3.2.S.4 Control of Drug Substance

3.2.S.4.1 Specification

Test		Specification	Methods
Appearance		White crystal or crystalline powder	DAB
Identi-fication	A.Optical Rotation,°	+26.5 ~ +29.0 °	Ph. Eur. 2.2.7
	B. IR	corresponds to standard	Ph. Eur.2.2.24
	C. Ninhydrin reaction	Violet	DAB
	D. Mercuric acetate reaction	White Precipitation	DAB
	E. Molybdophosphoric acidreaction	Yellow Precipitation	DAB
Purity	1) Clarity / Coloration	Clear, Colourless	Ph. Eur.2.2.1, 2.2.2(II)
	2) pH	6.0 ~ 7.0	Ph. Eur. 2.2.3
	3) Related substance (TLC)	Spots other than the main spots in the test solution are not larger than the spots obtained in the standard solution	Ph. Eur. 2.2.27
	4) Chloride	≤ 300 ppm	Ph. Eur.2.2.4
	5) Sulfate	≤ 200 ppm	Ph. Eur.2.4.13
	6) Ammonium	≤ 400 ppm	DAB N 2.4.1
	7) Iron	≤ 30 ppm	Ph. Eur.2.4.9
	8) Heavy metals	≤ 10 ppm	Ph. Eur.2.4.8
	9) Water	≤ 7.0 %	Ph. Eur.2.5.12
	10) Sulfated ash	≤ 0.2 %	Ph. Eur. 2.4.14
Assay		98.0 ~ 102.0 %	Ph. Eur. 2.2.20
Residual solvent(Methanol)		≤ 3,000 ppm	In-house

3.2.S.4.2 Analytical Procedures

1) Appearance: White crystal or crystalline powder

Take 1 g of sample in a watch glass placed on a white paper or a white paper.

Identification

2) Identification, B can be omitted when A, C, D, E was carried out and C,D,E can be omitted when A, B was carried out.

2.1) A. Specific rotation: +26.5 ~ +29.0 °

Dissolve 2.0 g of sample (as anhydrous) in 25 mL of 6 mol / L HCl solutionand measure the D line of the sodium (589.3 nm) at 20 ±0.5℃ and100 mm length. (Ph. Eur. 2.2.7)

2.2) B. IR:The IR Spectrum of sample corresponds to L-ornithine-L-Aspartate standard. Sample and Standard have the same absorption spectrum as Standard when measured according to the potassium bromide method (KBr) of the infrared spectral method. (Ph. Eur. 2.2.24)

2.3) C. Ninhydrin reaction: Violet

Add 0.5 mL of ninhydrin solution (2.5 mg / mL) to 2 mL of the solution (5 mg / mL) prepared by dissolving 0.1 g of the sample in 20 mL of water, heat in a water bath for 10 minutes, and check the reaction. (DAB)

2.4) D. Mercuric II acetate reaction: White Precipitation

To 0.5 mL of the solution (5 mg/mL) in which 0.1 g of the sample is dissolved in 20 mL of water, 0.5 mL of Mercuric II acetate solution (100 mg / mL) is added, and the reaction state is checked. (DAB)

2.5) E. Molybdophosphoric acid: Yellow Precipitation

Add 1 mL of a molybdophosphoric acid solution (50 mg/mL) to 2 mL of the solution (5 mg / mL) obtained by dissolving 0.1 g of the sample in 20 mL of water and confirm the reaction state. (DAB)

3) Purity

Test solution: Dissolve 2.5 g of sample in 100 mL ofwater

3.1) Clarity and coloration: Clear / Colourless

Using identical test-tubes of colourless, transparent, neutral glass with a flat base and an internal diameter of 15-25 mm, compare Test solution to be examined with a reference suspension freshly prepared as described below. Ensure that the depths of the layers in the 2 test-tubes are the same (about 40 mm). Compare the liquids in diffused daylight 5 min after preparation of the reference suspension, viewing vertically against a black background. Test solution is considered clear if its clarity is the same as that of water R or of the solvent used, or if its opalescence is not more pronounced than that of reference suspension I (Ph. Eur.2.2.1)

Using identical tubes of colourless, transparent, neutral glass with a flat base and an internal diameter of 15 mm to 25 mm, compare Test solution to be examined with water R or the solvent or the reference solution (see Tables of reference solutions) the depth of the layer being 40 mm. Compare the colours in diffused daylight viewing vertically against a white background. Test solution is colourless if it has the appearance of water R or the solvent or is not more intensely coloured than reference solution B9 (Ph. Eur. 2.2.2(II))

3.2) pH: 6.0 ~ 7.0

Weigh 2.5 g of the sample, dissolve in water to make 100 mL, and measure the pH. (Ph. Eur. 2.2.3)

3.3) Related substance: Spots other than the main spots in the test solution are not larger than the spots obtained in the standard solution

Precisely weighed 2.5 g of this solution into a 100mL volumetric flask, add 100 mL of water and dissolve. Use this solution as the sample solution. Take exactly 2 mL of the sample solution and dilute it with 100 mL of water. Use this solution as the standard solution. Dissolve 5 μL of the above sample solution and standard solution on a thin plate made of silica gel for thin layer chromatography according to European Pharmacopoeia Thin Layer Chromatography. Next, it is developed at about 10 cm using water: acetic acid (98%): 1-butanol = 25: 25: 50 as a developing solvent, and then dried at 110 ° C for 15 minutes. When the ninhydrin solution is evenly sprayed and dried at 110 ° C for 10 minutes.

* Ninhydrin solution: 0.2 g of ninhydrin is completely dissolved in 94 mL of n-butanol and 6 mL ofacetic acid. Shade to keep

3.4) Chloride: ≤ 300 ppm

▪Sample solution: Take 6.7 mL ofTest solution and dilute with 15 mL of water.

▪To 15 mL of the Sample solution add 1 mL of dilute nitric acid R and pour the mixture as a single addition into a test-tube containing 1 mL of silver nitrate solution R2. Prepare a standard in the same manner using 10 mL of chloride standard solution (5 ppm CI) R and 5 mL of water R. Examine the tubes laterally against a black background. After standing for 5 min protected from light, any opalescence in the Sample solution is not more intense than that in the standard. (Ph. Eur. 2.4.4)

3.5) Sulfate: ≤ 200 ppm

▪Sample solution: Weigh 0.75 g of the sample, dissolve in water to make 15 mL.

▪All solutions used for this test must be prepared with distilled water R. Add 3 mL of a 250 g/L solution of barium chloride R to 4.5 mL of sulfate standard solution (10 ppm SO4) R1. Shake and allow to stand for 1 min. To 2.5 mL of this suspension add 15 mL of the Sample solution and 0.5 mL of acetic acid R. Prepare a standard in the same manner using 15 mL of sulfate standard solution (10 ppm SO4) R instead of the prescribed solution. After 5 min, any opalescence in the test solution is not more intense than that in the standard. (Ph. Eur. 2.4.13)

3.6) Ammonium: ≤ 400 ppm

▪Sample solution

25 mg of this product is placed on a watch glass with a diameter of 60 mm and dissolved or suspended by adding 0.5 ml of water R. 0.30 g heavy magnesium oxide R is added to the solution or suspension.

▪Standard solution

In the same way, a reference mixture of 0.10 ml ammonium solution (100 ppm) R, 0.5 water R and 0.30 g heavy magnesium oxide R is prepared at the same time.

▪Immediately after mixing, a second watch glass with a diameter of 60 mm, on the inner surface of which a red litmus paper R moistened with a drop of water R had previously been attached, is placed edge by edge on the first watch glass. The test and reference mix are warmed to 40 ℃ for 15 minutes. The litmus paper over the test mixture must not turn blue more intensely than the litmus paper over the reference mixture. (DAB N 2.4.1)

3.7) Iron: ≤ 30 ppm

▪Sample solution

Dissolve 0.33 g of sample in 10 mL of dilute hydrochloric acid, add 10 mL of methyl isobutyl ketone each time, and extract it strongly (3 times) for 3 minutes. Separately, separate the methyl isobutyl ketone layer, add 10 mL of water, extrude strongly for 3 minutes, and use the aqueous layer as the Sample solution.

▪Add 2 mL of a 200 g/L solution of citric acid monohydrate R and 0.1 mL of thioglycollic acid R. Mix, make alkaline with ammonia R and dilute to 20mL with water R. Prepare a standard in the same manner, using 10 mL of iron standard solution (1 ppm Fe) R. After 5 min, any pink colour in the test solution is not more intense than that in the standard. (Ph. Eur. 2.4.9)

3.8) Heavy metals: ≤ 10 ppm

▪Test solution: Place the 2 g of sample to be examined in a silica crucible with 4 mL of a 250 g/L solution of magnesium sulfate R in dilute sulfuric acid R. Mix using a fine glass rod. Heat cautiously. If the mixture is liquid, evaporate gently to dryness on a water-bath. Progressively heat to ignition and continue heating until an almost white or at most greyish residue is obtained. Carry out the ignition at a temperature not exceeding 800 °C. Allow to cool. Moisten the residue with a few drops of dilute sulfuric acid R. Evaporate, ignite again and allow to cool. The total period of ignition must not exceed 2 h. Take up the residue in 2 quantities, each of 5 mL, of dilute hydrochloric acid R. Add 0.1 mL of phenolphthalein solution R, then concentrated ammonia R until a pink colour is obtained. Cool, add glacial acetic acid R until the solution is decolorised and add 0.5 mL in excess. Filter if necessary and wash the filter. Dilute to 20 mL with water R.

▪Reference solution (standard).

Prepare as described for the Test solution, using the 2 mL of lead standard solution (10 ppm Pb) R instead of the substance to be examined. To 10 mL of the solution obtained add 2 mL of the Test solution.

▪Monitor solution. Prepare as described for the Test solution, adding to the substance to be examined the volume of lead standard solution (10 ppm Pb) R prescribed for preparation of the reference solution. To 10 mL of the solution obtained add 2 mL of the test solution.

▪Blank solution.

A mixture of 10 mL of water R and 2 mL of the test solution,

▪To 12 mL of each solution, add 2 mL of buffer solution pH 3.5 R. Mix and add to 1.2 mL of thioacetamide reagent R. Mix immediately. Examine the solutions after 2 min.

▪System suitability:

The reference solution shows a slight brown colour compared to the blank solution, the monitor solution is at least as intense as the reference.

▪Result: any brown colour in the test solution is not more intense than that in the reference solution. If the result is difficult to judge, filter the solutions through a suitable membrane filter (nominal pore size 0.45 um). Carry out the filtration slowly and uniformly, applying moderate and constant pressure to the piston. Compare the spots on the filters obtained with the different solutions. (Ph. Eur. 2.4.8)

3.9) Water: ≤ 7.0 %

Take 0.200g sample and test with Karl Fischer method. (Ph. Eur. 2.5.12) The sample is dissolved in 10mL of formamide at 50° C, the solution is mixed with 20mL of methanol(anhydrous) and cooled to room temperature. Perform a blank test.

3.10) Sulfated ash: ≤ 0.2 %

Ignite a suitable crucible (for example, silica, platinum. porcelain or quartz) at 600 ± 50 ℃ for 30 min, allow to cool in a desiccator over silica gel or other suitable desiccant an weigh. Place the 2.0 g of sample to be examined in the crucible and weigh. Moisten the substance be examined with a small amount of sulfuric acid R (usually 1 mL) and heat gently at as low a temperature as until the sample is thoroughly charred. After cooling, moisten the residue with a small amount of sulfuric acid R (usually 1 mL), heat gently until white fumes are no longer evolved and ignite at 600 + 50 ℃ until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Allow the crucible to cool in a desiccator over silica gel or other suitable desiccant, weigh it again and calculate the percentage of residue. If the amount of the residue so obtained exceeds the prescribed limit, repeat the moistening with sulfuric acid R and ignition, as previously, for 30min periods until 2 consecutive weighings do not differ by more than 0.5 mg or until the percentage of residue complies with the prescribed limit. (Ph. Eur. 2.4.14)

4) Assay (Anhydrous): 98.0 ~ 102.0 %

Precisely weigh 70 mg of the sample, dissolve in 5 mL of formic acid, add 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid. (Potentiometric titration)0.1 mol/L Perchloric acid 1 mL = 8.84 mg C9H19N3O6

5) Residual solvent (MeOH): ≤ 3,000 ppm（0.3%）

Test solution

Take approximately 0.2g of this product, weigh it accurately, place it in a top empty bottle, add 2ml of water precisely to dissolve, and seal.

Reference solution

Take an appropriate amount of methanol, accurately weigh it, and dilute it with water to make a solution containing 75 μ g of methanol per 1ml. Precisely measure 2ml and place it in a top empty bottle, seal it.

Chromatographic conditions

A capillary column with 6% cyanopropylphenyl-94% dimethylpolysiloxane (or similar polarity) as the stationary liquid is used as the chromatographic column; Start at 35 ℃ and maintain for 20 minutes; The inlet temperature is 150 ℃; The detector temperature is 250 ℃; The equilibrium temperature of the headspace bottle is 80 ℃, and the equilibrium time is 40 minutes.

Measurement method

Accurately measure the test solution and the reference solution, inject them into the headspace separately, and record the chromatogram.

Limit

According to the external standard method and peak area calculation, the residual amount of methanol should be ≤ 3000ppm（0.3%）。

▪Operation

Gas chromatography		Condition
Detector		Flame ionization(FID)
Column		DB-624 Capillary(G43), 0.25 mm x 30 m, 1.4μm
Temp.	Detector	250 ℃
	Column temperature	Start at 35 ℃ and maintain for 20 minutes
	inlet	150 ℃
Carrier gas		nitrogen
Split Ratio		20 : 1
Flows		1) Hydrogen : 40 mL/min, 2) Air : 400.0 mL/min

Head-space	Condition
Sample equilibration temperature	80 ℃
Quantitative loop temperature	100 ℃
Transmission line temperature	110 ℃
Equilibrating Time	40 min
Injection Time	1 min(mL)

▪Calculation

$$\text{Residue of MeOH (ppm)} = \frac{At \times Ws \times 1 \times 10^6}{As \times 1000 \times Wt} \times \frac{Ps}{100}$$

At：Peak area of residual solvent in the sample solution

As : Peak area of residual solvent in standard solution

1: Dilution factor of sample

1000 : Dilution of standard solution

Wt : Weight of Sample (mg)

Ws : Weight of Standard (mg)

Ps : Purity of Standard

3.2.S.4.3 Validation of Analytical Procedures

1) 1) All tests on the product are in accordance with the German Pharmacopoeia and Methods Validation is omitted.

2) Verification of GC method for residual solvent (Methonol)

Test method: 3.2.S.4.2

Test Item	Criteria	Validation results
System suitability test	The peak area measurement value RSD% (n=6) of repeated injection shall not exceed 10.0%; the number of theoretical plates (N) ≥ 5000	Peak area repeatability RSD of 0.9% The minimum number of theoretical plates is 17842
Specificity	The blank solvent has no interference with methanol detection, and no other impurity peaks in the test solution interfere with each known impurity peak.	No interference
Detection of Quantitation	S/N is about 10, which can meet the testing requirements; Take the limit of quantification solution for 6 consecutive injections, and calculate the RSD of the peak area ≤ 10.0% and the RSD of the retention time ≤ 10.0%.	The limit of quantification is 1.510μg/ml, which is equivalent to the percentage content of the test product of 0.0015% The RSD for the limit of quantification precision was 2.6% RSD for retention time is 0%
Detection of Limit	S/N is about 3, which can meet the testing requirements	The detection limit is 0.453μg/ml, which is equivalent to 0.00045% of the test sample
Linearity and range	R≤0.990; The Y-axis intercept is within 25% of the 100% response value; Response factor RSD≤10%	Linear equation: y =665.4x + 0.4822 R is 1 The percentage of Y-intercept to 100% response value is 0.91% Linear range 0.00150~0.3765mg/ml Response factor RSD of 2.1%
Accuracy test	According to the external standard method, the detected amount and recovery rate of each impurity were calculated. The average recovery rate is between 80% and 120%, and the relative standard deviation should not exceed 10.0%	50% recovery was 108%, RSD (n=3) of 0% 100% recovery was 108%, RSD (n=3) of 0.6% 150% recovery was 108%, RSD (n=3) of 0.6% The average recovery was 108%, and the RSD (n=9) was 0.5%
Precision	Repeatability: The RSD of the peak area for 6 consecutive injections of the reference solution is ≤10.0%; the RSD of the	Repeatability: The RSD of the reference solution is 0.6% The RSD of the test solution is 1.0%

	impurity content of the 6 samples of the test solution is ≤10.0%. Intermediate precision: Impurity content of the test solution RSD% (n=6) ≤ 10.0%, RSD% (n=12) ≤ 15.0%	Intermediate precision: The RSD of the test solution (n=6) is 0.7% The RSD of the test solution (n=12) is 2.0%
Durability	Under each condition, the RD of methanol content in the solution of the spiked test sample shall not exceed 15.0%)	When there is a slight change in the measurement conditions, the theoretical plate number (N) of the reference substance is more than 5000, and the RD is less than 10.0%, all of which meet the requirements, and the measurement results are within the acceptable range. Including: different flow rates (2.8~3.2ml/min), different detector temperatures (245~255℃), different inlet temperatures (145~155℃), different headspace equilibration times (35~45min), Different headspace temperature (75 ~ 85 ℃).

3.2.S.4.4 Batch Analysis

1) Batch Tested

3 consecutive batches were adopted to analyze batches and tested items are conformed. We report the data related to three batches as follows.

	Batch Number	Batch Size	Manufacture Date	Analysis Date
PV1	C552202010	400.84 kg	2022.02.08	2022.02.10~2022.02.20
PV2	C552202011	400.84 kg	2022.02.09	2022.02.10~2022.02.20
PV3	C552202012	400.84 kg	2022.02.09	2022.02.11~2022.02.20

All three production batches produce were in Jingjing Pharmaceutical Co., Ltd. And conformity with the specification.

2) Result of Test

All three production batches studied were in conformity with the specification.

Batch number			C552202010	C552202011	C552202012
Manufacturing date			2022.02.08	2022.02.09	2022.02.09
Batch quantity			400.84kg	400.84kg	400.84kg
Testing Item		Standad	Result	Result	Result
Description		White crystal or crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder
pH		6.0 ~ 7.0	6.3	6.3	6.3
Identification	IR	should be consistent with the standard infrared spectrum	Consistent with standard infrared spectrum	Consistent with standard infrared spectrum	Consistent with standard infrared spectrum
	Optical Rotation,°	+26.5 ~ +29.0	28.0	28.3	28.3
Clarity of the solution		≤No. 1 Turbidity Standard	＜No. 1 Turbidity Standard	＜No. 1 Turbidity Standard	＜No. 1 Turbidity Standard
color of solution		≤ B9	＜B9	＜B9	＜B9
Wate %		≤ 7.0	0.81	0.66	0.77

Residue on ignition	≤ 0.2	0.02	0.03	0.01
Chloride,ppm	≤ 300	< 300	< 300	< 300
Sulfate(ppm)	≤ 200	< 200	< 200	< 200
Ammonium ,ppm	≤ 400	< 400	< 400	< 400
Heavy metal,ppm	≤ 10	< 10	< 10	< 10
Iron, ppm	≤ 30	< 30	< 30	< 30
Other Amino Acids,%	Spots other than the main spots in the test solution are not larger than the spots obtained in the standard solution	Compliance	Compliance	Compliance
Residual solvent methanol ppm	≤3000	91	95	89
Assay, %	98.0 ~ 102.0	98.6	98.9	99.0

3.2.S.4.5 Justification of Specifications

The specification of L-Ornithine-L-Aspartate is set in accordance with DAB in “Ornithinaspartat”. The acceptance criterion of residual solvent is set base on the permitted limit recommended in ICH Q3C.The analytical procedures are based on the DAB and Ph. Eur. general chapters. Residual solvent testing proceeds to the validated test method (3.2.S.4.3).