isopropyl alcohol control product reserve liquid: precision weigh isopropyl alcohol about 1000mg, put in 20ml measuring bottle, dissolve with solvent and dilute to the scale, shake well, ready. (About 50000 $\mu$ g/ml)

Ethyl acetate reference product reserve liquid: precision weigh about 1000mg of ethyl acetate, place in 20ml measuring bottle, dissolve and dilute with solvent to scale, shake well, ready. (Approx. 50000 $\mu$ g/ml)

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Secondary butanol reference product reserve liquid: precision weigh secondary butanol about 1000mg, place in 20ml bottle, dissolve and dilute with solvent to scale, shake well, then get. (About 50000 $\mu$ g/ml)

Tetrahydrofuran reference product reserve liquid: accurately weigh tetrahydrofuran about 144mg, put it in 20ml bottle, dissolve it with solvent and dilute it to the scale, shake well, and then get. (About 7200 $\mu$ g/ml)

Toluene control product reserve liquid: precision weigh toluene about 178mg, place in 20ml bottle, dissolve and dilute with solvent to scale, shake well, ready. (About 8900 $\mu$ g/ml)

N-methylpyrrolidone reference product reserve liquid: accurately weigh N-methylpyrrolidone about 106mg, place in 20ml bottle, dissolve with solvent and dilute to the scale, shake well, ready. (About 5300 $\mu$ g/ml)

Mixed reference product reserve liquid: precision measure ethanol reference product reserve liquid, isopropyl alcohol reference product reserve liquid, ethyl acetate reference product reserve liquid, secondary butanol reference product reserve liquid, tetrahydrofuran reference product reserve liquid, toluene reference product reserve liquid, N-methylpyrrolidone reference product reserve liquid 1.0ml each, put in 20ml bottle, dilute with solvent to the scale, shake well, ready. (Ethanol about 2500 $\mu$ g/ml, isopropyl alcohol about 2500 $\mu$ g/ml, ethyl acetate about 2500 $\mu$ g/ml, secondary butanol about 2500 $\mu$ g/ml, tetrahydrofuran about 360 $\mu$ g/ml, toluene about 445 $\mu$ g/ml, N-methylpyrrolidone about 265 $\mu$ g/ml)

Reference product solution: accurately measure 1.0ml of the mixed reference product reserve solution, place it in a 10ml bottle, dilute it with solvent to the scale, shake well, and obtain. (Ethanol about 250 $\mu$ g/ml, isopropyl alcohol about 250 $\mu$ g/ml, ethyl acetate about 250 $\mu$ g/ml, secondary butanol about 250 $\mu$ g/ml, tetrahydrofuran about 36 $\mu$ g/ml, toluene about 44.5 $\mu$ g/ml, N-methylpyrrolidone about 26.5 $\mu$ g/ml)

### ➤ **Injection Sequence**

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After the blank solution, the chromatogram was recorded by continuously feeding 6 samples of the sample solution.

### ➤ Measurement results

Results of system suitability test

Name of solvent	Parameter values	Reference solution injection						RSD/ %
		1	2	3	4	5	6	
Ethanol	RT (min)	8.229	8.233	8.225	8.222	8.244	8.246	0.13
	Peak area	213.3847	212.0271	212.0469	214.1431	214.5440	210.2753	0.8
	Separation	/	/	/	/	/	/	/
Isopropyl alcohol	RT (min)	9.598	9.602	9.592	9.588	9.615	9.615	0.12
	Peak area	234.4403	232.1335	233.3802	235.5531	235.4593	230.1003	1.0
	Separation	7.7	7.7	7.5	7.7	7.6	7.7	/
Ethyl acetate	RT (min)	14.220	14.226	14.216	14.212	14.237	14.238	0.08
	Peak area	182.3676	180.9274	182.5747	183.0741	182.9102	178.8448	0.9
	Separation	27.0	27.1	26.8	27.1	26.9	27.2	/
Secondary butanol	RT (min)	14.509	14.513	14.506	14.500	14.525	14.526	0.08
	Peak area	278.1483	273.9799	275.4820	280.5112	278.0600	276.1267	0.9
	Separation	1.8	1.8	1.8	1.8	1.8	1.8	/
tetrahydrofuran	RT (min)	14.807	14.809	14.801	14.798	14.823	14.822	0.08
	Peak area	36.6421	36.1685	36.3647	36.9569	36.3910	36.6089	0.8
	Separation	1.9	1.9	1.8	1.9	1.9	1.9	/
Toluene	RT (min)	20.138	20.136	20.128	20.129	20.149	20.149	0.05
	Peak area	96.0328	94.9365	96.0452	96.5924	96.2772	94.3475	1.0
	Separation	37.8	37.8	37.7	37.6	37.7	37.7	/
N-m	RT (min)	31.455	31.450	31.447	31.453	31.460	31.454	0.0

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ethy lpyr rolid one								15
	Peak area	25.8942	25.3002	24.9786	25.4332	24.8897	23.9517	2.7
	Separation	94.9	94.5	94.5	94.1	94.4	94.4	/

Conclusion: There was no interference in the blank solution. In the chromatogram obtained for 6 consecutive days, peaks of ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene and n-methylpyrrolidone appeared successively, and the separation degree between each adjacent solvent peak was greater than 1.5 (the requirement was greater than 1.5). The RSD of ethanol peak retention time was 0.13% (required less than 1.0%), and the RSD of peak area was 0.8% (required less than 10%). The RSD of isopropyl alcohol peak retention time was 0.12% (NMT 1.0%), and the RSD of peak area was 1.0% (NMT 10%); The RSD of the ethyl acetate peak was 0.08% (NMT 1.0%), and the RSD of the peak area was 0.9% (NMT 10%); The RSD of secondary butanol was 0.08% (NMT 1.0%), and the RSD of peak area was 0.9% (NMT 10%); The RSD of the peak retention time of tetrahydrofuran was 0.08% (NMT 1.0%), and the RSD of the peak area was 0.8% (NMT 10%); The RSD of toluene peak was 0.05% (NMT 1.0%), and the RSD of peak area was 1.0% (NMT 10%); The RSD of the N-methylpyrrolidone peak was 0.015% (NMT 1.0%), and the RSD of the peak area was 2.7% (NMT 10%); All the above meet the verification requirements, indicating that the method has good applicability in system.

### **b. Specificity**

#### ➤ **Acceptance Criteria**

There is no interference in the blank solution. In the control solution, the peaks of ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene and n-methylpyrrolidone come out successively, and the separation degree between the adjacent peaks should be greater than 1.5; The separation degree of each solvent peak and adjacent peaks in the specific mixed solution and the test product solution is NLT 1.5, the retention time of each known solvent peak in the specific mixed solution and the test product solution should be consistent with the retention time of each solvent positioning solution, and other solvent positioning solutions (benzene, piperidine) have no interference with the target solvent peak.

#### ➤ **Solution preparation**

Blank solution: solvent (N, n-dimethylformamide).

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Ethanol reference stock: Share ethanol reference stock under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Isopropyl alcohol reference reserve: Share isopropyl alcohol reference reserve under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Ethyl acetate reference reserve: Share the ethyl acetate reference reserve under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Secondary butanol reference product reserve: Share secondary butanol versus reference product reserve solution under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Tetrahydrofuran reference storage solution: Share tetrahydrofuran reference storage solution under "4.1 System Suitability". (Approx. 7200µg/ml)

Toluene reference reserve: Share toluene reference reserve under "4.1 System Suitability". (Approx. 8900µg/ml)

N-methylpyrrolidone reference stock: Share the N-methylpyrrolidone reference stock under "4.1 System Suitability". (Approx. 5300µg/ml)

Piperidine control product reserve liquid: precision weigh piperidine about 1000mg, place in 20ml bottle, dissolve with solvent and dilute to scale, shake well, ready. (Approx. 50000µg/ml)

Benzene reference product reserve liquid: precision weigh about 40mg benzene, put it in 200ml bottle, dissolve it with solvent and dilute it to the scale, shake well; Then accurately measure 1ml, put it in 10ml measuring bottle, dissolve it with solvent and dilute it to the scale, shake well, and then get. Get it now. (About 20µg/ml)

Reference product reserve liquid: precision measure ethanol reference product reserve liquid, isopropyl alcohol reference product reserve liquid, ethyl acetate reference product reserve liquid, secondary butanol reference product reserve liquid, tetrahydrofuran reference product reserve liquid, toluene reference product reserve liquid, N-methylpyrrolidone reference product reserve liquid 1.0ml each, put in 20ml bottle, dilute with solvent to scale, shake well, ready. (Ethanol about 2500µg/ml, isopropyl alcohol about

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2500 $\mu$ g/ml, ethyl acetate about 2500 $\mu$ g/ml, secondary butanol about 2500 $\mu$ g/ml, tetrahydrofuran about 360 $\mu$ g/ml, toluene about 445 $\mu$ g/ml, N-methylpyrrolidone about 265 $\mu$ g/ml)

Control product solution: accurately measure 1.0ml of control product reserve solution, place it in 10ml bottle, dilute it with solvent to scale, shake well, and obtain. (Ethanol about 250 $\mu$ g/ml, isopropyl alcohol about 250 $\mu$ g/ml, ethyl acetate about 250 $\mu$ g/ml, secondary butanol about 250 $\mu$ g/ml, tetrahydrofuran about 36 $\mu$ g/ml, toluene about 44.5 $\mu$ g/ml, N-methylpyrrolidone about 26.5 $\mu$ g/ml)

Mixed control product reserve liquid: Take a precise measure of 1.0ml each of ethanol reference liquid, isopropyl alcohol reference liquid, ethyl acetate reference liquid, secondary butanol reference liquid, tetrahydrofuran reference liquid, toluene reference liquid, N-methylpyrrolidone reference liquid, piperidine reference liquid and benzene reference liquid, put them in a 20ml bottle and dilute them to the scale with solvent. Shake well, then get. (Ethanol about 2500 $\mu$ g/ml, isopropyl alcohol about 2500 $\mu$ g/ml, ethyl acetate about 2500 $\mu$ g/ml, secondary butanol about 2500 $\mu$ g/ml, tetrahydrofuran about 360 $\mu$ g/ml, toluene about 445 $\mu$ g/ml, N-methylpyrrolidone about 265 $\mu$ g/ml, piperidine about 2500 $\mu$ g/ml, benzene about 1 $\mu$ g /ml)

Ethanol positioning solution: accurately measure 0.1ml of ethanol reference product reserve liquid, place it in 20ml measuring bottle, dilute it with solvent to scale, shake well, and get. (About 250 $\mu$ g/ml)

Isopropyl alcohol positioning solution: accurately measure 0.1ml of isopropyl alcohol reference product reserve solution, place it in 20ml measuring bottle, dilute it with solvent to the scale, shake well, and obtain. (About 250 $\mu$ g/ml)

Ethyl acetate positioning solution: accurately measure 0.1ml of ethyl acetate reference product reserve solution, place it in a 20ml measuring bottle, dilute it with solvent to the scale, shake well, and obtain. (About 250 $\mu$ g/ml)

Secondary butanol positioning solution: accurately measure secondary butanol reference product reserve liquid 0.1ml, place it in 20ml measuring

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bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 250 $\mu$ g/ml)

Tetrahydrofuran positioning solution: precisely measure tetrahydrofuran reference product reserve liquid 0.1ml, place in 20ml measuring bottle, dilute with solvent to scale, shake well, ready. (about 36 $\mu$ g/ml)

Toluene positioning solution: accurately measure toluene reference product reserve liquid 0.1ml, place in 20ml measuring bottle, dilute with solvent to scale, shake well, ready. (About 44.5 $\mu$ g/ml)

N-methylpyrrolidone positioning solution: accurately measure 0.1ml of N-methylpyrrolidone reference product reserve solution, place it in 20ml measuring bottle, dilute it with solvent to the scale, shake well, and obtain. (About 26.5 $\mu$ g/ml)

Piperidine positioning solution: precisely measure 0.1ml of piperidine control product reserve, place it in a 20ml measuring bottle, dilute it with solvent to the scale, shake well, and obtain. (About 250 $\mu$ g/ml)

Benzene positioning solution: Accurately measure 0.1ml of benzene reference product reserve liquid, place it in a 20ml measuring bottle, dilute it with solvent to the scale, shake well, and obtain. (About 0.1 $\mu$ g/ml)

Test product solution: Accurately weigh about 0.5g of this product, place it in 10ml measuring bottle, dissolve it with solvent and dilute it to the scale, shake well, and then get. (About 50mg/ml)

Specific mixed solution: Accurately weigh about 0.5g of this product, place it in 10ml measuring bottle, add 1.0ml of mixed control product reserve solution, dissolve it with solvent and dilute it to the scale, shake well, and then get.

### **➤ Injection Sequence**

After the blank solution, the positioning solution, the reference solution, the test product solution and the specific mixed solution were injected respectively, and the chromatogram was recorded.

### **➤ Measurement resultss**

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Name of solution		Concentration (µg/ml)	RT (min)	Separation
Solution name	Component name			
Ethanol locating solution	Ethanol	250.278	8.167	/
Isopropyl alcohol positioning solution	Isopropyl alcohol	250.410	9.516	/
Ethyl acetate positioning solution	Ethyl acetate	248.695	14.149	/
Secondary butanol positioning solution	Sec-butanol	250.585	14.429	/
Tetrahydrofuran locator solution	tetrahydrofuran	36.588	14.731	/
Toluene locator solution	Toluene	50.025	20.058	/
N-methylpyrrolidone positioning solution	N-methylpyrrolidon e	27.6875	31.386	/
Piperidine positioning solution	Piperidine	0.1033	/	/
Benzene locator solution	benzene	251.4725	/	/
Reference solution	Ethyl alcohol	250.278	8.151	/
	Isopropyl alcohol	250.410	9.503	8.2
	Ethyl acetate	248.695	14.143	28.3
	sec-butanol	250.585	14.429	1.8
	tetrahydrofuran	36.588	14.729	1.9
	Toluene	50.025	20.061	37.7
	N-methylpyrrolidon e	27.6875	31.388	95.5
Sample solution	Ethanol	/	8.166	/
	Isopropyl alcohol	/	/	/
	Ethyl acetate	/	/	/
	sec-butanol	/	/	/
	tetrahydrofuran	/	/	/
	Toluene	/	/	/
	N-methylpyrrolidon e	/	/	/
	Piperidine	/	/	/
Specific mixed solution	benzene	/	/	/
	Ethanol	250.278	8.149	/
	Isopropyl alcohol	250.410	9.504	8.2

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	Ethyl acetate	248.695	14.145	28.2
	sec-butanol	250.585	14.429	1.8
	tetrahydrofuran	36.588	14.728	1.9
	Toluene	50.025	20.061	37.5
	N-methylpyrrolidone	27.6875	31.390	94.3

Conclusion: There was no interference in the blank solution. In the control solution, the peaks of ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene and n-methylpyrrolidone appeared successively, and the separation degree between each adjacent solvent peak was greater than 1.5 (the requirement was greater than 1.5). The separation degree between individual solvent peaks and adjacent peaks in the specific mixed solution and the test solution is NLT 1.5, the retention time of each known solvent peak in the specific mixed solution and the test solution is consistent with the retention time of each solvent-locating solution, and other solvent-locating solutions (benzene, piperidine) have no interference with the target solvent peak. All of the above meet the verification requirements, indicating that the method has good specificity.

### **(4.3) Limits of quantification and detection**

#### **➤ Acceptance Criteria**

The S/N of each solvent peak in 6 LOQ solution maps should be NLT 10, the RSD of retention time should be no more than 2.0%, and the RSD of peak area should be no more than 10%. The S/N of each solvent peak in the 3-needle Limit of detection (LOD) solution atlas should be NLT 3.

#### **➤ Solution Preparation**

Blank solution: solvent (N, n-dimethylformamide).

Ethanol reference stock: Share ethanol reference stock under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Isopropyl alcohol reference stock: Share isopropyl alcohol reference stock under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Ethyl acetate reference reserve: Share the ethyl acetate reference reserve under "4.1 System Suitability". (Approx. 50,000 µg/ml)

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Secondary butanol reference product reserve: Share secondary butanol versus reference product reserve solution under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Tetrahydrofuran reference storage solution: Share tetrahydrofuran reference storage solution under "4.1 System Suitability". (Approx. 7200µg/ml)

Toluene reference reserve: Share toluene reference reserve under "4.1 System Suitability". (Approx. 8900µg/ml)

N-methylpyrrolidone reference stock: Share the N-methylpyrrolidone reference stock under "4.1 System Suitability". (Approx. 5300µg/ml)

Quantitative limited stock solution: Precise measurement of ethanol reference liquid 0.2ml, isopropyl alcohol reference liquid 0.2ml, ethyl acetate reference liquid 0.2ml, secondary butanol reference liquid 0.15ml, tetrahydrofuran reference liquid 1ml, toluene reference liquid 0.4ml, N-methylpyrrolidone reference liquid 1ml, Place in the same 10ml measuring bottle, dilute with solvent and set the volume to the scale, shake well, then measure 1ml precisely, place in 20ml measuring bottle, dilute with solvent and set the volume to the scale, shake well, then obtain.

Limit of quantitation (LOQ) solution: accurately measure 1ml of limited quantitation reserve liquid, place it in 10ml measuring bottle, dilute it with solvent and set the volume to the scale, shake well, and then get. (Dispense 6 parts in parallel)

Limit of detection (LOD) solution: Accurately measure the first part of the limit of quantification solution 3ml, place it in a 10ml measuring bottle, dilute it with solvent to the scale, shake well, and get.

### **➤ Injection Sequence**

After the blank solution, 6 parts of limited quantitation solution and 3 needles of limited detection solution were injected respectively, and the chromatogram was recorded.

### **➤ Measurement resultss**

Results of limit of quantitation test

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Substances Name	Solution name	Concentration /( $\mu\text{g}/\text{ml}$ )	Equivalent to the percentage % of the concentration of the test product solution	RT (min)	Peak area	S/N	
Ethanol	Limited quantitative Solution 1	5.006	0.01	8.261	4.7576	12.2	
	Limited quantitative Solution 2			8.263	5.0132	15.7	
	Limited quantitative Solution 3			8.254	4.8740	14.7	
	Limited quantitative Solution 4			8.256	4.7694	13.7	
	Limited quantitative Solution 5			8.255	5.0251	15.6	
	Limited quantitative solution 6			8.260	4.9149	14.3	
	RSD (%)			0.05	2.4	/	
Verdict: The S/N of ethanol in 6 limited quantification solution chromatograms was between 12.2-15.7 (NLT 10 required), the RSD of retention time was 0.05% (no more than 2.0% required), the RSD of peak area was 2.4% (no more than 10% required), and the limited quantification concentration of ethanol was 5.006 $\mu\text{g}/\text{ml}$ . Equivalent to 0.01% of the concentration of the test product; The above are in line with the verification requirements.							
Isopro pyl alcohol 1	Limited quantitative Solution 1	5.008	0.01	9.613	5.3135	11.9	
	Limited quantification solution 2			9.617	5.1179	15.2	
	Limited quantification			9.613	4.8142	14.2	

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	n solution 3							
	Limited quantitative Solution 4			9.613	4.7635	13.2		
	Limited quantitative Solution 5			9.611	5.1899	14.8		
	Limited quantitative solution 6			9.614	4.7570	13.8		
	RSD (%)			0.021	5	/		
	Verdict: The S/N of isopropyl alcohol in 6 limited quantification solution chromatograms ranged from 11.9 to 15.2 (NLT 10 required), the RSD of retention time was 0.021% (no more than 2.0% required), the RSD of peak area was 5% (no more than 10% required), and the limited quantification concentration of isopropyl alcohol was 5.008µg/ml. Equivalent to 0.01% of the concentration of the test product; The above are in line with the verification requirements.							
	Limit of quantification Solution 1			14.230	3.7080	11.2		
Ethyl acetate	Limited quantitative Solution 2	4.974	0.01	14.222	3.6889	14.2		
	Limited quantitative Solution 3			14.227	3.7113	13.4		
	Limited quantitative Solution 4			14.222	3.6914	12.4		
	Limited quantitative Solution 5			14.229	3.7151	13.8		
	Limited quantitative solution 6			14.219	3.6656	12.9		
	RSD (%)			0.04	0.6	/		
	Verdict: The S/N of ethyl acetate in 6 limited quantification solution chromatograms was between 11.2-14.2 (NLT 10 required), the RSD of							

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	retention time was 0.04% (no more than 2.0% required), the RSD of peak area was 0.6% (no more than 10% required), and the limited quantification concentration of ethyl acetate was 4.974 $\mu$ g/ml. Equivalent to 0.01% of the concentration of the test product; The above are in line with the verification requirements.						
SEC-b utanol	Limited quantification solution 1	3.759	0.008	14.518	4.1822	10.6	
	Limited quantitative Solution 2			14.519	4.1487	13.8	
	Limited quantitative Solution 3			14.524	4.1204	12.8	
	Limited quantitative Solution 4			14.517	4.1730	12.2	
	Limited quantitative Solution 5			14.517	4.0965	13.1	
	Limited quantitative solution 6			14.514	4.0401	12.3	
RSD (%)			0.023	1.3	/		
Verdict: The S/N of secondary butanol in 6 limited quantitation solution chromatograms ranged from 10.6 to 13.8 (NLT 10 required), the RSD of retention time was 0.023% (no more than 2.0% required), the RSD of peak area was 1.3% (no more than 10% required), and the limited quantitation concentration of secondary butanol was 3.759 $\mu$ g/ml. Equivalent to the concentration of the test product 0.008%; The above are in line with the verification requirements.							
Tetrahydrou ran	Limited quantitative Solution 1	3.659	0.007	14.810	4.2917	13.4	
	Limited quantitative Solution 2			14.813	4.1655	16.8	
	Limited quantitative			14.814	4.1972	15.7	

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	Solution 3					
	Limited quantitative Solution 4		14.813	4.1060	14.8	
	Limited quantitative Solution 5		14.810	4.1467	16.2	
	Limited quantitative solution 6		14.811	4.2390	15.4	
	RSD (%)		0.012	1.6	/	
	Conclusion: The S/N of tetrahydrofuran in 6 limited quantification solution chromatograms was between 13.4-16.8 (NLT 10), the RSD of retention time was 0.012% (no more than 2.0%), the RSD of peak area was 1.6% (no more than 10%), and the limiting concentration of tetrahydrofuran was 3.659µg/ml. Equivalent to the concentration of the test product 0.007%; The above are in line with the verification requirements.					
	Limited quantitative Solution 1		20.134	4.0267	13.7	
Toluene	Limited quantitative Solution 2	2.001	20.134	4.0244	17.5	
	Limited quantitative Solution 3		20.135	4.0668	16.5	
	Limited quantitative Solution 4		20.134	3.9945	15.5	
	Limited quantitative Solution 5		20.134	4.0965	17.1	
	Limited quantitative solution 6		20.133	4.1527	16.2	
	RSD (%)		0.004	1.5	/	
	Verdict: The S/N of toluene in 6 limited quantification solution chromatograms was between 13.7-17.5 (NLT 10), the RSD of retention time was 0.004% (no more than 2.0%), the RSD of peak area was 1.5% (no more					

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	than 10%), and the limited quantification concentration of toluene was 2.001 $\mu$ g/ml. Equivalent to 0.004% of the concentration of the test product; All the above meet the verification requirements.						
N-methylpyrrolidone	Limited quantitative Solution 1	2.769	0.006	31.457	3.2895	10.3	
	Limited quantitative Solution 2			31.460	3.4857	14.6	
	Limited quantitative Solution 3			31.463	3.5691	13.9	
	Limited quantitative Solution 4			31.463	3.3893	12.8	
	Limited quantitative Solution 5			31.459	3.5359	14.3	
	Limited quantitative solution 6			31.459	3.3758	13.2	
RSD (%)			0.008	4	/		
Verdict: The S/N of N-methylpyrrolidone in 6 limited quantification solution chromatograms ranged from 10.3-14.6 (NLT 10 required), the RSD of retention time was 0.008% (no more than 2.0% required), the RSD of peak area was 4% (no more than 10% required), and the limited quantification concentration of N-methylpyrrolidone was 2.769 $\mu$ g/ml. Equivalent to the concentration of the test product 0.006%; The above are in line with the verification requirements.							

### Test results of detection limits

Substances Name	Solution name		Concentration /( $\mu$ g/ml)	Equivalent to the percentage /% of the concentration of the test product solution	RT (min)	Peak area	S/N
Ethanol	Detection limit solution	1	1.502	0.003	8.290	1.9664	3.7
		2			8.277	1.953	3.5

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		3			5 8.263 4	1.932 4	3.2
Conclusion: The S/N of ethanol in the 3-needle detection limit solution chromatogram was between 3.2-3.7 (the requirement was NLT 3), and the detection limit concentration of ethanol was 1.502µg/ml, which was equivalent to the concentration of test product 0.003%. All the above are in line with the verification requirements.							
Isopropyl alcohol	Detection limit solution	1	1.502	0.003	9.655	2.680 9	4.7
		2			9.633	2.605 1	4.0
		3			9.614	2.612 5	4.0
Conclusion: The S/N of isopropyl alcohol in the 3-needle detection limit solution chromatogram was between 4.0-4.7 (the requirement was NLT 3), and the detection limit concentration of isopropyl alcohol was 1.502µg/ml, which was equivalent to the concentration of test product 0.003%. All the above were in line with the verification requirements.							
Ethyl acetate	Detection limit solution	1	1.492	0.003	14.26 3	2.238 2	4.7
		2			14.24 3	2.208 0	3.9
		3			14.23 4	2.056 4	3.8
Conclusion: The S/N of ethyl acetate in the 3-needle detection limit solution chromatogram was between 3.8-4.7 (required to be NLT 3), and the detection limit concentration of ethyl acetate was 1.492µg/ml, which was equivalent to the concentration of test product 0.003%. All the above were in line with the verification requirements.							
SEC-butanol	Detection limit solution	1	1.128	0.002	14.55 3	2.370 6	4.2
		2			14.53 0	2.270 6	3.7
		3			14.51 4	2.285 8	3.6
Conclusion: The S/N of secondary butanol in the 3-needle detection limited solution chromatogram was between 3.6-4.2 (the requirement was NLT 3), and the detection limited concentration of secondary butanol was 1.128µg/ml, which was equivalent to the concentration of test product 0.002%. All of the above are in line with the verification requirements.							
Tetrahydrofuran	Detection limit solution	1	1.098	0.002	14.84 1	2.205 5	5.0
		2			14.83 7	1.921 5	4.2
		3			14.81 2	2.128 6	4.3
Conclusion: The S/N of tetrahydrofuran in the 3-needle detection limit solution chromatogram was between 4.2-5.0 (the requirement was NLT 3), and the detection limit concentration of tetrahydrofuran was 1.098µg/ml, which was equivalent to the concentration of test product 0.002%. All the above are in line with the verification							

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		requirements.						
Toluene	Detection limit solution	1	0.600	0.001	20.15 7	2.264 3	5.3	
		2			20.14 6	2.171 2	4.7	
		3			20.13 5	2.140 3	4.4	
Conclusion: The S/N of toluene in the 3-needle detection limit solution chromatogram was between 4.4-5.3 (NLT 3), and the detection limit concentration of toluene was 0.600 $\mu$ g/ml, which was equivalent to the concentration of test product 0.001%. All the above are in line with the verification requirements.								
N-methylpyrrolidone	Detection limit solution	1	0.831	0.002	31.48 2	2.320 9	4.8	
		2			31.47 4	1.785 2	3.5	
		3			31.46 1	2.560 3	4.4	
Conclusion: The S/N of N-methylpyrrolidone in 3-needle limited solution chromatogram ranges from 3.5-4.8 (NLT 3 is required), and the detection limited concentration of N-methylpyrrolidone is 0.831 $\mu$ g/ml, which is equivalent to the concentration of test product 0.002%. All the above were in line with the verification requirements.								

### **(4.4) Linearity and range**

#### **➤ Acceptance Criteria**

In equivalent to the LOQ concentration of each solvent ~200% limit concentration range, relatively uniform take 6 concentration points, the impurity concentration as the horizontal coordinate, the peak area as the vertical coordinate, the regression coefficient R of the linear graph should NLT 0.990, the RSD of the response factor should not be more than 10%, Y-axis intercept absolute value accounted for 100% of the response value of the percentage should be less than 25%.

#### **➤ Solution Preparation**

Blank solution: solvent (N, n-dimethylformamide).

Ethanol reference stock: Share ethanol reference stock under "4.1 System Suitability". (Approx. 50,000  $\mu$ g/ml)

Isopropyl alcohol reference reserve: Share isopropyl alcohol reference reserve under "4.1 System Suitability". (Approx. 50,000  $\mu$ g/ml)

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Ethyl acetate reference reserve: Share the ethyl acetate reference reserve under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Secondary butanol reference product reserve: Share secondary butanol versus reference product reserve solution under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Tetrahydrofuran reference storage solution: Share tetrahydrofuran reference storage solution under "4.1 System Suitability". (Approx. 7200µg/ml)

Toluene reference reserve: Share toluene reference reserve under "4.1 System Suitability". (Approx. 8900µg/ml)

N-methylpyrrolidone reference stock: Share the N-methylpyrrolidone reference stock under "4.1 System Suitability". (Approx. 5300µg/ml)

Linear reserve liquid: precision measure ethanol reference product reserve liquid, isopropyl alcohol reference product reserve liquid, ethyl acetate reference product reserve liquid, secondary butanol reference product reserve liquid, tetrahydrofuran reference product reserve liquid, toluene reference product reserve liquid, N-methylpyrrolidone reference product reserve liquid 1.0ml each, placed in a 20ml bottle, diluted with solvent to the scale, shake well, ready. (Ethanol about 2500µg/ml, isopropyl alcohol about 2500µg/ml, ethyl acetate about 2500µg/ml, secondary butanol about 2500µg/ml, tetrahydrofuran about 360µg/ml, toluene about 445µg/ml, N-methylpyrrolidone about 265µg/ml)

Linear solution 1 (LOQ solution) : Share the first limit of quantification solution under "4.3 limits of quantification and detection limits"

Linear solution 2 (30% limit concentration) : Accurately measure 0.3ml of linear reserve solution, place it in 10ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain.

Linear solution 3 (80% limit concentration) : Accurately measure 0.8ml linear reserve solution, place it in a 10ml measuring bottle, dilute it with solvent to the scale, shake well, and obtain.

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Linear solution 4 (100% limit concentration) : precisely measure 1.0ml of linear reserve liquid, place it in a 10ml measuring bottle, dilute it with solvent to the scale, shake well, and get.

Linear solution 5 (150% limit concentration) : precision measure 1.5ml linear reserve solution, place it in 10ml measuring bottle, dilute it with solvent to the scale, shake well, ready.

Linear solution 6 (200% limit concentration) : precision measure 2.0ml linear reserve solution, place it in 10ml measuring bottle, dilute it with solvent to the scale, shake well, ready.

### ➤ **Injection Sequence**

After the blank solution, the linear solution of each concentration was injected respectively, and the chromatogram was recorded.

### ➤ **Measurement resultss**

Results of linear and range tests

Substances Name	Solution name	The percentage /% equivalent to the concentration of the solution of the test product	Concentration /( $\mu\text{g}/\text{ml}$ )	Peak area	Response factor
Ethanol	Linear solution 1	0.0100	5.006	4.1186	0.8228
	Linear Solution 2	0.1502	75.083	73.1266	0.9739
	Linear Solution 3	0.4004	200.222	187.5907	0.9369
	Linear solution 4	0.5006	250.278	233.5749	0.9333
	Linear Solution 5	0.7508	375.416	333.7332	0.8890
	Linear solution 6	1.0011	500.555	470.8123	0.9406
	RSD/% of the response factor			6	

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		<p style="text-align: center;"><b>乙醇线性与范围</b></p> <p style="text-align: center;"><math>y = 0.923x + 0.7951</math> <math>R^2 = 0.9982</math></p>			
	Linear graph				
	Linear equations	$Y = 0.923 + 0.7951 x$			
	Correlation coefficient R	0.999			
	Absolute value of Y-axis intercept as a percentage /% of 100% response value	0.34			
		The verdict: In the concentration range of 5.006μg/ml ~ 500.555μg/ml, with the concentration as the horizontal coordinate and the peak area as the vertical coordinate as the standard curve, the obtained linear equation is $y=0.923x+0.7951$ , and the correlation coefficient is 0.999 (the requirement is NLT 0.990). The RSD of the response factor is 6% (NMT 10%), and the absolute value of the Y-axis intercept accounts for 0.34% of the 100% response value (required within 25%); All the above meet the verification requirements, indicating that the analytical method has a good linear relationship in the concentration range of 5.006μg/ml ~ 500.555μg/ml for ethanol detection.			
Isopropyl Alcohol	Linear Solution 1	0.0100	5.008	4.8550	0.9694
	Linear Solution 2	0.1502	75.123	72.2137	0.9613
	Linear solution 3	0.4007	200.328	196.5378	0.9811
	Linear solution 4	0.5008	250.410	240.5275	0.9605
	Linear Solution 5	0.7512	375.615	355.4473	0.9463
	Linear solution 6	1.0016	500.820	504.4516	1.0073
	RSD/% of the response factor		2.2		

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		<p style="text-align: center;"><b>异丙醇线性与范围</b></p> <p style="text-align: center;"><math>y = 0.9917x - 3.6091</math> <math>R^2 = 0.9979</math></p>			
	Linear graph				
	Linear equations	$Y = 0.9917-3.6091 x$			
	Correlation coefficient R	0.999			
	Absolute value of Y-axis intercept as a percentage /% of 100% response value	1.5			
	<p>Conclusion: Isopropyl alcohol in the concentration range of 5.008μg/ml ~ 500.820μg/ml, with the concentration as the horizontal coordinate and the peak area as the vertical coordinate as the standard curve, the obtained linear equation is <math>y=0.9917x-3.6091</math>, and the correlation coefficient is 0.999 (the requirement is NLT 0.990). RSD of the response factor was 2.2% (NMT 10%), and the absolute value of the Y-axis intercept accounted for 1.5% of the 100% response value (required within 25%); All of the above meet the verification requirements, indicating that the analytical method has a good linear relationship in the concentration range of 5.008μg/ml ~ 500.820μg/ml.</p>				
Ethy 1 acet ate	Linear solution 1	0.0099	4.974	3.8354	0.7711
	Linear Solution 2	0.1492	74.609	56.1242	0.7522
	Linear Solution 3	0.3979	198.956	152.6369	0.7672
	Linear solution 4	0.4974	248.695	185.6191	0.7464
	Linear Solution 5	0.7461	373.043	274.8493	0.7368
	Linear solution 6	0.9948	497.390	388.8026	0.7817
	RSD/% of the response factor		2.3		

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		<p style="text-align: center;"><b>乙酸乙酯线性与范围</b></p> <p style="text-align: center;"><math>y = 0.7696x - 2.3</math> <math>R^2 = 0.9981</math></p>			
	Linear graph				
	Linear equations	$Y = 0.7696-2.3 x$			
	Correlation coefficient R	0.999			
	Absolute value of Y-axis intercept as a percentage /% of 100% response value	1.2			
	<p>The verdict: Ethyl acetate in the concentration range of 4.974μg/ml ~ 497.390μg/ml, with the concentration as the horizontal coordinate and the peak area as the vertical coordinate as the standard curve, the obtained linear equation is <math>y=0.7696x-2.3</math>, the correlation coefficient is 0.999 (the requirement is NLT 0.990). The RSD of the response factor was 2.3% (NMT 10%), and the absolute value of the Y-axis intercept accounted for 1.2% of the 100% response value (required within 25%); All of the above meet the verification requirements, indicating that the analytical method has a good linear relationship in the concentration range of 4.974μg/ml ~ 497.390μg/ml.</p>				
Sec-butanol	Linear solution 1	0.0075	3.759	4.2595	1.1332
	Linear Solution 2	0.1504	75.176	87.9476	1.1699
	Linear Solution 3	0.4009	200.468	240.1952	1.1982
	Linear solution 4	0.5012	250.585	291.3112	1.1625
	Linear Solution 5	0.7518	375.878	435.1142	1.1576
	Linear solution 6	1.0023	501.170	615.3318	1.2278
	RSD/% of the response factor		2.9		

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		<p style="text-align: center;"><b>仲丁醇线性与范围</b></p> <p style="text-align: center;"><math>y = 1.2102x - 4.7656</math> <math>R^2 = 0.998</math></p>			
	Linear graph				
	Linear equations	$Y = 1.2102-4.7656 x$			
	Correlation coefficient R	0.999			
	Absolute value of Y-axis intercept as a percentage /% of 100% response value	1.6			
<p>The verdict: In the concentration range of 3.759μg/ml ~ 501.170μg/ml, with the concentration as the horizontal coordinate and the peak area as the vertical coordinate as the standard curve, the obtained linear equation is <math>y=1.2102x-4.7656</math>, and the correlation coefficient is 0.999 (the requirement is NLT 0.990). The RSD of the response factor was 2.9% (NMT 10%), the absolute value of the Y-axis intercept accounted for 1.6% of the 100% response value (required within 25%); All of the above meet the verification requirements, indicating that the analytical method has a good linear relationship in the concentration range of 5.045μg/ml ~ 504.54μg/ml.</p>					
Tetrahydron	Linear Solution 1	0.0073	3.659	4.3828	1.1979
	Linear Solution 2	0.0220	10.976	11.9074	1.0848
	Linear solution 3	0.0585	29.270	32.1304	1.0977
	Linear solution 4	0.0732	36.588	38.7867	1.0601
	Linear Solution 5	0.1098	54.881	57.9125	1.0552
	Linear solution 6	0.1464	73.175	80.0393	1.0938
	RSD/% of the response factor		5		

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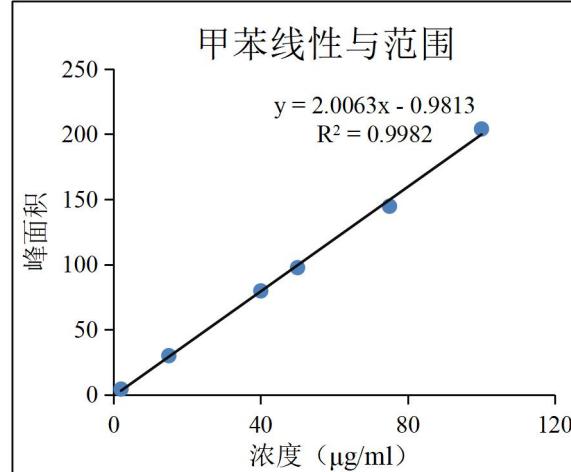
		<p style="text-align: center;">四氢呋喃线性与范围</p> <p style="text-align: center;"><math>y = 1.0779x + 0.0623</math> <math>R^2 = 0.999</math></p>			
	Linear graph				
	Linear equations	$Y = 1.0779 + 0.0623 x$			
	Correlation coefficient R	0.9995			
	Absolute value of Y-axis intercept as a percentage /% of 100% response value	0.16			
<p>The verdict: In the concentration range of 3.659<math>\mu\text{g}/\text{ml}</math> ~ 73.175<math>\mu\text{g}/\text{ml}</math>, with the concentration as the horizontal coordinate and the peak area as the vertical coordinate as the standard curve, the obtained linear equation is <math>y=1.0779x+0.0623</math>, and the correlation coefficient is 0.9995 (the requirement is NLT 0.990). The RSD of the response factor is 5% (NMT 10%), and the absolute value of the Y-axis intercept accounts for 0.16% of the 100% response value (required within 25%); All the above met the verification requirements, indicating that the analytical method for the detection of tetrahydrofuran has a good linear relationship in the concentration range of 3.659<math>\mu\text{g}/\text{ml}</math> ~ 73.175<math>\mu\text{g}/\text{ml}</math>.</p>					
Toluene	Linear solution 1	0.0040	2.001	4.2723	2.1351
	Linear Solution 2	0.0300	15.008	29.9444	1.9953
	Linear Solution 3	0.0800	40.020	79.6923	1.9913
	Linear solution 4	0.1001	50.025	97.5511	1.9500
	Linear solution 5	0.1501	75.038	144.6969	1.9283
	Linear solution 6	0.2001	100.050	204.0250	2.0392
	RSD/% of the response factor	4			

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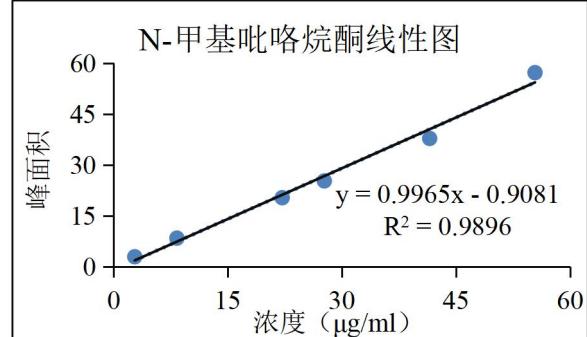
		 <p style="text-align: center;">甲苯线性与范围</p> <p style="text-align: center;"><math>y = 2.0063x - 0.9813</math> <math>R^2 = 0.9982</math></p>			
	Linear graph				
	Linear equations	$Y = 2.0063 - 0.9813 x$			
	Correlation coefficient R	0.999			
	Absolute value of Y-axis intercept as a percentage /% of 100% response value	1.1			
	<p>The verdict: The concentration of toluene in the range of 2.001μg/ml ~ 100.050μg/ml, with the concentration as the horizontal coordinate and the peak area as the vertical coordinate as the standard curve, the obtained linear equation is <math>y=2.0063x-0.9813</math>, the correlation coefficient is 0.999 (the requirement is NLT 0.990). RSD of response factor is 4% (NMT 10%), the absolute value of Y-axis intercept accounts for 1.1% of 100% response value (required within 25%); All the above meet the verification requirements, indicating that the analytical method has a good linear relationship in the concentration range of 2.001μg/ml ~ 100.050μg/ml.</p>				
N-m ethylpyrrolidine	Linear Solution 1	0.0055	2.769	2.9231	1.0557
	Linear Solution 2	0.0166	8.306	8.4062	1.0120
	Linear Solution 3	0.0443	22.150	20.3112	0.9170
	Linear solution 4	0.0554	27.688	25.2571	0.9122
	Linear Solution 5	0.0831	41.531	37.7702	0.9094
	Linear solution 6	0.1108	55.375	57.1453	1.0320
	RSD/% of the response factor		7		

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	Linear graph	
	Linear equations	$Y = 0.9965 - 0.9081 \times$
	Correlation coefficient R	0.995
	Absolute value of Y-axis intercept as a percentage /% of 100% response value	3.6
<p>The verdict: N-methylpyrrolidone was obtained in the concentration range of 2.769<math>\mu</math>g/ml ~ 55.375<math>\mu</math>g/ml, with the concentration as the horizontal coordinate and the peak area as the vertical coordinate as the standard curve. The obtained linear equation was <math>y=0.9965x-0.9081</math>, and the correlation coefficient was 0.995 (the requirement was NLT 0.990). RSD of response factor was 7% (NMT 10%), the absolute value of Y-axis intercept accounted for 3.6% of 100% response value (required within 25%); All the above met the verification requirements, indicating that the analytical method has a good linear relationship in the concentration range of 2.769<math>\mu</math>g/ml ~ 55.375<math>\mu</math>g/ml for the detection of N-methylpyrrolidone.</p>		

### (4.5) Accuracy

#### ➤ Brief Description

Add each solvent reference in the limit concentration range of 30%~150% to the test product, the recovery rate of each solvent is required to be between 85% ~ 115%, and the recovery RSD NMT 10%.

#### ➤ Solution preparation

Blank solution: solvent (N, n-dimethylformamide).

Ethanol reference stock: Share ethanol reference stock under "4.1 System Suitability". (Approx. 50,000  $\mu$ g/ml)

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Isopropyl alcohol reference reserve: Share isopropyl alcohol reference reserve under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Ethyl acetate reference reserve: Share the ethyl acetate reference reserve under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Secondary butanol reference product reserve: Share secondary butanol versus reference product reserve solution under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Tetrahydrofuran reference storage solution: Share tetrahydrofuran reference storage solution under "4.1 System Suitability". (Approx. 7200µg/ml)

Toluene reference reserve: Share toluene reference reserve under "4.1 System Suitability". (Approx. 8900µg/ml)

N-methylpyrrolidone reference stock: Share the N-methylpyrrolidone reference stock under "4.1 System Suitability". (Approx. 5300µg/ml)

Recovery reserve: Share linear reserve under "4.4 linear and range"

Control product solution: accurately measure the recovery rate of the reserve liquid 1.0ml, put it in a 10ml measuring bottle, and then dilute it with solvent to the scale, shake well, and then obtain.

Test product solution: Accurately weigh about 0.5g of this product, place it in 10ml measuring bottle, dissolve it with solvent and dilute it to the scale, shake well, ready. (Parallel preparation of 2 servings)

30% sample recovery solution: precision weigh about 0.5g of this product, precision weigh, put in 10ml measuring bottle, then precision measure the recovery of 0.3ml of reserve liquid placed in the same measuring bottle, dissolve with solvent and dilute to the scale, shake well, and then get. (Parallel preparation of 3 parts)

100% sample recovery solution: precision weigh about 0.5g of this product, precision weigh, put in 10ml measuring bottle, then precision measure the recovery of reserve liquid 1.0ml put in the same measuring bottle, dissolve with solvent and dilute to the scale, shake well, and then get. (Parallel preparation of 3 parts)

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150% sample recovery solution: precision weigh about 0.5g of this product, precision weigh, put in 10ml measuring bottle, then precision measure the recovery reserve liquid 1.5ml put in the same measuring bottle, dissolve with solvent and dilute to the scale, shake well, and then get. (Parallel preparation of 3 parts)

### ➤ **Injection procedure**

After the blank solution, the control product solution, the test product solution and the recovery solution of each concentration were injected respectively, and the chromatogram was recorded.

### ➤ **Measurement resultss**

Accuracy test results

Substance Name	Solution name	Original quantity in test product (μg/ml)	Amount added (μg/ml)	Measured amount (μg/ml)	Recovery rate /%				
Ethanol	30% sample recovery solution 1	51.2415	75.0833	122.8447	95.4				
	30% addition recovery solution 2	51.3641	75.0833	124.7496	97.7				
	30% addition recovery solution 3	51.2293	75.0833	123.6445	96.4				
	100% sample recovery solution 1	51.1619	250.2775	303.9775	101.0				
	100% addition recovery solution 2	51.1169	250.2775	304.6158	101.3				
	100% addition recovery solution 3	51.3560	250.2775	305.8432	101.7				
	150% addition recovery solution 1	51.4449	375.4163	466.2005	110.5				
	150% addition recovery solution 2	51.2569	375.4163	438.8007	103.2				
	150% addition recovery solution 3	51.1138	375.4163	440.8515	103.8				
	Average recovery /%				101.2				
Recovery rate RSD/%					5				
Conclusion: Ethanol was detected in the samples, and 75.0833μg/ml-375.4163μg/ml ethanol control was added into the samples. The recoveries of 9 samples were all in the range of 95.4%-110.5% (the requirement was 85%-115%), and the average recovery was 101.2%. The recovery RSD was 5% (the requirement was not more than 10%); All the above met the verification requirements, indicating that the method was accurate in detecting solvent ethanol in YA2304.									
Substances Name	Solution name	Original amount in test product	Amount added (μg/ml)	Measured amount (μg/ml)	Recovery rate /%				

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		( $\mu\text{g}/\text{ml}$ )			
Isopropyl alcohol	30% sample recovery solution 1	Not detected	75.1230	74.0095	98.5
	30% sample recovery solution 2	Not detected	75.1230	74.8906	99.7
	30% sample recovery solution 3	Not detected	75.1230	74.0543	98.6
	100% sample recovery solution 1	Not detected	250.4100	252.7144	100.9
	100% sample recovery solution 2	Not detected	250.4100	253.0627	101.1
	100% sample recovery solution 3	Not detected	250.4100	253.9289	101.4
	150% sample recovery solution 1	Not detected	375.6150	409.5334	109.0
	150% sample recovery solution 2	Not detected	375.6150	387.2121	103.1
	150% sample recovery solution 3	Not detected	375.6150	387.0754	103.1
	Average recovery rate /%				101.7
Recovery rate RSD/%					4
Conclusions: Isopropyl alcohol was not detected in the samples, and 75.1230 $\mu\text{g}/\text{ml}$ -375.6150 $\mu\text{g}/\text{ml}$ isopropyl alcohol was added into the samples. The recoveries of 9 samples were all in the range of 98.5%-109.0% (the requirements were in the range of 85%-115%), and the average recovery was 101.7%. The recovery RSD was 4% (the requirement was not more than 10%); All the above met the verification requirements, indicating that the method was accurate in detecting isopropyl alcohol in YA2304.					
Substances Name	Solution name	Original amount in test product ( $\mu\text{g}/\text{ml}$ )	Amount added ( $\mu\text{g}/\text{ml}$ )	Measured amount ( $\mu\text{g}/\text{ml}$ )	Recovery rate /%
Ethyl acetate	30% sample recovery solution 1	Not detected	74.6085	73.3037	98.3
	30% sample recovery solution 2	Not detected	74.6085	74.2183	99.5
	30% sample recovery solution 3	Not detected	74.6085	73.1270	98.0
	100% sample recovery solution 1	Not detected	248.6950	250.6196	100.8
	100% recovery solution 2	Not detected	248.6950	251.0619	101.0
	100% sample recovery solution 3	Not detected	248.6950	252.0282	101.3
	150% sample recovery solution 1	Not detected	373.0425	402.2260	107.8
	150% sample recovery solution 2	Not detected	373.0425	383.3969	102.8
	150% sample recovery solution 3	Not detected	373.0425	384.7227	103.1
	Average recovery rate /%				101.4
Recovery rate RSD/%					3.0
Conclusions: No ethyl acetate was detected in the samples. 74.6085 $\mu\text{g}/\text{ml}$ -373.0425 $\mu\text{g}/\text{ml}$ ethyl acetate was added into the samples.					

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	The recoveries of 9 samples were all in the range of 98.0%-107.8% (the requirement was 85%-115%), and the average recoveries were 101.4%. The recovery RSD was 3.0% (no more than 10% required); All the above met the verification requirements, indicating that the method was accurate in detecting ethyl acetate in YA2304.				
Substances Name	Solution name	Original quantity in test product (µg/ml)	Amount added (µg/ml)	Measured amount (µg/ml)	Recovery rate /%
sec-butano 1	30% sample recovery solution 1	Not detected	75.1755	74.5260	99.1
	30% sample recovery solution 2	Not detected	75.1755	75.1045	99.9
	30% sample recovery solution 3	Not detected	75.1755	74.1757	98.7
	100% sample recovery solution 1	Not detected	250.5850	254.1755	101.4
	100% sample recovery solution 2	Not detected	250.5850	254.9211	101.7
	100% sample recovery solution 3	Not detected	250.5850	255.8160	102.1
	150% sample recovery solution 1	Not detected	375.8775	412.4938	109.7
	150% sample recovery solution 2	Not detected	375.8775	389.1479	103.5
	150% sample recovery solution 3	Not detected	375.8775	390.5969	103.9
	Average recovery rate /%				102.2
	Recovery rate RSD/%				4
	Conclusions: No secondary butanol was detected in the samples. 75.1755µg/ml-375.8775µg/ml of secondary butanol was added into the samples. The recoveries of 9 samples were all in the range of 98.7%-109.7% (the requirement was 85%-115%), and the average recoveries were 102.2%. The RSD of recovery was 4% (NMT 10%); All the above met the verification requirements, indicating that the method was accurate in detecting secondary butanol in YA2304.				
Substances Name	Solution name	Original amount in test product (µg/ml)	Amount added (µg/ml)	Measured amount (µg/ml)	Recovery rate /%
tetrahydrofuran	30% sample recovery solution 1	Not detected	10.9763	10.8178	98.6
	30% sample recovery solution 2	Not detected	10.9763	10.9247	99.5
	30% sample recovery solution 3	Not detected	10.9763	10.7783	98.2
	100% sample recovery solution 1	Not detected	36.5875	36.8776	100.8
	100% sample recovery solution 2	Not detected	36.5875	37.0832	101.4
	100% sample recovery solution 3	Not detected	36.5875	36.9783	101.1
	150% sample recovery solution 1	Not detected	54.8813	58.3538	106.3

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Toluene	150% recovery solution 2	Not detected	54.8813	56.3252	102.6
	150% sample recovery solution 3	Not detected	54.8813	56.8066	103.5
	Average recovery rate /%				101.3
	Recovery rate RSD/%				2.6
	Conclusions: Tetrahydrofuran was not detected in the test products, and 10.9763µg/ml-54.8813µg/ml of tetrahydrofuran was added into the test products. The recovery rates of 9 samples were all in the range of 98.2%-106.3% (the requirement was 85%-115%), and the average recovery rate was 101.3%. The recovery RSD was 2.6% (the requirement was not more than 10%); All the above met the verification requirements, indicating that the method was accurate in detecting tetrahydrofuran in YA2304.				
	Substances Name	Solution name	Original amount in test product (µg/ml)	Amount added (µg/ml)	Measured amount (µg/ml)
	30% sample recovery solution 1	Not detected	15.0075	14.9077	99.3
	30% sample recovery solution 2	Not detected	15.0075	15.0652	100.4
	30% sample recovery solution 3	Not detected	15.0075	14.8431	98.9
	100% sample recovery solution 1	Not detected	50.0250	50.4010	100.8
	100% sample recovery solution 2	Not detected	50.0250	50.3329	100.6
	100% sample recovery solution 3	Not detected	50.0250	50.7148	101.4
	150% sample recovery solution 1	Not detected	75.0375	80.9908	107.9
	150% sample recovery solution 2	Not detected	75.0375	76.8254	102.4
	150% sample recovery solution 3	Not detected	75.0375	77.1206	102.8
Average recovery rate /%				101.6	
Recovery rate RSD/%				2.7	
Conclusions: Toluene was not detected in the test products, and toluene control products of 15.0075µg/ml-75.0375µg/ml were added into the test products. The recoveries of 9 samples were all in the range of 98.9%-107.9% (the requirements were in the range of 85%-115%), and the average recoveries were 101.6%. The recovery RSD was 2.7% (no more than 10% required); All the above met the verification requirements, indicating that the method was accurate in detecting toluene in YA2304.					
Substances Name	Solution name	Original amount in test product (µg/ml)	Amount added (µg/ml)	Measured amount (µg/ml)	Recovery rate /%
N-methylpyrrolidone	30% sample recovery solution 1	Not detected	8.3063	7.5231	90.6
	30% sample recovery solution 2	Not detected	8.3063	7.7719	93.6
	30% sample recovery solution 3	Not detected	8.3063	8.1034	97.6

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100% sample recovery solution 1	Not detected	27.6875	26.8509	97.0
100% sample recovery solution 2	Not detected	27.6875	27.1560	98.1
100% sample recovery solution 3	Not detected	27.6875	26.9525	97.3
150% sample recovery solution 1	Not detected	41.5313	46.2093	111.3
150% sample recovery solution 2	Not detected	41.5313	42.9870	103.5
150% sample recovery solution 3	Not detected	41.5313	39.6163	95.4
Average recovery rate /%				98.3
Recovery rate RSD/%				7
Conclusion: N-methylpyrrolidone was not detected in the samples, and 8.3063 $\mu$ g/ml-41.5313 $\mu$ g/ml of N-methylpyrrolidone was added into the samples. The recoveries of 9 samples were all in the range of 90.6%-111.3% (the requirement was 85%-115%), and the average recoveries were 98.3%. The recovery RSD was 7% (no more than 10% required); All the above met the verification requirements, indicating that the method was accurate in detecting N-methylpyrrolidone in YA2304.				

### **(4.6) Precision**

Precision refers to the proximity between the results obtained by multiple sampling of the same uniform test product under specified test conditions, and its precision is judged by examining repeatability and reproducibility.

#### **(4.6.1) Repeatability**

##### **➤ Acceptance Criteria**

6 repeatable sample solutions were prepared by analyst A on date A and measured by instrument A; The blank solution should be free from interference; In the chromatogram obtained from the control product solution, ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene, n-methylpyrrolidone peaks in turn, and the separation degree between each adjacent peak should be greater than 1.5. The RSD of each solvent in the 6 repeatable test product solutions NMT 10%.

##### **➤ Solution preparation**

Blank solution: solvent (N, n-dimethylformamide).

Ethanol reference stock: Share ethanol reference stock under "4.1 System

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Suitability". (Approx. 50,000 µg/ml)

Isopropyl alcohol reference reserve: Share isopropyl alcohol reference reserve under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Ethyl acetate reference reserve: Share the ethyl acetate reference reserve under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Secondary butanol reference product reserve: Share secondary butanol versus reference product reserve solution under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Tetrahydrofuran reference storage solution: Share tetrahydrofuran reference storage solution under "4.1 System Suitability". (Approx. 7200µg/ml)

Toluene reference reserve: Share toluene reference reserve under "4.1 System Suitability". (Approx. 8900µg/ml)

N-methylpyrrolidone reference stock: Share the N-methylpyrrolidone reference stock under "4.1 System Suitability". (Approx. 5300µg/ml)

Repeatable reference product reserve liquid: precision measure ethanol reference product reserve liquid, isopropyl alcohol reference product reserve liquid, ethyl acetate reference product reserve liquid, secondary butanol reference product reserve liquid, tetrahydrofuran reference product reserve liquid, toluene reference product reserve liquid, N-methylpyrrolidone reference product reserve liquid 1.0ml each, put in 20ml bottle, dilute with solvent to scale, shake well, and obtain. (Ethanol about 2500µg/ml, isopropyl alcohol about 2500µg/ml, ethyl acetate about 2500µg/ml, secondary butanol about 2500µg/ml, tetrahydrofuran about 370µg/ml, toluene about 445µg/ml, N-methylpyrrolidone about 265µg/ml)

Reference product solution: Accurately measure 1.0ml of the duplicate reference product reserve solution, place it in a 10ml bottle, dilute it with solvent to the scale, and shake well. (Ethanol about 250µg/ml, isopropyl alcohol about 250µg/ml, ethyl acetate about 250µg/ml, secondary butanol about 250µg/ml, tetrahydrofuran about 37µg/ml, toluene about 44.5µg/ml, N-methylpyrrolidone about 26.5µg/ml)

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Repeatable test product solution: accurately weigh about 0.5g of this product, accurately weigh it, put it in 10ml measuring bottle, then accurately measure 1.0ml of repeatable control product reserve liquid, put it in the same measuring bottle, dissolve it with solvent and dilute it to the scale, shake well, and obtain. (Parallel preparation of 6 parts)

### ➤ **Injection procedure**

After the blank solution, the control solution and 6 repetitive test product solutions were injected respectively, and the chromatogram was recorded.

### ➤ **Measurement resultss**

Results of repeatability - system suitability test

Name of solvent	Degree of separation	
	Reference solution -1	Control solution -2
Ethanol	/	/
Isopropyl alcohol	8.5	8.5
Ethyl acetate	28.6	28.6
sec-butanol	1.8	1.8
tetrahydrofuran	1.9	1.9
Toluene	37.8	37.6
N-methylpyrrolidone	93.7	93.9

Conclusion: The blank solution has no interference; Ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene and n-methylpyrrolidone peaked in sequence in the chromatogram obtained from the control solution, and the separation degree between each adjacent solvent peak was greater than 1.5 (the requirement was greater than 1.5). The suitability of the system meets the requirements.

Repeatability test results

Solution name Name of solvent	Residual solvent content /%						
	Repetiti ve test produc t solutio n 1	Repetiti ve test produc t solutio n 2	Repetiti ve test produc t solutio n 3	Repetiti ve test produc t solutio n 4	Repetiti ve test produc t solutio n 5	Repetiti ve test produc t solutio n 6	RSD/ % (n=6)
Ethanol	0.5754	0.5806	0.5779	0.5758	0.5773	0.5687	0.7
Isopropyl	0.4952	0.4992	0.4955	0.4953	0.4918	0.4882	0.8

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alcohol							
Ethyl acetate	0.5128	0.5167	0.5124	0.5123	0.5097	0.5048	0.8
Sec-butyl alcohol	0.4972	0.4949	0.4925	0.4977	0.4880	0.4893	0.9
tetrahydrofuran	0.0756	0.0750	0.0748	0.0753	0.0740	0.0738	1.0
Toluene	0.1033	0.1043	0.1031	0.1034	0.1027	0.1013	1.0
N-methylpyrrolidone	0.0592	0.0584	0.0583	0.0579	0.0574	0.0559	2.0

Verdict: The RSD of ethanol content was 0.7% (required less than 10%), the RSD of isopropyl alcohol content was 0.8% (required less than 10%), the RSD of ethyl acetate content was 0.8% (required less than 10%), and the RSD of secondary butanol content was 0.9% (required less than 10%). RSD of tetrahydrofuran was 1.0% (NMT 10%), RSD of toluene was 1.0% (NMT 10%), and RSD of N-methylpyrrolidone was 2.0% (NMT 10%); All the above met the verification requirements, indicating that the method had good repeatability.

### **(4.6.2) Reproducibility**

#### **➤ Acceptance Criteria**

6 reproducible sample solutions were prepared by Analyst B on date B and determined using analytical laboratory instruments. The blank solution should be free of interference; In the chromatogram obtained for 6 consecutive days, ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene, n-methylpyrrolidone peaks in turn, and the separation degree between each adjacent peak should be greater than 1.5. The RSD of the peak retention time of each solvent peak should NMT 1.0%, and the RSD of the peak area should NMT 10%. The RSD of each solvent in 6 reproducible sample solutions NMT 10%;

In the results of 12 repeatability and reproducibility tests, the RSD of each solvent content NMT 10%.

#### **➤ Solution preparation**

Finerenone

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Hinye Pharmaceutical Co., Ltd.

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Blank solution: solvent (N, n-dimethylformamide).

Ethanol reference product reserve liquid: precision weigh ethanol about 1000mg, put in 20ml bottle, dissolve with solvent and dilute to the scale, shake well, ready. (About 50000 $\mu$ g/ml)

Isopropyl alcohol reference product reserve liquid: precision weigh isopropyl alcohol about 1000mg, place in 20ml bottle, dissolve with solvent and dilute to the scale, shake well, ready. (About 50000 $\mu$ g/ml)

Ethyl acetate reference product reserve liquid: precision weigh about 1000mg of ethyl acetate, place in 20ml measuring bottle, dissolve and dilute with solvent to scale, shake well, ready. (Approx. 50000 $\mu$ g/ml)

Secondary butanol reference product reserve liquid: precision weigh secondary butanol about 1000mg, place in 20ml bottle, dissolve and dilute with solvent to scale, shake well, then get. (About 50000 $\mu$ g/ml)

Tetrahydrofuran reference product reserve liquid: accurately weigh tetrahydrofuran about 144mg, put it in 20ml bottle, dissolve it with solvent and dilute it to the scale, shake well, and then get. (About 7200 $\mu$ g/ml)

Toluene control product reserve liquid: precision weigh toluene about 178mg, place in 20ml bottle, dissolve and dilute with solvent to scale, shake well, ready. (About 8900 $\mu$ g/ml)

N-methylpyrrolidone reference product reserve liquid: accurately weigh N-methylpyrrolidone about 106mg, place in 20ml measuring bottle, dissolve and dilute with solvent to scale, shake well, then obtain. (About 5300 $\mu$ g/ml)

Repeatable reference product reserve liquid: precision measure ethanol reference product reserve liquid, isopropyl alcohol reference product reserve liquid, ethyl acetate reference product reserve liquid, secondary butanol reference product reserve liquid, tetrahydrofuran reference product reserve liquid, toluene reference product reserve liquid, N-methylpyrrolidone reference product reserve liquid 1.0ml each, put in a 20ml bottle, dilute with solvent to the scale, shake well, and obtain. (Ethanol about 2500 $\mu$ g/ml, isopropyl alcohol about 2500 $\mu$ g/ml, ethyl acetate about 2500 $\mu$ g/ml, secondary butanol about 2500 $\mu$ g/ml, tetrahydrofuran about 2500 $\mu$ g/ml, toluene about 2500 $\mu$ g/ml, N-methylpyrrolidone about 2500 $\mu$ g/ml)

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secondary butanol about 2500 $\mu$ g/ml, tetrahydrofuran about 360 $\mu$ g/ml, toluene about 445 $\mu$ g/ml, N-methylpyrrolidone about 265 $\mu$ g/ml)

Reference solution: Accurately measure 1.0ml of the reproducible reference reserve solution, place it in a 10ml measuring bottle, dilute it with solvent to the scale, shake well, and obtain. (Ethanol about 250 $\mu$ g/ml, isopropyl alcohol about 250 $\mu$ g/ml, ethyl acetate about 250 $\mu$ g/ml, secondary butanol about 250 $\mu$ g/ml, tetrahydrofuran about 36 $\mu$ g/ml, toluene about 44.5 $\mu$ g/ml, N-methylpyrrolidone about 26.5 $\mu$ g/ml)

Reproducible test product solution: accurately weigh about 0.5g of this product, accurately weigh it, place it in 10ml measuring bottle, then accurately measure 1.0ml of reproducible control product reserve liquid, place it in the same measuring bottle, dissolve it with solvent and dilute it to the scale, shake well, and obtain. (Parallel preparation of 6 servings)

### ➤ **Injection procedure**

Reproducibility - System suitability: After the blank solution, continuous injection 6 for the sample solution, record the chromatogram.

Reproducibility - Sample testing: After the blank solution, continuously inject 2 samples for the image solution and 6 reproducible test solution, and record the chromatogram.

### ➤ **Measurement results**

Reproducibility - system suitability test results

Name of solvent	Parameter values	Reference solution was injected						RSD /%
		1	2	3	4	5	6	
Ethanol	RT (min)	8.529	8.529	8.529	8.525	8.527	8.529	0.020
	Peak area	222.63 71	225.75 16	225.22 03	224.29 74	223.14 25	221.57 64	0.8
	Separation	/	/	/	/	/	/	/
Isopropyl alcohol	RT (min)	9.933	9.932	9.932	9.928	9.930	9.932	0.019
	Peak area	221.84 10	224.06 69	225.77 57	223.69 06	221.82 37	221.34 30	0.8

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	Separation	8.8	8.7	8.7	8.7	8.8	8.9	/
Ethyl acetate	RT (min)	14.546	14.545	14.546	14.543	14.546	14.547	0.010
	Peak area	184.41 56	187.26 00	187.34 29	185.48 64	185.10 12	183.09 72	0.9
	Separation	29.0	28.9	28.8	28.9	29.0	29.1	/
Secondary butanol	RT (min)	14.815	14.815	14.816	14.813	14.816	14.817	0.010
	Peak area	291.66 44	297.07 76	297.74 93	295.10 50	294.48 60	290.67 89	1.0
	Separation	1.7	1.7	1.7	1.7	1.7	1.7	/
tetrahydrofuran	RT (min)	15.125	15.123	15.124	15.122	15.123	15.126	0.010
	Peak area	39.618 2	41.084 2	40.338 7	40.160 9	41.254 7	39.426 3	1.9
	Separation	2.0	2.0	2.0	2.0	2.0	2.0	/
Toluene	RT (min)	20.400	20.398	20.398	20.398	20.401	20.401	0.008
	Peak area	94.967 9	96.218 0	96.464 1	95.508 1	95.164 9	94.401 7	0.9
	Separation	37.4	37.2	37.4	37.3	37.2	37.4	/
N-methylpyrrolidone	RT (min)	31.551	31.549	31.549	31.549	31.551	31.552	0.005
	Peak area	32.709 8	32.704 6	32.738 0	31.226 5	33.035 4	32.010 2	3.1
	Separation	92.4	92.6	92.5	92.8	92.7	92.6	/
Conclusion: There was no interference in the blank solution. In the chromatogram obtained for 6 consecutive days, peaks of ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene and n-methylpyrrolidone appeared successively, and the separation degree between each adjacent solvent peak was greater than 1.5 (the requirement was greater than 1.5). The RSD of ethanol peak retention time was 0.020% (required less than 1.0%), and the RSD of peak area was								

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0.8% (required less than 10%). The RSD of isopropyl alcohol peak retention time was 0.019% (NMT 1.0%), and the RSD of peak area was 0.8% (NMT 10%); The RSD of the ethyl acetate peak was 0.010% (NMT 1.0%), and the RSD of the peak area was 0.9% (NMT 10%); The RSD of secondary butanol was 0.010% (NMT 1.0%), and the RSD of peak area was 1.0% (NMT 10%); The RSD of the peak retention time of tetrahydrofuran was 0.010% (NMT 1.0%), and the RSD of the peak area was 1.9% (NMT 10%); The RSD of toluene peak was 0.008% (NMT 1.0%), and the RSD of peak area was 0.9% (NMT 10%); The RSD of the N-methylpyrrolidone peak was 0.005% (NMT 1.0%), and the RSD of the peak area was 2.1% (NMT 10%); The suitability of the system met the verification requirements.

### Precision (repeatability and reproducibility) test results

Solution name Solvent name		Residual solvent content /%						RSD (n=6)	
		Repeatability							
		1	2	3	4	5	6		
Repeatability	Ethanol	0.5754	0.5806	0.5779	0.5758	0.5773	0.5687	0.7%	
	Isopropyl alcohol	0.4952	0.4992	0.4955	0.4953	0.4918	0.4882	0.8%	
	Ethyl acetate	0.5128	0.5167	0.5124	0.5123	0.5097	0.5048	0.8%	
	sec-butanol	0.4972	0.4949	0.4925	0.4977	0.4880	0.4893	0.9%	
	tetrahydrofuran	0.0756	0.0750	0.0748	0.0753	0.0740	0.0738	1.0%	
	Toluene	0.1033	0.1043	0.1031	0.1034	0.1027	0.1013	1.0%	
	N-methylpyrrolidone	0.0592	0.0584	0.0583	0.0579	0.0574	0.0559	2.0%	
Reproducibility	Ethanol	0.5943	0.6102	0.6295	0.5872	0.5492	0.5650	5%	
	Isopropyl alcohol	0.4836	0.4946	0.5138	0.4762	0.4493	0.4600	5%	
	Ethyl acetate	0.5049	0.5139	0.5255	0.4973	0.4681	0.4797	5%	
	sec-butanol	0.4795	0.4912	0.5135	0.4720	0.4461	0.4571	6%	
	tetrahydrofuran	0.0775	0.0804	0.0815	0.0782	0.0723	0.0731	5%	
	Toluene	0.0996	0.1014	0.1042	0.0982	0.0926	0.0947	5%	
	N-methylpyrrolidone	0.0502	0.0505	0.0541	0.0490	0.0475	0.0492	5%	
Precision RSD		4%	4%	4%	4%	4%	9%	/	

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(n=12)								
Verdict: The RSD of ethanol content in 6 reproducible samples was 0.7% (required less than 10%), the RSD of isopropyl alcohol content was 0.8% (required less than 10%), the RSD of ethyl acetate content was 0.8% (required less than 10%) and the RSD of secondary butanol content was 0.9% (required less than 10%). RSD of tetrahydrofuran was 1.0% (NMT 10%), RSD of toluene was 1.0% (NMT 10%), and RSD of N-methylpyrrolidone was 2.0% (NMT 10%); All the above met the verification requirements, indicating that the method has good reproducibility. The reproducibility and intermediate precision 12 results showed that RSD of ethanol content was 4% (NMT 10%), RSD of isopropyl alcohol content was 4% (NMT 10%), RSD of ethyl acetate content was 4% (NMT 10%), and RSD of secondary butanol content was 4% (NMT 10%). RSD of tetrahydrofuran was 4% (NMT 10%), RSD of toluene was 4% (NMT 10%), and RSD of N-methylpyrrolidone was 9% (NMT 10%); All the above meet the verification requirements, indicating that the method is of good precision.								

### **(4.7) Durability**

#### ➤ **Brief Description**

By changing the chromatographic conditions (changing the initial temperature, flow rate, column) and normal chromatographic conditions to test the solvent content of each test product solution to achieve, the chromatographic diagram obtained by the control product solution, methyl ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene, n-methylpyrrolidone peaks in turn, the separation degree between the adjacent peaks should be greater than 1.5. Under different conditions of the same chromatographic parameters, the RSD of each solvent content to be measured in the test product solution should not be 10%.

Changes in chromatographic conditions for durability test

Chromatographic Parameters	Standard conditions	Variable Values
Flow rate	3.5 ml/min	3.4ml/min, 3.6ml/min
Initial column temperature	40 °C	35°C, 45°C
Column	Column 1	Column 2

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	(DB-624, 60m*0.53mm, 3.0µm, SN: US1501943H, Serial number: CXGC-1)	(DB-624, 60m*0.53mm, 3.0µm, SN: US1111842H, Serial number: YLGC-2)
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### ➤ **Solution preparation**

Blank solution: solvent (N, n-dimethylformamide).

Ethanol reference stock: Share ethanol reference stock under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Isopropyl alcohol reference reserve: Share isopropyl alcohol reference reserve under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Ethyl acetate reference reserve: Share the ethyl acetate reference reserve under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Secondary butanol reference product reserve: Share secondary butanol versus reference product reserve solution under "4.1 System Suitability". (Approx. 50,000 µg/ml)

Tetrahydrofuran reference storage solution: Share tetrahydrofuran reference storage solution under "4.1 System Suitability". (Approx. 7200µg/ml)

Toluene reference reserve: Share toluene reference reserve under "4.1 System Suitability". (Approx. 8900µg/ml)

N-methylpyrrolidone reference stock: Share the N-methylpyrrolidone reference stock under "4.1 System Suitability". (Approx. 5300µg/ml)

Durable reference product reserve liquid: precision measure ethanol reference product reserve liquid, isopropyl alcohol reference product reserve liquid, ethyl acetate reference product reserve liquid, secondary butanol reference product reserve liquid, tetrahydrofuran reference product reserve liquid, toluene reference product reserve liquid, N-methylpyrrolidone reference product reserve liquid 1.0ml each, put in 20ml bottle, dilute with solvent to the scale, shake well, ready. (Ethanol about 2500µg/ml, isopropyl alcohol about 2500µg/ml, ethyl acetate about 2500µg/ml, secondary butanol about 2500µg/ml, tetrahydrofuran about 360µg/ml, toluene about 445µg/ml, N-methylpyrrolidone about 265µg/ml)

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Durability reference product solution: accurately measure 1.0ml of the durability reference product reserve solution, place it in a 10ml measuring bottle, dilute it with solvent to the scale, shake well, and obtain. (Ethanol about 250 $\mu$ g/ml, isopropyl alcohol about 250 $\mu$ g/ml, ethyl acetate about 250 $\mu$ g/ml, secondary butanol about 250 $\mu$ g/ml, tetrahydrofuran about 36 $\mu$ g/ml, toluene about 44.5 $\mu$ g/ml, N-methylpyrrolidone about 26.5 $\mu$ g/ml)

Durability test product solution: precision weigh about 0.5g of this product, precision weigh, put in 10ml measuring bottle, then precision measure the durability control product reserve liquid 1.0ml put in the same measuring bottle, dissolve with solvent and dilute to the scale, shake well, and then get. (Parallel preparation of 2 servings)

### ➤ **Injection procedure**

Under each chromatographic condition, after the blank solution, the control product solution and the test product solution were injected respectively, and the chromatogram was recorded.

### ➤ **Measurement resultss**

Results of durability - system suitability test

Name of solvent	Sample injection Number of times	Degree of separation						
		Ethan ol	Isopro pyl alcoh ol	Ethyl acetate	sec-b utano 1	tetrah ydrof uran	Tolue ne	N-methy lpyrrolid one
Normal conditions	1	/	8.8	28.9	1.8	1.9	37.9	94.7
	2	/	8.7	29.0	1.8	1.9	37.9	95.0
Flow rate (3.4ml/min)	1	/	8.9	29.3	1.8	2.0	38.1	93.6
	2	/	8.8	29.0	1.8	2.0	38.2	94.0
Flow rate (3.6ml/min)	1	/	8.7	28.9	1.8	1.9	37.9	96.0
	2	/	8.5	28.6	1.8	1.8	37.8	95.7
Initial column temperature (35 ° C)	1	/	9.2	29.6	1.9	1.8	38.4	94.7
	2	/	9.3	29.8	1.9	1.8	38.4	94.7

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Initial column temperature (45 ° C)	1 2	/	8.2 8.3	28.3 28.3	1.7 1.7	2.0 2.1	37.5 37.4	94.4 94.7
Column 2	1	/	8.8	29.2	1.8	1.9	38.0	93.6
	2	/	8.9	29.3	1.8	1.9	37.8	94.0

### Durability test results

Changing conditions		Number of injections	Residual solvent content /%						
			Ethanol	Isopropyl alcohol	Ethyl acetate	sec-butanol	tetrahydrofuran	Toluene	N-methylpyrrolidone
Velocity of flow	3.4 ml/min	1	0.5963	0.5123	0.5227	0.5077	0.0764	0.1053	0.0556
		2	0.5942	0.5115	0.5232	0.5096	0.0768	0.1052	0.0554
	3.5 ml/min	1	0.5810	0.4949	0.5091	0.4944	0.0745	0.1025	0.0576
		2	0.5730	0.4880	0.5019	0.4884	0.0733	0.1009	0.0609
	3.6 ml/min	1	0.5735	0.4939	0.5041	0.4911	0.0735	0.1021	0.0556
		2	0.5790	0.4967	0.5095	0.4972	0.0743	0.1025	0.0589
	RSD/% (n=6)	1.8	2.1	1.8	1.8	2.0	1.8	4	
	35 °C	1	0.5791	0.4968	0.5101	0.4956	0.0743	0.1033	0.0605
		2	0.5811	0.4963	0.5098	0.5016	0.0735	0.1029	0.0618
	40 °C	1	0.5810	0.4949	0.5091	0.4944	0.0745	0.1025	0.0576
		2	0.5730	0.4880	0.5019	0.4884	0.0733	0.1009	0.0609
	45 °C	1	0.5829	0.4985	0.5117	0.5002	0.0738	0.1039	0.0613
		2	0.5793	0.4966	0.5114	0.4977	0.0738	0.1032	0.0591
at	RSD/% (n=6)	0.6	0.8	0.8	1.0	0.7	1.0	2.6	

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u re									
C ol u m n	Colu mn 1	1	0.5963	0.5123	0.5227	0.5077	0.0764	0.105 3	0.0556
		2	0.5942	0.5115	0.5232	0.5096	0.0768	0.105 2	0.0554
	Colu mn 2	1	0.5669	0.4898	0.5009	0.4891	0.0723	0.102 0	0.0576
		2	0.5752	0.4921	0.5071	0.4964	0.0750	0.102 4	0.0559
	RSD/% (n=4)	2.5	2.5	2.2	2.0	2.8	1.8	1.8	
Conclusion: The blank solution has no interference; Ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene and n-methylpyrrolidone peaks in the chromatogram obtained from the control solution with the same chromatographic parameters under different conditions, and the separation degree between each adjacent solvent peak is greater than 1.5 (the requirement is greater than 1.5). RSDS of each solvent content in the test product solution under different conditions of the same chromatographic parameters were all less than 10% (the requirement is not greater than 10%); All the above meet the requirements, indicating that the method has good durability.									

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### III. Summary of Method Validation

#### Summaries of Method Validation of Residual Solvents in Finerenone

<b>Project</b>	<b>Acceptable criteria</b>	<b>Verification of results</b>
System suitability	<p>The blank solution should be free of interference; In the chromatogram obtained for 6 consecutive days, ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene and n-methylpyrrolidone appeared successively, and the separation degree between each adjacent solvent peak was greater than 1.5 (the requirement was greater than 1.5). The RSD of the peak retention time of each solvent peak should NMT 1.0%, and the RSD of the peak area should NMT 10%.</p>	<p>There was no interference in the blank solution. In the chromatogram obtained from the irradiation solution for 6 consecutive years, the peaks of ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene and n-methylpyrrolidone appeared successively, and the separation degree between each adjacent solvent peak was greater than 1.5 (the requirement was greater than 1.5). The RSD of ethanol peak retention time was 0.13% (NMT 1.0%), and the RSD of peak area was 0.8% (NMT 10%). The RSD of isopropyl alcohol peak retention time was 0.12% (NMT 1.0%), and the RSD of peak area was 1.0% (NMT 10%); The RSD of the ethyl acetate peak was 0.08% (NMT 1.0%), and the RSD of the peak area was 0.9% (NMT 10%); The RSD of secondary butanol was 0.08% (NMT 1.0%), and the RSD of peak area was 0.9% (NMT 10%); The RSD of the peak retention time of tetrahydrofuran was 0.08% (NMT 1.0%), and the RSD of the peak area was 0.8% (NMT 10%); The RSD of toluene peak was 0.05% (NMT 1.0%), and the RSD of peak area was 1.0% (NMT 10%); The RSD of the N-methylpyrrolidone peak was 0.015% (NMT 1.0%), and the RSD of the peak area was 2.7% (NMT 10%); All the above meet the verification requirements, indicating that the method has good applicability in system.</p>
Specificity	<p>There was no interference in the blank solution. In the control solution, the peaks of ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene and n-methylpyrrolidone appeared</p>	<p>There was no interference in the blank solution. In the control solution, the peaks of ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene and n-methylpyrrolidone appeared successively, and the separation degree between the peaks of each adjacent solvent was greater than 1.5 (the requirement was greater than 1.5). The separation degree between individual solvent peaks and adjacent peaks in the specific mixed solution and the test solution is NLT 1.5, the retention time of each known solvent</p>

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	<p>successively, and the separation degree between the adjacent peaks should be greater than 1.5; The separation degree of each solvent peak and adjacent peaks in the specific mixed solution and the test product solution is NLT 1.5, the retention time of each known solvent peak in the specific mixed solution and the test product solution should be consistent with the retention time of each solvent positioning solution, and the positioning solution of other solvents (piperidine, benzene) has no interference with the target solvent peak.</p>	<p>peak in the specific mixed solution and the test solution is consistent with the retention time of each solvent positioning solution, and other solvent positioning solutions (benzene, piperidine) have no interference with the target solvent peak.</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Solution Name</th><th>Component name</th><th>Component concentration (<math>\mu\text{g}/\text{ml}</math>)</th><th>Rt (min)</th><th>Degree of separation from the previous peak</th><th>The percentage equivalent to the concentration of the principal component in the test solution</th></tr> </thead> <tbody> <tr> <td rowspan="7">Specific mixed solution</td><td>Ethanol</td><td>250.278</td><td>8.149</td><td>/</td><td>0.5%</td></tr> <tr> <td>Isopropyl alcohol</td><td>250.410</td><td>9.504</td><td>8.2</td><td>0.5%</td></tr> <tr> <td>Ethyl acetate</td><td>248.695</td><td>14.145</td><td>28.2</td><td>0.5%</td></tr> <tr> <td>sec-butanol</td><td>250.585</td><td>14.429</td><td>1.8</td><td>0.5%</td></tr> <tr> <td>tetrahydrofuran</td><td>36.588</td><td>14.728</td><td>1.9</td><td>0.072%</td></tr> <tr> <td>Toluene</td><td>50.025</td><td>20.061</td><td>37.5</td><td>0.089%</td></tr> <tr> <td>N-methylpyrrolidone</td><td>27.6875</td><td>31.390</td><td>94.3</td><td>0.053%</td></tr> </tbody> </table>							Solution Name	Component name	Component concentration ( $\mu\text{g}/\text{ml}$ )	Rt (min)	Degree of separation from the previous peak	The percentage equivalent to the concentration of the principal component in the test solution	Specific mixed solution	Ethanol	250.278	8.149	/	0.5%	Isopropyl alcohol	250.410	9.504	8.2	0.5%	Ethyl acetate	248.695	14.145	28.2	0.5%	sec-butanol	250.585	14.429	1.8	0.5%	tetrahydrofuran	36.588	14.728	1.9	0.072%	Toluene	50.025	20.061	37.5	0.089%	N-methylpyrrolidone	27.6875	31.390	94.3	0.053%
Solution Name	Component name	Component concentration ( $\mu\text{g}/\text{ml}$ )	Rt (min)	Degree of separation from the previous peak	The percentage equivalent to the concentration of the principal component in the test solution																																													
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	Toluene	50.025	20.061	37.5	0.089%																																													
	N-methylpyrrolidone	27.6875	31.390	94.3	0.053%																																													
Limits of quantification, detection	<p>The S/N of each solvent peak in the 6 LOQ solution maps should be NLT 10, the RSD of retention time should be no more than 2.0%, and the RSD of peak area should be no more than 10%. The S/N of each solvent peak in the 3-needle Limit of detection (LOD) solution atlas should be NLT 3.</p>	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th rowspan="2">Component Name</th><th colspan="2">Detection Limits</th><th colspan="3">Limit of quantitation</th></tr> <tr> <th colspan="2">Concentration (equivalent to the percentage of solution concentration of the test product)</th><th colspan="3">Concentration (equivalent to the test product solution concentration percentage)</th></tr> </thead> <tbody> <tr> <td>Ethyl alcohol</td><td colspan="2">1.502<math>\mu\text{g}/\text{ml}</math> (0.003%)</td><td colspan="3">5.006<math>\mu\text{g}/\text{ml}</math> (0.01%)</td></tr> <tr> <td>Isopropyl alcohol</td><td colspan="2">1.502<math>\mu\text{g}/\text{ml}</math> (0.003%)</td><td colspan="3">5.008<math>\mu\text{g}/\text{ml}</math> (0.01%)</td></tr> <tr> <td>Ethyl</td><td colspan="2">1.492<math>\mu\text{g}/\text{ml}</math> (0.003%)</td><td colspan="3">4.974<math>\mu\text{g}/\text{ml}</math> (0.01%)</td></tr> </tbody> </table>							Component Name	Detection Limits		Limit of quantitation			Concentration (equivalent to the percentage of solution concentration of the test product)		Concentration (equivalent to the test product solution concentration percentage)			Ethyl alcohol	1.502 $\mu\text{g}/\text{ml}$ (0.003%)		5.006 $\mu\text{g}/\text{ml}$ (0.01%)			Isopropyl alcohol	1.502 $\mu\text{g}/\text{ml}$ (0.003%)		5.008 $\mu\text{g}/\text{ml}$ (0.01%)			Ethyl	1.492 $\mu\text{g}/\text{ml}$ (0.003%)		4.974 $\mu\text{g}/\text{ml}$ (0.01%)															
Component Name	Detection Limits		Limit of quantitation																																															
	Concentration (equivalent to the percentage of solution concentration of the test product)		Concentration (equivalent to the test product solution concentration percentage)																																															
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Ethyl	1.492 $\mu\text{g}/\text{ml}$ (0.003%)		4.974 $\mu\text{g}/\text{ml}$ (0.01%)																																															

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		acetate		
		sec-butanol	1.128 $\mu\text{g}/\text{ml}$ (0.002%)	3.759 $\mu\text{g}/\text{ml}$ (0.008%)
		tetrahydrofuran	1.098 $\mu\text{g}/\text{ml}$ (0.002%)	3.659 $\mu\text{g}/\text{ml}$ (0.007%)
		Toluene	0.600 $\mu\text{g}/\text{ml}$ (0.001%)	2.001 $\mu\text{g}/\text{ml}$ (0.004%)
		N-methylpyrrolidone	0.831 $\mu\text{g}/\text{ml}$ (0.002%)	2.769 $\mu\text{g}/\text{ml}$ (0.006%)
Linearity and range	<p>Within the limit concentration range of LOQ concentration ~200% of each solvent, 6 concentration points were selected fairly uniformly, with the concentration of each solvent as the horizontal coordinate and the peak area as the vertical coordinate. The regression coefficient R of the obtained linear graph should NLT 0.990, and the RSD of the response factor should not be more than 10%. The absolute Y-axis intercept accounted for 100% of the response value of the percentage should be less than 25%.</p>	Component Names	Concentration (equivalent to the concentration percentage of the test product solution)	Linear equation
		Ethanol	5.006 $\mu\text{g}/\text{ml}$ to 500.555 $\mu\text{g}/\text{ml}$ (0.0100% to 1.0011%)	$Y = 0.923 + 0.7951x$
		Isopropyl alcohol	5.008 $\mu\text{g}/\text{ml}$ to 500.820 $\mu\text{g}/\text{ml}$ (0.0100% to 1.0016%)	$Y = 0.9917 - 3.6091x$
		Ethyl acetate	4.974 $\mu\text{g}/\text{ml}$ ~497.390 $\mu\text{g}/\text{ml}$ (0.0099%~0.9948%)	$Y = 0.7696 - 2.3x$
		sec-butanol	3.759 $\mu\text{g}/\text{ml}$ ~501.170 $\mu\text{g}/\text{ml}$ (0.0075%~1.0023%)	$Y = 1.2102 - 4.7656x$
		tetrahydrofuran	3.659 $\mu\text{g}/\text{ml}$ to 73.175 $\mu\text{g}/\text{ml}$ (0.0073% to 0.1464%)	$Y = 1.0779 + 0.0623x$
		Toluene	2.001 $\mu\text{g}/\text{ml}$ ~100.050 $\mu\text{g}/\text{ml}$ (0.0040%~0.2001%)	$Y = 2.0063 - 0.9813x$
		N-methylpyrrolidone	2.769 $\mu\text{g}/\text{ml}$ to 55.375 $\mu\text{g}/\text{ml}$ (0.0055% to 0.1108%)	$Y = 0.9965 - 0.9081x$

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Accuracy	<p>Each solvent with a limit concentration range of 30% to 150% was added to the test product, and the recovery rate of each solvent was required to be between 85% and 115%, and the recovery RSD NMT 10%.</p>	<b>Component name</b>	<b>Labeling condition</b>	<b>Average recovery rate</b>	<b>RSD</b>
		Ethanol	Plus 75.0833µg/ml-375.4163µg/ml	101.2%, n=9	5%, n=9
		Isopropyl alcohol	Added 75.1230µg/ml-375.6150µg/ml	101.7%, n=9	4%, n=9
		Ethyl acetate	Marked 74.6085µg/ml-373.0425µg/ml	101.4%, n=9	3.0%, n=9
		sec-butanol	Added 75.1755µg/ml-375.8775µg/ml	102.2%, n=9	4%, n=9
		tetrahydrofuran	Added 10.9763µg/ml-54.8813µg/ml	101.3%, n=9	2.6%, n=9
		Toluene	Marked 15.0075µg/ml-75.0375µg/ml	101.6%, n=9	2.7%, n=9
		N-methylpyrrolidone	Added 8.3063µg/ml-41.5313µg/ml	%, n=9	7%. n=9
Precision	<p>Repeatability and reproducibility: RSD of each solvent in 6 repeatability (or reproducibility) test solution NMT 10%.</p> <p>Precision: In the results of 12 repeatability and intermediate precision tests, the RSDS of each solvent content NMT 10%.</p>	<b>Component name</b>	<b>Repeatability</b>	<b>Reproducibility</b>	<b>Precision</b>
		Ethanol	0.7%, n=6	5%, n=6	4%, n=12
		Isopropyl alcohol	0.8%, n=6	5%, n=6	4%, n=12
		Ethyl acetate	0.8%, n=6	5%, n=6	4%, n=12
		sec-butanol	0.9%, n=6	6%, n=6	4%, n=12
		tetrahydrofuran	1.0%, n=6	5%, n=6	4%, n=12

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		Toluene 1.0%, n=6	5%, n=6	4%, n=12
	N-methylpyrrolidone 2.0%, n=6	5%, n=6	9%, n=12	
Durability	<p>By changing the chromatographic conditions (changing the initial temperature, flow rate, column) and normal chromatographic conditions to test the solvent content of each test product solution to achieve, the chromatographic diagram obtained by the control product solution, ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene, n-methylpyrrolidone peaks in turn, the separation degree between the adjacent peaks should be greater than 1.5. Under different conditions of the same chromatographic parameters, the RSD of the content of each solvent to be measured in the test product solution NMT 10%.</p>	<p>The initial flow rate was <math>3.5\text{ml}/\text{min}\pm0.1\text{ml}/\text{min}</math>, the initial column temperature was <math>40^\circ\text{C}\pm5^\circ\text{C}</math>, and the chromatographic columns with different batches were investigated as follows:</p> <p>System applicability: no interference from blank solution; Ethanol, isopropyl alcohol, ethyl acetate, secondary butanol, tetrahydrofuran, toluene and n-methylpyrrolidone peaked in sequence in the chromatogram obtained from the control solution under different conditions of the same chromatographic parameters, and the separation degree between each adjacent solvent peak was greater than 1.5.</p> <p>Test product solution: RSDS of each solvent content in test product solution under the same chromatographic parameters and different conditions were all less than 10%.</p>		

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### **3.2.S.4.3.4 Methodological validation of genotoxic impurities in finished products**

#### **I. Analytical Procedures**

Chromatographic conditions The capillary column with (50%-phenyl)-methylpolysiloxane (Agilent DB-17 MS, 30m\*0.25mm, 0.25 $\mu$ m or similar polarity) as the fixed liquid was used as the chromatographic column. The initial temperature was 50°C, maintained for 3min, and the temperature was heated to 230°C at the rate of 20°C per minute. Maintenance for 5 minutes; Inlet temperature 250°C; The injection volume is 1 $\mu$ l. (Recommended carrier gas is helium, carrier gas flow rate is 1.50ml per minute, no shunt injection, transmission line temperature 250°C)

Mass spectrum conditions A triple quadrupole tandem mass spectrometry was used as the detector, an electron bombardment source (EI) was used, the ion source temperature was 230°C, and the collision gas was nitrogen. It is recommended to set scanning parameters according to the following table:

Component name	Time to start scanning (min)	Collection method	Dwell time (ms)	Channel 1 Detect ion pairs (m/z) and CE	Channel 2 Detect ion pairs (m/z) and CE
Dimethyl sulfate	5.00	MRM	40	66.00 > 48.00* 15.00 V	95.00 > 43.00 0.00 V
Diethyl sulfate		MRM	40	124.90 > 45.10* 12.00 V	45.10 > 43.10 18.00 V
Diisopropyl sulfate		MRM	40	87.00 > 45.10* 6.00 V	69.00 > 41.10 12.00 V
Di-sec-butyl sulfate		MRM	40	101.00 > 45.10* 6.00 V	59.00 > 43.00 30.00 V

Note: Bands \* are quantitative ion pairs.

The suitability of the system requires that in the chromatogram of the reference product solution, dimethyl sulfate, diethyl sulfate, diisopropyl

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sulfate and di-sec-butyl sulfate peak in turn. The signal-to-noise ratio of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and di-sec-butylsulfate should NLT 10 in the chromatogram under quantitative ion of the sensitive solution.

### **Assay**

The solvent, the sensitivity solution, the reference solution and the test product solution were each 1 $\mu$ l, and the samples were injected respectively, and the chromatogram was recorded. If there are peaks of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and disec-butyl sulfate in the chromatogram of the test product solution, the content of each sulfate impurity is calculated according to the external standard method and the peak area.

The calculation formula is:

$$\text{Assay (ppm)} = \frac{C_{\text{Reference}} \times A_{\text{test}} \times V}{W \times A_{\text{Reference}}}$$

$C_{\text{reference}}$  is the concentration of the reference solution (ng/ml);

$V$  is the dilution volume (ml) of the test product solution;

$A_{\text{test}}$  is the peak area of each sulfate impurity in the test product solution;

$A_{\text{reference}}$  is the peak area of each sulfate impurity in the control product solution;

$W$  is the weight of the test product (mg).

Limit test product solution chromatogram, if there are dimethyl sulfate, diethyl sulfate, diisopropyl sulfate, dibutyl sulfate peak, according to the external standard method to calculate the peak area, containing dimethyl sulfate, diethyl sulfate, diisopropyl sulfate, dibutyl sulfate NMT 75ppm, the sum of genotoxic impurities NMT 250ppm.

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### **II. Validation Results and Discussion**

#### **a. System applicability**

##### **➤ Acceptance Criteria**

The applicability of the system is achieved by examining the sensitivity solution and the reference solution. Take the sensitivity solution to inject 1 needle and the reference solution to inject 6 needles continuously. The signal-to-noise ratio of each impurity peak in the sensitivity solution should NLT 10; The RSD for the retention time of each impurity in the luminous solution NMT 1%, and the RSD for the peak area NMT 10%.

##### **➤ Solution preparation**

Solvent: acetonitrile

Dimethyl sulfate reserve liquid: Take about 15mg of dimethyl sulfate reference product, accurately weigh it, place it in a 100ml bottle, dissolve it with acetonitrile and dilute it to the scale, shake well, and obtain.

Diethyl sulfate reserve liquid: Take about 15mg of diethyl sulfate reference product, accurately weigh, place in 100ml measuring bottle, dissolve with acetonitrile and dilute to the scale, shake well, ready.

Diisopropyl sulfate reserve liquid: Take about 15mg of diisopropyl sulfate control product, accurately weigh, place in 100ml measuring bottle, dissolve with acetonitrile and dilute to the scale, shake well, ready.

Diisopropyl sulfate reserve liquid: Take diisopropyl sulfate reference product about 15mg, precision weigh, put in 100ml bottle, dissolve with acetonitrile and dilute to the scale, shake well, ready.

Control product reserve liquid: precisely measure dimethyl sulfate reserve liquid, diethyl sulfate reserve liquid, diisopropyl sulfate reserve liquid and disec-butyl sulfate reserve liquid 1ml each, in the same 20ml measuring bottle, dilute with acetonitrile to the scale, shake well, ready.

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Blank solution: solvent (acetonitrile).

Sensitivity solution: Accurately measure the control product reserve liquid 10 $\mu$ l into 5ml measuring bottle, dilute with acetonitrile to the scale, shake well, ready.

Control product solution: precisely measure the control product storage solution 100 $\mu$ l into 5ml measuring bottle, dilute it with acetonitrile to the scale, shake well, ready.

### ➤ **Injection Sequence**

After the blank solution, the sensitivity solution was injected with 1 needle, the control solution was injected with 6 needles continuously, and the chromatogram was recorded.

### ➤ **Measurement results**

Names of Components		Reference solution						RS D/%	Sensitivity solution signal-to-noise ratio
		1	2	3	4	5	6		
Dime thyl sulfate	Retention time	6.468	6.467	6.468	6.462	6.462	6.462	0.05	110.87
	Peak area	1862900	1915489	1888346	1880640	1871134	1756625	2.9	
Diethyl sulfate	Retention time	7.769	7.769	7.769	7.769	7.769	7.769	0	179.58
	Peak area	2072058	2108223	2137147	2096655	2111452	1988683	2.5	
Diisopropyl sulfate	Retention time	8.204	8.204	8.204	8.204	8.204	8.204	0	54.31
	Peak area	1416125	1450026	1482225	1468136	1455242	1362652	3.0	
Di-sec-butyl sulfate	Retention time	9.507	9.507	9.512	9.507	9.507	9.507	0.021	43.75
	Peak area	662276	669903	668544	665793	635428	575463	6	
Conclusion: The SNR of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and disec-butyl sulfate in the sensitivity solution were 110.87, 179.58, 54.31 and 43.75, respectively, all of which were NLT 10.6 The RSD of the retention time of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and di-sec-butylsulfate in the irradiation solution were									

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0.05%, 0%, 0% and 0.021%, respectively, and were all less than 1%. The RSD of the peak area of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and di-sec-butylsulfate in the irradiation solution were 2.9%, 2.5%, 3.0% and 6%, respectively, all of which were less than 10%, indicating that the system applicability of the method for the detection of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and di-sec-butylsulfate met the requirements.

### **(4.2) Specificity**

#### **➤ Acceptance Criteria**

The retention time of the impurities in the specific mixed solution and the reference solution should be consistent with the retention time in the impurity localization solution. The peak area of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and dibutyl sulfate in the blank solution NMT 20% of the average value of the impurity peak area in the mass spectrum of the 6 limited quantification solutions.

#### **➤ Solution preparation**

Blank solution: Blank solution as prepared under 4.1.

Dimethyl sulfate reserve solution: dimethyl sulfate reserve solution prepared under item 4.1.

Diethyl sulfate storage solution: diethyl sulfate storage solution prepared under item 4.1.

Diisopropyl sulfate reserve solution: diisopropyl sulfate reserve solution prepared under item 4.1.

Disec-butyl sulfate storage solution: disec-butyl sulfate storage solution prepared under item 4.1.

Reference product reserve solution: the reference product reserve solution prepared under item 4.1.

Reference solution: the same reference solution prepared under item 4.1.

Dimethyl sulfate positioning solution: accurately measure 1ml of dimethyl sulfate reserve solution, place it in a 20ml measuring bottle, dilute it with acetonitrile to the scale, and shake well; Precision measure 100 $\mu$ l of the above solution, place it in a 5ml measuring bottle, dilute it with acetonitrile to

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the scale, shake well, and get.

Diethyl sulfate positioning solution: accurately measure 1ml of diethyl sulfate reserve solution, place it in 20ml measuring bottle, dilute it with acetonitrile to the scale, and shake well; Precision measure 100 $\mu$ l of the above solution, place it in a 5ml measuring bottle, dilute it with acetonitrile to the scale, shake well, and get.

Diisopropyl sulfate positioning solution: accurately measure 1ml of diisopropyl sulfate reserve solution, place it in a 20ml measuring bottle, dilute it with acetonitrile to the scale, and shake well; Precision measure 100 $\mu$ l of the above solution, place it in 5ml measuring bottle, dilute it with acetonitrile to the scale, shake well, and get.

Disec-butyl sulfate positioning solution: accurately measure 1ml of disec-butyl sulfate reserve solution, place it in a 20ml measuring bottle, dilute it with acetonitrile to the scale, and shake well; Precisely measure the above solution 100 $\mu$ l, place it in a 5ml measuring bottle, dilute it with acetonitrile to the scale, shake well, and get.

Test product solution: Take about 10mg of this product, weigh it accurately, put it in 5ml measuring bottle, add acetonitrile to appropriate amount, swirl to dissolve, then dilute it with acetonitrile to scale, shake well, ready.

Specific mixed solution: Take about 10mg of this product, accurately weigh it, place it in 5ml bottle, add an appropriate amount of acetonitrile to dissolve, then precisely add 100 $\mu$ l of control product reserve, dilute it with acetonitrile to the scale, shake well, ready.

### ➤ **Injection Sequence**

Blank solution, reference solution, dimethyl sulfate localization solution, diethyl sulfate localization solution, diisopropyl sulfate localization solution, di-sec-butyl sulfate localization solution, test product solution and specific mixed solution were injected into 1 needle successively, and the chromatogram was recorded.

### ➤ **Measurement result**

Name of solution	Component	Rt (min)	MRM quantifies
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	name		ions Counter peak area
Reference solution	Dimethyl sulfate	6.462	1604486
	Diethyl sulfate	7.769	2271452
	Diisopropyl sulfate	8.204	1468496
	Di-sec-butyl sulfate	9.507	594890
Solution for the test product	Dimethyl sulfate	Not detected	Not detected
	Diethyl sulfate	Not detected	Not detected
	Diisopropyl sulfate	Not detected	Not detected
	Di-sec-butyl sulfate	Not detected	Not detected
Specific mixed solution	Dimethyl sulfate	6.468	1905636
	Diethyl sulfate	7.769	2396344
	Diisopropyl sulfate	8.204	1722683
	Di-sec-butyl sulfate	9.507	803619
Dimethyl sulfate locator solution	Dimethyl sulfate	6.462	1917908
Diethyl sulfate locating solution	Diethyl sulfate	7.769	2286745
Diisopropyl sulfate positioning solution	Diisopropyl sulfate	8.204	1438239
Di-sec-butyl sulfate positioning solution	Di-sec-butyl sulfate	9.507	622794
Blank solution	Dimethyl sulfate	Not detected	Not detected
	Diethyl sulfate	Not detected	Not detected
	Diisopropyl sulfate	Not detected	Not detected
	Di-sec-butyl sulfate	Not detected	Not detected
Items	Average of the peak area of the 6 parts limited	Ratio of the response value of the peak area of each component of the blank solution to the peak area of each	

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	quantification solution	component of the 6-part limited quantification solution /%
Dimethyl sulfate	188679	Not detected
Diethyl sulfate	216723	Not detected
Diisopropyl sulfate	139179	Not detected
Di-sec-butyl sulfate	56839	Not detected

Conclusion: The retention time of the impurities in the specific mixed solution and the reference solution is consistent with the retention time of the impurities in the localization solution. No dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and disec-butyl sulfate were detected in the blank solution, indicating that the specificity of the method met the requirements.

### **(4.3) Limits of quantification and detection**

#### ➤ **sketch**

Limits of quantification and detection are achieved by testing the signal-to-noise ratio of a reference solution diluted with a certain concentration. It is required that the SNR of all impurities in the 6 limited quantitation solutions should NLT 10, the RSD of the retention time of each impurity should not be more than 1.0%, and the RSD of the peak area of each impurity should not be more than 10%. The signal-to-noise ratio of every impurity in the mass spectrogram of the detection limited solution should NLT 3.

#### ➤ **Solution preparation**

Blank solution: Blank solution as prepared under 4.1.

Reference stock solution: the same as the reference stock solution prepared under item 4.1.

Limited quantitation solution: Accurately measure the control product reserve solution 10µl into 5ml measuring bottle, dilute it with acetonitrile to the scale, shake well, and then obtain. Prepare 6 parts in parallel.

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Detection limit solution: Accurately measure the first limit of quantification solution 1.5ml into 5ml measuring bottle, dilute with acetonitrile to the scale, shake well, and then get.

### ➤ **Injection procedure**

Take the blank solution and inject 1 needle, the detection limited solution and inject 3 needles continuously, 6 parts of the limited quantitative solution and inject 1 needle respectively, and record the chromatogram.

### ➤ **Measurement results**

Results of limit of quantitation test

To be measured Ingredients	Solution name	Concentration / (ng/ml)	Equivalent to test product solution concentration percentage / ppm	Retention time /min	Peak area	S/N
Dimethyl sulfate	Limited quantitative Solution 1	14.9352	7.5	6.468	193946	149.94
	Limited quantification solution 2	14.9352		6.462	191459	150.64
	Limited quantitative Solution 3	14.9352		6.462	184126	150.55
	Limited quantitative Solution 4	14.9352		6.462	187606	158.45
	Limited quantitative Solution 5	14.9352		6.462	181354	154.75
	Limited quantitative solution 6	14.9352		6.468	193581	143.07
RSD%			0.05	2.8	/	
Verdict: The limited quantification concentration of dimethyl sulfate was 14.9352ng/ml, which was equivalent to 7.5ppm of the solution concentration. The RSD of the retention time in 6 parts of the solution was						

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	0.05%, less than 1.0%, the RSD of the peak area was 2.8%, less than 10%, and the S/N ranged from 143.07 to 158.45, all of which were greater than 10. It shows that this method has a good response to the detection of dimethyl sulfate.					
Diethyl sulfate	Limited quantitative solution 1	15.3648	7.7	7.769	222581	207.1 3
	Limited quantitative Solution 2	15.3648		7.769	213753	167.3 3
	Limited quantitative Solution 3	15.3648		7.769	217018	179.4 8
	Limited quantitative Solution 4	15.3648		7.769	211589	180.5 1
	Limited quantitative Solution 5	15.3648		7.769	213217	152.9 4
	Limited quantitative solution 6	15.3648		7.769	222181	191.6 7
	RSD%			0	2.2	/
	Conclusion: The limit of quantitation concentration of diethyl sulfate was 15.3648ng/ml, which was equivalent to 7.7ppm of the solution, the RSD of retention time in 6 parts of the solution was 0% (less than 1.0%), the RSD of peak area was 2.2% (less than 10%), and the S/N ranged from 152.94 to 207.13, all of which were greater than 10. The results indicated that the method had a good response for the detection of diethyl sulfate.					
Diisopropyl sulfate	Limited quantitative solution 1	15.1417	7.6	8.204	139949	71.56
	Limited quantitative Solution 2	15.1417		8.204	139261	60.74
	Limited quantitative Solution 3	15.1417		8.204	140461	64.91
	Limited	15.1417		8.204	139189	64.38

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	quantitative Solution 4						
	Limited quantitative Solution 5	15.1417		8.204	136057	52.55	
	Limited quantitative solution 6	15.1417		8.204	140157	67.31	
	RSD%			0	1.2	/	
	Conclusion: The limited quantification concentration of diisopropyl sulfate was 15.1417ng/ml, which was equivalent to 7.6ppm of the test solution. The RSD of the retention time in 6 parts of the limited quantification solution was 0%, less than 1.0%, the RSD of the peak area was 1.2%, less than 10%, and the S/N ranged from 52.55 to 71.56, all of which were greater than 10. The results indicated that the response of this method was good for the detection of diisopropyl sulfate.						
	Limited quantitative Solution 1	15.3638		9.507	57846	76.76	
	Limited quantitative Solution 2	15.3638		9.507	58220	59.11	
Di-sec- butyl sulfate	Limited quantitative Solution 3	15.3638	7.7	9.507	56479	61.59	
	Limited quantitative Solution 4	15.3638		9.507	57117	58.01	
	Limited quantitative Solution 5	15.3638		9.507	55235	56.30	
	Limited quantitative solution 6	15.3638		9.507	56138	60.63	
	RSD%			0	2.0	/	
	Conclusion: The limited quantitation concentration of disec-butylsulfate was 15.3638ng/ml, which was equivalent to 7.7ppm of the solution, the RSD of the retention time in 6 parts of the limited quantitation solution was 0%, less than 1.0%, the RSD of the peak area was 2.0%, less than 10%, and						

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	the S/N ranged from 56.30 to 76.76, all of which were greater than 10. The results indicated that the method had a good response to the detection of di-sec-butylsulfate.
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### Detection limit test results

Impurities Name	Detection limit solution injection times	Concentration /(ng/ml)	Equivalent to test product solution concentration percentage /ppm	S/N
Dimethyl sulfate	1	4.4806	2.2	46.09
	2			40.09
	3			47.76
	Conclusion: The detection limit concentration of dimethyl sulfate is 4.4806ng/ml, which is equivalent to 2.2ppm of the test solution, and the S/N in the detection limit solution for 3 consecutive stiches ranges from 40.09 to 47.76, all of which are greater than 3. It shows that this method has a good response to the detection of dimethyl sulfate.			
Diethyl sulfate	1	4.6094	2.3	53.85
	2			55.62
	3			57.13
	Conclusion: The detection limit concentration of diethyl sulfate was 4.6094ng/ml, which was equivalent to 2.3ppm of the test solution. S/N in the detection limit solution for 3 consecutive needles ranged from 53.85 to 57.13, all of which were greater than 3. It indicates that this method has a good response to the detection of diethyl sulfate.			
Diisopropyl sulfate	1	4.5425	2.3	18.46
	2			20.78
	3			18.67
	Conclusion: The detection limit concentration of diisopropyl sulfate was 4.5425ng/ml, which was equivalent to 2.3ppm of the test solution, and the S/N in the detection limit solution for 3 consecutive stiches ranged from 18.46 to 20.78, all of which were greater than 3. It indicates that this method has a good response to the detection of diisopropyl sulfate.			
Di-sec-butyl sulfate	1	4.6091	2.3	16.69
	2			26.77
	3			19.04

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	Conclusion: The detection limit concentration of disec-butyl sulfate is 4.6091ng/ml, which is equivalent to 2.3ppm of the test solution, and the S/N in the detection limit solution for 3 consecutive stiches is between 16.69 and 26.77, all of which are greater than 3. It indicates that this method has a good response to the detection of di-sec-butyl sulfate.
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### **(4.4) Linearity and range**

#### **➤ Acceptance Criteria**

In the equivalent of the impurities LOQ~200% limit concentration range, more uniform take 6 concentration points, the impurity concentration as the horizontal coordinate, the impurity peak area response value as the ordinate, as a linear regression curve, the regression coefficient R of the linear graph should be NLT 0.990, the Y-axis intercept is within 25% of the 100% response value.

#### **➤ Solution preparation**

Blank solution: Blank solution as prepared under 4.1.

Linear Solution 1 (LOQ) : Accurately measure the control product reserve solution 10μl into 5ml measuring bottle, dilute it with acetonitrile to the scale, shake well, and obtain.

Linear solution 2: Accurately measure the control product reserve solution 50μl into a 5ml measuring bottle, dilute it with acetonitrile to the scale, shake well, ready.

Linear solution 3: Accurately measure the control product reserve liquid 80μl into 5ml measuring bottle, dilute it with acetonitrile to the scale, shake well, ready.

Linear solution 4: Precisely measure the control product reserve liquid 100μl into 5ml measuring bottle, dilute it with acetonitrile to the scale, shake well, ready.

Linear solution 5: Precisely measure the control product reserve liquid

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150 $\mu$ l into 5ml measuring bottle, dilute it with acetonitrile to the scale, shake well, ready.

Linear solution 6: Accurately measure the control product reserve liquid 200 $\mu$ l into 5ml measuring bottle, dilute it with acetonitrile to the scale, shake well, ready.

### ➤ Injection Sequence

Take a blank solution and inject 1 needle, each linear solution from low concentration to high concentration of 1 needle, record the chromatogram.

### ➤ Measurement results

Results of linear and range tests

impurity Name	Solution name	Equivalent to test product solution concentration percentage / ppm	Concentration of test substance (ng/ml)	Peak area
Dimethyl sulfate	Linear solution 1	7.5	14.9352	189843
	Linear Solution 2	37.3	74.6760	955650
	Linear Solution 3	59.7	119.4816	1503384
	Linear solution 4	74.7	149.3520	1990355
	Linear Solution 5	112.0	224.0280	2901824
	Linear solution 6	149.4	298.7040	3941888
	Linear Chart	<p>硫酸二甲酯线性图</p> <p><math>y = 13215x - 26946</math></p> <p><math>R^2 = 0.9993</math></p>		
	Linear equations	$y=13215x-26946$		
	Correlation coefficient R	0.9997		
	Absolute value of Y-axis intercept as a percentage of	1.4		

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	100% response (%)			
The verdict: In the concentration range of 14.9352~298.7040ng/ml, the linear equation is $y=13215x-26946$ , the correlation coefficient is 0.9997, greater than 0.990, and the absolute value of y-intercept accounts for 1.4% of 100% response value, less than 25%. It indicates that dimethyl sulfate meets the requirements linearly in the concentration range of 14.9352-298.7040 ng/ml.				
Diethyl sulfate	Linear Solution 1	7.7	15.3648	225866
	Linear Solution 2	38.4	76.8240	1184267
	Linear Solution 3	61.5	122.9184	1937152
	Linear solution 4	76.8	153.6480	2377909
	Linear Solution 5	115.2	230.4720	3628004
	Linear solution 6	153.6	307.2960	5096780
	Linear Chart			
	Linear equations	$y=16542x-91014$		
	Correlation coefficient R	0.9990		
	Absolute value of Y-axis intercept as a percentage of 100% response (%)	3.7		
	The verdict: In the concentration range of 15.3648~307.2960ng/ml, the linear equation of diethyl sulfate is $y=16542x - 91014$ , the correlation coefficient is 0.9990, greater than 0.990, and the absolute value of y-intercept accounts for 3.7% of 100% response value, less than 25%. It indicates that diethyl sulfate meets the requirements linearly in the concentration range of 15.3648~307.2960ng/ml.			
	Diisopropyl sulfate	Linear Solution 1	7.6	15.1417
	Diisopropyl sulfate	Linear Solution 2	37.9	75.7085
		Linear Solution 3	60.6	121.1336
		Linear solution 4	75.7	151.4170
		Linear Solution 5	113.6	227.1255
				2376454

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	Linear solution 6	151.4	302.8340	3366201	
	Linear Chart	<p style="text-align: center;">硫酸二异丙酯线性图</p> <p style="text-align: center;"><math>y = 11126x - 84402</math> <math>R^2 = 0.9973</math></p>			
	Linear equations	$y=11126x-84402$			
	Correlation coefficient R	0.9986			
	Absolute value of Y-axis intercept as a percentage of 100% response (%)	5.3			
	<p>The verdict: In the concentration range of 15.1417~302.8340ng/ml, the linear equation of diisopropyl sulfate is <math>y=11126x-84402</math>, the correlation coefficient is 0.9986, greater than 0.990, and the absolute value of y-intercept is 5.3% of 100% response value, less than 25%. It indicates that diisopropyl sulfate meets the requirements linearly in the concentration range of 15.1417~302.8340ng/ml.</p>				
Disec-butyl sulfate	Linear Solution 1	7.7	15.3638	54810	
	Linear Solution 2	38.4	76.8189	311128	
	Linear solution 3	61.5	122.9102	528954	
	Linear solution 4	76.8	153.6377	638145	
	Linear Solution 5	115.2	230.4566	988606	
	Linear solution 6	153.6	307.2754	1416224	
Disec-butyl sulfate	Linear Chart	<p style="text-align: center;">硫酸二仲丁酯线性图</p> <p style="text-align: center;"><math>y = 4616.2x - 41084</math> <math>R^2 = 0.9964</math></p>			

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	Linear equations	$Y = 4616.2 x - 41084$
	Correlation coefficient R	0.9982
	Absolute value of Y-axis intercept as a percentage of 100% response (%)	6.1
The verdict: In the concentration range of 15.3638~307.2754ng/ml, the linear equation of disec-butyl sulfate is $y=4616.2x-41084$ , the correlation coefficient is 0.9982, greater than 0.990, and the absolute value of y-intercept accounts for 6.1% of 100% response value, less than 25%. It indicates that di-sec-butylsulfate meets the requirements linearly in the concentration range of 15.3638~307.2754ng/ml.		

### **(4.5) Repeatability**

#### **➤ Acceptance Criteria**

Repeatability is achieved by examining the repeatability of the results of each component to be tested in the repeatable test product solution. Prepare 6 parts of repeatable test product solution with 100% limit of the components to be tested. The RSD of the content of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and disec-butyl sulfate in the 6 parts of repeatable test product solution shall NMT 20%.

#### **➤ Preparation of solution**

Blank solution: Blank solution as prepared under 4.1.

Reference stock solution: the same as the reference stock solution prepared under item 4.1.

Reference solution: the same as the reference solution prepared under 4.1.

Repeated test product solution: take about 10mg of this product, accurately weigh it, place it in a 5ml measuring bottle, add acetonitrile to dissolve, then add the control product reserve solution of 100μl, dilute with

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acetonitrile to the scale, shake well, and get. 6 parts were prepared in parallel.

### ➤ **Injection procedure**

1 needle was injected into blank solution, 2 needles were injected into control solution continuously, and 1 needle was injected into 6 repetitive test product solutions respectively, and the chromatogram was recorded.

### ➤ **Measurement results**

Name of solution	Content (ppm)			
	Dimethyl sulfate	Diethyl sulfate	Diisopropyl sulfate	Di-sec-butyl sulfate
Repetitive test product solution 1	64.4	72.2	67.4	67.9
Repetitive test product solution 2	68.5	74.3	69.9	71.2
Repetitive test product solution 3	63.1	72.4	67.6	67.8
Repetitive test product solution 4	59.3	69.5	64.7	64.7
Repeated test product solution 5	55.5	74.6	68.6	70.2
Repetitive test product solution 6	72.3	75.7	73.0	76.8
Average	63.9	73.1	68.5	69.8
RSD/%	10	4	5	6
Verdict: The RSD of the content of dimethyl sulfate was 10%, less than 20%, the RSD of the content of diethyl sulfate was 4%, less than 20%, the RSD of the content of diisopropyl sulfate was 5%, less than 20%, and the RSD of the content of disec-butyl sulfate was 6%, less than 20%. The results indicated that the reproducibility of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and disec-butylsulfate met the requirements.				

### **(4.6) Accuracy**

#### ➤ **Brief Description**

Accuracy is achieved by adding each impurity reference in the range of 30% limit concentration to 150% limit concentration of each impurity in the

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test product solution, and determining the recovery rate between the theoretical addition amount and the actual detection amount. The recovery rate of each impurity in the test solution should be between 80.0% and 120.0%, and the RSD of the recovery rate of each impurity NMT 15%.

### ➤ **Solution preparation**

Blank solution: Blank solution as prepared under 4.1.

Reference stock solution: the same as the reference stock solution prepared under item 4.1.

Reference solution: the same reference solution prepared under item 4.1.

Test product solution: Take about 10mg of this product, weigh it accurately, place it in a 5ml measuring bottle, add an appropriate amount of acetonitrile to dissolve, dilute it with acetonitrile to the scale, shake well, and get. Prepare 2 parts in parallel.

30% standard test product solution: Take about 10mg of this product, accurately weigh it, place it in a 5ml measuring bottle, add an appropriate amount of acetonitrile to dissolve, accurately add the control product reserve liquid 30 $\mu$ l, dilute it with acetonitrile to the scale, shake well, and get. Dispense 3 parts in parallel.

100% standard test product solution: take about 10mg of this product, weigh it accurately, place it in a 5ml measuring bottle, add acetonitrile and swirl to dissolve, add 100 $\mu$ l of control product reserve liquid precisely, dilute it with acetonitrile to the scale, shake well, and get. Dispense 3 parts in parallel

150% standard test product solution: Take about 10mg of this product, accurately weigh it, place it in a 5ml measuring bottle, add acetonitrile and scroll to dissolve, accurately add the control product reserve liquid 150 $\mu$ l, dilute it with acetonitrile to the scale, shake well, and get. Dispense 3 parts in parallel.

### ➤ **Injection procedure**

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After the blank solution, 2 needles were injected into the control solution continuously, 2 samples of test product solution and 9 samples of each concentration added standard test product solution were injected into 1 needle successively, and the chromatogram was recorded.

### ➤ Results of determination

Impurities Name	Solution name	Peak area	Content		Average content	
Dimethylsulfate	Sample solution 1	Not detected	Not detected		Not detected	
	Test product solution 2	Not detected	Not detected			
	Solution name	Peak area	Amount available (ng)	Amount added (ng)	Measured amount (ng)	Recovery rate (%)
	30% standard test solution 1	562037	Not detected	0.2317	0.2176	93.94
	30% labeled test product solution 2	566838	Not detected	0.2317	0.2195	94.74
	30% labeled test product solution 3	550588	Not detected	0.2317	0.2132	92.02
	100% labeled test product solution 1	1958356	Not detected	0.7722	0.7583	98.19
	100% labeled test product solution 2	1957586	Not detected	0.7722	0.7580	98.15
	100% labeled test product solution 3	1922011	Not detected	0.7722	0.7442	96.37

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	150% labeled test product solution 1	291749 2	Not detected	1.1584	1.1297	97.52
	150% labeled test product solution 2	293070 1	Not detected	1.1584	1.1348	97.96
	150% labeled test product solution 3	293539 5	Not detected	1.1584	1.1366	98.12
	Average recovery rate (%)	96.33				
	RSD(%)	2.4				
	Verdict: No dimethyl sulfate was detected in the test products. The average recovery rate was 96.33% when 30% limit concentration ~150% limit concentration of dimethyl sulfate was added to the test products, the recovery rate was 92.02%~98.19%, 80%~120%, and the RSD of recovery was 2.4%. Less than 15%, indicating that the accuracy of this method in detecting dimethyl sulfate in YA2304 meets the requirements.					
impurity Name	Solution name	Peak area	Content		Average content	
Diet hyl sulfate	Sample solution 1	Not detected	Not detected		Not detected	
	Test product solution 2	Not detected	Not detected			
Diet hyl sulfate	Solution name	Peak area	Available amount (ng)	Amount added (ng)	Measured amount (ng)	Recovery rate (%)
	30% standard test solution 1	642815	Not detected	0.2333	0.2185	93.66
	30% labeled test product solution 2	655611	Not detected	0.2333	0.2229	95.53
	30% labeled test	622481	Not	0.2333	0.2116	90.70

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	product solution 3		detected			
	100% labeled test product solution 1	2310732	Not detected	0.7776	0.7855	101.01
	100% labeled test product solution 2	2348224	Not detected	0.7776	0.7982	102.65
	100% labeled test product solution 3	2383474	Not detected	0.7776	0.8102	104.19
	150% labeled test solution 1	3686778	Not detected	1.1665	1.2532	107.44
	150% labeled test product solution 2	3668959	Not detected	1.1665	1.2472	106.92
	150% labeled test product solution 3	3657077	Not detected	1.1665	1.2432	106.57
	Average recovery rate (%)			100.96		
	RSD(%)			7		
	Verdict: Diethyl sulfate was not detected in the test products, and the average recovery rate was 100.96% when 30% limit concentration ~150% limit concentration of diethyl sulfate was added to the test products, the recovery rate was 90.70%~107.44%, 80%~120%, and the RSD of recovery was 7%. Less than 15%, indicating that the accuracy of this method in detecting diethyl sulfate in YA2304 meets the requirements.					
Impurities Name	Solution name	Peak area	Content		Average content	
Diisopropyl sulfonate	Test product solution 1	Not detected	Not detected		Not detected	
	Test product solution 2	Not detected	Not detected			

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	d				
Solution name	Peak area	Amount available (ng)	Amount added (ng)	Measured amount (ng)	Recovery rate (%)
30% standard test solution 1	438674	Not detected	0.2210	0.2129	96.31
30% labeled test product solution 2	442129	Not detected	0.2210	0.2145	97.07
30% labeled test product solution 3	432713	Not detected	0.2210	0.2100	95.00
100% labeled test product solution 1	1567591	Not detected	0.7367	0.7607	103.25
100% labeled test product solution 2	1579099	Not detected	0.7367	0.7662	104.01
100% labeled test product solution 3	1603299	Not detected	0.7367	0.7780	105.60
150% labeled test product solution 1	2495306	Not detected	1.1051	1.2108	109.57
150% labeled test product solution 2	2447580	Not detected	1.1051	1.1877	107.47
150% labeled test product solution 3	2475123	Not detected	1.1051	1.2010	108.68
Average recovery rate (%)		103.00			
RSD(%)		6			
Verdict: Diisopropyl sulfate was not detected in the test product. The average recovery of diisopropyl sulfate was 103.00% when the limit concentration of 30% ~150% was added to the test product. The recovery of each solution with added standard was 95.00%~109.57%, 80%~120%,					

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	and the RSD of recovery was 6%. Is less than 15%, indicating that the accuracy of this method in detecting diisopropyl sulfate in YA2304 meets the requirements.					
Impurities Name	Solution name	Peak area	Content		Average content	
Di-isec-butyl sulfonate	Sample solution 1	Not detected	Not detected		Not detected	
	Test product solution 2	Not detected	Not detected			
	Solution name	Peak area	Amount available (ng)	Amount added (ng)	Measured amount (ng)	Recovery rate (%)
	30% standard test solution 1	159119	Not detected	0.2306	0.2053	89.03
	30% labeled test product solution 2	160074	Not detected	0.2306	0.2065	89.57
	30% labeled test product solution 3	164782	Not detected	0.2306	0.2126	92.20
	100% labeled test product solution 1	583727	Not detected	0.7687	0.7532	97.98
	100% labeled test product solution 2	602244	Not detected	0.7687	0.7771	101.09
	100% labeled test product solution 3	603209	Not detected	0.7687	0.7783	101.25
	150% labeled test product solution 1	947602	Not detected	1.1530	1.2227	106.04

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150% labeled test product solution 2	934005	Not detected	1.1530	1.2052	104.52
150% labeled test product solution 3	942394	Not detected	1.1530	1.2160	105.46
Average recovery rate (%)	98.57				
RSD(%)	7				
Verdict: Di-sec-butylsulfate was not detected in the test product. The average recovery was 98.57% when 30% ~150% limit concentration of di-sec-butylsulfate was added to the test product. The recovery was 89.03%~106.04%, 80%~120%, and the RSD of recovery was 7%. Less than 15%, indicating that the accuracy of this method in detecting di-sec-butylsulfate in YA2304 meets the requirements.					

### **(4.7) Durability**

#### ➤ Brief Description

Durability is achieved by changing the chromatographic conditions (starting temperature, inlet temperature, carrier gas flow rate) and testing the content of various impurities in the test solution with 100% limit concentration of impurities under normal chromatographic conditions. Under different conditions with the same chromatographic parameters, the signal-to-noise ratio of each impurity peak in the sensitivity solution should NLT 10, and the RSD of each impurity content in the durability test product NMT 20%.

Changes in chromatographic conditions of durability test

Chromatographic Parameters	Standard condition	Variable Values
Starting temperature	50 °C	45 °C, 55 °C
Carrier gas flow rate	1.5 ml/min	1.4ml/min, 1.6ml/min
Inlet temperature	250 °C	245 °C, 255 °C

#### ➤ Solution preparation

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Blank solution: Blank solution as prepared under 4.1.

Sensitivity solution: Sensitivity solution prepared under item 4.1.

Reference stock solution: the same as the reference stock solution prepared under item 4.1.

Reference solution: the same reference solution prepared under item 4.1.

Durability test product solution: Take about 10mg of this product, accurately weigh it, place it in a 5ml measuring bottle, add an appropriate amount of acetonitrile, swirl to dissolve, then add 100 $\mu$ l of control product reserve solution, dilute with acetonitrile to the scale, shake well, and get. Parallel preparation of 2 parts.

### ➤ **Injection procedure**

Under each durability chromatographic condition, after the blank solution, the sensitivity solution was injected with 1 needle, the control solution was injected with 2 needles continuously, and the 2 durability test solution was injected with 1 needle each, and the chromatographic diagram was recorded.

### ➤ **Measurement resultss**

Results of durability - system suitability test

Chromatographic Condition s	Paramete r values	Sensitivity solution signal-to-noise ratio			
		Dimethyl sulfate	Diethyl sulfate	Diisopropyl sulfate	Di-sec-butyl sulfate
Initial temperat ure (° C)	45	114.84	95.45	73.79	88.31
	50	122.23	111.70	74.11	79.46
	55	81.76	115.68	77.89	79.38
Inlet temperat	245	93.93	112.23	80.10	93.18
	250	122.23	111.70	74.11	79.46

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ure (° C)	255	79.80	89.71	70.61	63.19
Flow rate (ml/min)	1.4	70.22	125.68	70.44	69.16
	1.5	122.23	111.70	74.11	79.46
	1.6	53.48	92.02	69.59	67.67

Conclusion: The SNR of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and disec-butyl sulfate in the sensitivity solution were all greater than 10 under the conditions of normal chromatographic parameters, changing the initial temperature, carrier gas flow rate, inlet temperature and column. The suitability of the system met the requirements.

### Durability - Sample test results

Chromatographic Conditions	Parameter values	Sample number	Content (ppm)			
			Dimethyl sulfate	Diethyl sulfate	Diisopropyl sulfate	Di-sec-butyl sulfate
Initial temperature (° C)	45	1	65.1	65.7	63.8	65.3
		2	70.9	68.8	64.5	66.0
	50	1	63.6	70.3	65.2	66.9
		2	68.1	71.9	66.6	67.1
	55	1	65.5	69.6	64.5	64.3
		2	69.3	69.7	63.9	62.8
	RSD/%		5	3.0	1.7	2.6
	245	1	66.6	70.6	66.5	67.9
		2	72.0	71.7	66.8	67.6
	250	1	63.6	70.3	65.2	66.9
		2	68.1	71.9	66.6	67.1
Inlet temperature (° C)	255	1	66.7	68.9	63.6	64.0
		2	73.1	70.3	64.6	64.3
	RSD/%		6	1.6	2.0	2.6
	1.4	1	67.5	68.7	63.2	64.3
		2	73.8	70.0	64.2	64.5
	1.5	1	63.6	70.3	65.2	66.9
		2	68.1	71.9	66.6	67.1
Flow rate (ml/min)	1.6	1	67.9	67.9	63.9	64.4

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	2	74.2	69.6	64.5	64.0
	RSD/%	6	2.0	1.9	2.2

Conclusion: The RSDS of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and disec-butyl sulfate were 5%, 3.0%, 1.7% and 2.6%, respectively, when the initial column temperature of the two durability test solutions was 45°C, 50°C and 55°C, all of which were less than 20%. RSDS of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and di-sec-butylsulfate were 6%, 2.0%, 1.9% and 2.2%, respectively, at the flow rates of 1.4ml/min, 1.5ml/min and 1.6ml/min for durability test solutions, all of which were less than 20%. The RSDS of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and di-sec-butylsulfate were 6%, 1.6%, 2.0% and 2.6%, respectively, when the temperature of the 2 durability test solutions was 245°C, 250°C and 255°C, respectively, and were all less than 20%. The durability of the method met the requirements.

### **(4.8) Solution stability**

#### **➤ Brief Description**

Each solution is placed at room temperature, and the sensitivity solution, the reference solution and the 100% labeled test product solution are injected at different times. The RSD of the peak area response value of each impurity in the sensitivity solution, the reference solution and the 100% labeled test product solution NMT 10%.

#### **➤ Solution preparation**

Blank solution: Blank solution as prepared under 4.1.

Reference stock solution: the same as the reference stock solution prepared under item 4.1.

Sensitivity solution: Sensitivity solution as prepared under item 4.1.

Reference solution: the same as the reference solution prepared under item 4.1.

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100% standard test product solution: take about 10mg of this product, accurately weigh it, place it in a 5ml measuring bottle, add acetonitrile appropriate amount 4 vortex to dissolve, accurately add the reference product reserve solution 100 $\mu$ l, dilute it with acetonitrile to the scale, shake well, and get.

### ➤ **Injection Sequence**

Each solution was placed at room temperature, the sensitivity solution, the control solution and the 100% standard test solution were injected with 1 needle at 0h, 1.5h, 3.0h, 4.6h, 6.1h, 7.7h, 9.2h, 10.7h, 12.3h, 13.8h and 15.3h respectively, and the chromatogram was recorded.

### ➤ **Measurement result**

Stability test results of sensitivity solution

Time point (h)	Impurity peak area response value			
	Dimethyl sulfate	Diethyl sulfate	Diisopropyl sulfate	Di-sec-butyl sulfate
0	190403	188948	119008	44718
1.5	188412	187452	120001	45940
3.0	184496	189022	119921	46351
4.6	176755	186169	117991	45666
6.1	171826	183690	117573	44664
7.7	168783	186301	117518	44549
9.2	162656	180525	112729	43637
10.7	159591	175216	111716	43572
12.3	155049	171937	107037	42250
13.8	151390	169294	105405	40626
15.3	148599	166753	101765	39844
RSD/%	9	5	6	5

Conclusion: The RSD of the peak area response of dimethyl sulfate in the sensitive solution is 9%, not more than 10%; The RSD of the peak area response of diethyl sulfate was 5%, less than 10%; The RSD of the peak area response of diisopropyl sulfate was 6%, less than 10%; The RSD of the peak area response of disec-butyl

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sulfate is 5%, not more than 10%, indicating that the stability of the impurities in the sensitive solution meets the requirements, that is, stable within 15.3 h.

### Stability test results of reference solution

Time point (h)	Impurity peak area response value			
	Dimethyl sulfate	Diethyl sulfate	Diisopropyl sulfate	Di-sec-butyl sulfate
0	2072209	2282778	1414237	546582
1.5	2010329	2212930	1351951	514373
3.0	1952305	2180200	1337131	514936
4.6	1859120	2130727	1304841	498932
6.1	1817613	2118867	1290160	497691
7.7	1746971	2088184	1262308	481474
9.2	1746533	2027443	1225627	471398
10.7	1698288	1992264	1199260	456705
12.3	1628748	1944962	1172415	454139
13.8	1596695	1922525	1148778	438631
15.3	1545509	1886615	1133133	431864
RSD/%	10	7	8	8

Conclusion: The RSD of the peak area response of dimethyl sulfate in the control solution is 10%, not more than 10%; The RSD of the peak area response of diethyl sulfate was 7%, less than 10%; The RSD of the peak area response of diisopropyl sulfate was 8%, less than 10%; The RSD of the peak area response of disec-butyl sulfate was 8%, not more than 10%, indicating that the stability of the impurities in the control solution met the requirements, that is, stable within 15.3 h.

### Stability test results of 100% labeled test product solution

Time point (h)	Impurity peak area response value			
	Dimethyl sulfate	Diethyl sulfate	Diisopropyl sulfate	Di-sec-butyl sulfate
0	2086277	2331842	1461126	571654
1.5	2012763	2227395	1374888	525995
3.0	1942224	2187200	1347851	519604
4.6	1871331	2157936	1331608	516497
6.1	1835089	2160943	1321617	512831
7.7	1745523	2107728	1280936	494456
9.2	1765301	2068140	1260113	487783
10.7	1696135	2023748	1223767	473809
12.3	1646900	1993164	1201867	462912

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13.8	1599533	1951026	1178292	448921
15.3	1546866	1931126	1163169	448232
RSD/%	10	6	8	8

Conclusion: The RSD of peak area response of dimethyl sulfate in 100% standard test solution is 10%, not more than 10%; The RSD of the peak area response of diethyl sulfate was 6%, less than 10%; The RSD of the peak area response of diisopropyl sulfate was 8%, less than 10%; The RSD of the peak area response of disec-butylsulfate was 8%, not more than 10%, indicating that the stability of impurities in 100% standard test solution met the requirements, that is, stable within 15.3 h.

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### III. Summary of Method Validation

Summary of the results of the verification of the method of genotoxic impurities in the finished product

<b>Project</b>	<b>Acceptable criteria</b>	<b>Verification result</b>		
System suitability	The signal-to-noise ratio of each impurity peak in the sensitivity solution should NLT 10; The RSD of the retention time of each impurity in the luminous solution NMT 1%, and the RSD of the peak area response value NMT 10%.	The SNR of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and disec-butyl sulfate in the sensitivity solution were 110.87, 179.58, 54.31 and 43.75, respectively, and were NLT 10. The RSD of the retention time of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and di-sec-butylsulfate in the irradiation solution were 0.05%, 0%, 0% and 0.021%, respectively, and were not greater than 1%. The RSD of the peak area of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and di-sec-butylsulfate in the irradiation solution were 2.9%, 2.5%, 3.0% and 6%, respectively, all of which were less than 10%, indicating that the system applicability of the method for the detection of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and di-sec-butylsulfate met the requirements.		
Specificity	The retention time of each impurity in the specific mixed solution and the control solution should be consistent with the retention time in the impurity localization solution. The peak area response value of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and disec-butyl sulfate in the blank solution NMT 20% of the average value of the peak area response of each impurity in the mass spectrum of 6 limited quantification solutions.	The retention time of each impurity in the specific mixed solution and the reference solution is consistent with the retention time of each impurity localization solution. Dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and disec-butyl sulfate were not detected in the blank solution, indicating that the specificity of the method met the requirements.		
Limits of quantification and detection	It is required that the SNR of each impurity in the 6 limited quantitation solutions should NLT 10, the RSD of the retention time of each impurity should not be more than 1.0%, and the RSD of the peak	<b>Impurities</b>	<b>Detection Limits</b>	<b>Limit of quantitation</b>
		Dimethyl	Concentration (equivalent to the percentage of solution concentration of the test product)	Concentration (equivalent to the test product solution concentration percentage)
		Dimethyl	4.4806ng/ml (2.2ppm)	14.9352ng/ml (7.5ppm)

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n	area response value of each impurity should not be more than 10%. The signal-to-noise ratio of every impurity in the mass spectrogram of the detection limited solution should be NLT 3.	sulfate				
		Diethyl sulfate	4.6094ng/ml (2.3ppm)	15.3648ng/ml (7.7ppm)		
		Diisopropyl sulfate	4.5425ng/ml (2.3ppm)	15.1417ng/ml (7.6ppm)		
		Di-sec-butyl sulfate	4.6091ng/ml (2.3ppm)	15.3638ng/ml (7.7ppm)		
Linearity vs. Range	The regression coefficient R of the obtained linear graph should be NLT 0.990, and the Y-intercept should be less than 25% of the 100% response value.	<b>Impurities</b>	<b>Concentration (equivalent to the percentage of solution concentration of the test product)</b>	<b>Linear equation</b>		
		Dimethyl sulfate	14.9352ng/ml~298.7040ng/ml (7.5ppm~149.4ppm)	y=13215x-26946		
		Diethyl sulfate	15.3648ng/ml to 307.2960ng/ml (7.7ppm to 153.6ppm)	y=16542x-91014		
		Diisopropyl sulfate	15.1417ng/ml to 302.8340ng/ml (7.6ppm to 151.4ppm)	y=11126x-84402		
		Di-sec-butyl sulfate	15.3638ng/ml to 307.2754ng/ml (7.7ppm to 153.6ppm)	Y = 4616.2 x - 41084		
Repeatability	The RSD of the content of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and di-sec-butyl sulfate in the solution of 6 repeatable test products should NMT 20%.	<b>Impurities</b>	Dimethyl sulfate	Diethyl sulfate	Diisopropyl sulfate	Di-sec-butyl sulfate
		Repeatability	10%, n=6	4%, n=6	5%, n=6	6%, n=6
Accuracy	It is required that the recovery rate of each impurity in the solution of each added standard test product should be between 80.0% and 120.0%, and the RSD of each impurity recovery rate NMT 15%.	<b>Impurities</b>	<b>Marking condition</b>	<b>Average recovery rate</b>	<b>RSD</b>	
		Dimethyl sulfate	Added 0.2317ng to 1.1584ng	96.33%, n=9	2.4%, n=9	
		Diethyl sulfate	Added 0.2333ng to 1.1665ng	100.96%, n=9	7%, n=9	
		Diisopropyl sulfate	Added 0.2210ng to 1.1051ng	103.00%, n=9	6%, n=9	
		Di-sec-butyl sulfate	Added 0.2306ng to 1.1530ng	98.57%, n=9	7%, n=9	
Durabil	Under different conditions of	The initial column temperature was 50°C±5°C, the flow rate of carrier gas was				

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ity	the same chromatographic parameters, the signal-to-noise ratio of each impurity peak in the sensitivity solution should NLT 10, and the RSD of each impurity content in the durability test product NMT 20%.	1.5ml/min±0.1ml/min, and the inlet temperature was 250°C±5°C. The results were as follows: System applicability: Under different chromatographic conditions, the signal-to-noise ratio of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and disec-butyl sulfate in the sensitivity solution were all greater than 10, and the system applicability met the requirements. Test product solution: under different chromatographic conditions, the RSDS of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and di-sec-butylsulfate in the durability test product solution were not more than 20%, which met the verification requirements.
Stabilit y of solutio n	Each solution is placed at room temperature, and the sensitivity solution, the reference solution and the 100% labeled test product solution are injected at different times. The RSD of the peak area response value of each impurity in the sensitivity solution, the reference solution and the 100% labeled test product solution NMT 10%.	The RSDS of the peak area response of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and disec-butylsulfate were 9%, 5%, 6% and 5%, respectively, within 15.3h after the sensitivity solution was placed at room temperature, and were all less than 10%. The verification requirements were met, indicating that the sensitive solution was stable within 15.3h at room temperature. The RSD of the peak area response of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and disec-butyl sulfate were 10%, 7%, 8% and 8%, respectively, within 15.3h when the control solution was placed at room temperature. The verification requirements were met, indicating that the control solution was stable within 15.3h at room temperature. The RSD of peak area response of dimethyl sulfate, diethyl sulfate, diisopropyl sulfate and disec-butyl sulfate were 10%, 6%, 8% and 8%, respectively, and none of them were greater than 10%, after 100% standard test solution was placed at room temperature for 15.3h. It met the verification requirements, indicating that the 100% labeled test solution was stable within 15.3h at room temperature. The above solutions do not need to be used in the new preparation.

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### **3.2.S.4.3.5 Method Validation of assay in Finerenone**

#### **I. Analytical Procedures**

Please refer to the analytical procedures of assay in Finerenone in section 3.2.S.4.2.

#### **II. Validation Results and Discussion**

##### **a. Selection of Detection Wavelength**

###### **➤ Acceptance Criteria**

The selection of detection wavelength is through the ultraviolet scanning of the principal component at 200nm ~ 400nm wavelength, and the principal component is required to have a large absorption at 251nm.

###### **➤ Solution Preparation**

Solvent acetonitrile: water (30:70, v/v)

Blank solution: solvent.

Reference solution: Accurately weigh YA2304 about 10mg of reference product, place it in a 25ml bottle, add an appropriate amount of solvent to dissolve by ultrasound, dilute it with solvent to scale, and shake well; Accurately measure 1ml of the above solution, place it in a 20ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 0.02mg/ml)

###### **➤ Injection procedure**

After the chromatographic system is balanced, take blank solution and reference solution to inject 1 needle each, and record the chromatogram.

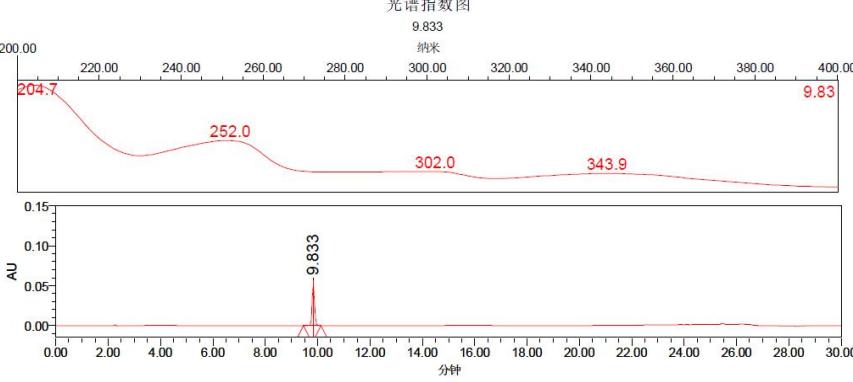
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### ➤ Measurement results

Name of solution	Test substance	Retention time (min)	Whether there is a large absorption at 251nm
Reference solution	Finerenone	9.833	is
Reference solution wavelength scan	 <p>光谱指指数图 9.833 纳米 200.00 220.00 240.00 260.00 280.00 300.00 320.00 340.00 360.00 380.00 400.00 204.7 252.0 302.0 343.9 样品名称 对照品溶液; 样品瓶 1:A,2; 进样 1; 采集日期 2024/3/26 13:50:30 CST</p> <p>AU</p> <p>分钟</p>		

Conclusion: The principal component has greater absorption at 251nm.

### (4.2) System applicability

#### ➤ Acceptance Criteria

The blank solution should be free from interference; The relative standard deviation of the main peak area should NMT 2%, the relative standard deviation of the main peak retention time should NMT 2.0%, the theoretical plate number should NLT 3000 according to the phenalidone peak, and the trailing factor NMT 1.5.

#### ➤ Solution preparation

Blank solution: solvent.

Reference solution: Prepare it by the same method as the reference solution under "4.1 wavelength selection". (Approx. 0.02mg/ml)

#### ➤ Injection procedure

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After the chromatographic system was balanced, the blank solution was injected with 1 needle, the control solution was injected with 6 needles again, and the chromatogram was recorded.

### ➤ **Measurement resultss**

Investigation parameters	Control solution was injected						RSD
	1	2	3	4	5	6	
Retention time (min)	9.852	9.848	9.850	9.863	9.862	9.861	0.07%
Main peak area	362915	362748	362771	365085	364069	364169	0.27%
Main peak trailing factor	1.18	1.17	1.18	1.18	1.18	1.18	/
Main peak theoretical plate number	76308	76957	77076	77267	77520	77334	/

Conclusion: the blank solution has no interference; The RSD of the main peak retention time was 0.07% (required not to exceed 2.0%), the RSD of the peak area was 0.27% (required not to exceed 2.0%), and the theoretical plate number of the peak of finalidone was NLT 76308 (NLT 3000). The trailing factor was in the range of 1.17~1.18 (the requirement NMT 1.5). All above meet the requirements, indicating that the method has good applicability in the system.

### **(4.3) Linearity and range**

### ➤ **Acceptance Criteria**

In the concentration range equivalent to 60%~140% of the control solution, a relatively uniform selection of 5 concentration points, with the concentration as the horizontal coordinate and the peak area as the vertical coordinate as the linear regression curve, the correlation coefficient (R) of the regression curve is NLT 0.998, the RSD of the response factor should NMT 2.0%. The absolute value of Y-axis intercept accounts for less than 2% of 100% response value.

### ➤ **Solution preparation**

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Blank solution: solvent.

YA2304 reference product reserve liquid: Accurately weigh YA2304 reference product about 10mg, place in 25ml measuring bottle, add appropriate amount of solvent to dissolve by ultrasound, dilute with solvent to scale, shake well, and then get. (About 0.4mg/ml)

Linear solution ① : Accurately measure 0.6ml of YA2304 reference product reserve liquid, place it in a 20ml measuring bottle, dilute it with solvent to the scale, shake well, and obtain. (about 12 $\mu$ g/ml)

Linear solution ② : Accurately measure 0.8ml of YA2304 reference product reserve liquid, place it in 20ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (about 16 $\mu$ g/ml)

Linear solution ③ : Accurately measure YA2304 reference product reserve liquid 1.0ml, place it in a 20ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. (About 20 $\mu$ g/ml)

Linear solution ④ : precisely measure 1.2ml of YA2304 reference product reserve liquid, place it in a 20ml measuring bottle, dilute it with solvent to the scale, shake well, and get. (about 24 $\mu$ g/ml)

Linear solution ⑤ : Accurately measure 1.4ml of YA2304 reference product reserve liquid, place it in 20ml measuring bottle, dilute it with solvent to the scale, shake well, and get. (about 28 $\mu$ g/ml)

### **➤ Injection procedure**

After the chromatographic system was balanced, the blank solution was injected with 1 needle, and the 5 linear solutions were injected with 1 needle

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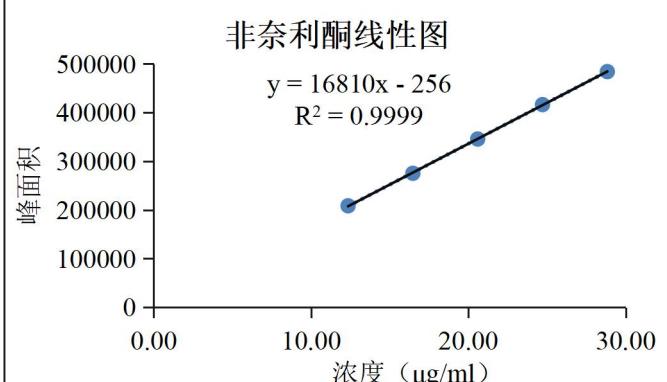
Version: 001

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each from low concentration to high concentration, and the chromatogram was recorded.

### ➤ Measurement resultss

Results of linear and range tests

Name of solution	Equivalent to the percentage of the concentration of the control solution	Concentration (μg/ml)	Peak area	Response factor
Linear solution ①	61.79%	12.3576	208458	16869
Linear solution ②	82.38%	16.4768	275246	16705
Linear solution ③	102.98%	20.5960	345639	16782
Linear solution ④	123.58%	24.7152	416368	16847
Linear solution ⑤	144.17%	28.8344	484119	16790
Response factor RSD	0.4%			
Linear graph				
Linear equations	$y=16810x-256$			
Slope	16810			
Correlation coefficient r	0.99995			
Absolute value of Y-axis intercept as a percentage of 100% response	0.076%			

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value	
Conclusion: When Finerenone was in the concentration range of 12.3576 $\mu$ g/ml~28.8344 $\mu$ g/ml, the linear equation was $y=16810x-256$ , the correlation coefficient $r$ was 0.99995 (NLT 0.998), and the RSD of the response factor was 0.4% (NMT 2.0%). The absolute value of Y-axis intercept as a percentage of 100% response value is 0.076% (no more than 2.0% required). All of the above meet the requirements, indicating that under this method, when Finerenone is in the concentration range of 12.3576 $\mu$ g/ml to 28.8344 $\mu$ g/ml, the linear relationship between peak area and concentration is good.	

### **(4.4) Accuracy**

#### **➤ Brief Description**

Add YA2304 reference in the range of 80% to 120% reference solution concentration to the solvent; The recovery rate is required to be between 98% and 101%, and the RSD of recovery rate NMT 2.0%.

#### **➤ Solution preparation**

Blank solution: solvent

Reference solution: Prepare it by the same method as the reference solution under "4.1 wavelength selection". 2 copies were prepared in parallel, in which the control solution ② was prepared by sharing the control product reserve liquid under "4.3 linearity and range". (Approx. 0.02mg/ml)

80% recovery solution: precision weigh YA2304 reference product about 8mg, put it in 25ml bottle, add appropriate amount of solvent to dissolve by ultrasound, dilute with solvent to scale, shake well; Accurately measure 1ml of the above solution, place it in a 20ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. Prepare 3 servings in parallel (about 0.016mg/ml)

100% recovery solution: accurately weigh YA2304 reference product about

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10mg, put it in 25ml bottle, add appropriate amount of solvent to dissolve by ultrasound, dilute it with solvent to scale, shake well; Accurately measure 1ml of the above solution, place it in a 20ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. Prepare 3 servings in parallel (about 0.02mg/ml)

120% recovery solution: accurately weigh YA2304 reference product about 12mg, place it in 25ml bottle, add appropriate amount of solvent to dissolve by ultrasound, dilute it with solvent to scale, shake well; Accurately measure 1ml of the above solution, place it in a 20ml measuring bottle, dilute it with solvent to the scale, shake well, and then obtain. Prepare 3 servings in parallel (about 0.024mg/ml)

### ➤ **Injection procedure**

After the chromatographic system was balanced, the blank solution was injected with 1 needle, the control solution (1) was injected with 6 needles, the control solution (2) was injected with 2 needles, the 9 recovery solutions were injected with 1 needle each, and the chromatogram was recorded.

### ➤ **Measurement resultss**

Name of solution	Amount to add ( $\mu\text{g}/\text{ml}$ )	Measured quantity ( $\mu\text{g}/\text{ml}$ )	Recovery rate	Average recovery rate	RSD
80% recovery solution -1	16.0417	16.1074	100.4%	99.9%	0.6%
80% recovery solution -2	16.0038	15.9873	99.9%		
80% recovery solution -3	16.0597	16.0472	99.9%		
100% recovery solution -1	20.7416	20.7942	100.3%		
100% recovery	20.6977	20.6882	100.0%		

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solution -2					
100% recovery solution -3	20.6897	20.7189	100.1%		
120% recovery solution -1	24.3747	24.2658	99.6%		
120% recovery solution -2	24.2889	23.9431	98.6%		
120% recovery solution -3	24.4963	24.5611	100.3%		
Conclusion: 16.0038 $\mu$ g/ml~24.4963 $\mu$ g/ml of YA2304 was added into the solvent. The recoveries were 98.6%~100.4% (the requirement was 98%~101%), and the RSD of the recovery was 0.6% (the requirement was not more than 2.0%). All the above met the verification requirements, indicating the accuracy of the method was good.					

### **(4.5) Precision**

Precision refers to the proximity between the results obtained by multiple sampling of the same uniform test product under specified test conditions, and its precision is judged by examining repeatability and intermediate precision.

#### **(4.5.1) Repeatability**

##### **➤ Acceptance Criteria**

The analyst A prepared 6 repeatable sample solutions on the date A, measured by instrument A, requiring the first reference solution to be continuously sampled for 6 times, the relative standard deviation of the main peak area should NMT 2%, the relative standard deviation of the main peak retention time should NMT 2.0%, and the theoretical plate number should NLT 3000 calculated according to the peak of Finerenone. The trailing factor NMT 1.5; The RSD of principal component in 6 repeatable test product solutions NMT 1.0%.

##### **➤ Solution preparation**

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Blank solution: solvent

Reference solution: Prepare it by the same method as the reference solution under "4.1 wavelength selection". Prepare 2 parts in parallel. (Approx. 0.02mg/ml)

Repeatable test product solution: accurately weigh about 10mg of this product, place it in 25ml measuring bottle, add appropriate amount of solvent and ultrasonic to dissolve, dilute it with solvent to scale, shake well; Accurately measure 1ml of the above solution, place it in a 20ml measuring bottle, dilute it with solvent to the scale, shake well, and then get. Prepare 6 parts in parallel. (Approx. 0.02mg/ml)

### ➤ **Injection procedure**

After the chromatographic system was balanced, the blank solution was injected with 1 needle, the control solution (1) was injected with 6 needles, the control solution (2) was injected with 2 needles, each of the 6 repetitive test solutions was injected with 1 needle, and the chromatogram was recorded.

### ➤ **Measurement results**

Results of repeatability - system suitability test

Investigation parameters	Control solution was injected						RSD
	1	2	3	4	5	6	
Retention time (min)	9.882	9.884	9.883	9.886	9.883	9.885	0.015 %
Main peak area	340753	340305	340824	341335	340663	340895	0.10%
Main peak trailing factor	1.18	1.19	1.18	1.19	1.19	1.19	/
Main peak theoretical plate	79832	79779	80060	79587	79934	79660	/

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number							
Conclusion: the blank solution has no interference; The RSD of the main peak retention time was 0.015% (required not to exceed 2.0%), the RSD of the peak area was 0.10% (required not to exceed 2.0%), and the theoretical plate number of the peak of finalidone was NLT 79587 (NLT 3000). The trailing factor is in the range of 1.18~1.19 (the requirement NMT 1.5). The above all meet the verification requirements, indicating that the suitability of the system meets the requirements.							

### Repeatability test results

Investigation items	Solution name						RSD
	Repetitive test product solution 1	Repetitive test product solution 2	Repetitive test product solution 3	Repetitive test product solution 4	Repetitive test product solution 5	Repetitive test product solution 6	
Principal component content	99.1%	99.3%	98.9%	100.0%	99.7%	99.3%	0.5%
Conclusion: The RSD of principal component in 6 repeatable samples was 0.5% (the requirement NMT 1.0%), which met the verification requirements, indicating that the method had good repeatability.							

### **(4.5.2) Intermediate precision**

#### ➤ **sketch**

6 intermediate precision test product solutions were prepared by Analyst B on date B. The relative standard deviation of the main peak area should NMT 2%, the relative standard deviation of the main peak retention time should NMT 2.0%, the theoretical plate number should NLT 3000, and the trailing factor NMT 1.5. The RSD of principal component in 6 intermediate precision test product solutions NMT 1.0%.

For the results of 12 tests of repeatability and intermediate precision, the RSD of principal component content NMT 2.0%.

#### ➤ **Solution preparation**

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Blank solution: thinner

Reference solution: Prepare it by the same method as the reference solution under "4.1 wavelength selection". 2 copies were prepared in parallel, in which the control solution (1) shared the control solution under "4.1 wavelength selection". (Approx. 0.02mg/ml)

Intermediate precision test product solution: accurately weigh about 10mg of this product, place it in a 25ml measuring bottle, add an appropriate amount of diluent to dissolve by ultrasound, dilute it with diluent to the scale, and shake well; Precision measure 1ml of the above solution, place it in a 20ml measuring bottle, dilute it with diluent to the scale, shake well, and then get.

Prepare 6 parts in parallel. (Approx. 0.02mg/ml)

### ➤ **Injection procedure**

After the chromatographic system is balanced, take blank solution into 1 needle, control solution 1 into 6 needles, control solution 2 into 2 needles, 6 intermediate precision test solution 1 needle each, record the chromatogram.

### ➤ **Measurement results**

Results of intermediate precision - system suitability test

Investigation parameters	Control solution was injected						RSD
	1	2	3	4	5	6	
Retention time (min)	9.809	9.798	9.788	9.785	9.796	9.795	0.09%
Main peak area	344187	345304	345625	345620	345536	345986	0.19%
Main peak trailing factor	1.23	1.23	1.23	1.23	1.23	1.22	/
Main peak theoretical plate number	50253	48230	48071	47066	48322	48600	/

Conclusion: the blank solution has no interference; The RSD of the main peak

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retention time was 0.09% (required not to exceed 2.0%), the RSD of the peak area was 0.19% (required not to exceed 2.0%), and the theoretical plate number of the peak of finalidone was NLT 47066 (NLT 3000). The trailing factor was in the range of 1.22~1.23 (the requirement NMT 1.5). The above all meet the verification requirements, indicating that the suitability of the system meets the requirements.

### Precision test results

Investigation items	Repetitive test product solution						RSD (n=6)	RSD (n=12)
	1	2	3	4	5	6		
Principal component content	99.1%	99.3%	98.9%	100.0 %	99.7%	99.3%	0.5%	
Items to examine	Intermediate precision test product solution						RSD (n=6)	0.4%
	1	2	3	4	5	6		
Principal component content	99.5%	99.0%	99.7%	99.4%	99.5%	98.8%	0.4%	

Conclusion: The RSD of principal component in 6 intermediate precision test solutions was 0.4% (the requirement NMT 1.0%), which met the verification requirements, indicating that the intermediate precision of the method was good. The RSD of principal component in 12 repetitive sample solutions and intermediate precision sample solutions was 0.4% (the requirement NMT 2.0%), which met the verification requirements, indicating that the method was of good precision.

### **(4.6) Stability of the solution**

#### ➤ **Acceptance Criteria**

Each solution is placed under the 5°C injector, the control solution and the test product solution are injected at different time points, and the theoretical plate number in the control solution is required to be NLT 3000 according to the peak of Finerenone, and the tail factor NMT 1.5; The RSD of the main peak-peak area between the control solution and the test solution NMT 2.0%, and no new impurities interfering with the detection of principal components should be added.

#### ➤ **Solution preparation**

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Blank solution: solvent.

Reference solution: Share the reference solution under "4.2 System Suitability". (Approx. 0.02mg/ml)

Test product solution: accurately weigh about 10mg of this product, put it in 25ml measuring bottle, add appropriate amount of solvent to dissolve by ultrasound, dilute it with solvent to scale, shake well; Accurately measure 1ml of the above solution, place it in a 20ml measuring bottle, dilute it with solvent to the scale, shake well, and then get. (About 0.02mg/ml)

### ➤ **Injection procedure**

After the chromatographic system was balanced, the control solution placed in the 5°C injector was injected at 0h, 2h, 4h, 6h, 12h, 24h, 39h, 54h and 75h, and the chromatogram was recorded. (Chromatograms of reference solution 0h and 2h share the chromatograms of reference solution 1 and 5 with repeated injection of 6 needles under "4.2 System Suitability", respectively)

After the chromatographic system was balanced, the test solution placed in the 5°C injector was injected at 0h, 2h, 4h, 10h, 22h, 37h, 52h and 73h respectively, and the chromatogram was recorded.

### ➤ **Results of Determination**

Stability test results of solution

Reference solution				Test product solution	
Inspection time	Main peak area	Main peak trailing factor	Main peak theoretical plate number	Survey time	Main peak area
0h	362915	1.18	76308	0h	346965
2h	364069	1.18	77520	2h	347156
4h	364017	1.18	76537	4h	348093
6h	364517	1.18	77200	10h	351717
12h	365302	1.18	77107	22h	355761
24h	366661	1.18	79369	37h	347277

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39h	363491	1.19	79861	52h	350903
54h	365063	1.19	81912	73h	360143
75h	369263	1.20	82877	/	/
RSD	0.6%	/	/	/	1.4%

Verdict: ① The control solution was placed under the sample injector at 5°C for 75h, the peak area RSD of the main peak area was 0.6% (required not to exceed 2.0%), the theoretical plate number of the peak of finalidone was NLT 76308 (NLT 3000), and the trailing factor was in the range of 1.18~1.20 (required not to exceed 1.5). And no impurities interfering with the detection of Finerenone content were produced, indicating that the reference solution was stable within 75h after being placed under the 5°C injector. ② The sample solution was placed under the 5°C injector for 73h, the RSD of the main peak area was 1.4% (required not to exceed 2.0%), and no impurities interfered with the detection of fineridone content, indicating that the sample solution was stable within 73h under the 5°C injector.

### **(4.7) Durability**

#### ➤ **Brief Description**

Durability refers to the determination conditions have a small change, the Measurement resultss are not affected by the degree of tolerance, by examining a single factor to change the chromatographic conditions (change column temperature, wavelength, flow rate, different columns) and normal chromatographic conditions to test the content of the test product solution to achieve. In the chromatogram obtained from the reference product solution, the number of theoretical plates should NLT 3000 according to the phenalidone peak, and the trailing factor NMT 1.5. Single factor change chromatographic conditions and normal chromatographic conditions under the test sample solution, the RSD of the principal component content should NMT 2.0%.

Table 3.2.S.4.3.7-10 Changes in chromatographic conditions for durability

test

Chromatogra	Standard conditions	Variable Values
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Parameter	Value	Value
Column temperature	20 °C	22°C, 18°C
Wavelength	251 nm	249 nm, 253 nm
Flow rate	1.0 ml/min	0.95ml/min, 1.05 ml/min
Chromatographic column	YMC Trairt C18 (3μm, 4.6mm x 150mm) (serial number: 129ZB00034; Serial number: ZJC18-35)	YMC Trairt C18 (3μm, 4.6mm x 150mm) (serial number: 117EB10004; Serial number: CXC18-05)

### ➤ Solution preparation

Blank solution: solvent.

Reference solution: Prepare it by the same method as the reference solution under "4.1 wavelength selection". 2 copies were prepared in parallel, in which the reference solution under "4.5.2 intermediate precision" was shared for durability of different chromatographic columns, and the rest were shared under "4.5.1 repeatability". (Approx. 0.02mg/ml)

Test product solution: Prepare the test product solution under "4.3 specific properties" by the same method. Two samples were prepared in parallel, in which, except for the intermediate precision test solution ① and ② under "4.5.2 Intermediate precision" for durability of different chromatocolumns, the rest were shared with the repetitive test solution ① and ② under "4.5.1 repeatability". (Approx. 0.02mg/ml)

### ➤ Injection procedure

Under the conditions of different durability (normal conditions, column temperature, flow rate, wavelength, different column), after the chromatographic system was balanced, the blank solution was injected with 1 needle, 2 control solution and 2 test solution were injected with 2 needles respectively, and the chromatogram was recorded.

### ➤ Measurement results

Inspection	Parameter	Reference solution ①	Control solution ②	Test product solution
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conditions	values	Main peak trailing factor	Main peak theoretic al plate number	Main peak trailing factor	Main peak theoretic al plate number	Principal compone nt content	RSD of content		
Normal conditions	1.18	80373	1.19	80074					
Normal conditions	1.18	80414	1.19	80543					
Wavelength	249nm	1.16	73280	1.17	74216	99.5%	0.23%		
		1.17	74786	1.17	75205				
	253nm	1.17	73925	1.17	73825	99.8%			
		1.17	74339	1.17	74802				
Column temperature	18 °C	1.20	80421	1.20	80180	99.5%	0.23%		
		1.19	79968	1.20	80426				
	22 °C	1.19	84752	1.19	84360	99.6%			
		1.18	83317	1.19	84331				
Flow rate	0.95 ml/min	1.21	88884	1.21	88706	100.0%	0.23%		
		1.21	88534	1.21	89418				
	1.05 ml/min	1.18	78257	1.18	78226	99.9%			
		1.19	78526	1.19	78432				
Column 2		1.18	84401	1.18	84466	99.4%			
		1.18	85388	1.18	84993				

Conclusion: Under the conditions of column temperature, wavelength, flow rate, different chromatographic columns and normal conditions, the theoretical plate number calculated by the peak of fenelidone is NLT 73280 (NLT 3000), and the trailing factor is in the range of 1.16~1.21 (NMT 1.5). Under the condition of single factor change of color, the sample solution was tested under normal chromatographic conditions, and the RSD of principal component was 0.23% (not more than 2.0%). The above are in line with the verification requirements, indicating that the method has good durability.

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### III. Summary of Method Validation

#### Summaries of Method Validation of Assay in Finerenone

Item	Acceptable criteria	Verification of results
Wavelength selection	The principal component has large absorption at 251nm wavelength	<p>The principal component has greater absorption at 251nm. In the control solution, the retention time of Finerenone was 9.833min, and the spectral diagram of the control solution was as follows:</p> <p>光谱指指数图 9.833 纳米 200.00 220.00 240.00 260.00 280.00 300.00 320.00 340.00 360.00 380.00 400.00 204.7 252.0 302.0 343.9 样品名称 对照品溶液; 样品瓶 1:A,2; 进样 1; 采集日期 2024/3/26 13:50:30 CST</p> <p>AU 0.15 0.10 0.05 0.00 0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 分钟 9.833</p>
System applicability	The blank solution should be free of interference; The relative standard deviation of the main peak area should NMT 2%, the relative standard deviation of the main peak retention time should NMT 2.0%, the theoretical plate number should NLT 3000 according to the phenalidone peak, and the	<p>The blank solution has no interference; The RSD of the main peak retention time was 0.07% (required not to exceed 2.0%), the RSD of the peak area was 0.27% (required not to exceed 2.0%), and the theoretical plate number of the peak of fenalidone was NLT 76308 (required NLT 3000). The trailing factor was in the range of 1.17~1.18 (the requirement NMT 1.5). All above meet the requirements, indicating that the method has good applicability in the system.</p>

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	trailing factor NMT 1.5.												
Linearity and range	In the concentration range equivalent to 60%~140% of the control solution, 5 concentration points were evenly selected, and the concentration was taken as the horizontal coordinate and the peak area was taken as the vertical coordinate as the linear regression curve. The correlation coefficient (R) of the regression curve was NLT 0.998, and the RSD of the response factor should NMT 2.0%. The absolute value of Y-axis intercept accounts for less than 2% of 100% response value.												
		<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: center;"><b>Impurities</b></th><th style="text-align: center;"><b>Concentration (equivalent to the percentage of solution concentration of the test product)</b></th><th colspan="2" style="text-align: center;"><b>Linear equation</b></th></tr> </thead> <tbody> <tr> <td style="text-align: center;">YA2304</td><td style="text-align: center;">12.3576<math>\mu</math>g/ml~28.8344<math>\mu</math>g/ml (61.79%~144.17%)</td><td colspan="2" style="text-align: center;"><math>y=16810x-256</math></td></tr> </tbody> </table>				<b>Impurities</b>	<b>Concentration (equivalent to the percentage of solution concentration of the test product)</b>	<b>Linear equation</b>		YA2304	12.3576 $\mu$ g/ml~28.8344 $\mu$ g/ml (61.79%~144.17%)	$y=16810x-256$	
<b>Impurities</b>	<b>Concentration (equivalent to the percentage of solution concentration of the test product)</b>	<b>Linear equation</b>											
YA2304	12.3576 $\mu$ g/ml~28.8344 $\mu$ g/ml (61.79%~144.17%)	$y=16810x-256$											
Accuracy	Add YA2304 reference in the range of 80% reference solution concentration to 120% reference solution concentration to the thinner; The recovery rate is required to be between 98% and 101%, and the RSD of recovery rate NMT 2.0%.	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: center;"><b>Impurities</b></th><th style="text-align: center;"><b>Marking condition</b></th><th style="text-align: center;"><b>Average recovery rate</b></th><th style="text-align: center;"><b>RSD</b></th></tr> </thead> <tbody> <tr> <td style="text-align: center;">YA2304</td><td style="text-align: center;">Added 16.0038<math>\mu</math>g/ml to 24.4963<math>\mu</math>g/ml</td><td style="text-align: center;">99.9%, n=9</td><td style="text-align: center;">0.6%, n=9</td></tr> </tbody> </table>				<b>Impurities</b>	<b>Marking condition</b>	<b>Average recovery rate</b>	<b>RSD</b>	YA2304	Added 16.0038 $\mu$ g/ml to 24.4963 $\mu$ g/ml	99.9%, n=9	0.6%, n=9
<b>Impurities</b>	<b>Marking condition</b>	<b>Average recovery rate</b>	<b>RSD</b>										
YA2304	Added 16.0038 $\mu$ g/ml to 24.4963 $\mu$ g/ml	99.9%, n=9	0.6%, n=9										
Precision	Repeatability and intermediate precision: the RSD of principal component in 6 repeatability (or intermediate precision) test product solution NMT 1.0%. Precision: For the results of 12 repeatability and intermediate precision tests, the RSD of	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: center;"><b>Principal Component</b></th><th style="text-align: center;"><b>Repeatability</b></th><th style="text-align: center;"><b>Intermediate precision</b></th><th style="text-align: center;"><b>Precision</b></th></tr> </thead> <tbody> <tr> <td style="text-align: center;">YA2304</td><td style="text-align: center;">0.5%, n=6</td><td style="text-align: center;">0.4%, n=6</td><td style="text-align: center;">0.4%, n=</td></tr> </tbody> </table>				<b>Principal Component</b>	<b>Repeatability</b>	<b>Intermediate precision</b>	<b>Precision</b>	YA2304	0.5%, n=6	0.4%, n=6	0.4%, n=
<b>Principal Component</b>	<b>Repeatability</b>	<b>Intermediate precision</b>	<b>Precision</b>										
YA2304	0.5%, n=6	0.4%, n=6	0.4%, n=										

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	principal component content NMT 2.0%.	
Solution stability	<p>Each solution was placed in the sample injector at 5°C, the control solution and the test product solution were injected at different times, and the theoretical plate number in the control solution was required to be NLT 3000 according to the peak of Finerenone, and the tail factor NMT 1.5; The RSD of the main peak-peak area between the control solution and the test solution NMT 2.0%, and no new impurities interfering with the detection of principal components should be added.</p>	<p>The control product solution: Placed under the 5°C injector for 75h, the peak area RSD of the main peak area is 0.6% (required not to exceed 2.0%), the theoretical plate number of the peak of finalidone is NLT 76308 (required NLT 3000), and the trailing factor is in the range of 1.18~1.20 (required not to exceed 1.5). And no impurities interfered with the detection of Finerenone content, indicating that the control solution was stable within 75h after being placed under the 5°C sampler.</p> <p>Test solution: The RSD of main peak area was 1.4% (required not to exceed 2.0%) within 73h after being placed under the 5°C injector, and no impurities interfered with the detection of Finerenone content, indicating that the test solution was stable within 73h after being placed under the 5°C injector.</p> <p>The above solutions do not need new preparation for temporary use.</p>
Durability	<p>Under the conditions of single factor change column temperature, wavelength, flow rate, different chromatographic columns and normal chromatographic conditions, the theoretical plate number calculated according to the peak of Finerenone should NLT 3000, and the trailing factor NMT 1.5.</p> <p>Under single factor change chromatographic conditions and normal chromatographic conditions, the RSD of the principal component content should NMT 2.0%.</p>	<p>The column temperature was <math>20^{\circ}\text{C} \pm 2^{\circ}\text{C}</math>, the wavelength was <math>251\text{nm} \pm 2\text{nm}</math>, the flow rate was <math>1.0\text{ml/min} \pm 0.05\text{ml/min}</math>, and the chromatographic column with different batch NO.s was investigated as follows:</p> <p>System applicability: In the chromatogram obtained from the control product solution, the theoretical plate number calculated by the peak of Finerenone was NLT 73280 (required NLT 3000), and the trailing factor was in the range of 1.16~1.21 (required not to exceed 1.5). The suitability of the system met the verification requirements.</p> <p>Test product solution: The test product solution was tested under normal chromatographic conditions under single factor color change condition, and the RSD of principal component was 0.23% (no more than 2.0% required); The</p>

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	above are in line with the verification requirements.
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### **3.2.S.4.4 Batch Analyses**

#### **Analytical Data of Three Consecutive Batches**

Analytical data of three commercial scale batches (Batch No.: 231201, 240101 and 240102) are summarized in 3.2.S.4.4 *Table 1*. All batches were fully tested according to the analytical procedures in section 3.2.S.4.2.

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Table 3.2.S.4.4-1 Batch Analysis Results of Finerenone

Batch No.		Batch No.: 231201	Batch No.: 240101	Batch No.: 240102
Batch size		11.085kg	9.925kg	10.215kg
Date of manufacture		Jan. 06, 2024	Jan. 19, 2024	Jan. 30, 2024
Site of manufacture		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.		
Items	Specifications	Results	Results	Results
Characters	white to yellow powder	white powder	white powder	white powder
	Solubility: dissolved in methanol, slightly soluble in ethanol, acetonitrile and acetone, slightly soluble in isopropyl alcohol, and almost insoluble in water	Complies	Complies	Complies
Identification	IR: The infrared light absorption pattern of this product should be consistent with that of the control product	Complies	Complies	Complies
	HPLC: In the chromatogram recorded under the enantiomer determination, the retention time of the main peak of the test solution should be consistent with the retention time of the main peak of the system suitability solution	Complies	Complies	Complies
Related substances I	YA2304-10: NMT 0.10%	Not detected	Not detected	Not detected
	YA2304-12: NMT 0.15%	Not detected	Not detected	Not detected
	YA2304-14: NMT 0.15%	Not detected	Not detected	Not detected

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	YA2304-15: NMT 0.15%	Not detected	Not detected	Not detected
	YA2304-16: NMT 0.15%	Not detected	Not detected	Not detected
	YA2304-17: NMT 0.15%	Not detected	Not detected	Not detected
	YA2304-18: NMT 0.15%	Not detected	Not detected	Not detected
	YA2304-19: NMT 0.15%	< 0.05%	< 0.05%	< 0.05%
	Other single impurities: NMT 0.10%	< 0.05%	< 0.05%	< 0.05%
	Total impurity: NMT 1.0%	< 0.05%	< 0.05%	< 0.05%
enantiomers	YA2304-20: NMT 0.15%	0.03%	0.03%	0.02%
Residual solvents	Ethanol NMT 0.5%	0.1%	0.2%	0.1%
	Isopropyl alcohol NMT 0.5%	Not detected	Not detected	Not detected
	Ethyl acetate NMT 0.5%	Not detected	Not detected	Not detected
	Secondary butanol NMT 0.5%	Not detected	Not detected	Not detected
	Tetrahydrofuran NMT 0.072%	Not detected	Not detected	Not detected
	Toluene NMT 0.089%	Not detected	Not detected	Not detected
	N-methylpyrrolidone NMT 0.053%	Not detected	Not detected	Not detected
genotoxic impurities	Dimethyl sulfate NMT 75ppm	Not detected	Not detected	Not detected
	Diethyl sulfate NMT 75ppm	Not detected	Not detected	Not detected
	Diisopropyl sulfate NMT 75ppm	Not detected	Not detected	Not detected

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	Disec-butyl sulfate NMT 75ppm	Not detected	Not detected	Not detected
	Sum of genotoxic impurities NMT 250ppm	Not detected	Not detected	Not detected
Water	NMT 0.5%	0.05%	0.04%	0.2%
Residue on ignition	NMT 0.1%	Complies	Complies	Complies
Heavy metals	NMT 10ppm	Complies	Complies	Complies
Microbial limits	TAMC: NMT $10^3$ CFU/g	< 10 CFU/g	< 10 CFU/g	<10 CFU/g
	TYMC: NMT $10^2$ CFU/g	< 10 CFU/g	< 10 CFU/g	<10 CFU/g
	Absence of <i>Escherichia coli</i> (1g)	Not detected	Not detected	Not detected
Assay	98.0% to 102.0% (anhydrous substance).	99.8%	99.8%	99.7%

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### **Batch Determination**

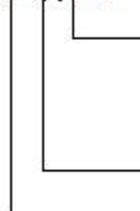
The Batch No. of commercial production of APIs can be divided into two cases:  
Batch No. of intermediates and Batch No. of the final drug substance (API).

1) Batch No. composition of intermediate: product code + year + month + serial number (D+YY+MM+ZZ). For example, the Batch No. of an intermediate is D220901, where D is the product code, 22 is the year, 09 is the month, 01 is the serial number, indicating the first batch of intermediate of a specific API in September 2022.

2) Batch No. composition of the final drug substance (API): year + month + product code + serial number (YYYY+MM+X+ZZ), in which the year is 4 digits, the month is 2 digits, and the product code is 1 or 2 letters (2 letters are W+ the other letter). For example, the Batch No. of an API is 202209D01, in which 2022 is the year; 09 is the month; D is the product code; 01 is the serial number, representing the first batch of a specific API in September 2022.

Batch No. composition of API manufactured from trial production in technology transfer stage and process validation: 6 digits.

e.g.: **220901**



represents the sequence of this API  
manufactured in this workshop in this month  
(the first batch)  
represents the month (September)  
represents the year (2022)

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**Certificate of Analysis (COA) of three Commercial Scale Batches**

COA of Finerenone (Batch No.: 231201)

COA of Finerenone (Batch No.: 240101)

COA of Finerenone (Batch No.: 240102)

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### **3.2.S.4.5 Justification of Specification**

The specification of Finerenone was established by the applicant according to the Quality Guidelines and Multidisciplinary Guidelines of ICH. The analytical procedures of all of the tests (Characters, Identification, Related substances, Residual solvents, Loss and Dry, Residue on ignition, Heavy metals, Microbial limits and Assay) were established by the applicant and the test methods have been well validated.

### **Appearance**

A qualitative test performed to know the colour of the API. White to yellow powder by visual inspection.

### **Solubility**

Solubility is a basic phenomenon and commonly evaluated parameter which helps in pre-formulation studies and to know the dissolution of the API in different solvents. dissolved in methanol, slightly soluble in ethanol, acetonitrile and acetone, slightly soluble in isopropyl alcohol, and almost insoluble in water.

### **Infrared Absorption Spectrophotometry**

This is a specific test for identification of drug substances by IR using validated method. The IR spectra of the substance to be examined should comply with that of the Finerenone CRS. Examine by *Standard operating procedure of infrared absorption spectrophotometry*.

### **High Performance Liquid Chromatography**

In the chromatogram recorded under the enantiomer determination, the retention time of the main peak of the test solution should be consistent with the retention time of the main peak of the system suitability solution. (*See Enantiomer*).

### **Related substances**

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The *related substances* of Finerenone was established by the applicant according to the *ICH Q3A(R2): Impurities in New Drug Substances*. The threshold of Specified Impurity including all of the Impurity (YA2304-14, YA2304-15, YA2304-19): NMT 0.15%, YA2304-10: NMT 0.10%; Unspecified Impurity was established by the Identified threshold : NMT 0.10%.

### **Enantiomers**

The *Enantiomers* of Finerenone was established by the applicant according to the *ICH Q3A(R2): Impurities in New Drug Substances*. The threshold of Specified Impurity including all of the YA2304-20: NMT 0.15%.

### **Residual solvents**

The *residual solvents* of Finerenone was established by the applicant according to the *ICH Q3C(R8) Impurities: Guideline for Residual Solvents*. Ethanol NMT 0.5 %; Tetrahydrofuran NMT 0.072%; Toluene NMT 0.089%; N-methylpyrrolidone NMT 0.053%.

### **Water**

NMT 0.5%. Examine in accordance with Standard operating procedure of Water by the Karl Fischer determination.

### **Residue on ignition**

NMT 0.1%. Examine by *Standard operating procedure of residue on ignition determination*.

### **Heavy metals**

NMT 10ppm. Examine by *Standard operating procedure of heavy metals determination*.

### **Microbial Limits**

The drug substance Finerenone is a non-sterile API. The applicant established microbial limits test in the Finerenone specification according to chapter *Microbiological examination of non-sterile products microbial enumeration tests* and *Microbiological examination of non-sterile products control bacteria tests* :

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- TAMC is NMT  $10^3$  CFU/g;
- TYMC is NMT  $10^2$  CFU/g;
- *E.coli* is absent in each 1 g of sample.

### **Assay**

A quantitative test performed to know the actual content of API and the acceptance criteria has calculated based on the content specified and unspecified impurities controlled. It contains NLT 98.0% and NMT 102.0% of C<sub>21</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>, calculated on the anhydrous substance basis.

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### 3.2.S.5 Reference Standards or Materials

#### I. Reference Standards used for Testing Finerenone

In-house working standards has been established. Their basic information is as below.

Table 3.2.S.5-1 Reference Standards used for Testing of Finerenone

No.	Name of reference standard	Category	Batch No.	Content (%)	Source of reference standard
1	YA2304-10	Working standard	DTA304	99.77%	Purchased <sup>*1</sup>
2	YA2304-12	Working standard	23081801-C	99.0%	Selfmade
3	YA2304-14	Working standard	23082301-C	99.6%	Selfmade
4	YA2304-15	Working standard	23082903-C	99.3%	Selfmade
5	YA2304-16	Working standard	23082201-C	93.7%	Selfmade
6	YA2304-17	Working standard	DMW857	99.30%	Purchased <sup>*1</sup>
7	YA2304-18	Working standard	23082802-C	98.9%	Selfmade
8	YA2304-19	Working standard	23080701-A	99.4%	Selfmade
9	YA2304-20	Working standard	23080401-A	99.2%	Selfmade
10	YA2304-52	Working standard	24022801-C	90.8%	Selfmade
11	Dimethyl sulfate	Working standard	C15128674	99.759%	Purchased <sup>*2</sup>
12	Diethyl sulfate	Working standard	C14721434	99.024%	Purchased <sup>*2</sup>

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13	Diisopropyl sulfate	Working standard	DLX318	97%	Purchased* <sup>1</sup>
14	Di-sec-butyl sulfate	Working standard	23110207-H	99.7%	Selfmade
15	YA2304 (Finerenone)	Working standard	23091802-D	99.7%	Selfmade

\*1: Reference standard of YA2304-10, YA2304-17, Diisopropyl sulfate was purchased from Shanghai Bidd Pharmaceutical Technology Co., LTD.

\*2: Reference standard of Dimethyl sulfate, Diethyl sulfatewas purchased from Shanghai McLean Biochemical Technology Co., LTD.

The in-house prepared Finerenone (batch No. 23091802-D) is employed as working standard. It has been fully characterized in terms of chemical structure and purity. Its content value has been assigned by the applicant according to Finerenone working standard.

## **II. Description and Establishment of Working Standards**

YA2304-10 working standard (batch No. DTA304), YA2304-17 working standard (batch No. DMW857), Dimethyl sulfate working standard (batch No. C15128674), Diethyl sulfate working standard (batch No. C14721434), Diisopropyl sulfate working standard (batch No. DLX318) was purchased from other companies.

The description (preparation, specification and analytical procedures) of Finerenone(YA2304) working standard, YA2304-12 working standard, YA2304-14 working standard, YA2304-15 working standard, YA2304-16 working standard, YA2304-18 working standard, YA2304-19 working standard, YA2304-20 working standard, YA2304-52 working standard and Di-sec-butyl sulfate working standard are as follows.

### **(1) YA2304-10 working standard**

The YA2304-10 reference product used in this application was derived fr om Shanghai BidD Medical Technology Co., LTD. It was named D-dibenz

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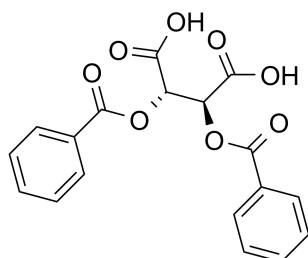
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oyl tartaric acid on the physical label and (2S,3S)-2, 3-BIS (beznoyloxy)succinic acid on the manufacturer's report. The reference product on the invoice is called (2S,3S)-2, 3-bis (benzoyloxy) succinic acid. The applicant has confirmed its chemical structure and the content is 99.77% according to the report form provided by the manufacturer.

Sample lot No.: DTA304

Structural formula:



Molecular formula: C<sub>18</sub>H<sub>14</sub>O

Molecular weight: 358.3

### **(1.1) Structural analysis of YA2304-10 reference**

The chemical structure of the reference product was confirmed by the applicant by means of <sup>1</sup>H NMR, <sup>13</sup>C NMR, mass spectrometry and specific curl. <sup>1</sup>H NMR, specific curl and mass spectrometry were provided by Shanghai Bider Medical Technology Co., LTD., and <sup>13</sup>C NMR was commissioned by Changsha Kangpeng Pharmaceutical Co., LTD. The relevant test results and structural analysis are detailed as follows:

#### **a. Nuclear Magnetic Resonance (NMR)**

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

Solvent: DMSO;

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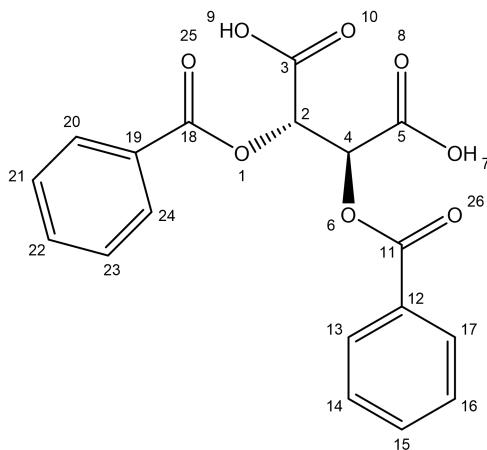
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Reference: TMS ( $^1\text{H}$  spectrum);

Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

The number of the hydrocarbon atom in the structure (non-systematic naming, only used for hydrocarbon magnetic spectrum analysis)



The test data and analytical results of hydrocarbon spectra are shown in Table 3.2.S.5-2 and Table 3.2.S.5-3.

Table 3.2.S.5-2  $^1\text{H}$  NMR chemical shift value and correlation spectrum of the tested sample (from high field to low field)

Peak No.	Delta ppm	Peak pattern	Coupling constant (J)	Proton number	Attribution
1	5.95	s	/	2H	2, 4 - CH
2	7.62	t	/	4H	21,23,14,16 - CH
3	7.73	d	7.4	2H	15, 22 - CH
4	8.06	d	7.3	4H	13,17,20,24 - CH
5	13.97	s	/	2H	9, 7 - OH

Table 3.2.S.5-3  $^{13}\text{C}$ NMR chemical shift value and correlation spectrum (from high field to low field) of the tested sample

Peak No.	Delta ppm	Number of carbon	Attribution
----------	-----------	------------------	-------------

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		atoms	
1	71.973	2	2, 4 - C
2	128.954	2	12-3 C
3	129.517	4	14,16,21,23 - C
4	129.901	4	13,17,22,24 - C
5	134.591	2	15, 22 - C
6	165.176	2	11-16 C
7	167.686	2	3, 5 - C

<sup>1</sup>H-NMR(DMSO-d<sub>6</sub>, 400Hz) showed 14 hydrogen protons, and the hydrogen displacement of each group could be reasonably attributed. <sup>13</sup>C-NMR(DMSO-d<sub>6</sub>, 100Hz) showed 18 carbon atoms, and the carbon displacement of each group could be reasonably assigned. The NMR data were in good agreement with the target compound.

### **b, Mass spectrometry (MS)**

Testing instrument: LC-MS (Manufacturer's spectrum)

Analytical method: /

Test data:

Table 3.2.S.5-4 MS data of the tested sample

Mass-charge ratio (m/z)	Corresponding ion	Spare note
357.1	[M-H] <sup>-</sup>	Molecular ion peak in negative ion mode

In the negative ion mode, the mass charge ratio m/z is 357.1 peak, which is attributed to the molecular ion peak in the negative ion mode, consistent with the molecular weight of the target molecule 358.3.

### **c, specific curl**

Test data:

Table 3.2.S.5-5 Specific curl of the tested sample

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Specific Curl
116.129 (C=1.31 ETOH)

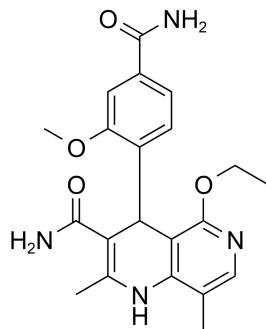
The relative rotation value of the control substance is +116.129°, which can confirm that the optical rotation configuration is right-handed structure, which is consistent with the target molecule.

### **(2) YA2304-12 working standard**

The reference product YA2304-12 used in this study was self-made by Tianyi Hengyi Pharmaceutical Co., LTD. It was named 4-(4-aminoformyl-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthyl-3-formamide on the physical label and the reference product report. The applicant confirmed its chemical structure and content calibration, and the content was calculated according to 99.0%.

Sample lot No.: 23081801-C

Structural formula:



Molecular formula: C<sub>21</sub>H<sub>24</sub>O<sub>4</sub>N

Molecular weight: 396.45

#### **(2.1) Preparation method of YA2304-12**

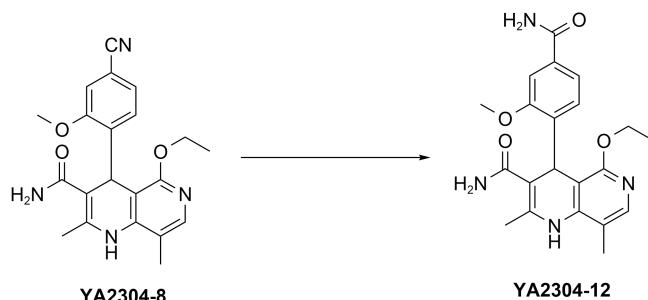
Add YA2304-8(4.2g), potassium carbonate (2.2g) and DMSO(20 mL) into a 250 mL flask, slowly add 30% hydrogen peroxide (6 mL) under agitation, stir at room temperature for 0.5 h, add water (60 mL) into the reaction system, stir for 0.5 h, and filter. Rinse with water (100 mL) and dry at 50 °C to obtain YA2304-12, white solid (4.3 g).

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### (2.2) Structural analysis of YA2304-12 reference

The chemical structure of the reference product was confirmed by<sup>1</sup> the applicant by means of H NMR,<sup>13</sup> C NMR and mass spectrometry. The mass spectrometry was tested by the applicant by himself,<sup>1</sup> and the H NMR and<sup>13</sup> C NMR were tested by Changsha Kangpeng Pharmaceutical Co., LTD. The relevant test results and structural analysis are detailed as follows:

#### a. Nuclear Magnetic Resonance (NMR)

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

Solvent: DMSO;

Reference material: TMS (<sup>1</sup>H spectrum);

Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

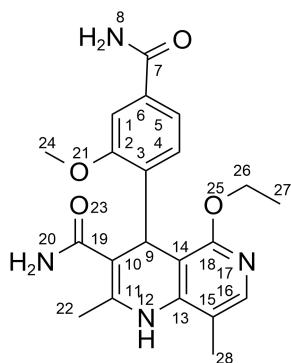
The number of the hydrocarbon atom in the structure (non-systematic naming, only used for hydrocarbon magnetic spectrum analysis)

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The test data and analytical results of hydrocarbon spectra are shown in Table 3.2.S.5-6 and Table 3.2.S.5-7.

Table 3.2.S.5-6  $^1\text{H}$  NMR chemical shift value and correlation spectrum (from high field to low field) of the tested sample

Peak No.	Delta ppm	Peak pattern	Coupling constant (J)	Proton number	Attribution
1	1.063-1.098	t	6.8	3H	27-CH <sub>3</sub>
2	2.137	s	/	3H	28-CH <sub>3</sub>
3	2.249	s	/	3H	22-CH <sub>3</sub>
4	3.841	s	/	3H	24-CH <sub>3</sub>
5	4.000-4.041	m	6.8	2H	26-CH <sub>2</sub>
6	5.326	s	/	1H	9-CH
7	6.672	bs	/	2H	20-NH <sub>2</sub>
8	7.048-7.068	d	8.0	1H	4-CH
9	7.278	s	/	2H	8-NH <sub>2</sub>
10	7.289-7.313	dd	7.6, 1.4	1H	5-CH
11	7.371-7.377	d	1.3	1H	1-CH
12	7.553	s	/	1H	16-CH
13	7.709	s	/	1H	12-NH
14	7.865	s	/	2H	8-NH <sub>2</sub>

Table 3.2.S.5-7  $^{13}\text{C}$  NMR chemical shift values and correlation spectra (from high field to low field) of the tested sample

Peak No.	Delta ppm	Number of carbon atoms	Attribution

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1	14.198	1	28-bit carbon
2	14.795	1	27-bit carbon
3	18.769	1	22-place carbon
4	31.950	1	9-bit carbons
5	56.170	1	24-bit carbon
6	60.989	1	26-bit carbon
7	104.515	1	14-bit carbon
8	105.682	1	10-bit carbon
9	110.334	1	3-place carbons
10	111.895	1	15-place carbon
11	120.433	1	1 bit carbon
12	130.041	1	5-bit carbon
13	133.912	1	4-place carbons
14	139.612	1	6-place carbons
15	139.784	1	11-bit carbon
16	144.399,	1	13-place carbon
17	144.441	1	16-bit carbon
18	155.225	1	2-bit carbons
19	159.845	1	18-bit carbon
20	168.212	1	19-carbon
21	170.093	1	7-bit carbons

<sup>1</sup>H-NMR(DMSO-d<sub>6</sub>, 400Hz) showed 24 hydrogen protons, and the hydrogen displacement of each group could be reasonably attributed. <sup>13</sup>C-NMR(DMSO-d<sub>6</sub>, 100Hz) showed 21 carbon atoms, and the carbon displacement of each group could be reasonably assigned. The NMR data were in good agreement with the target compounds.

**b, Mass spectrometry (MS)**

Detection instrument: Vanquish-TSQ QUANTIS liquid chromatography-mass spectrometry

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Hinxe Pharmaceutical Co., Ltd.

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Version: 001

Date: Sep. 2024

Analytical method: Chinese Pharmacopoeia 2020 Edition of the four General Rules 0512 and 0431

Test data:

Table 3.2.S.5-8 MS data of the tested sample

Mass-charge ratio (m/z)	Corresponding ion	Spare note
395.19	[M-H] <sup>-</sup>	Molecular ion peak in negative ion mode

In the negative ion mode, the mass-charge ratio m/z is 395.19 peak, which is attributed to the molecular ion peak in the negative ion mode, which is consistent with the molecular weight of the target molecule 396.45.

### **(2.3) Content calibration**

#### **Properties**

This product should be white to white powder.

#### **Purity**

Determination according to high performance liquid chromatography (General Rule 0512 of the Fourth part of Chinese Pharmacopoeia, 2020 Edition).

Chromatographic conditions: octadecylsilane bonded silica gel was used as filler (XBridge Shield RP18, 150×4.6mm, 3.5μm or equivalent column performance); A Welch Ghost-Buster Column (4.6×50mm, or equivalent ghost-buster column) was installed after the mixer of the pump and before the sampler. 0.03% phosphoric acid solution was used as mobile phase A and acetonitrile as mobile phase B; Gradient elution is performed according to the following table; Column temperature is 25°C; The detection wavelength was 251nm; The flow rate was 1.0mL per minute; The injection volume was 5μl.

Time /min	Mobile phase A/%	Mobile phase B/%
0	95	5
16.5	50	50

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17	20	80
22	20	80
22.5	95	5
35	95	5

Solvent 30% acetonitrile

Take an appropriate amount of this product from the test product solution, weigh it accurately, dissolve and dilute it with solvent to make a solution containing YA2304-12 about 0.25mg per 1ml.

Method 5 $\mu$ l of the test solution was accurately measured and injected into the liquid chromatograph, and the chromatogram was recorded. The main peak purity should NLT 95.0% according to area normalization method.

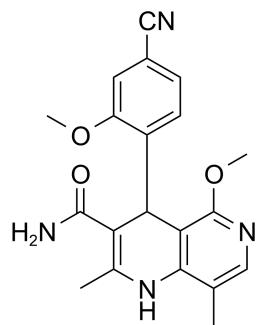
The content is calculated according to the purity.

### **(3) YA2304-14 working standard**

The reference product YA2304-14 used in this study was self-made by Tianyi Hengyi Pharmaceutical Co., LTD. It was named 4-(4-cyano-2-methoxyphenyl)-5-methoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthyl-3-formamide on the physical label and the reference product report. The applicant confirmed its chemical structure and content calibration, and the content was calculated according to 99.6%.

Sample lot No.: 23082301-C

Structural formula:



Molecular formula: C<sub>20</sub>H<sub>20</sub>O<sub>3</sub>N

Molecular weight: 364.40

Finerenone

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Hinye Pharmaceutical Co., Ltd.

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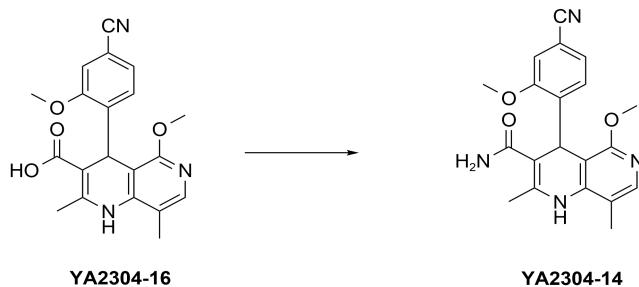
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Date: Sep. 2024

### (3.1) Preparation method of YA2304-14

Add YA2304-16 (2.0g) and tetrahydrofuran (12mL) into 250 mL flask, stir and add CDI (1.2g) in batches, stir for 1 hour, add DMAP (0.07g), and heat up to 50 °C. After 2.5 h of agitation, hamethyldisilazane (3.9 g) was added to the reaction system, the temperature was raised to reflux reaction for 3 h, and the temperature was lowered to 0 ~ 10 °C. Tetrahydrofuran (1.2 g) and water (1.0 g) were added to the reaction system, and the temperature was raised to 50 °C for 20 minutes, and the temperature was lowered to 0 ~ 10 °C for 30 minutes, and then filtered. Washed with water (30 mL) and dried at 50 °C, YA2304-14, white solid (0.8 g) was obtained.



### (3.2) Structural analysis of YA2304-14 reference

The chemical structure of the reference product was confirmed by the applicant through <sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectrometry, among which the mass spectrometry was tested by the applicant himself, and the <sup>1</sup>H NMR and <sup>13</sup>C NMR commissioned Changsha Kangpeng Pharmaceutical Co., LTD for testing. the relevant test results and structural analysis are detailed as follows:

#### a. Nuclear Magnetic Resonance (NMR)

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

Solvent: DMSO;

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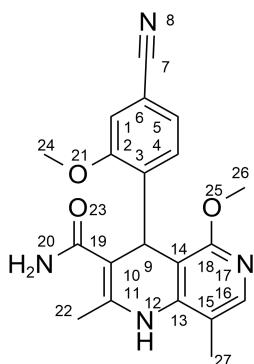
Date: Sep. 2024

Reference: TMS ( $^1\text{H}$  spectrum);

Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

The number of the hydrocarbon atom in the structure (non-systematic naming, only used for hydrocarbon magnetic spectrum analysis)



The test data of hydrocarbon spectra and the analytical results are shown in Table 3.2.S.5-9 and Table 3.2.S.5-10.

Table 3.2.S.5-9  $^1\text{H}$  NMR chemical shift value and correlation spectrum of the tested sample (from high field to low field)

Peak No.	Delta ppm	Peak pattern	Coupling constant (J)	Proton number	Attribution
1	2.141	s	/	3H	27-CH <sub>3</sub>
2	2.188	s	/	3H	22-CH <sub>3</sub>
3	3.616	s	/	3H	26-CH <sub>3</sub>
4	3.824	s	/	3H	24-CH <sub>3</sub>
5	5.406	s	/	1H	9-CH
6	6.730-6.828	bs	/	2H	20-NH <sub>2</sub>
7	7.098-7.117	d	7.6	1H	4-CH
8	7.269-7.292	dd	7.6, 1.2	1H	5-CH
9	7.400-7.403	d	1.2	1H	1-CH
10	7.597	s	/	1H	16-CH
11	7.738	s	/	1H	12-NH

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Table 3.2.S.5-10  $^{13}\text{C}$  NMR chemical shift values and correlation spectra (from high field to low field) of the tested sample

Peak No.	Delta ppm	Number of carbon atoms	Attribution
1	14.199	1	27-place carbon
2	18.398	1	22-place carbon
Solvent Peak	25.581	/	THF
3	32.977	1	9-bit carbon
4	53.267	1	24-place carbon
5	56.789	1	26-bit carbon
Solvent peak	67.482	/	THF
6	103.281	1	14-bit carbon
7	105.921	1	10-bit carbon
8	110.056	1	3-place carbons
9	112.209	1	15-place carbon
10	115.155	1	1 bit carbon
11	119.383	1	7-bit carbon
12	125.343	1	5-bit carbons
13	130.958	1	4-place carbons
14	138.328	1	11-bit carbon
15	141.802	1	6-bit carbons
16	144.545	1	13-place carbon
17	144.926	1	16-bit carbon
18	156.284	1	2-bit carbons
19	160.130	1	18-bit carbon
20	170.327	1	19-carbon

$^1\text{H-NMR}(\text{DMSO-d}_6, 400\text{Hz})$  showed 20 hydrogen protons, and the hydrogen displacement of each group could be reasonably attributed.

$^{13}\text{C-NMR}(\text{DMSO-d}_6, 100\text{Hz})$  showed 20 carbon atoms, and the carbon displacement of each group could be reasonably assigned. The NMR data

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were in good agreement with the target compound.

### **b, Mass spectrometry (MS)**

Detection instrument: Vanquish-TSQ QUANTIS liquid chromatography-mass spectrometry

Analytical method: Chinese Pharmacopoeia 2020 Edition of the four General Rules 0512 and 0431

Test data:

Table 3.2.S.5-11 MS data of the tested sample

Mass-charge ratio (m/z)	Corresponding ion	Spare note
365.26	[M+H] <sup>+</sup>	Molecular ion peak in positive ion mode

In the positive ion mode, the mass charge ratio m/z was detected to be 365.26, which was attributed to the molecular ion peak in the positive ion mode, consistent with the molecular weight of the target molecule 364.40.

### **(3.3) Content calibration**

#### **Properties**

This product should be white to white powder.

#### **purity**

Determination according to high performance liquid chromatography (General Rule 0512 of the Fourth part of Chinese Pharmacopoeia, 2020 Edition).

Chromatographic conditions: octadecylsilane bonded silica gel was used as filler (XBridge Shield RP18, 150\*4.6mm, 3.5μm or equivalent performance column); Welch Ghost-Buster Column (4.6×50mm, or equivalent ghost-buster column) was installed after the mixer of the pump and before the sampler. 0.03% phosphoric acid solution was used as mobile phase A and acetonitrile as mobile phase B; Gradient elution is performed according to the

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following table; Column temperature is 25°C; The detection wavelength was 251nm; The flow rate was 1.0mL per minute; The injection volume was 5μl.

Time /min	Mobile phase A/%	Mobile phase B/%
0	95	5
16.5	50	50
17	20	80
22	20	80
22.5	95	5
35	95	5

Solvent 70% acetonitrile

Take an appropriate amount of this product from the test product solution, weigh it accurately, dissolve and dilute it with solvent to make a solution containing YA2304-14 about 0.25mg per 1ml.

Method 5μl of the test solution was accurately measured and injected into the liquid chromatograph, and the chromatogram was recorded. The main peak purity should NLT 95.0% according to area normalization method.

The content is calculated according to the purity.

### **(4) YA2304-15 working standard**

The reference product YA2304-15 used in this study was derived from Tianyi Hengyi Pharmaceutical Co., LTD. It was named 4-(4-cyano-2-methoxyphenyl)-5-isopropoxy-2, 8-dimethyl-1, 4-dihydro-1, 6-naphthyl-3-formamide on the physical label and the reference product report. The applicant confirmed its chemical structure and content calibration, and the content was calculated according to 99.3%.

Sample lot No.: 23082903-C

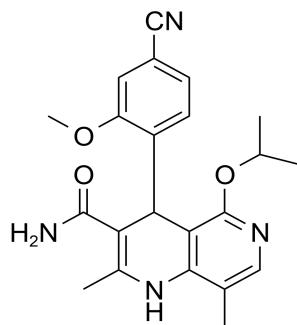
Structural formula:

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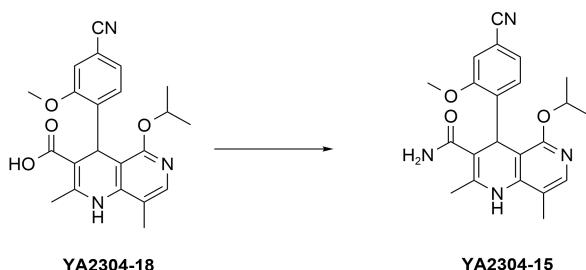


Molecular formula: C<sub>22</sub>H<sub>24</sub>O<sub>3</sub>N

Molecular weight: 392.46

### **(4.1) Preparation method of YA2304-15**

Add YA2304-18(2.0g) and tetrahydrofuran (21mL) into 250 mL flask, add CDI (2.0g) in batches under agitation, add DMAP (0.11g) after 1 hour of agitation, heat up to 50°C, add hexamethyldisilazane (6.3g) into the reaction system after 2.5 h of agitation. Heating up to reflux reaction for 3 h, cooling down to 0~10°C, adding tetrahydrofuran (1.2 g) and water (1.0 g) to the reaction system, stirring at 80 °C for 2 h, cooling down to 0~10°C for 30 minutes, filtering, washing with water (30 mL), drying at 50 °C, YA2304-15 was obtained. White solid (2.6g).



### **(4.2) Structural analysis of YA2304-15 reference**

The chemical structure of the reference product was confirmed by<sup>1</sup> the applicant by means of HNMR,<sup>13</sup> CNMR and mass spectrometry, among which the mass spectrometry was tested by the applicant himself,<sup>1</sup> HNMR and<sup>13</sup> CNMR commissioned Changsha Kangpeng Pharmaceutical Co., LTD for testing. The relevant test atlas and contract for testing are detailed in section

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3.2.R.7.S, and the relevant test results and structural analysis are detailed as follows:

### a. Nuclear Magnetic Resonance (NMR)

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

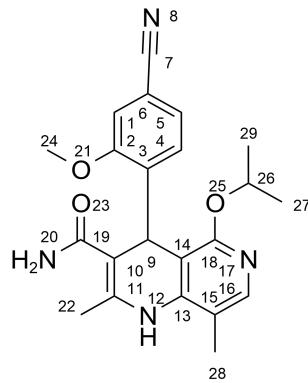
Solvent: DMSO;

Reference: TMS ( $^1\text{H}$  spectrum);

Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

The number of the hydrocarbon atom in the structure (non-systematic naming, only used for hydrocarbon magnetic spectrum analysis)



The test data and analytical results of hydrocarbon spectra are shown in Table 3.2.S.5-12 and Table 3.2.S.5-13.

Table 3.2.S.5-12  $\text{HN}^1\text{MR}$  chemical shift value and correlation spectrum of the tested sample (from high field to low field)

Peak No.	Delta ppm	Peak pattern	Coupling constant (J)	Proton number	Attribution
1	0.733-0.748	t	6	3H	27-CH <sub>3</sub>
2	1.178-1.193	t	6	3H	29-CH <sub>3</sub>

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3	2.124	s	/	3H	28-CH <sub>3</sub>
4	2.203	s	/	3H	22-CH <sub>3</sub>
Solvent peak	3.583-3.616	m	/	/	THF
5	3.835	s	/	3H	24-CH <sub>3</sub>
6	4.956-4.987	m	/	1H	26-CH
7	5.361	s	/	1H	9-CH
8	6.773-6.705	bs	/	2H	20-NH <sub>2</sub>
9	7.168-7.188	d	8.0	1H	4-CH
10	7.274-7.297	dd	8.0, 1.2	1H	5-CH
11	7.370-7.373	d	1.2	1H	1-CH
12	7.557	s	/	1H	16-CH
13	7.675	s	/	1H	12-NH

Table 3.2.S.5-13 CN<sup>13</sup>MR chemical shift values and correlation spectra (from high field to low field) of the tested sample

Peak sequence number	Delta ppm	Number of carbon atoms	Attribution
1	14.182	1	28-bit carbon
2	18.556	1	22-place carbon
3	21.708	1	27-place carbon
4	22.365	1	29-place carbon
Solvent Peak	25.586	/	THF
5	32.442	1	9-bit carbon
6	56.405	1	24-place carbon
7	67.002	1	26-bit carbon
Solvent Peak	67.478	/	THF
8	104.245	1	14-bit carbon
9	105.756	1	10-carbon
10	109.875	1	3-place carbons
11	111.510	1	15-place carbon

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12	114.313	1	1 bit carbon
13	119.462	1	7-bit carbon
14	125.154	1	5-bit carbons
15	131.563	1	4-place carbons
16	138.558	1	11-bit carbon
17	142.476	1	6-bit carbons
18	144.662	1	13-place carbon
19	144.726	1	16-bit carbon
20	156.101	1	2-bit carbons
21	159.440	1	18-bit carbon
22	170.174	1	19-bit carbon

<sup>1</sup>H-NMR(DMSO-d<sub>6</sub>, 400Hz) showed 24 hydrogen protons, and the hydrogen displacement of each group could be reasonably attributed. <sup>13</sup>C-NMR(DMSO-d<sub>6</sub>, 100Hz) showed 22 carbon atoms, and the carbon displacement of each group could be reasonably attributed, and the NMR data were consistent with the target compound.

### **b, Mass spectrometry (MS)**

Test instrument: Vanquish-TSQ QUANTIS liquid chromatography-mass spectrometry

Analytical method: Chinese Pharmacopoeia 2020 Edition of the four General Rules 0512 and 0431

Test data:

Table 3.2.S.5-14 MS data of the tested sample

Mass-charge ratio (m/z)	Corresponding ion	Spare note
393.29	[M+H] <sup>+</sup>	Molecular ion peak in positive ion mode

In the positive ion mode, the mass-charge ratio m/z is 393.29 peak, which is attributed to the molecular ion peak in the positive ion mode, consistent with

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the molecular weight of the target molecule 392.46.

### **(4.3) Content calibration**

#### **Properties**

This product should be white to white powder.

#### **Purity**

Determination according to high performance liquid chromatography (General Rule 0512 of the Fourth part of Chinese Pharmacopoeia, 2020 Edition).

Chromatographic conditions: octadecylsilane bonded silica gel was used as filler (XBridge Shield RP18, 150×4.6mm, 3.5μm or equivalent performance column); A Welch Ghost-Buster Column (4.6×50mm, or equivalent ghost-buster column) was installed after the mixer of the pump and before the sampler. 0.03% phosphoric acid solution was used as mobile phase A and acetonitrile as mobile phase B; Gradient elution is performed according to the following table; Column temperature is 25°C; The detection wavelength was 251nm; The flow rate was 1.0mL per minute; The injection volume was 5μl.

Time /min	Mobile phase A/%	Mobile phase B/%
0	95	5
16.5	50	50
17	20	80
22	20	80
22.5	95	5
35	95	5

Solvent 70% acetonitrile

Take an appropriate amount of this product from the test product solution, weigh it accurately, dissolve and dilute it with solvent to make a solution containing YA2304-15 about 0.25mg per 1ml.

The test solution of 5μl was accurately measured and injected into the liquid chromatograph, and the chromatogram was recorded. The main peak purity

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was NLT 95.0% according to the area normalization method.

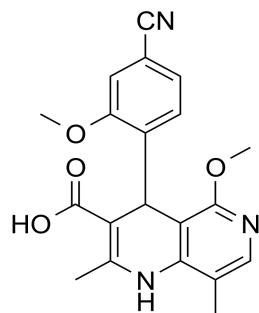
The content is calculated according to the purity.

### **(5) YA2304-16 working standard**

The reference product YA2304-16 used in this study was derived from Tianyi Hengyi Pharmaceutical Co., LTD. It was named 4-(4-cyano-2-methoxyphenyl)-5-methoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthyl-3-carboxylic acid on the physical label and the reference product report. The applicant confirmed its chemical structure and content calibration, and the content was calculated according to 93.7%.

Sample lot No.: 23082201-C

Structural formula:



Molecular formula: C<sub>20</sub>H<sub>19</sub>O<sub>4</sub>N

Molecular weight: 365.39

#### **(5.1) Preparation method of YA2304-16**

Add YA2304-50(4.8g), tetrahydrofuran (60 mL) and water (30 mL) into 250 mL flask, stir and cool to 0~5 °C, slowly add 7.3% NaOH aq.(12.5 g) into the reaction system, and react at 0~5 °C for 3 h. Sodium acetate (0.9 g) and toluene (50 mL) were added into the reaction system, and the solution was divided after stirring at 0 ~ 5 °C for 20 minutes. The lower aqueous phase was treated with 10% NaOH aq. Adjust the pH to 6.5~7.0, stir at 0~5 °C for 30 minutes, filter, rinse with water (100 mL), dry at 50 °C, YA2304-16, light

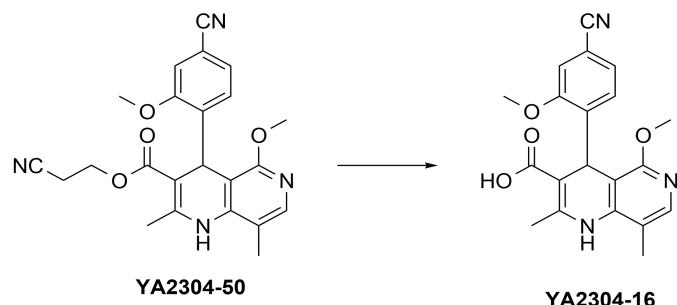
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yellow solid (3.2 g) was obtained.



### **(5.2) Structural analysis of YA2304-16 reference**

The chemical structure of the reference product was confirmed by the applicant by means of  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and mass spectrometry. The mass spectrometry was tested by the applicant by himself, and the  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR were tested by Changsha Kangpeng Pharmaceutical Co., LTD. The relevant test results and structural analysis are detailed as follows:

#### **a. Nuclear Magnetic Resonance (NMR)**

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

Solvent: DMSO;

Reference material: TMS ( $^1\text{H}$  spectrum);

Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

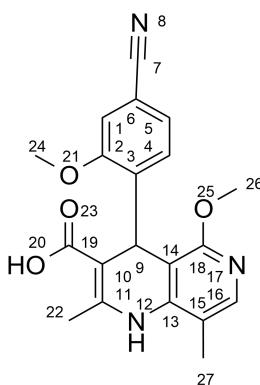
The number of the hydrocarbon atom in the structure (non-systematic naming, only used for hydrocarbon magnetic spectrum analysis)

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The test data and analytical results of hydrocarbon spectra are shown in Table 3.2.S.5-15 and Table 3.2.S.5-16.

Table 3.2.S.5-15  $^1\text{H}$  NMR chemical shift value and correlation spectrum of the tested sample (from high field to low field)

Peak No.	Delta ppm	Peak pattern	Coupling constant (J)	Proton number	Attribution
1	2.160	s	/	3H	27-CH <sub>3</sub>
2	2.504	s	/	3H	22-CH <sub>3</sub>
3	3.650	s	/	3H	24-CH <sub>3</sub>
4	3.766	s	/	3H	26-CH <sub>3</sub>
5	5.370	s	/	1H	9-CH
6	7.248-7.262	t	/	2H	4, 5 - CH
7	7.340-7.343	d	/	1H	1-CH
8	7.606	s	/	1H	16-CH
9	8.202	s	/	1H	12-NH
10	11.481	s	/	1H	20-COOH

Table 3.2.S.5-16  $^{13}\text{C}$  NMR chemical shift values and correlation spectra (from high field to low field) of the tested sample

Peak No.	Delta ppm	Number of carbon atoms	Attribution
1	14.174	1	27-place carbon
2	19.272	1	Twenty-two carbon
3	34.223	1	9-bit carbons

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4	56.352	1	24-place carbon
5	56.453	1	26-bit carbon
6	100.037	1	14-bit carbon
7	103.771	1	10-bit carbon
8	110.034	1	3-place carbons
9	112.438	1	15-place carbon
10	115.416	1	1 bit carbon
11	119.430	1	7-bit carbon
12	124.497	2	4, 5-place carbon
13	141.344	1	6-place carbon
14	144.232	2	13,16 carbon
15	147.519	1	11-bit carbon
16	157.660	1	2-bit carbons
17	160.155	1	18-bit carbon
18	168.962	1	19-carbon

<sup>1</sup>H-NMR(DMSO-d<sub>6</sub>, 400Hz) showed 19 hydrogen protons, and the hydrogen displacement of each group could be reasonably attributed. <sup>13</sup>C-NMR(DMSO-d<sub>6</sub>, 100Hz) showed 20 carbon atoms, and the carbon displacement of each group could be reasonably assigned. The NMR data were in good agreement with the target compound.

### **b, Mass spectrometry (MS)**

Detection instrument: Vanquish-TSQ QUANTIS liquid chromatography-mass spectrometry

Analytical method: Chinese Pharmacopoeia 2020 Edition of the four General Rules 0512 and 0431

Test data:

Table 3.2.S.5-17 MS data of the tested sample

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Mass-charge ratio (m/z)	Corresponding ion	Spare note
366.26	[M+H] <sup>+</sup>	Molecular ion peak in positive ion mode

In the positive ion mode, the detected mass-charge ratio m/z is 366.26 peak, which is attributed to the molecular ion peak in the positive ion mode, consistent with the molecular weight of the target molecule 365.39.

### **(5.3) Content calibration**

#### **Properties**

This product should be like white to yellow powder.

#### **purity**

Determination according to high performance liquid chromatography (General Rule 0512 of the Fourth part of Chinese Pharmacopoeia, 2020 Edition).

Chromatographic conditions: octadecylsilane bonded silica gel was used as filler (XBridge Shield RP18, 150×4.6mm, 3.5μm or equivalent performance column); 20mM ammonium acetate solution (pH8.1) (weigh ammonium acetate about 1.54g, dissolve in 1000ml pure water, adjust the pH to 8.1 with ammonia water) was used as mobile phase A, acetonitrile was used as mobile phase B; Gradient elution was performed according to the following table; Column temperature is 25°C; The detection wavelength was 251nm; The flow rate was 1.0mL per minute; The injection volume was 5μl.

Time /min	Mobile phase A/%	Mobile phase B/%
0	95	5
16.5	50	50
17	20	80
22	20	80
22.5	95	5
35	95	5

Solvent acetonitrile

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Sample solution take appropriate amount of this product, weigh it accurately, dissolve and dilute it with solvent to make a solution containing YA2304-16 about 0.25mg per 1ml.

Method 5 $\mu$ l of the test solution was accurately measured and injected into the liquid chromatograph, and the chromatogram was recorded. The main peak purity should NLT 90.0% according to area normalization method.

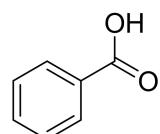
The content is calculated according to the purity.

### **(6) YA2304-17 working standard**

The control product YA2304-17 used in this application is from Shanghai Bide Pharmaceutical Technology Co., LTD., which is named Benzoic acid on the physical label, which is named Benzoic acid on the manufacturer's photo report list, and the control product name is Benzoic acid on the invoice. The applicant has confirmed its chemical structure, and according to the report provided by the manufacturer, the content is calculated by 99.30%.

Sample lot No.: DMW857

Structural formula:



Molecular formula: C<sub>7</sub>H<sub>6</sub>O

Molecular weight: 122.12

#### **(6.1) Structural analysis of YA2304-17 reference**

The chemical structure of the reference product was confirmed by<sup>1</sup> the applicant by means of H NMR,<sup>13</sup> CNMR and mass spectrometry, among which the mass spectrometry was tested by the applicant himself, <sup>1</sup>H NMR was provided by Shanghai Bider Medical Technology Co., LTD., and <sup>13</sup>C NMR

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commissioned Changsha Kangpeng Medical Co., LTD to test. The relevant test results and structural analysis are detailed as follows:

### a. Nuclear Magnetic Resonance (NMR)

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

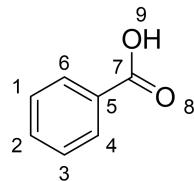
Solvent: DMSO;

Reference: TMS ( $^1\text{H}$  spectrum);

Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

The number of the hydrocarbon atom in the structure (non-systematic naming, only used for hydrocarbon magnetic spectrum analysis)



The test data and analytical results of hydrocarbon spectra are shown in Table 3.2.S.5-18 and Table 3.2.S.5-19.

Table 3.2.S.5-18  $^1\text{H}$  NMR chemical shift value and correlation spectrum of the tested sample (from high field to low field)

Peak No.	Delta ppm	Peak pattern	Coupling constant ( $J$ )	Proton number	Attribution
1	5.74 ~ 7.39	m	/	2H	1, 3 - CH
2	7.71 ~ 7.54	m	/	1H	2-CH
3	7.94	ddd	7.4, 2.9, 1.6	2H	4, 6 - CH
4	12.94	s	0.8Hz	1H	9-OH

Table 3.2.S.5-19  $^{13}\text{C}$  NMR chemical shift values and correlation spectra of the

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tested sample (from high field to low field)

Peak No.	Delta ppm	Number of carbon atoms	Attribution
1	128.992	2	1, 3-place C
2	129.727	2	4,6 C's
3	131.222	1	5 digit C's
4	133.286	1	2-bit C
5	167.796	1	7-bit C

<sup>1</sup>H-NMR(DMSO-d<sub>6</sub>, 400Hz) showed 6 hydrogen protons, and the hydrogen displacement of each group could be reasonably attributed. <sup>13</sup>C-NMR(DMSO-d<sub>6</sub>, 100Hz) showed 7 carbon atoms, and the carbon displacement of each group could be reasonably assigned. The NMR data were in good agreement with the target compound.

### b, Mass spectrometry (MS)

Detection instrument: Vanquish-TSQ QUANTIS liquid chromatography-mass spectrometry

Analytical method: Chinese Pharmacopoeia 2020 Edition of the four General Rules 0512 and 0431

Test data:

Table 3.2.S.5-20 MS data of the tested sample

Mass-charge ratio (m/z)	Corresponding ion	Spare note
121.08	[M-H] <sup>-</sup>	Molecular ion peak in negative ion mode

In the negative ion mode, the mass-charge ratio m/z was detected as 121.08 peak, which was attributed to the molecular ion peak in the negative ion mode, consistent with the molecular weight of the target molecule 122.12.

### (7) YA2304-18 working standard

The reference product YA2304-18 used in this study was derived from T

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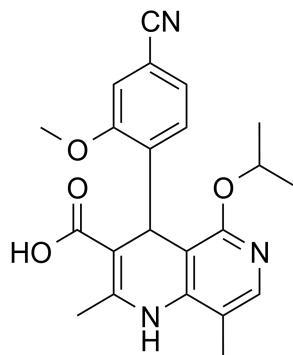
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iandi Hengyi Pharmaceutical Co., LTD. It was named 4-(4-cyano-2-methoxyphenyl)-5-isopropoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthyl-3-carboxylic acid on the physical label and the reference product report. The applicant confirmed its chemical structure and content calibration, and the content was calculated according to 98.9%.

Sample lot No.: 23082802-C

Structural formula:



Molecular formula: C<sub>22</sub>H<sub>23</sub>O<sub>4</sub>N

Molecular weight: 393.44

### **(7.1) Preparation method of YA2304-18**

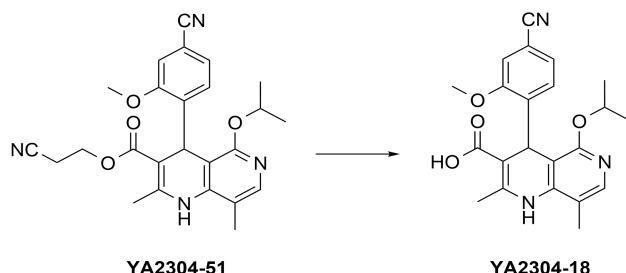
Add YA2304-51(6.0 g), tetrahydrofuran (36 mL) and water (18 mL) into 250 mL flask, stir and cool to 0~5°C, slowly add 7.3% NaOH aq.(14.7 g) into the reaction system, and react at 0~5°C for 3 h. Sodium acetate (1.1g) and toluene (45 mL) were added into the reaction system, stirring at 0~5 °C for 20 minutes before separating the solution, and the lower aqueous phase was treated with 10% NaOH aq. Adjust the pH to 6.5~7.0, stir at 0~5 °C for 30 minutes, filter, rinse with water (100 mL), dry at 50 °C, YA2304-18, white solid (4.5 g) was obtained.

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### (7.2) Structural analysis of YA2304-18 reference

The chemical structure of the reference product was confirmed by<sup>1</sup> the applicant by means of H NMR,<sup>13</sup> C NMR and mass spectrometry. The mass spectrometry was tested by the applicant by himself,<sup>1</sup> and the H NMR and<sup>13</sup> C NMR were tested by Changsha Kangpeng Pharmaceutical Co., LTD. For details about the relevant test graphs and contract, see section 3.2.R.7.S. The relevant test results and structural analysis are detailed as follows:

#### a. Nuclear Magnetic Resonance (NMR)

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

Solvent: DMSO;

Reference: TMS (<sup>1</sup>H spectrum);

Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

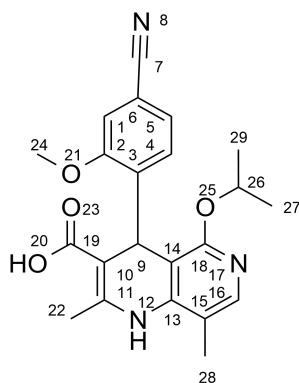
The number of the hydrocarbon atom in the structure (non-systematic naming, only used for hydrocarbon magnetic spectrum analysis)

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The test data and analytical results of hydrocarbon spectra are shown in Table 3.2.S.5-21 and Table 3.2.S.5-22.

Table 3.2.S.5-21  $^1\text{H}$  NMR chemical shift value and correlation spectrum of the tested sample (from high field to low field)

Peak No.	Delta ppm	Peak pattern	Coupling constant (J)	Proton number	Attribution
1	0.829-0.845	d	6.4	3H	27-CH <sub>3</sub>
2	1.209-1.224	d	6.4	3H	29-CH <sub>3</sub>
3	2.144	s	/	3H	28-CH <sub>3</sub>
4	2.383	s	/	3H	22-CH <sub>3</sub>
5	3.727	s	/	3H	24-CH <sub>3</sub>
6	4.968-5.029	m	/	1H	26-CH
7	5.307	s	/	1H	9-CH
8	7.275-7.313	d	/	3H	1, four, five - CH
9	7.570	s	/	1H	16-CH
10	8.119	s	/	1H	12-NH
11	11.429	s	/	1H	20-COOH

Table 3.2.S.5-22  $^{13}\text{C}$  NMR chemical shift value and correlation spectrum (from high field to low field) of the tested sample

Peak No.	Delta ppm	Number of carbon atoms	Attribution
1	14.141	1	28-bit carbon
2	19.361	1	22-place carbon

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3	21.817	1	27-place carbon
4	22.400	1	26-place carbon
5	34.719	1	9-bit carbons
6	56.188	1	24-place carbon
7	67.091	1	26-bit carbon
8	99.901	1	10-bit carbon
9	104.274	2	3,14 carbon
10	109.867	1	15-position carbon
11	111.681	1	1 bit carbon
12	119.520	1	7-bit carbon
13	124.126	1	5-bit carbons
14	132.412	1	4-place carbons
15	141.553	1	6-place carbons
16	144.164	1	13-place carbon
17	144.836	1	16-bit carbon
18	147.451	1	11 bit carbon
19	157.793	1	2-bit carbons
20	159.446	1	18-bit carbon
21	169.063	1	19-bit carbon

<sup>1</sup>H-NMR(DMSO-d<sub>6</sub>, 400Hz) showed 23 hydrogen protons, and the hydrogen displacement of each group could be reasonably attributed.

<sup>13</sup>C-NMR(DMSO-d<sub>6</sub>, 100Hz) showed 22 carbon atoms, and the carbon displacement of each group could be reasonably assigned. The NMR data were in good agreement with the target compound.

### **b, Mass spectrometry (MS)**

Detection instrument: Vanquish-TSQ QUANTIS liquid chromatography-mass spectrometry

Analysis methods: General Rules 0512 and 0431 of the fourth edition of

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Chinese Pharmacopoeia (2020 edition)

Test data:

Table 3.2.S.5-23 MS data of the tested sample

Mass-charge ratio (m/z)	Corresponding ion	Spare note
394.31	[M+H] <sup>+</sup>	Molecular ion peak in positive ion mode

In the positive ion mode, the mass-charge ratio m/z is 394.31 peak, which is attributed to the molecular ion peak in the positive ion mode, consistent with the molecular weight of the target molecule 393.44.

### **(7.3) Content calibration**

#### **Properties**

This product should be white to white powder.

#### **Purity**

Determination according to high performance liquid chromatography (General Rule 0512 of the Fourth part of Chinese Pharmacopoeia, 2020 Edition).

Chromatographic conditions: octadecylsilane bonded silica gel was used as filler (XBridge Shield RP18, 150×4.6mm, 3.5μm or equivalent performance column); 20mM ammonium acetate solution (pH 8.1) (weigh ammonium acetate about 1.54g, dissolve in 1000ml pure water, adjust the pH to 8.1 with ammonia water) was used as mobile phase A, acetonitrile was used as mobile phase B; Gradient elution was performed according to the following table; Column temperature is 25°C; The detection wavelength was 251nm; The flow rate was 1.0mL per minute; The injection volume was 5μl.

Time /min	Mobile phase A/%	Mobile phase B/%
0	95	5
16.5	50	50
17	20	80

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22	20	80
22.5	95	5
35	95	5

Solvent acetonitrile

Sample solution take appropriate amount of this product, weigh it accurately, dissolve and dilute it with solvent to make a solution containing YA2304-18 about 0.25mg per 1ml.

Method 5 $\mu$ l of the test solution was accurately measured and injected into the liquid chromatograph, and the chromatogram was recorded. The main peak purity should NLT 95.0% according to area normalization method.

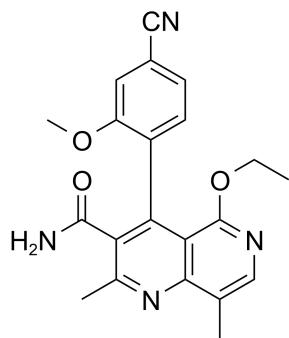
The content is calculated according to the purity.

### **(8) YA2304-19 working standard**

The reference product YA2304-19 used in this study was from Tiandi Hengyi Pharmaceutical Co., LTD. It was named 4-(4-cyano-2-methoxyphenyl)-5-ethoxy-2, 8-dimethyl-1, 6-naphthyl-3-formamide on the physical label and the reference product report. The applicant confirmed its chemical structure and content calibration, and the content was calculated according to 99.4%.

Sample lot No.: 23080701-A

Structural formula:



Molecular formula: C<sub>21</sub>H<sub>20</sub>O<sub>3</sub>N

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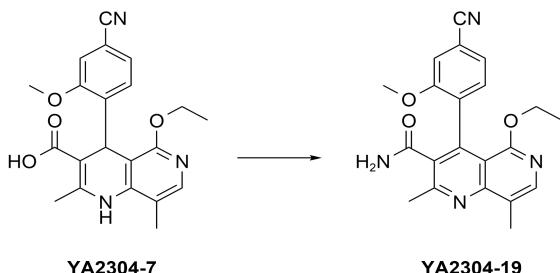
Version: 001

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Molecular weight: 376.42

### **(8.1) Preparation method of YA2304-19**

Add YA2304-7(20.0g), CDI (12.0g) and tetrahydrofuran (100 mL) into 250 mL flask, stir to dissolve, control the temperature by 15~20°C, add DMAP (0.64g), stir for 10 minutes, rise to 50 °C and react for 2 h, add hamethyldisilazane (37.4g), stir for 10 minutes. Overnight reaction at 60°C until the reaction of raw material is complete. Drop to 0~5°C, add water (10.4 g) and tetrahydrofuran (16.0 g) mixed liquid crystallization, temperature rise reflux stirring for 3 h beating, temperature drop to 0~5°C stirring for 2 h after filtration, mother liquor concentrated to dry, column purification (EA/n-heptane =1/1), concentration, recrystallization (EA/ n-heptane =1/5), vacuum drying, Yellow granular solid YA2304-19 was obtained.



### **(8.2) Structural analysis of YA2304-19 reference**

The chemical structure of the reference product was confirmed by<sup>1</sup> the applicant by means of H NMR,<sup>13</sup> C NMR and mass spectrometry. The mass spectrometry was tested by the applicant by himself,<sup>1</sup> and the H NMR and<sup>13</sup> C NMR were tested by Changsha Kangpeng Pharmaceutical Co., LTD. The relevant test results and structural analysis are detailed as follows:

#### **a. Nuclear Magnetic Resonance (NMR)**

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

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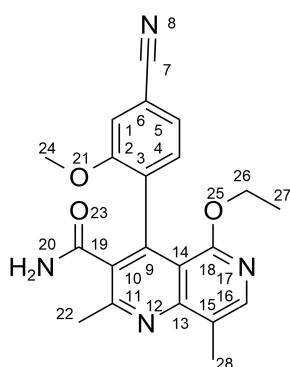
Solvent: DMSO;

Reference: TMS ( $^1\text{H}$  spectrum);

Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

The number of the hydrocarbon atom in the structure (non-systematic naming, only used for hydrocarbon magnetic spectrum analysis)



The test data and analytical results of hydrocarbon spectra are shown in Table 3.2.S.5-24 and Table 3.2.S.5-25.

Table 3.2.S.5-24  $^1\text{H}$  NMR chemical shift value and correlation spectrum of the tested sample (from high field to low field)

Peak No.	Delta ppm	Peak pattern	Coupling constant (J)	Proton number	Attribution
1	0.702-0.737	t	/	3H	27-CH <sub>3</sub>
2	2.508	s	/	3H	28-CH <sub>3</sub>
3	2.719	s	/	3H	22-CH <sub>3</sub>
4	3.669	s	/	3H	24-CH <sub>3</sub>
5	3.945-4.049	m	/	2H	26-CH <sub>2</sub>
6	7.318-7.338	d	/	1H	4-CH
7	7.461-7.465	d	/	1H	5-CH
8	7.524-7.527	d	/	2H	20-NH <sub>2</sub>
9	7.735	s	/	1H	16-CH
10	8.041-8.043	d	/	1H	1-CH

Table 3.2.S.5-25  $^{13}\text{C}$  NMR chemical shift value and correlation spectrum  
 (from high field to low field) of the tested sample

Peak No.	Delta ppm	Number of ownership
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		carbon atoms	
1	13.585	1	28-bit carbon
2	14.761	1	27-bit carbon
3	24.055	1	22-place carbon
4	56.565	1	24-place carbon
5	61.687	1	26-bit carbon
6	111.026	1	14-bit carbon
7	111.838	1	10-bit carbon
8	113.883	1	7-bit carbons
9	119.443	1	15-bit carbon
10	122.968	1	1 bit carbon
11	124.052	1	6-place carbon
12	130.463	1	5-bit carbon
13	133.312	1	4-place carbons
14	133.765	1	11-bit carbon
15	139.899	1	3-bit carbons
16	142.589	1	13-bit carbon
17	151.139	1	16-bit carbon
18	157.750	1	2-bit carbons
19	158.515	1	18-bit carbon
20	159.484	1	9-bit carbons
21	168.540	1	19-place carbon

<sup>1</sup>H-NMR(DMSO-d<sub>6</sub>, 400Hz) showed 20 hydrogen protons, and the hydrogen displacement of each group could be reasonably attributed.

<sup>13</sup>C-NMR(DMSO-d<sub>6</sub>, 100Hz) showed 21 carbon atoms, and the carbon displacement of each group could be reasonably assigned. The NMR data were in good agreement with the target compounds.

### **b, Mass spectrometry (MS)**

Detection instrument: Vanquish-TSQ QUANTIS liquid chromatography-mass spectrometry

Analytical method: Chinese Pharmacopoeia 2020 Edition of the four General Rules 0512 and 0431

Test data:

Table 3.2.S.5-26 MS data of the tested sample

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Mass-charge ratio (m/z)	Corresponding ion	Spare note
377.26	[M+H] <sup>+</sup>	Molecular ion peak in positive ion mode

In the positive ion mode, the mass-charge ratio m/z is detected to be 377.26 peak, which is attributed to the molecular ion peak in the positive ion mode, consistent with the molecular weight of the target molecule 376.42.

### **(8.3) Content calibration**

#### **Properties**

This product should be like white to yellow powder.

#### **Purity**

Determination according to high performance liquid chromatography (General Rule 0512 of the Fourth part of Chinese Pharmacopoeia, 2020 Edition).

Chromatographic conditions: octadecylsilane bonded silica gel was used as filler (XBridge Shield RP18, 150×4.6mm, 3.5μm or equivalent performance column); A Welch Ghost-Buster Column (4.6×50mm, or equivalent ghost-buster column) was installed after the mixer of the pump and before the sampler. 0.03% phosphoric acid solution was used as mobile phase A and acetonitrile as mobile phase B; Gradient elution is performed according to the following table; Column temperature is 25°C; The detection wavelength was 251nm; The flow rate was 1.0mL per minute; The injection volume was 5μl.

Time /min	Mobile phase A/%	Mobile phase B/%
0	95	5
16.5	50	50
17	20	80
22	20	80
22.5	95	5
35	95	5

Solvent 70% acetonitrile

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Take an appropriate amount of this product from the test product solution, weigh it accurately, dissolve and dilute it with solvent to make a solution containing YA2304-19 about 0.25mg per 1ml.

Method 5 $\mu$ l of the test solution was accurately measured and injected into the liquid chromatograph, and the chromatogram was recorded. The main peak purity should NLT 95.0% according to area normalization method.

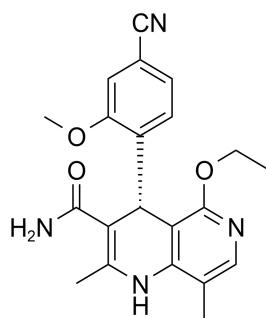
The content is calculated according to the purity.

### **(9) YA2304-20 working standard**

The reference product YA2304-20 used in this study was derived from Tianyi Hengyi Pharmaceutical Co., LTD. It was named (4R)-4-(4-cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthyl-3-formamide on the physical label and the reference product report. The applicant confirmed its chemical structure and content calibration, and the content was calculated according to 99.2%.

Sample lot No.: 23080401-A

Structural formula:



Molecular formula: C<sub>21</sub>H<sub>22</sub>O<sub>3</sub>N

Molecular weight: 378.43

### **(9.1) Preparation method of YA2304-20**

Take YA2304-9 mother liquor (600ml, obtained by combining multiple batches of small experiments), control the temperature by 15~20°C, add 9% potassium phosphate solution to pH>7.5, and add water (200ml). Water bath

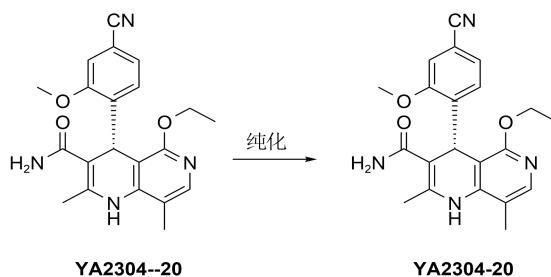
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temperature control 50°C, decompression concentration until no large fraction, a large number of white solid precipitation. Heat preservation 20~25°C, filtration, wet product added with ethanol (300ml), reflux dissolution at high temperature, filtration, concentration under reduced pressure to about 110g, reflux at high temperature for 1 hour, cooling to 0~5°C, crystallization for 2 h, filtration, vacuum drying at 50°C, YA2304-20 (13.0g) was obtained.



### (9.2) Structural analysis of YA2304-20 reference product

The chemical structure of the reference product was confirmed by<sup>1</sup> the applicant by means of H NMR,<sup>13</sup> C NMR, mass spectrometry and chiral liquid chromatography. The mass spectrometry and chiral liquid chromatography were tested by the applicant by himself,<sup>1</sup> and the H NMR and<sup>13</sup> C NMR were tested by Changsha Kangpeng Pharmaceutical Co., LTD. The relevant detection spectra and contract for commissioned testing are detailed in section 3.2.R.7.S. The relevant test results and structural analysis are listed as follows:

#### a. Nuclear Magnetic Resonance (NMR)

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

Solvent: DMSO;

Reference: TMS (<sup>1</sup>H spectrum);

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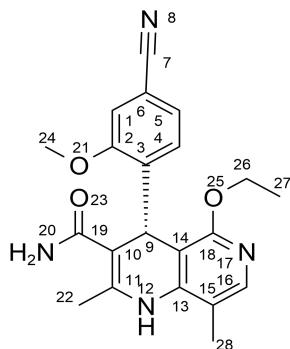
Version: 001

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Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

The number of hydrocarbon atoms in the structure (non-systematic naming, only used for hydrocarbon magnetic spectrum analysis)



The test data and analytical results of hydrocarbon spectra are shown in Table 3.2.S.5-27 and Table 3.2.S.5-28

Table 3.2.S.5-27  $^1\text{H}$  NMR chemical shift value and correlation spectrum of the tested sample (from high field to low field)

Peak No.	Delta ppm	Peak pattern	Coupling constant (J)	Proton number	Attribution
1	1.038-1.073	t	7.2	3H	27-CH <sub>3</sub>
2	2.128	s	/	3H	28-CH <sub>3</sub>
3	2.207	s	/	3H	22-CH <sub>3</sub>
4	3.836	s	/	3H	24-CH <sub>3</sub>
5	4.010-4.034	m	7.2	2H	26-CH <sub>2</sub>
6	5.396	s	/	1H	9-CH
7	6.710-6.785	bs	/	2H	20-NH <sub>2</sub>
8	7.160-7.180	d	7.6	1H	4-CH
9	7.275-7.298	dd	7.6, 1.4	1H	5-CH
10	7.377-7.380	d	1.3	1H	1-CH
11	7.561	s	/	1H	16-CH
12	7.706	s	/	1H	12-NH

Table 3.2.S.5-28  $^{13}\text{C}$  NMR chemical shift value and correlation spectrum (from high field to low field) of the tested sample

Peak No.	Delta ppm	Number of carbon atoms	Attribution
1	14.196	1	28-bit carbon

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2	14.734	1	27-bit carbon
3	18.521	1	22-place carbon
4	32.868	1	9-bit carbons
5	56.505	1	24-place carbon
6	61.006	1	26-bit carbon
7	103.643	1	14-bit carbon
8	105.771	1	10-bit carbon
9	109.986	1	2-place carbons
10	111.911	1	15-place carbon
11	114.583	1	1 bit carbon
12	119.432	1	7-bit carbon
13	125.223	1	5-bit carbons
14	131.366	1	4-site carbon
15	138.643	1	11-bit carbon
16	142.151	1	7-bit carbons
17	144.669	2	13,16 carbon
18	156.137	1	3-bit carbon
19	159.846	1	18-bit carbon
20	170.202	1	19-bit carbon

<sup>1</sup>H-NMR(DMSO-d<sub>6</sub>, 400Hz) showed 22 hydrogen protons, and the hydrogen displacement of each group could be reasonably attributed. <sup>13</sup>C-NMR(DMSO-d<sub>6</sub>, 100Hz) showed 21 carbon atoms, and the carbon displacement of each group could be reasonably assigned. The NMR data were in good agreement with the target compounds.

### b, Mass spectrometry (MS)

Detection instrument: Vanquish-TSQ QUANTIS liquid chromatography-mass spectrometry

Analytical method: Chinese Pharmacopoeia 2020 Edition of the four General Rules 0512 and 0431

Test data:

Table 3.2.S.5-29 MS data of the tested sample

Mass-charge ratio (m/z)	Corresponding ion	Spare note
379.28	[M+H] <sup>+</sup>	Molecular ion peak in positive ion mode

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In the positive ion mode, the detected mass-charge ratio m/z is 379.28 peak, which is attributed to the molecular ion peak in the positive ion mode, consistent with the molecular weight of the target molecule 378.43.

### **c. Chiral liquid chromatography**

Testing instrument: Waters Acquity Arc

Analytical methods: General Rule 0512 of the fourth part of Chinese Pharmacopoeia (2020 Edition)

Test data:

Table 3.2.S.5-30 Retention time of the tested sample

Sample	Retention time (min)
YA2304-20 Reference product (lot No.: 23080401-A)	24.858
Control Product YA2304 (lot No.: 23091802-D)	20.240

According to the results under 3.2.S.4.3.3 Specific attribute of product enantiomer methodology verification, the retention time of the main peak in the control product solution (YA2304-20 positioning solution) was 24.858min, and the retention time of the main peak in YA2304 positioning solution was 20.240min. The separation degree between YA2304 peaks and YA2304-20 peaks in the system suitability solution was 3.0.

### **d. Configuration confirmation**

According to the structural analysis of the compound, Finerenone contains a chiral center, and Finerenone C9 is in the S configuration. According to the preparation process of YA2304, Finerenone is salted out with the resolution agent during the resolution step and separated after filtration, while the enantiomer YA2304-20 is dissolved in the filtrate. YA2304-20 reference product was prepared by caustic collection of filtrate and then refined.

Finerenone has been proved by single crystal diffraction that C9 has an S configuration (see section 3.2.S.3.1 Structure and Physical and Chemical

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Properties for details). Based on the confirmed planar structure and the principle that the retention time of enantiomers is different under chiral chromatography conditions, the results detected by the chiral liquid chromatography above are as follows: It can be confirmed that the reference product YA2304-20 is the enantiomer of fenelidone.

### **(9.3) Content calibration**

#### **Properties**

This product should be white to white powder.

#### **Moisture**

Take about 0.1g~0.2g of this product, according to the water determination method (" Chinese Pharmacopoeia "2020 edition of the fourth part of the general rule 0832 coulometric titration method) determination, water content shall not exceed 2.0%.

#### **Purity**

Determination according to high performance liquid chromatography (General Rule 0512 of the Fourth part of Chinese Pharmacopoeia, 2020 Edition).

Solvent acetonitrile-water (30:70)

Take an appropriate amount of this product from the test product solution, dissolve it with a solvent and dilute it into a solution containing about 0.5mg per 1ml.

System suitability reserve liquid precision measure 0.6ml of test product solution, place it in 10ml measuring bottle, dilute it with solvent to scale, shake well, and then obtain.

Precision weigh the system suitability solution YA2304 reference product about 20mg, put it into a 20ml measuring bottle, then accurately add the system suitability reserve solution 1.0ml, dissolve it with solvent and dilute it to the scale, shake well, and then obtain.

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Chromatographic conditions with silica gel surface covalently bonded cellulose - III - (3, 5-dichlorophenyl carbamate) as filler column (CHIRALPAK IC, 250×4.6mm, 5μm or equivalent performance); With 20mM ammonium acetate buffer (pH adjusted to 3.1 with phosphoric acid) -acetonitrile (70:30) as mobile phase, iso-degree elution was performed for 40min; The flow rate was 1.0ml per minute. Column temperature was 40°C; The detection wavelength was 251nm; Injection volume 5μl.

System suitability requirements In the system suitability solution chromatogram, the separation degree of YA2304 peaks and YA2304-20 peaks should be greater than 1.5.

The test product solution and the system suitability solution were precisely measured and injected into the chromatograph respectively to record the chromatogram. According to the area normalization method, the main peak purity should NLT 95.0%.

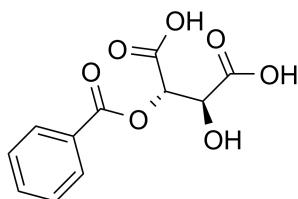
The content is measured by purity x (1- moisture).

### **(10) YA2304-52 working standard**

The reference product YA2304-52 used in this study was derived from Tianyi Hengyi Pharmaceutical Co., LTD. It was named D-monobenzoyl taric acid on the physical label and the reference product report. The applicant confirmed its chemical structure and content calibration, and the content was calculated according to 90.8%.

Sample lot No.: 24022801-C

Structural formula:



Molecular formula: C<sub>11</sub>H<sub>10</sub>O

Finerenone

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Hinxe Pharmaceutical Co., Ltd.

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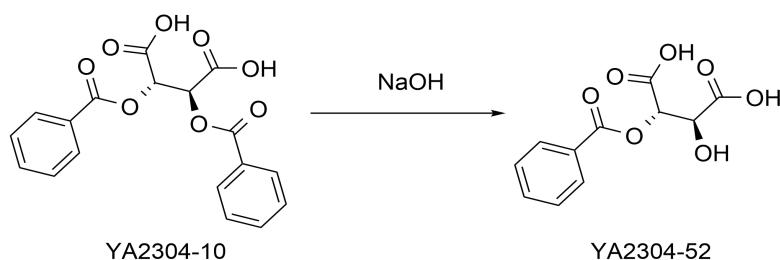
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Molecular weight: 254.19

### (10.1) Preparation method of YA2304-52

YA2304-10-Lf1126185589 (5.0g), sodium hydroxide (1.6g), methanol (20ml), and water (20ml) were added to the reaction bottle at 45°C for 2h, then lowered to room temperature for filtration, and the pH of the filtrate was adjusted to about 7. The chromatography was prepared by reversed-phase column under medium pressure and purified, and fractions were collected. Freeze-dried 0.72g white solid, YA2304-52.



### (10.2) Structural analysis of YA2304-52 reference

The chemical structure of the reference product was confirmed by the applicant through  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, mass spectrometry, specific curl and other testing means.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR commissioned Changsha Kangpeng Pharmaceutical Co., LTD for testing. The relevant test atlas and commissioned test contract are detailed in section 3.2.R.7.S. The relevant test results and structural analysis are detailed as follows:

#### a. Nuclear Magnetic Resonance (NMR)

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

Solvent: DMSO;

Reference: TMS ( $^1\text{H}$  spectrum);

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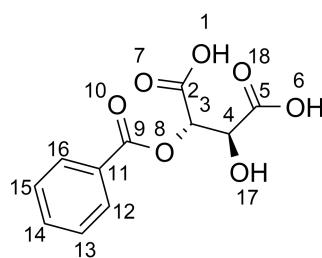
Version: 001

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Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

The number of the hydrocarbon atom in the structure (non-systematic naming, only used for hydrocarbon magnetic spectrum analysis)



The test data and analytical results of hydrocarbon spectra are shown in Table 3.2.S.5-31 and Table 3.2.S.5-32

Table 3.2.S.5-31  $^1\text{H}$  NMR chemical shift value and correlation spectrum of the tested sample (from high field to low field)

Peak No.	Delta ppm	Peak pattern	Coupling constant (J)	Proton number	Attribution
1	4.251	s	/	1H	17-OH
2	4.520-4.525	d	/	1H	4-CH
3	5.329-5.335	d	/	1H	3-CH
4	7.449-7.488	t	7.6	2H	13, 15 - CH
5	7.596-7.633	t	7.6	1H	14-CH
6	7.977-7.998	t	7.6	2H	, 12 dec - CH

Table 3.2.S.5-32  $^{13}\text{C}$  NMR chemical shift value and correlation spectrum (from high field to low field) of the tested sample

Peak No.	Delta ppm	Carbon atom number	Attribution
1	73.791	1	4 digit C
2	77.398	1	3-bit C
3	128.666	2	13,15 C's
4	129.110	1	11 bit C's
5	129.701	2	12,16 C's
6	133.891	1	14 bit C
7	168.029	1	9 digit C's

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8	177.157	1	5 digit C's
9	178.400	1	2-bit C

<sup>1</sup>H-NMR(DMSO-d<sub>6</sub>, 400Hz) showed 8 hydrogen protons, 2 carboxyl hydrogen did not peak, and the hydrogen displacement of each group could be reasonably attributed. <sup>13</sup>C-NMR(DMSO-d<sub>6</sub>, 100Hz) showed 11 carbon atoms, and the carbon displacement of each group could be reasonably assigned. The NMR data were in good agreement with the target compound.

### **b. Mass spectrometry (MS)**

Testing instrument: Waters Acquity Arc-QDA liquid chromatography-mass spectrometry

Analytical methods: General Rules 0512 and 0431 of the Fourth part of Chinese Pharmacopoeia (2020 Edition)

Test data:

Table 3.2.S.5-33 MS data of the tested sample

Mass-charge ratio (m/z)	Corresponding ion	Spare note
253.17	[M-H] <sup>-</sup>	Molecular ion peak in negative ion mode

In the negative ion mode, the mass-charge ratio m/z is 253.17 peak, which is attributed to the molecular ion peak in the negative ion mode, which is consistent with the molecular weight of the target molecule 254.19.

### **c. Confirmation of configuration**

Combined with the analysis of the preparation process of the reference product, the two chiral centers in YA2304-52 were introduced from the raw material YA2304-10 used in its preparation, and the two chiral centers were not affected in the subsequent ester hydrolysis reaction when the hydroxyl group was replaced and left. The molecular configuration of the reference product YA2304-52 and the raw material YA2304-10 is the same. The specific

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rotation of raw material YA2304-10 (batch NO.: Lf1126185589) is 114.1°, that is, the configuration should be (2S,3S). Combined with the preparation technology, it can be confirmed that the reference product YA2304-52 is the configuration of (2S,3S), that is, the absolute configuration expressed in the structural formula by classical chemical correlation method.

### **(10.3) Content calibration**

#### **Properties**

This product should be like white powder.

#### **Purity**

Determination according to high performance liquid chromatography (General Rule 0512 of the Fourth part of Chinese Pharmacopoeia, 2020 Edition).

Chromatographic conditions: Philomon Comixsil RP-100 (4.6mm×250mm, 5μm) or equivalent performance column); 0.1% sulfuric acid solution as mobile phase A, acetonitrile as mobile phase B; Gradient elution is performed according to the following table; The flow rate is 1.0ml per minute; Column temperature was 30°C; Sample plate temperature is 5°C; The detection wavelength was 210nm; And the injection volume was 20μl.

Time (min.)	Mobile phase A (%)	Mobile phase B (%)
0	86	14
8.5	86	14
20	35	65
23	35	65
25	20	80
27	20	80
28	86	14
40	86	14

Solvent 50% acetonitrile

Take an appropriate amount of this product from the test product solution, weigh it accurately, dissolve and dilute it with solvent to make a solution

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containing YA2304-52 about 2mg per 1ml.

The test product solution was accurately measured 20 $\mu$ l and injected into the liquid chromatograph, and the chromatogram was recorded. The main peak purity should NLT 90.0% according to area normalization method.

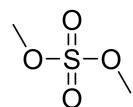
The content is calculated according to the purity.

### **(11) Dimethyl sulfate working standard**

The reference product of Dimethyl sulfate used in this application comes from Shanghai McLean Biochemical Technology Co., LTD. It is named Dimethyl sulfate on the physical label, Dimethyl sulfate on the manufacturer's report, and Dimethyl sulfate on the invoice. The chemical structure of Dimethyl sulfate was confirmed by the applicant, and the content was 99.759% according to the manufacturer's report.

Sample lot No.: C15128674

Structural formula:



Molecular formula: C<sub>2</sub>H<sub>6</sub>O<sub>4</sub>S

Molecular weight: 126.13

#### **(11.1) Structure analysis of dimethyl sulfate reference**

The chemical structure of the reference product was confirmed by<sup>1</sup> the applicant by means of H NMR, <sup>13</sup> C NMR and mass spectrometry. The mass spectrometry was tested by the applicant by himself,<sup>1</sup> and the H NMR and <sup>13</sup> C NMR were tested by Changsha Kangpeng Pharmaceutical Co., LTD. The relevant detection graphs and the contract for entrusted testing are detailed in section 3.2.R.7.S. The relevant test results and structural analysis are detailed as follows:

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### a. Nuclear Magnetic Resonance (NMR)

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

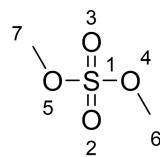
Solvent: CDCl<sub>3</sub>;

Reference: TMS (<sup>1</sup>H spectrum);

Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

The number of the hydrocarbon atom in the structure (non-systematic naming, only used for hydrocarbon magnetic spectrum analysis)



The test data and analytical results of hydrocarbon spectra are shown in Table 3.2.S.5-35 and Table 3.2.S.5-36

Table 3.2. <sup>1</sup>H NMR chemical shift value and correlation spectrum (from high field to low field) of the tested sample S.5-35

Peak No.	Delta ppm	Peak pattern	Coupling constant (J)	Proton number	Attribution
1	3.914-3.915	d	0.6	6H	6, 7 - CH

Table 3.2.S.5-36 <sup>13</sup>C NMR chemical shift value and correlation spectrum (from high field to low field) of the tested sample

Peak No.	Delta ppm	Number of carbon atoms	Attribution
1	58.654	2	6, 7-place C

<sup>1</sup>H-NMR(CDCl<sub>3</sub>, 400Hz) shows 6 hydrogen protons, and the hydrogen displacement of each group can be reasonably attributed; <sup>13</sup>C-NMR(CDCl<sub>3</sub>,

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100Hz) showed 2 carbon atoms, and the carbon displacement of each group could be reasonably assigned. The NMR data corresponded well with the target compound.

### **b, Mass spectrometry (MS)**

Testing instrument: Agilent 8890-7010B gas chromatogram-mass spectrometry

Analytical methods: 0521 and 0431 of the Four General Rules of Chinese Pharmacopoeia (2020 Edition)

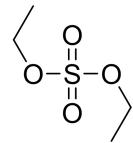
The results were consistent with dimethyl sulfate in the standard spectrum library.

### **(12) diethyl sulfate working standard**

The reference product of Diethyl sulfate used in this study came from Shanghai McLean Biochemical Technology Co., LTD. It was named as Diethyl sulfate on the physical label, Diethyl sulfate on the manufacturer's report and Diethyl sulfate on the invoice. The chemical structure of the product was confirmed by the applicant and its content was 99.024% according to the manufacturer's report.

Sample lot No.: C14721434

Structural formula:



Molecular formula: C<sub>4</sub>H<sub>10</sub>O<sub>4</sub>S

Molecular weight: 154.18

### **(12.1) Structure analysis of diethyl sulfate reference**

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The chemical structure of the reference product was confirmed by<sup>1</sup> the applicant by means of H NMR,<sup>13</sup> C NMR and mass spectrometry. The mass spectrometry was tested by the applicant by himself,<sup>1</sup> and the H NMR and<sup>13</sup> C NMR were tested by Changsha Kangpeng Pharmaceutical Co., LTD. For details about the relevant detection graphs and contract, see section 3.2.R.7.S. The relevant test results and structural analysis are detailed as follows:

### a. Nuclear Magnetic Resonance (NMR)

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

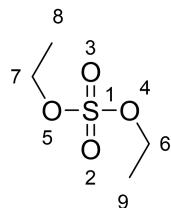
Solvent: CDCl<sub>3</sub>;

Reference: TMS (<sup>1</sup>H spectrum);

Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

The number of the hydrocarbon atom in the structure (non-systematic naming, only used for hydrocarbon magnetic spectrum analysis)



The test data and analytical results of hydrocarbon spectra are shown in Table 3.2.S.5-37 and Table 3.2.S.5-38.

Table 3.2.S.5-37 <sup>1</sup>H NMR chemical shift value and correlation spectrum of the tested sample (from high field to low field)

Peak	Delta ppm	Peak	Coupling	Proton	Attributio
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No.		pattern	constant ( <i>J</i> )	number	n
1	1.366-1.401	t	7.2	6H	8 or 9 - CH
2	4.265-4.318	q	7.1	4H	6, 7 - CH

Table 3.2.S.5-38  $^{13}\text{C}$  NMR chemical shift value and correlation spectrum (from high field to low field) of the tested sample

Peak No.	Delta ppm	Number of carbon atoms	Attribution
1	14.470	2	8, 9-place C
2	69.426	2	Six, seven C's

$^1\text{H}$ -NMR( $\text{CDCl}_3$ , 400Hz) showed 10 hydrogen protons, and the hydrogen displacement of each group could be reasonably attributed.  $^{13}\text{C}$ -NMR( $\text{CDCl}_3$ , 100Hz) showed 4 carbon atoms, and the carbon displacement of each group could be reasonably assigned. The NMR data corresponded well with the target compound.

### b, Mass spectrometry (MS)

Testing instrument: Agilent 8890-7010B gas chromatogram-mass spectrometry

Analytical methods: 0521 and 0431 of the Four General Rules of Chinese Pharmacopoeia (2020 Edition)

Detection result: It was consistent with diethyl sulfate in standard spectrum library.

### (13) diisopropyl sulfate working standard

The reference product of Diisopropyl Sulfate used in this research application came from Shanghai Bidd Pharmaceutical Technology Co., LTD. It was named diisopropyl sulfate on the physical label, diisopropyl sulfate on the manufacturer's report, and diisopropyl sulfate on the invoice. The chemical structure of the product was confirmed by the applicant. Acco

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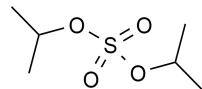
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rding to the report provided by the manufacturer, the content was 97%.

Sample lot No.: DLX318

Structural formula:



Molecular formula: C<sub>6</sub>H<sub>14</sub>O<sub>4</sub>S

Molecular weight: 182.23

### **(13.1) Structure analysis of diisopropyl sulfate reference**

The chemical structure of the reference product was confirmed by the applicant through <sup>1</sup> H NMR, <sup>13</sup> C NMR and mass spectrometry, among which the mass spectrometry was tested by the applicant himself, <sup>1</sup> H NMR was provided by Shanghai Bider Medical Technology Co., LTD., <sup>13</sup> C NMR was tested by Changsha Kangpeng Medical Co., LTD. The relevant detection spectra and the contract for commissioning are detailed in section 3.2.R.7.S, and the relevant test results and structural analysis are detailed as follows:

#### **a. Nuclear Magnetic Resonance (NMR)**

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

Solvent: CDCl<sub>3</sub>;

Reference: TMS (<sup>1</sup>H spectrum);

Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

The number of the hydrocarbon atom in the structure (non-systematic n

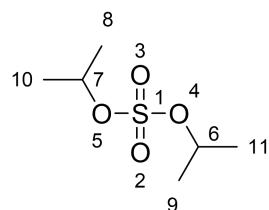
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aming, only used for hydrocarbon magnetic spectrum analysis)



The test data and analytical results of hydrocarbon spectra are shown in Table 3.2.S.5-39 and Table 3.2.S.5-40.

Table 3.2.S.5-39  $^1\text{H}$  NMR chemical shift value and correlation spectrum of the tested sample (from high field to low field)

Peak No.	Delta ppm	Peak pattern	Coupling constant ( $J$ )	Proton number	ownership
1	1.43-1.44	d	/	12H	8,9,10,11 - CH
2	4.86-4.93	dt	/	2H	6, 7 - CH

Table 3.2.S.5-40  $^{13}\text{C}$  NMR chemical shift values and correlation spectra (from high field to low field) of the tested sample

Peak No.	Delta ppm	Number of carbon atoms	Attribution
1	22.518	4	8,9,10,11 digit C's
2	79.885	2	6,7 C's

$^1\text{H-NMR}(\text{CDCl}_3, 400\text{Hz})$  showed 14 hydrogen protons, and the hydrogen displacement of each group could be reasonably attributed.  $^{13}\text{C-NMR}(\text{CDCl}_3, 100\text{Hz})$  showed 6 carbon atoms, and the carbon displacement of each group could be reasonably assigned. The NMR data corresponded well with the target compound.

### b, Mass spectrometry (MS)

Testing instrument: Agilent 8890-7010B gas chromatogram-mass spectrometry

Analytical methods: 0521 and 0431 of the Four General Rules of Chinese Pharmacopoeia (2020 Edition)

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Test data:

Table 3.2.S.5-41 MS data of the tested sample

Mass-charge ratio (m/z)	Corresponding ion	Spare note
183.0000	[M] <sup>+</sup>	Molecular ion peak
167.1000	[M-CH <sub>3</sub> ] <sup>+</sup>	Fragment ion peak
138.9000	[M-CHCH <sub>3</sub> CH <sub>3</sub> ] <sup>+</sup>	Fragment ion peak

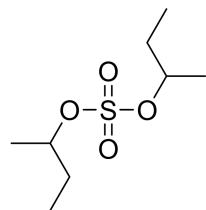
The molecular weight of the target is 182.23, and the molecular ion peak of the mass-charge ratio of 183.0000 was detected, which is consistent with the target molecule. The mass-charge ratio of 167.1000 and 138.9000 was detected, which is attributed to the molecular fragment peak after cracking off a methyl group and a propyl group, which is consistent with the target molecule.

### **(14) disec-butylsulfate working standard**

The reference product of sec-butyl sulfate used in the process of this study was from Tiandi Hengyi Pharmaceutical Co., LTD. It was named as sec-butyl sulfate on the physical label and the reference product report. The applicant confirmed its chemical structure and content calibration, and the content was calculated according to 99.7%.

Sample lot No.: 23110207-H

Structural formula:



Molecular formula: C<sub>8</sub>H<sub>18</sub>O<sub>4</sub>S

Molecular weight: 210.29

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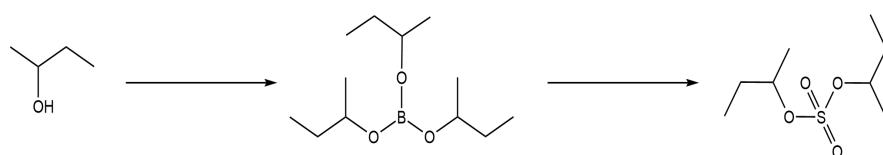
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### (14.1) Preparation method of disec-butyl sulfate

Boric acid (30 g), secondary butanol (360 g) and toluene (200 ml) were added into the reaction bottle, and the reaction was continued for 1 h after the reflux water separation reaction to anhydrous formation at 135°C. Control the temperature below 55°C and concentrate under pressure (until the concentration weighs 100 g), add dichloromethane (100 ml) to dissolve, cool down to 0°C, drop concentrated sulfuric acid (55 g), after dripping, keep warm at 25~30°C for 24 h, add dichloromethane, filter, wash organic layer three times, wash saturated salt water once, dry anhydrous sodium sulfate. Control the temperature below 50°C vacuum concentration without distillate, light yellow oil that is di-sec-butylsulfate.



### (14.2) Analysis of the structure of disec-butylsulfate reference

The chemical structure of the reference product was confirmed by the applicant through  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, mass spectrometry and other detection means. The mass spectrometry was tested by the applicant, and the  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR were tested by Changsha Kangpeng Pharmaceutical Co., LTD., the relevant detection spectrum and the contract for entrusted testing are detailed in section 3.2.R.7.S. The relevant test results and structural analysis are as follows:

#### a. Nuclear Magnetic Resonance (NMR)

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

Solvent:  $\text{CDCl}_3$ ;

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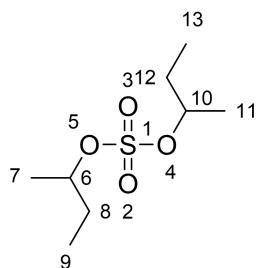
Date: Sep. 2024

Reference: TMS ( $^1\text{H}$  spectrum);

Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

The number of the hydrocarbon atom in the structure (non-systematic naming, only used for hydrocarbon magnetic spectrum analysis)



The test data and analytical results of hydrocarbon spectra are shown in Table 3.2.S.5-42 and Table 3.2.S.5-43

Table 3.2.S.5-42  $^1\text{H}$  NMR chemical shift value and correlation spectrum of the tested sample (from high field to low field)

Peak No.	Delta ppm	Peak pattern	Coupling constant ( $J$ )	Proton number	Attribution
1	0.963-1.000	t	7.2	6H	9, 13 - CH
2	1.412-1.428	d	6.4	6H	7, 11 - CH
3	1.679-1.781	m	/	4H	8, 12 - CH
4	4.695-4.742	m	/	2H	6, 10 - CH

Table 3.2.S.5-43  $^{13}\text{C}$  NMR chemical shift value and correlation spectrum

(from high field to low field) of the tested sample

Peak No.	Delta ppm	Number of carbon atoms	Attribution
1	9.175	1	9 Place C
2	9.194	1	13 bit C
3	19.820	1	7 digit C's
4	19.839	1	11 bit C
5	29.239	1	8-bit C

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6	29.291	1	12 bit C
7	84.508	2	6,10 C's

<sup>1</sup>H-NMR(CDCl<sub>3</sub>, 400Hz) showed 18 hydrogen protons, and the hydrogen displacement of each group could be reasonably attributed. <sup>13</sup>C-NMR(CDCl<sub>3</sub>, 100Hz) showed 8 carbon atoms, and the carbon displacement of each group could be reasonably assigned. The NMR data corresponded well with the target compound.

### **b, Mass spectrometry (MS)**

Testing instrument: Agilent 8890-7010B gas chromatogram-mass spectrometry

Analytical methods: 0521 and 0431 of the Four General Rules of Chinese Pharmacopoeia (2020 Edition)

Test data:

Table 3.2.S.5-44 MS data of the tested sample

Mass-charge ratio (m/z)	Corresponding ion	Remarks
194.9987	[M-CH <sub>3</sub> ] <sup>+</sup>	Fragment ion peak
180.9999	[M-C <sub>2</sub> H <sub>5</sub> ] <sup>+</sup>	
152.9994	[M-CHCH <sub>2</sub> CH <sub>3</sub> CH <sub>3</sub> ] <sup>+</sup>	
57.1996	-CHCH <sub>2</sub> CH <sub>3</sub> CH <sub>3</sub> <sup>+</sup>	

The molecular weight of the target was 210.29, and the detected mass-to-charge ratio was 194.9987, 180.9999 and 152.9994, which belonged to the molecular fragment peak after splitting one methyl group, one ethyl group and one butyl group. The mass-to-charge ratio of 57.1996 was the butyl fragment peak, which was consistent with the target molecule.

### **(14.3) Content calibration**

### **Properties**

Finerenone

Confidential

Hinnye Pharmaceutical Co., Ltd.

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This product should be light yellow oily liquid.

### **Moisture**

Take about 0.1~0.3g of this product, according to the water determination method (" Chinese Pharmacopoeia "2020 edition of the fourth part of the general rule 0832 Coulometric titration method) determination, water content should not exceed 1.0%.

### **Purity**

Determination by gas chromatography (General Rule 0521 of the Fourth part of Chinese Pharmacopoeia, 2020 Edition).

The capillary column with 100% dimethylpolysiloxane (DB-1, 30m×0.32mm, 1 $\mu$ m or similar polarity) as fixed liquid was used as the chromatographic column; The initial column temperature was 50°C for 3min, and then it was heated up to 135°C at the rate of 10°C/min for 9min, and then heated up to 230°C at the rate of 20°C/min for 14.5min. Inlet temperature: 150 ° C; The detector is FID detector, the temperature is 250°C; (Recommended carrier gas nitrogen, carrier gas flow rate of 3.0ml/min; The air flow rate is 300ml/min; Hydrogen flow rate is 30ml/min; The injection volume is 1 $\mu$ l and the shunt ratio is 5:1)

Solvent acetonitrile

Preparation of test product solution: Take about 70mg of this product, weigh it accurately, put it in a 10ml measuring bottle, add acetonitrile to dissolve, dilute with acetonitrile to the scale, shake well, as test product solution.

Determination method: Precisely measure the above test product solution 1 $\mu$ l, inject it into the liquid chromatograph, record the chromatogram, according to the area normalization method, the main peak purity shall NLT 95.0%.

The content is measured by purity × (1-moisture).

### **(15) YA2304 working standard**

Finerenone

Confidential

Hinnye Pharmaceutical Co., Ltd.

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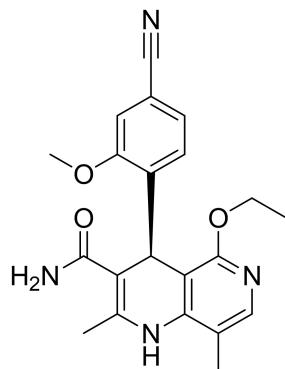
Version: 001

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The reference product YA2304 used in this study was derived from Tian di Hengyi Pharmaceutical Co., LTD. It was named Finerenone on the physical label and the reference product report. The chemical structure of YA2304 was confirmed by the applicant and its content was calibrated. The content was 99.7%.

Sample lot No.: 23091802-D

Structural formula:



Molecular formula: C<sub>21</sub>H<sub>22</sub>O<sub>3</sub>N

Molecular weight: 378.43

### **(15.1) Preparation method of YA2304**

YA2304-cp (613g) and ethanol (9.81kg) were added to the reactor, and the system was heated to 70-80°C and stirred to dissolve, and then filtered while hot. Most of the ethanol was concentrated under pressure at 55°C and the total volume of residual distillate was about 4L. The system was stirred at 30-40°C for about 15 h. The temperature was lowered to 0-5°C and stirred for 2.0 h. The filter cake was washed with 0-5°C ethanol (1.5kg), and the filter cake was vacuum-dried at 55°C to obtain white solid (534.8g).

### **(15.2) Structural analysis of YA2304**

The chemical structure of the reference product was confirmed by<sup>1</sup> the applicant by means of H NMR,<sup>13</sup> C NMR, mass spectrometry and chiral liquid

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chromatography. The mass spectrometry and chiral liquid chromatography were tested by the applicant by himself,<sup>1</sup> and the H NMR and<sup>13</sup> C NMR were tested by Changsha Kangpeng Pharmaceutical Co., LTD. The relevant test results and structural analysis are listed as follows:

#### **a. Nuclear Magnetic Resonance (NMR)**

Instrument model: Bruker Avance AV 400 superconducting pulse Fourier transform nuclear magnetic resonance spectrometer

Test conditions:

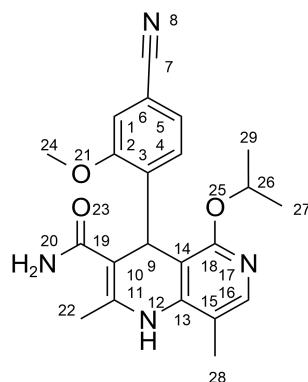
Solvent: DMSO;

Reference: TMS ( $^1\text{H}$  spectrum);

Resonance frequency of hydrogen spectrum: 400MHz

Resonant frequency of carbon spectrum: 100MHz

The number of the hydrocarbon atom in the structure (non-systematic naming, only used for hydrocarbon magnetic spectrum analysis)



The test data and analytical results of hydrocarbon spectra are shown in Table 3.2.S.5-45 and Table 3.2.S.5-46.

Table 3.2.S.5-45  $^1\text{H}$  NMR chemical shift value and correlation spectrum (from high field to low field) of the tested sample

Peak	Delta ppm	Peak	Coupling	Proton	Attributio
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No.		pattern	constant (J)	number	n
1	1.038-1.073	t	7.2	3H	27-CH <sub>3</sub>
2	2.128	s	/	3H	28-CH <sub>3</sub>
3	2.204	s	/	3H	22-CH <sub>3</sub>
4	3.835	s	/	3H	24-CH <sub>3</sub>
5	4.010-4.034	qd	7.2	2H	26-CH <sub>2</sub>
6	5.394	s	/	1H	9-CH
7	6.707-6.783	bs	/	2H	20-NH <sub>2</sub>
8	7.158-7.178	d	8.0	1H	4-CH
9	7.276-7.299	dd	8.0, 1.2	1H	5-CH
10	7.377-7.380	d	1.2	1H	1-CH
11	7.560-7.561	s	0.4	1H	16-CH
12	7.705	s	/	1H	12-NH

Table 3.2.S.5-46 <sup>13</sup>C NMR chemical shift value and correlation spectrum  
(from high field to low field) of the tested sample

Peak No.	Delta ppm	Number of carbon atoms	Attribution
1	14.202	1	28-bit carbon
2	14.739	1	27-bit carbon
3	18.521	1	Twenty-two carbon
4	32.872	1	9-bit carbons
5	56.509	1	24-place carbon
6	61.005	1	26-bit carbon
7	103.640	1	14-bit carbon
8	105.784	1	10-bit carbon
9	109.983	1	2-place carbons
10	111.909	1	15-place carbon
11	114.588	1	1 bit carbon
12	119.434	1	6-place carbon
13	125.223	1	5-bit carbon
14	131.363	1	4-place carbons
15	138.616	1	11-bit carbon
16	142.150	1	7-bit carbons
17	144.646	1	13-bit carbon
18	144.674	1	16-bit carbon
19	156.138	1	3-site carbon
20	159.843	1	18-bit carbon
21	170.190	1	19-bit carbon

<sup>1</sup>H-NMR(DMSO-d<sub>6</sub>, 400Hz) showed 22 hydrogen protons, and the hydrogen

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displacement of each group could be reasonably attributed.  $^{13}\text{C}$ -NMR(DMSO-*d*<sub>6</sub>, 100Hz) showed 21 carbon atoms, and the carbon displacement of each group could be reasonably assigned. The NMR data were in good agreement with the target compounds.

### **b. Mass spectrometry (MS)**

Detection instrument: Vanquish-TSQ QUANTIS liquid chromatography-mass spectrometry

Analytical method: Chinese Pharmacopoeia 2020 Edition of the four General Rules 0512 and 0431

Test data: See Table 3.2.S.5-47

Table 3.2.S.5-47 MS data of the tested sample

Mass-charge ratio (m/z)	Corresponding ion	Spare note
379.27	[M+H] <sup>+</sup>	Molecular ion peak in positive ion mode

In the positive ion mode, the mass-charge ratio m/z is detected as 379.27 peak, which is attributed to the molecular ion peak in the positive ion mode, consistent with the molecular weight of the target molecule 378.43.

### **c. Chiral liquid chromatography**

Testing instrument: Waters Acuity Arc

Analytical methods: General Rule 0512 of the fourth part of Chinese Pharmacopoeia (2020 Edition)

Test data:

Table 3.2.S.5-48 Retention time of the tested sample

Sample	Retention time (min)
YA2304-20 Reference product (lot No.: 23080401-A)	24.858
Control Product YA2304 (lot No.: 23091802-D)	20.240

According to the results under 3.2.S.4.3.3 Specific attribute of product

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enantiomer methodology verification, the retention time of the main peak in the control product solution (YA2304-20 positioning solution) was 24.858min, and the retention time of the main peak in YA2304 positioning solution was 20.240min. The separation degree between YA2304 peaks and YA2304-20 peaks in the system suitability solution was 3.0.

### **d. Configuration confirmation**

There is a chiral center in the molecular structure of finerenone, and C9 in the structure of Finerenone is S configuration. The applicant has carried out single crystal diffraction detection on the sample of Finerenone prepared by registered process (lot No.: 231101), and confirmed that its three-dimensional structure is consistent with the theory. The preparation process of the reference product in this batch is consistent with the registered process, so its configuration can be confirmed as S configuration.

### **(15.3) Content calibration**

#### **Properties**

This product should be white to yellow powder.

#### **Moisture**

Take about 0.1g~0.2g of this product, according to the water determination method (" Chinese Pharmacopoeia "2020 edition of the fourth part of the general rule 0832 Coulometric titration method) determination, water content should not exceed 0.5%.

#### **Incandescent residue**

Take about 1.0g of this product, according to the method of incandescent residue inspection (" Chinese Pharmacopoeia "2020 edition of the fourth part of the General Rule 0841) determination, residual residue shall not exceed 0.1%.

#### **Purity**

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Determination according to high performance liquid chromatography

(General Rule 0512 of the Fourth part of Chinese Pharmacopoeia, 2020 Edition).

Chromatographic conditions: octadecylsilane bonded silica gel was used as filler (XBridge Shield RP18, 150\*4.6mm, 3.5 $\mu$ m or equivalent performance column); Welch Ghost-Buster Column (4.6×50mm, or equivalent ghost-buster column) was installed after the mixer of the pump and before the sampler. 0.03% phosphoric acid solution was used as mobile phase A and acetonitrile as mobile phase B; Gradient elution is performed according to the following table; Column temperature is 25°C; The detection wavelength was 251nm; The flow rate was 1.0mL per minute; The injection volume was 5 $\mu$ L.

Time /min	Mobile phase A/%	Mobile phase B/%
0	95	5
16.5	50	50
17	20	80
22	20	80
22.5	95	5
35	95	5

Solvent 30% acetonitrile

Appropriate amount of this product is taken from the test product solution, accurately weighed, dissolved and diluted with solvent to make a solution containing Finerenone of about 0.25mg per 1ml.

The main peak purity was NLT 99.0% according to area normalization method. 5 $\mu$ L of test solution was injected into liquid chromatograph.

The content is measured by purity × (1 - moisture - incandescent residue).

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### 3.2.S.6 Container Closure System

#### I. Description of Container Closure System

The container closure system of Finerenone manufactured by Hinye Pharmaceutical Co., Ltd. consists of pharmaceutical grade low-density polyethylene (LDPE) bag as primary packaging and cardboard drum as secondary packaging.

The packaging procedure is as follows:

**Primary packaging:** The drug substance is weighed and packed with double-layered pharmaceutical grade low-density polyethylene (LDPE) bag according to the package size. Lock and seal with cable tie, and transfer to the outside packaging room through the transfer window.

**Secondary packaging:** Put it into cardboard drum as one bag per drum, and seal. Fill in packaging label, and affix it to the outside of the drum.

**Package size:** 0.5 kg/drum, 1.0 kg/drum, 2.0 kg/drum, 3.0 kg/drum, 5.0 kg/drum, 10.0 kg/drum.

#### II. Specification of Packaging Materials

##### Primary Packaging Material

The specification and analytical method of pharmaceutical grade low-density polyethylene (LDPE) bag established by the applicant is as follows:

Table 3.2.S.6-1 Acceptance Criteria of pharmaceutical grade low-density polyethylene (LDPE) bag

Test item	Acceptance criteria
Appearance	The surface should be smooth, uniform in color, and free of holes, foreign matters, odors, and conglutination. The heat-sealed part of the bag should be flat and free of defects.

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Test item		Acceptance criteria
Size		Length: 1100 ± 5mm; Width: 810 ± 5mm;
Test	density	0.910~0.935g/cm <sup>3</sup>
	Residue on ignition	NMT 0.1%
Extractables Test	Easy oxide	The difference between the titration volumes is NMT 1.5 mL.
	Heavy metals	NMT 1ppm.
	Non-volatile substances	Water The residue of the water extract is NMT 30.0 mg comparing to the blank solution.
	65% Ethanol	The residue of the 65% ethanol extract is NMT 30.0 mg comparing to the blank solution.
Microbial limit	n-Hexane	The residue of the n-hexane extract is NMT 30.0 mg comparing to the blank solution.
	Total aerobic microbial count (TAMC)	NMT 1000 CFU/100 cm <sup>2</sup> .
	Total yeasts and moulds count (TYMC)	NMT 100 CFU/100 cm <sup>2</sup> .
Escherichia coli		Absence of Escherichia coli

## **Test Methods for pharmaceutical grade low-density polyethylene (LDPE) bag**

### **Appearance**

Take an appropriate amount of sample, and look directly at it in a bright environment with natural light. The surface should be smooth, uniform in color, and free of holes, foreign matters, odors, and conglutination. The heat-sealed part of the bag should be flat and free of defects. Except for “NB046” which is black, all other parts should be transparent.

### **Size**

Take an appropriate amount of sample and measure the length and width with a ruler, which should comply with the acceptance criteria.

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Length: 1100 ± 5mm; Width: 810 ± 5mm.

### **Test**

#### **Density**

The sample should be placed in an environment with a temperature of (23±2)°C and a relative humidity of (50±5)% for more than 4h, and then perform the test under these conditions. Weigh accurately about 2g of sample, immerse it with anhydrous ethanol. (The density of the immersion liquid is generally less than the density of the sample. When the material density is greater than 1, use water as immersion liquid. When the material density is less than 1, use anhydrous ethanol).

Take an appropriate amount of the sample, place it on the balance, weigh accurately and record the mass in the air (a). Then place the sample in a certain amount of immersion liquid anhydrous ethanol with a known density ( $\rho_x$ ), weigh accurately and record the mass (b). The upper end of the sample should NLT 10 mm from the liquid surface, and air bubbles should not adhere to the surface of the sample. The density of the sample is calculated by the following formula. The density should be 0.910~0.935 g/cm<sup>3</sup>.

$$\rho = \frac{a \times \rho_x}{a - b}$$

#### **Residue on ignition:**

NMT 0.10 %, using 1.0 g. Examine by *Determination of Residue on Ignition*.

#### **Extractables Test**

Test solution: unless otherwise specified, take an appropriate amount of sample, and take three pieces of the sample with an inner surface area of about 600 cm<sup>2</sup> each (divided into small pieces of 3 cm in length and 0.3 cm in width), place these small pieces in three conical flasks with stoppers, respectively, add 200 mL of water (70°C ± 2°C), 65% ethanol (70°C ± 2°C),

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n-hexane ( $58^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ), respectively. After soaking for 2h, take it out, let it cool to room temperature, and compensate to original volume using the same solvent, and use the obtained solutions as the test solutions (water-extracting solution, ethanol-extracting solution, and n-hexane extracting solution).

Prepare each blank solution in same manner using the same batch of water, 65% ethanol, and n-hexane respectively.

### **Heavy metals**

Measure accurately 20 mL of the water-extracting solution, add 2 mL of acetate buffer solution (pH 3.5). Perform the test according to the "Operating Procedures for the Test of Heavy Metals" (Method 1). The heavy metals is NMT 1 ppm.

### **Easy oxide**

Precisely measure 20ml of the water-extracting solution, add 20ml of 0.002mol/L potassium permanganate titration solution and 1ml of dilute sulfuric acid, boil for 3min, and cool rapidly. Add 0.1 g of potassium iodide, allow to stand protected from light for 5min, and titrate with 0.01 mol/L sodium thiosulfate titration solution. When the titration is near the end point, add 5 drops of starch indicator solution, continue to titrate until it becomes colorless. Perform a blank titration using 20 mL of the bank solution, The difference between the titration volumes is NMT 1.5 mL.

### **Non-volatile substances**

Measure 100 mL of water-extracting solution, 65% ethanol-extracting solution, N-hexane extracting solution and the respective blank solution. Place them in an evaporating dish that has been dried to a constant weight, evaporate in a water bath, then dry at  $105^{\circ}\text{C}$  for 2h, and weigh the residue accurately after cooling.

The residue of the water extract is NMT 30.0 mg comparing to the blank

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solution. The residue of the 65% ethanol extract is NMT 30.0 mg comparing to the blank solution. The residue of the n-hexane extract is NMT 30.0 mg comparing to the blank solution.

### **Microbial limits**

Take the sample and press it on the inner surface with a sterilized metal plate with hole area of 20 cm<sup>2</sup>. Slightly moisten the sterile cotton swab with 0.9% sterile sodium chloride solution. Use a cotton swab to wipe the hole area of the plate 5 times, then use another cotton swab to wipe 5 times. That is to say, wipe each hole area with 2 cotton swabs 10 times totally. Wipe 5 hole areas with a total area of 100 cm<sup>2</sup>.

Cut each cotton swab immediately after being used, and put it into an conical flask (or large test tube) containing 30 mL of 0.9% sterile sodium chloride solution. Put all the cotton swabs into the conical flask (or large test tube), shake immediately for 1min to get the sample solution.

Perform the test with sample solution according to *Operating Procedures for the Test of Microbial Limit*. The acceptance criteria of total aerobic microbial count (TAMC) and total yeasts and moulds count (TYMC) are 1000 cfu/100 cm<sup>2</sup> and 100 cfu/100 cm<sup>2</sup>, respectively. *Escherichia coli* is not observed.

The copy of typical COA of pharmaceutical grade low-density polyethylene (LDPE) bag analyzed by Hinye Pharmaceutical Co., Ltd. and corresponding translation are annexed.

3.2.S.6 Annex 1 COA of pharmaceutical grade low-density polyethylene (LDPE) bag analyzed by Hinye Pharmaceutical Co., Ltd.(Batch No. )

3.2.S.6 Annex 2 English translation of COA of pharmaceutical grade low-density polyethylene (LDPE) bag analyzed by Hinye Pharmaceutical Co., Ltd.(Batch No. )

3.2.S.6 Annex 3 COA of pharmaceutical grade low-density polyethylene

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(LDPE) bag analyzed by Hinye Pharmaceutical Co., Ltd.(Batch No. )

3.2.S.6 Annex 4 English translation of COA of pharmaceutical grade low-density polyethylene (LDPE) bag analyzed by QHinye Pharmaceutical Co., Ltd.(Batch No. )

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## Secondary Packaging Material

The specification and analytical method of cardboard drum established by the applicant is as follows:

Table 3.2.S.6-2 Acceptance Criteria of Cardboard Drum

Test item		Acceptance criteria
Appearance	Surface	The surface is smooth, clean and free from pollution and other abnormal conditions.
	Size	Inner diameter × height: (310 mm ± 5 mm) × (410 mm ± 5 mm)
		Inner diameter × height: (450 mm ± 5 mm) × (500 mm ± 5 mm)
Material and construction		The sidewall is convolutely yellow paperboard. The cover and bottom are wood boards locking into sidewall with a stainless steel hoop.

## **Test Methods for Cardboard Drum**

### **Appearance**

#### **Surface**

Visual inspection. The surface is smooth, clean and free from pollution and other abnormal conditions.

#### **Size**

Measure the inner diameter and height of the cardboard with rule. The size should comply with the requirements below:

Material code	Inner diameter	Inner height
WB081	310 mm ± 5 mm	410 mm ± 5 mm
WB465	450 mm ± 5 mm	500 mm ± 5 mm

#### **Material**

Visual inspection. The sidewall is convolutely yellow paperboard. The cover and bottom are wood boards locking into sidewall with a stainless steel hoop.

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The copy of typical COA of cardboard drum analyzed by Hinye Pharmaceutical Co., Ltd. and corresponding translation are annexed.

*3.2.S.6 Annex 5 COA of cardboard drum analyzed by Hinye Pharmaceutical Co., Ltd.*

*3.2.S.6 Annex 6 English translation of COA of cardboard drum analyzed by Hinye Pharmaceutical Co., Ltd.*

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### **III. Annexes: COAs**

3.2.S.6 Annex 1 COA of pharmaceutical grade low-density polyethylene (LDPE) bag analyzed by Hinye Pharmaceutical Co., Ltd. (Batch No. )

3.2.S.6 Annex 2 English translation of COA of pharmaceutical grade low-density polyethylene (LDPE) bag analyzed by Hinye Pharmaceutical Co., Ltd.(Batch No. )

3.2.S.6 Annex 3 COA of pharmaceutical grade low-density polyethylene (LDPE) bag analyzed by Hinye Pharmaceutical Co., Ltd.(Batch No. )

3.2.S.6 Annex 4 English translation of COA of pharmaceutical grade low-density polyethylene (LDPE) bag analyzed by Hinye Pharmaceutical Co., Ltd.(Batch No. )

3.2.S.6 Annex 5 COA of cardboard drum analyzed by Hinye Pharmaceutical Co., Ltd.(Batch No. )

3.2.S.6 Annex 6 English translation of COA of cardboard drum analyzed by Hinye Pharmaceutical Co., Ltd. (Batch No. 1 )

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### **3.2.S.7 Stability**

#### **3.2.S.7.1 Stability Summary and Conclusions**

##### **Batch Tested**

Long-term and accelerated stability studies were carried out on three validation batches (batch No. 231201, 240101 and 240102, batch size: 10 kg).

Stress testing was carried out on one Commercial scale batch (batch No. 240101). It included the effect of high temperature (60 °C), high humidity (92.5% RH, 25°C) and illumination (visible light: 4500±500 LX; near ultraviolet: 90±5 µW/cm<sup>2</sup>, 25°C) on Finerenone without packaging.

The stability batch information and studies conducted are summarized in

Table 3.2.S.7.1-1.

##### **Stability Protocol of Long-term Stability**

Storage condition: 30°C±2°C/65%RH±5%RH.

Packaging: double-layered pharmaceutical grade low-density polyethylene (LDPE) bag.

Study period: 4 years for the three validation batches (batch No. 231201, 240101 and 240102).

Test points: 0, 3, 6, 9, 12, 18, 24, 36, 48 months for the validation batches (batch No. 231201, 240101 and 240102) as needed.

##### **Stability Protocol of Accelerated Stability**

Storage condition: 40°C±2°C/75%RH±5%RH

Packaging: double-layered pharmaceutical grade low-density polyethylene (LDPE) bag.

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Study period: 6 months.

Test points: 0, 3, 6 months for the validation batches (batch No.: 231201, 240101 and 240102).

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Table 3.2.S.7.1-1 Information of Stability Batches

Batch No.	Validation batches		
	231201	240101	240102
Batch size	10 kg	10 kg	10 kg
Manufacturing date	Jan. 2024	Jan. 2024	Jan. 2024
Stability studies	Stress test, Long-term, accelerated	Long-term, accelerated	Long-term, accelerated
starting time	Sept. 12, 2022	Sept. 12, 2022	Sept. 12, 2022
Testing frequency (time points)	Stress test: 0, 5, 15, 30D Long-term: 0, 3, 6, 9, 12, 18, 24, 36, 48, 60M; Accelerated: 0, 3, 6 M.	Long-term: 0, 3, 6, 9, 12, 18, 24, 36, 48 M; Accelerated: 0, 3, 6 M.	Long-term: 0, 3, 6, 9, 12, 18, 24, 36, 48, 60M; Accelerated: 0, 3, 6 M.
Status	Long-term: Ongoing Accelerated: Completed	Long-term: Ongoing Accelerated: Completed	Long-term: Ongoing Accelerated: Completed
Completed time points*	Long-term: 0, 3, 6 M; Accelerated: 0, 3, 6 M.	Long-term: 0, 3, 6 M; Accelerated: 0, 3, 6 M.	Long-term: 0, 3, 6 M; Accelerated: 0, 3, 6 M.

\* Stability data available are presented in section 3.2.S.7.3

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### **Stability Specification and Analytical Method**

The test items are listed in the table below. Process validation batch in the long-term stability study, The tests of particle size, microbial limits, XRD at 3, 9 and 18 months are omitted. The tests of Genotoxic impurities at 3, 9, 12 and 18 months are omitted. The stability specification is described in the table below. The analytical methods are referred to section 3.2.S.4.2. Validation of these analytical methods is described in section 3.2.S.4.3.

<b>Test Items</b>		<b>Acceptance Criteria</b>
Characters	Appearance	White to yellow powder
Tests	Particle size	Basically consistent with the results of 0 month
	Related substances	YA2304-10 NMT 0.10%; YA2304-12 NMT 0.15%; YA2304-14 NMT 0.15%; YA2304-15 NMT 0.15%; YA2304-16 NMT 0.15%; YA2304-17 NMT 0.15%; YA2304-18 NMT 0.15%; YA2304-19 NMT 0.15%;  Individual unspecified impurity NMT 0.10%; Total impurities NMT 1.0%
	Enantiomer s	YA2304-20 NMT 0.15%
	Genotoxic impurities	Dimethyl sulfate NMT 75ppm; Diethyl sulfate NMT 75ppm; Diisopropyl sulfate NMT 75ppm; Disec-butyl sulfate NMT 75ppm; The sum of genotoxic impurities NMT 250ppm
	Water	NMT 0.5%
	Microbial limits	TAMC: NMT $10^3$ CFU/g TYMC: NMT $10^2$ CFU/g Absence of Escherichia coli (1g)
	Assay	98.0% to 102.0% (anhydrous substance).
	Crystal form(XRD)	Basically consistent with the results of 0 month

### **Stability Study Conclusions, Proposed Storage Conditions and Re-test**

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### **Period**

The results of characters, related substances, loss on drying, assay, and hygroscopic weight gain of batch 240101 are qualified at condition of high temperature (60°C), high humidity (92.5% RH, 25°C) and photolysis (visible light:  $4500 \pm 500$  LX; near ultraviolet:  $90 \pm 5 \mu\text{W}/\text{cm}^2$ , 25°C) in 30 days. The Finerenone drug substance has a good stability under high temperature, high humidity and photolysis.

Currently 6 months of three validation batches (batch No. 231201、240101 and 240102) has been completed. All data presented at accelerated condition  $40^\circ\text{C} \pm 2^\circ\text{C} / 75\% \text{RH} \pm 5\% \text{RH}$  (6 months) and long-term condition  $30^\circ\text{C} \pm 2^\circ\text{C} / 65\% \text{RH} \pm 5\% \text{RH}$  (6 months) met the acceptance criteria, and there is no significant change.

There is no significant change at accelerated condition within 6 months. The data of long-term stability already conducted for 6 months met the acceptance criteria. No change trends are observed. The applicant request a retest period of 24 months for Finerenone packed in double-layered pharmaceutical grade low-density polyethylene (LDPE) bag. The storage condition is sealed storage.

### **3.2.S.7.2 Post-approval Stability Protocol and Stability Commitment**

We, Hinye Pharmaceutical Co., Ltd., hereby make the commitment that the long-term stability studies on validation batches (batch No.: 231201, 240101 and 240102) will be continued throughout the protocol period of 4 years, which covers the proposed re-test period. All the stability data obtained on these batches will be supplied to authority.

### **Stability Protocol of Long-term Stability**

Storage condition:  $30^\circ\text{C} \pm 2^\circ\text{C} / 75\% \text{RH} \pm 5\% \text{RH}$ .

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Packaging: double-layered pharmaceutical grade low-density polyethylene (LDPE) bag.

Study period: 4 years for the validation batches (batch No.: 231201, 240101 and 240102).

Test points: 0, 3, 6, 9, 12, 18, 24, 36 and 48 months as needed.

Test items: Appearance, related substances, Genotoxic impurities, Water, microbial limits, assay, particle size distribution, crystal form. The acceptance criteria and test points for each item are listed in the table below.

Analytical method: the same as the procedures described in section 3.2.S.4.2

*Analytical Procedures.*

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Table 3.2.S.7.2-1 Stability Specification and Test Points of Post-approval Stability Study

<b>Storage condition</b>		30°C±2°C / 65%RH±5%RH								
<b>Packaging</b>		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag								
<b>Items</b>	<b>Specifications</b>	<b>Time points (months)</b>								
		<b>0</b>	<b>3</b>	<b>6</b>	<b>9</b>	<b>12</b>	<b>18</b>	<b>24</b>	<b>36</b>	<b>48</b>
Appearance	White to yellow powder	√	√	√	√	√	√	√	√	√
Related substances	YA2304-10 NMT 0.10%; YA2304-12 NMT 0.15%; YA2304-14 NMT 0.15%; YA2304-15 NMT 0.15%; YA2304-16 NMT 0.15%; YA2304-17 NMT 0.15%; YA2304-18 NMT 0.15%; YA2304-19 NMT 0.15%; Individual unspecified impurity NMT 0.10%; Total impurities NMT 1.0%	√	√	√	√	√	√	√	√	√
Enantiomers	YA2304-20 NMT 0.15%	√	√	√	√	√	√	√	√	√

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Storage condition		30°C±2°C / 65%RH±5%RH								
Packaging		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag								
Items	Specifications	Time points (months)								
		0	3	6	9	12	18	24	36	48
Genotoxic impurities	Dimethyl sulfate NMT 75ppm; Diethyl sulfate NMT 75ppm; Diisopropyl sulfate NMT 75ppm; Disec-butyl sulfate NMT 75ppm; The sum of genotoxic impurities NMT 250ppm	√	✗	√	✗	✗	✗	√	√	√
Water	NMT 0.5%	√	√	√	√	√	√	√	√	√
Microbial limits	TAMC: NMT 10 <sup>3</sup> CFU/g; TYMC: NMT 10 <sup>2</sup> CFU/g; Absence of <i>Escherichia coli</i> (1g)	√	✗	√	✗	√	✗	√	√	√
Assay	98.0% to 102.0% (Anhydrous substance).	√	√	√	√	√	√	√	√	√
Particle size	Basically consistent with the results of 0 month	√	✗	√	✗	√	✗	√	√	√
Crystal form(XRD)	Basically consistent with the results of 0 month	√	✗	√	✗	√	✗	√	√	√

Note: the cell marked with √ indicates that the item will be tested at the corresponding time point; while the cell marked with ✗ indicates that the item will be omitted.

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### **3.2.S.7.3 Stability Data**

The stability results for the three validation batches (batch No. 231201, 240101 and 240102, batch size: 10 kg) are detailed below.

- long term stability studies: 3.2.S.7.3 *Table 1, Table 2, Table 3.*
- accelerated stability studies: 3.2.S.7.3 *Table 4, Table 5, Table 6.*

Stress testing was carried out on one batch of Finerenone (batch No. 240101). It included the effect of high temperature (60°C), high humidity (92.5%RH, 25°C) and illumination (visible light: 4500 ± 500 LX; near ultraviolet: 90±5 µW/cm<sup>2</sup>, 25°C).

- high temperature: 3.2.S.7.3 *Table 7;*
- high humidity: 3.2.S.7.3 *Table 8;*
- photostability: 3.2.S.7.3 *Table 9.*

Please note:

- “Not Detected” is abbreviated as ‘ND’;
- “NMT” is abbreviated as ‘NMT’.
- “Not Test” is abbreviated as ‘--’.

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### Long term stability studies

Table 3.2.S.7.3-1 Long-term Stability Data of Finerenone (Batch No. 231201)

<b>Batch No.</b>		231201		<b>Batch size</b>		10 kg					
<b>Primary package</b>		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag		<b>Storage condition</b>		30°C±2°C / 65%RH±5%RH					
<b>Manufacturing date</b>		Jan. 06, 2024		<b>Stability starting date</b>		Feb. 04, 2024					
<b>Manufacturing site</b>		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.									
<b>Items</b>	<b>Specifications</b>	<b>Time points (Months)</b>									
		0	3	6	9	12	18	24	36	48	
Appearance	White to yellow powder.	white powder	white powder	white powder							
Related substances	YA2304-10 NMT 0.10%;	ND	ND	ND							
	YA2304-12 NMT 0.15%;	ND	ND	ND							
	YA2304-14 NMT 0.15%;	ND	ND	ND							
	YA2304-15 NMT 0.15%;	ND	<0.05%	ND							
	YA2304-16 NMT 0.15%;	ND	ND	ND							
	YA2304-17 NMT 0.15%;	ND	ND	ND							
	YA2304-18 NMT 0.15%;	ND	ND	ND							
	YA2304-19 NMT 0.15%;	<0.05%	<0.05%	<0.05%							
	Other single impurities: NMT 0.10%	<0.05%	<0.05%	<0.05%							
	Total impurity: NMT 1.0%	<0.05%	<0.05%	<0.05%							

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<b>Batch No.</b>		231201			<b>Batch size</b>	10 kg				
<b>Primary package</b>		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag			<b>Storage condition</b>	30°C±2°C / 65%RH±5%RH				
<b>Manufacturing date</b>		Jan. 06, 2024			<b>Stability starting date</b>	Feb. 04, 2024				
<b>Manufacturing site</b>		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.								
<b>Items</b>	<b>Specifications</b>	<b>Time points (Months)</b>								
		<b>0</b>	<b>3</b>	<b>6</b>	<b>9</b>	<b>12</b>	<b>18</b>	<b>24</b>	<b>36</b>	<b>48</b>
enantiomers	YA2304-20: NMT 0.15%	0.03%	0.03%	0.04%						
genotoxic impurities	Dimethyl sulfate NMT 75ppm	ND	/	ND						
	Diethyl sulfate NMT 75ppm	ND	/	ND						
	Diisopropyl sulfate NMT 75 ppm	ND	/	ND						
	Disec-butyl sulfate NMT 75 ppm	ND	/	ND						
	Sum of genotoxic impurities NMT 250ppm	ND	/	ND						
Water	NMT 0.5%	0.05%	0.1%	0.05%						
Microbial limits	TAMC: NMT 10 <sup>3</sup> CFU/g	Complies	/	Complies						
	TYMC: NMT 10 <sup>2</sup> CFU/g	Complies	/	Complies						
	Absence of Escherichia coli (1g)	Complies	/	Complies						
Assay	98.0% to 102.0% (anhydrous substance).	99.8%	100.2%	100.7%						

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<b>Batch No.</b>		231201		<b>Batch size</b>	10 kg						
<b>Primary package</b>		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag			<b>Storage condition</b>		30°C±2°C / 65%RH±5%RH				
<b>Manufacturing date</b>		Jan. 06, 2024			<b>Stability starting date</b>		Feb. 04, 2024				
<b>Manufacturing site</b>		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.									
<b>Items</b>	<b>Specifications</b>	<b>Time points (Months)</b>									
		<b>0</b>	<b>3</b>	<b>6</b>	<b>9</b>	<b>12</b>	<b>18</b>	<b>24</b>	<b>36</b>	<b>48</b>	
Crystal form XRD	Basically consistent with the results of 0 month	Complies	/	Complies							
Particle size	Basically consistent with the results of 0 month	D90: 7.712μm	/	D90: 6.163μm							

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Table 3.2.S.7.3-2 Long-term Stability Data of Finerenone (Batch No. 240101)

<b>Batch No.</b>		240101		<b>Batch size</b>	10 kg					
<b>Primary package</b>		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag		<b>Storage condition</b>	30°C±2°C / 65%RH±5%RH					
<b>Manufacturing date</b>		Jan. 19, 2024		<b>Stability starting date</b>	Feb. 04, 2024					
<b>Manufacturing site</b>		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.								
<b>Items</b>	<b>Specifications</b>	<b>Time points (Months)</b>								
		0	3	6	9	12	18	24	36	48
Appearance	White to yellow powder.	white powder	white powder	white powder						
Related substances	YA2304-10 NMT 0.10%;	ND	ND	ND						
	YA2304-12 NMT 0.15%;	ND	ND	ND						
	YA2304-14 NMT 0.15%;	ND	ND	ND						
	YA2304-15 NMT 0.15%;	ND	ND	ND						
	YA2304-16 NMT 0.15%;	ND	ND	ND						
	YA2304-17 NMT 0.15%;	ND	ND	ND						
	YA2304-18 NMT 0.15%;	ND	ND	ND						
	YA2304-19 NMT 0.15%;	<0.05%	<0.05%	<0.05%						
	Other single impurities: NMT 0.10%	<0.05%	<0.05%	<0.05%						
Total impurity: NMT 1.0%	<0.05%	<0.05%	<0.05%							
enantiomers	YA2304-20: NMT 0.15%	0.03%	0.04%	0.04%						

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Batch No.		240101			Batch size		10 kg										
Primary package		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag			Storage condition		30°C±2°C / 65%RH±5%RH										
Manufacturing date		Jan. 19, 2024			Stability starting date		Feb. 04, 2024										
Manufacturing site		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.															
Items	Specifications	Time points (Months)															
		0	3	6	9	12	18	24	36	48							
genotoxic impurities	Dimethyl sulfate NMT 75ppm	ND	/	ND													
	Diethyl sulfate NMT 75ppm	ND	/	ND													
	Diisopropyl sulfate NMT 75 ppm	ND	/	ND													
	Disec-butyl sulfate NMT 75 ppm	ND	/	ND													
	Sum of genotoxic impurities NMT 250ppm	ND	/	ND													
Water	NMT 0.5%	0.04%	0.1%	0.04%													
Microbial limits	TAMC: NMT 10 <sup>3</sup> CFU/g	Complies	/	Complies													
	TYMC: NMT 10 <sup>2</sup> CFU/g	Complies	/	Complies													
	Absence of Escherichia coli (1g)	Complies	/	Complies													
Assay	98.0% to 102.0% (anhydrous substance).	99.8%	100.5%	99.5%													
Crystal form XRD	Basically consistent with the results of 0 month	Complies	/	Complies													

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<b>Batch No.</b>		240101		<b>Batch size</b>		10 kg											
<b>Primary package</b>		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag				<b>Storage condition</b>		30°C±2°C / 65%RH±5%RH									
<b>Manufacturing date</b>		Jan. 19, 2024		<b>Stability starting date</b>		Feb. 04, 2024											
<b>Manufacturing site</b>		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.															
<b>Items</b>	<b>Specifications</b>	<b>Time points (Months)</b>															
		0	3	6	9	12	18	24	36	48							
Particle size	Basically consistent with the results of 0 month	D90: 7.833μm	/	D90: 7.500μm													

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Table 3.2.S.7.3-3 Long-term Stability Data of Finerenone (Batch No. 240102)

Batch No.		240102		Batch size		10 kg					
Primary package		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag		Storage condition		30°C±2°C / 65%RH±5%RH					
Manufacturing date		Jan. 30, 2024		Stability starting date		Feb. 04, 2024					
Manufacturing site		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.									
Items	Specifications	Time points (Months)									
		0	3	6	9	12	18	24	36	48	
Appearance	White to yellow powder.	white powder	white powder	white powder							
Related substances	YA2304-10 NMT 0.10%;	ND	ND	ND							
	YA2304-12 NMT 0.15%;	ND	ND	ND							
	YA2304-14 NMT 0.15%;	ND	ND	ND							
	YA2304-15 NMT 0.15%;	<0.05%	<0.05%	<0.05%							
	YA2304-16 NMT 0.15%;	ND	ND	ND							
	YA2304-17 NMT 0.15%;	ND	ND	ND							
	YA2304-18 NMT 0.15%;	ND	ND	ND							
	YA2304-19 NMT 0.15%;	<0.05%	<0.05%	<0.05%							
	Other single impurities: NMT 0.10%	<0.05%	<0.05%	<0.05%							
Total impurity: NMT 1.0%	<0.05%	<0.05%	<0.05%								
enantiomers	YA2304-20: NMT 0.15%	0.02%	0.01%	0.02%							

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Batch No.		240102			Batch size		10 kg										
Primary package		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag			Storage condition		30°C±2°C / 65%RH±5%RH										
Manufacturing date		Jan. 30, 2024			Stability starting date		Feb. 04, 2024										
Manufacturing site		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.															
Items	Specifications	Time points (Months)															
		0	3	6	9	12	18	24	36	48							
genotoxic impurities	Dimethyl sulfate NMT 75ppm	ND	/	ND													
	Diethyl sulfate NMT 75ppm	ND	/	ND													
	Diisopropyl sulfate NMT 75 ppm	ND	/	ND													
	Disec-butyl sulfate NMT 75 ppm	ND	/	ND													
	Sum of genotoxic impurities NMT 250ppm	ND	/	ND													
Water	NMT 0.5%	0.2%	0.1%	0.1%													
Microbial limits	TAMC: NMT 10 <sup>3</sup> CFU/g	Complies	/	Complies													
	TYMC: NMT 10 <sup>2</sup> CFU/g	Complies	/	Complies													
	Absence of Escherichia coli (1g)	Complies	/	Complies													
Assay	98.0% to 102.0% (anhydrous substance).	99.7%	100.7%	99.5%													
Crystal form XRD	Basically consistent with the results of 0 month	Complies	/	Complies													

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<b>Batch No.</b>		240102		<b>Batch size</b>		10 kg											
<b>Primary package</b>		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag				<b>Storage condition</b>		30°C±2°C / 65%RH±5%RH									
<b>Manufacturing date</b>		Jan. 30, 2024		<b>Stability starting date</b>		Feb. 04, 2024											
<b>Manufacturing site</b>		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.															
<b>Items</b>	<b>Specifications</b>	<b>Time points (Months)</b>															
		0	3	6	9	12	18	24	36	48							
Particle size	Basically consistent with the results of 0 month	D90: 8.027μm	/	D90: 7.559μm													

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### Accelerated stability studies

Table 3.2.S.7.3-4 Accelerated Stability Data of Finerenone (Batch No. 231201)

<b>Batch No.</b>		231201		<b>Batch size</b>	10 kg									
<b>Primary package</b>		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag		<b>Storage condition</b>	40°C±2°C / 75%RH±5%RH									
<b>Manufacturing date</b>		Jan. 06, 2024		<b>Stability starting date</b>	Feb. 04, 2024									
<b>Manufacturing site</b>		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.												
<b>Items</b>	<b>Specifications</b>	<b>Time points (Months)</b>												
		<b>0</b>	<b>3</b>	<b>6</b>	<b>9</b>	<b>12</b>	<b>18</b>	<b>24</b>	<b>36</b>	<b>48</b>				
Appearance	White to yellow powder.	white powder	white powder	white powder										
Related substances	YA2304-10 NMT 0.10%;	ND	ND	ND										
	YA2304-12 NMT 0.15%;	ND	ND	ND										
	YA2304-14 NMT 0.15%;	ND	ND	ND										
	YA2304-15 NMT 0.15%;	ND	ND	ND										
	YA2304-16 NMT 0.15%;	ND	ND	ND										
	YA2304-17 NMT 0.15%;	ND	ND	ND										
	YA2304-18 NMT 0.15%;	ND	ND	ND										
	YA2304-19 NMT 0.15%;	<0.05%	<0.05%	<0.05%										
	Other single impurities: NMT 0.10%	<0.05%	<0.05%	<0.05%										
	Total impurity: NMT 1.0%	<0.05%	<0.05%	<0.05%										

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## Drug Master File of Finerenone

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<b>Batch No.</b>		231201			<b>Batch size</b>	10 kg									
<b>Primary package</b>		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag			<b>Storage condition</b>	40°C±2°C / 75%RH±5%RH									
<b>Manufacturing date</b>		Jan. 06, 2024			<b>Stability starting date</b>	Feb. 04, 2024									
<b>Manufacturing site</b>		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.													
<b>Items</b>	<b>Specifications</b>	<b>Time points (Months)</b>													
		<b>0</b>	<b>3</b>	<b>6</b>	<b>9</b>	<b>12</b>	<b>18</b>	<b>24</b>	<b>36</b>	<b>48</b>					
enantiomers	YA2304-20: NMT 0.15%	0.03%	0.03%	0.04%											
genotoxic impurities	Dimethyl sulfate NMT 75ppm	ND	/	ND											
	Diethyl sulfate NMT 75ppm	ND	/	ND											
	Diisopropyl sulfate NMT 75 ppm	ND	/	ND											
	Disec-butyl sulfate NMT 75 ppm	ND	/	ND											
	Sum of genotoxic impurities NMT 250ppm	ND	/	ND											
Water	NMT 0.5%	0.05%	0.1%	0.04%											
Microbial limits	TAMC: NMT 10 <sup>3</sup> CFU/g	Complies	/	Complies											
	TYMC: NMT 10 <sup>2</sup> CFU/g	Complies	/	Complies											
	Absence of Escherichia coli (1g)	Complies	/	Complies											
Assay	98.0% to 102.0% (anhydrous substance).	99.8%	100.8%	99.8%											

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## Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

<b>Batch No.</b>		231201		<b>Batch size</b>	10 kg						
<b>Primary package</b>		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag			<b>Storage condition</b>		40°C±2°C / 75%RH±5%RH				
<b>Manufacturing date</b>		Jan. 06, 2024			<b>Stability starting date</b>		Feb. 04, 2024				
<b>Manufacturing site</b>		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.									
<b>Items</b>	<b>Specifications</b>	<b>Time points (Months)</b>									
		<b>0</b>	<b>3</b>	<b>6</b>	<b>9</b>	<b>12</b>	<b>18</b>	<b>24</b>	<b>36</b>	<b>48</b>	
Crystal form XRD	Basically consistent with the results of 0 month	Complies	/	Complies							
Particle size	Basically consistent with the results of 0 month	D90: 7.712μm	/	D90: 6.235μm							

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## Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

Table 3.2.S.7.3-5 Accelerated Stability Data of Finerenone (Batch No. 240101)

Batch No.		240101		Batch size		10 kg					
Primary package		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag		Storage condition		40°C±2°C / 75%RH±5%RH					
Manufacturing date		Jan. 19, 2024		Stability starting date		Feb. 04, 2024					
Manufacturing site		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.									
Items	Specifications	Time points (Months)									
		0	3	6	9	12	18	24	36	48	
Appearance	White to yellow powder.	white powder	white powder	white powder							
Related substances	YA2304-10 NMT 0.10%;	ND	ND	ND							
	YA2304-12 NMT 0.15%;	ND	ND	ND							
	YA2304-14 NMT 0.15%;	ND	ND	ND							
	YA2304-15 NMT 0.15%;	ND	ND	ND							
	YA2304-16 NMT 0.15%;	ND	ND	ND							
	YA2304-17 NMT 0.15%;	ND	ND	ND							
	YA2304-18 NMT 0.15%;	ND	ND	ND							
	YA2304-19 NMT 0.15%;	<0.05%	<0.05%	<0.05%							
	Other single impurities: NMT 0.10%	<0.05%	<0.05%	<0.05%							
enantiomers	Total impurity: NMT 1.0%	<0.05%	<0.05%	<0.05%							
	YA2304-20: NMT 0.15%	0.03%	0.03%	0.05%							

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Date: Sep. 2024

Batch No.		240101			Batch size		10 kg										
Primary package		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag			Storage condition		40°C±2°C / 75%RH±5%RH										
Manufacturing date		Jan. 19, 2024			Stability starting date		Feb. 04, 2024										
Manufacturing site		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.															
Items	Specifications	Time points (Months)															
		0	3	6	9	12	18	24	36	48							
genotoxic impurities	Dimethyl sulfate NMT 75ppm	ND	/	ND													
	Diethyl sulfate NMT 75ppm	ND	/	ND													
	Diisopropyl sulfate NMT 75 ppm	ND	/	ND													
	Disec-butyl sulfate NMT 75 ppm	ND	/	ND													
	Sum of genotoxic impurities NMT 250ppm	ND	/	ND													
Water	NMT 0.5%	0.05%	0.1%	0.04%													
Microbial limits	TAMC: NMT 10 <sup>3</sup> CFU/g	Complies	/	Complies													
	TYMC: NMT 10 <sup>2</sup> CFU/g	Complies	/	Complies													
	Absence of Escherichia coli (1g)	Complies	/	Complies													
Assay	98.0% to 102.0% (anhydrous substance).	99.8%	100.6%	99.6%													
Crystal form XRD	Basically consistent with the results of 0 month	Complies	/	Complies													

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<b>Batch No.</b>		240101		<b>Batch size</b>		10 kg											
<b>Primary package</b>		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag				<b>Storage condition</b>		40°C±2°C / 75%RH±5%RH									
<b>Manufacturing date</b>		Jan. 19, 2024		<b>Stability starting date</b>		Feb. 04, 2024											
<b>Manufacturing site</b>		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.															
<b>Items</b>	<b>Specifications</b>	<b>Time points (Months)</b>															
		0	3	6	9	12	18	24	36	48							
Particle size	Basically consistent with the results of 0 month	D90: 7.833μm	/	D90: 7.227μm													

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Date: Sep. 2024

Table 3.2.S.7.3-6 Accelerated Stability Data of Finerenone (Batch No. 240102)

Batch No.		240102		Batch size		10 kg					
Primary package		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag		Storage condition		40°C±2°C / 75%RH±5%RH					
Manufacturing date		Jan. 30, 2024		Stability starting date		Feb. 04, 2024					
Manufacturing site		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.									
Items	Specifications	Time points (Months)									
		0	3	6	9	12	18	24	36	48	
Appearance	White to yellow powder.	white powder	white powder	white powder							
Related substances	YA2304-10 NMT 0.10%;	ND	ND	ND							
	YA2304-12 NMT 0.15%;	ND	ND	ND							
	YA2304-14 NMT 0.15%;	ND	ND	ND							
	YA2304-15 NMT 0.15%;	<0.05%	<0.05%	<0.05%							
	YA2304-16 NMT 0.15%;	ND	ND	ND							
	YA2304-17 NMT 0.15%;	ND	ND	ND							
	YA2304-18 NMT 0.15%;	ND	ND	ND							
	YA2304-19 NMT 0.15%;	<0.05%	<0.05%	<0.05%							
	Other single impurities: NMT 0.10%	<0.05%	<0.05%	<0.05%							
enantiomers	Total impurity: NMT 1.0%	<0.05%	<0.05%	<0.05%							
	YA2304-20: NMT 0.15%	0.02%	0.01%	0.02%							

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Version: 001

Date: Sep. 2024

Batch No.		240102			Batch size		10 kg										
Primary package		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag			Storage condition		40°C±2°C / 75%RH±5%RH										
Manufacturing date		Jan. 30, 2024			Stability starting date		Feb. 04, 2024										
Manufacturing site		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.															
Items	Specifications	Time points (Months)															
		0	3	6	9	12	18	24	36	48							
genotoxic impurities	Dimethyl sulfate NMT 75ppm	ND	/	ND													
	Diethyl sulfate NMT 75ppm	ND	/	ND													
	Diisopropyl sulfate NMT 75 ppm	ND	/	ND													
	Disec-butyl sulfate NMT 75 ppm	ND	/	ND													
	Sum of genotoxic impurities NMT 250ppm	ND	/	ND													
Water	NMT 0.5%	0.2%	0.1%	0.05%													
Microbial limits	TAMC: NMT 10 <sup>3</sup> CFU/g	Complies	/	Complies													
	TYMC: NMT 10 <sup>2</sup> CFU/g	Complies	/	Complies													
	Absence of Escherichia coli (1g)	Complies	/	Complies													
Assay	98.0% to 102.0% (anhydrous substance).	99.7%	100.7%	101.1%													
Crystal form XRD	Basically consistent with the results of 0 month	Complies	/	Complies													

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<b>Batch No.</b>		240102		<b>Batch size</b>		10 kg											
<b>Primary package</b>		double-layered pharmaceutical grade low-density polyethylene (LDPE) bag				<b>Storage condition</b>		40°C±2°C / 75%RH±5%RH									
<b>Manufacturing date</b>		Jan. 30, 2024		<b>Stability starting date</b>		Feb. 04, 2024											
<b>Manufacturing site</b>		API (II) Workshop, Hinye Pharmaceutical Co., Ltd.															
<b>Items</b>	<b>Specifications</b>	<b>Time points (Months)</b>															
		0	3	6	9	12	18	24	36	48							
Particle size	Basically consistent with the results of 0 month	D90: 8.027μm	/	D90: 7.246μm													

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Version: 001

Date: Sep. 2024

### Stress testing

Table 3.2.S.7.3-7 High Temperature Stress Test Data of Finerenone (Batch No. 231201)

<b>Batch No.</b>		240101	<b>Batch size</b>		10 kg		
<b>Manufacturing date</b>		Jan. 06, 2024	<b>Stability starting date</b>		Feb. 26, 2024		
<b>Degradation condition</b>		High temperature 60°C: place appropriate amount of substance without package into a container evenly, the thickness of substance is 3 ~ 5 mm. Store the culture dish into constant temperature oven at 60°C.					
<b>Items</b>	<b>Specifications</b>	<b>Time points</b>					
		<b>0d</b>	<b>5d</b>	<b>15d</b>	<b>30d</b>		
Appearance	White to yellow powder.	White powder	Off White powder	Yellowish powder	Yellowish powder		
Related substances	YA2304-10 NMT 0.10%;	ND	ND	ND	ND		
	YA2304-12 NMT 0.15%;	ND	<0.05%	<0.05%	<0.05%		
	YA2304-14 NMT 0.15%;	ND	<0.05%	<0.05%	<0.05%		
	YA2304-15 NMT 0.15%;	ND	ND	ND	ND		
	YA2304-16 NMT 0.15%;	ND	ND	ND	0.05%		
	YA2304-17 NMT 0.15%;	ND	ND	ND	ND		
	YA2304-18 NMT 0.15%;	ND	ND	ND	<0.05%		
	YA2304-19 NMT 0.15%;	<0.05%	<0.05%	0.07%	0.11%		
	Other single impurities: NMT 0.10%	<0.05%	<0.05%	<0.05%	0.06%		

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Date: Sep. 2024

<b>Batch No.</b>	240101	<b>Batch size</b>	10 kg					
<b>Manufacturing date</b>	Jan. 06, 2024	<b>Stability starting date</b>	Feb. 26, 2024					
<b>Degradation condition</b>	High temperature 60°C: place appropriate amount of substance without package into a container evenly, the thickness of substance is 3 ~ 5 mm. Store the culture dish into constant temperature oven at 60°C.							
<b>Items</b>	<b>Specifications</b>	<b>Time points</b>						
		<b>0d</b>	<b>5d</b>	<b>15d</b>	<b>30d</b>			
	Total impurity: NMT 1.0%	<0.05%	<0.05%	0.11%	0.22%			
Enantiomers	YA2304-20: NMT 0.15%	0.03%	ND	ND	ND			
Water	NMT 0.5%	0.05%	0.03%	0.05%	0.06%			
Assay	98.0% to 102.0% (anhydrous substance).	99.8%	100.2%	99.9%	99.9%			

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Version: 001

Date: Sep. 2024

Table 3.2.S.7.3-8 High Humidity Stress Test Data of Finerenone (Batch No. 231201)

<b>Batch No.</b>	231201	<b>Batch size</b>	10 kg		
<b>Manufacturing date</b>	Jan. 06, 2024	<b>Stability starting date</b>	Feb. 24, 2024		
<b>Degradation condition</b>	High humidity 92.5%RH: place appropriate amount of substance without package into a container evenly, the thickness of substance is 3 ~ 5 mm. Store the culture dish at condition 92.5%RH, 25°C.				
<b>Items</b>	<b>Specifications</b>	<b>Time points</b>			
		<b>0d</b>	<b>5d</b>	<b>15d</b>	<b>30d</b>
Appearance	White to yellow powder.	White powder	White powder	White powder	White powder
Related substances	YA2304-10 NMT 0.10%;	ND	ND	ND	ND
	YA2304-12 NMT 0.15%;	ND	<0.05%	<0.05%	<0.05%
	YA2304-14 NMT 0.15%;	ND	ND	<0.05%	ND
	YA2304-15 NMT 0.15%;	ND	<0.05%	<0.05%	<0.05%
	YA2304-16 NMT 0.15%;	ND	ND	ND	ND
	YA2304-17 NMT 0.15%;	ND	ND	ND	ND
	YA2304-18 NMT 0.15%;	ND	ND	ND	ND
	YA2304-19 NMT 0.15%;	<0.05%	<0.05%	<0.05%	<0.05%
	Other single impurities: NMT 0.10%	<0.05%	<0.05%	<0.05%	<0.05%
	Total impurity: NMT 1.0%	<0.05%	<0.05%	<0.05%	<0.05%

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Date: Sep. 2024

<b>Batch No.</b>	231201	<b>Batch size</b>	10 kg					
<b>Manufacturing date</b>	Jan. 06, 2024	<b>Stability starting date</b>	Feb. 24, 2024					
<b>Degradation condition</b>	High humidity 92.5%RH: place appropriate amount of substance without package into a container evenly, the thickness of substance is 3 ~ 5 mm. Store the culture dish at condition 92.5%RH, 25°C.							
<b>Items</b>	<b>Specifications</b>	<b>Time points</b>						
		<b>0d</b>	<b>5d</b>	<b>15d</b>	<b>30d</b>			
Enantiomers	YA2304-20: NMT 0.15%	0.03%	0.02%	0.02%	0.02%			
Water	NMT 0.5%	0.05%	0.03%	0.03%	0.03%			
Assay	98.0% to 102.0% (anhydrous substance).	99.8%	100.0%	100.3%	100.0%			
Hygroscopic gain (%)	NMT 5%	/	0.04%	0.02%	0.05%			

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Version: 001

Date: Sep. 2024

Table 3.2.S.7.3-9 Photostability Data of Finerenone (Batch No. 231201)

<b>Batch No.</b>		231201	<b>Batch size</b>	10 kg			
<b>Manufacturing date</b>		Jan. 04, 2024	<b>Stability starting date</b>	Feb. 24, 2024			
<b>Degradation condition</b>		Illumination (visible light: $4500 \pm 500$ LX; near ultraviolet: $90 \pm 5 \mu\text{W}/\text{cm}^2$ , $25^\circ\text{C}$ ): place appropriate amount of substance without package into a container evenly, the thickness of substance is 3 ~ 5 mm. Store the culture dish at condition: visible light: $4500 \pm 500$ LX; near ultraviolet: $90 \pm 5 \mu\text{W}/\text{cm}^2$ , $25^\circ\text{C}$ .					
<b>Items</b>	<b>Specifications</b>	<b>Time points</b>					
		<b>0d</b>	<b>5d</b>	<b>15d</b>	<b>30d</b>		
Appearance	White to yellow powder.	White powder	Off White powder	Yellowish powder	Yellowish powder		
Related substances	YA2304-10 NMT 0.10%;	ND	ND	ND	ND		
	YA2304-12 NMT 0.15%;	ND	<0.05%	<0.05%	<0.05%		
	YA2304-14 NMT 0.15%;	ND	<0.05%	<0.05%	<0.05%		
	YA2304-15 NMT 0.15%;	ND	<0.05%	<0.05%	<0.05%		
	YA2304-16 NMT 0.15%;	ND	<0.05%	<0.05%	<0.05%		
	YA2304-17 NMT 0.15%;	ND	ND	ND	ND		
	YA2304-18 NMT 0.15%;	ND	ND	ND	ND		
	YA2304-19 NMT 0.15%;	<0.05%	0.06%	0.14%	0.12%		
	Other single impurities: NMT 0.10%	<0.05%	0.08%	0.12%	0.11%		

## Drug Master File of Finerenone

Document: YA2304-Quality

Version: 001

Date: Sep. 2024

<b>Batch No.</b>	231201	<b>Batch size</b>	10 kg		
<b>Manufacturing date</b>	Jan. 04, 2024	<b>Stability starting date</b>	Feb. 24, 2024		
<b>Degradation condition</b>	Illumination (visible light: $4500 \pm 500$ LX; near ultraviolet: $90 \pm 5 \mu\text{W}/\text{cm}^2$ , $25^\circ\text{C}$ ): place appropriate amount of substance without package into a container evenly, the thickness of substance is 3 ~ 5 mm. Store the culture dish at condition: visible light: $4500 \pm 500$ LX; near ultraviolet: $90 \pm 5 \mu\text{W}/\text{cm}^2$ , $25^\circ\text{C}$ .				
<b>Items</b>	<b>Specifications</b>	<b>Time points</b>			
		<b>0d</b>	<b>5d</b>	<b>15d</b>	<b>30d</b>
	Total impurity: NMT 1.0%	<0.05%	0.14%	0.30%	0.22%
Enantiomers	YA2304-20: NMT 0.15%	0.03%	ND	ND	ND
Water	NMT 0.5%	0.05%	0.04%	0.07%	0.08%
Assay	98.0% to 102.0% (anhydrous substance).	99.8%	99.7%	99.1%	100.0%

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