Aspartate Ornithine Residual Solvent Analysis Method
Validation report

Jing Jing Pharmaceutical Co., Ltd.

List

Content

一. Validation Overview

To investigate the residual solvent (methanol) in ornithine aspartate, we validated the residual solvent (methanol) detection method. The verification items include: System suitability test、 specificity, Detection of Quantitation, Detection of Limit, Linearity and range，Accuracy test，Precision and durability. The verification content and results are as follows

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 impurity content of the 6 samples of the	Repeatability: The RSD of the reference solution is 0.6% The RSD of the test solution is 1.0% Intermediate precision:

	test solution is ≤10.0%. Intermediate precision: Impurity content of the test solution RSD% (n=6) ≤ 10.0%, RSD% (n=12) ≤ 15.0%	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 ℃).

二、Method validation content

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 ≤ 0.3%.

三、Verification project

1 System suitability test

1.1 Experimental conditions

1.1.1 Separation detection system

Chromatographic column: capillary column (6% cyanopropylphenyl-94% dimethylpolysiloxane as a stationary solution (DB-624 type, size: 30m×0.25mm×1.4μm)).

Column temperature: 35 ℃, run for 20 minutes.

Detector Type: FID

Detector temperature: 250℃

Inlet temperature: 150℃

Split ratio: 20:1

Carrier gas: nitrogen flow rate 3ml/min

Combustion gas: hydrogen flow 40ml/min

Combustion-supporting gas: air flow 400ml/min

1.1.2 Sampling system

Sample equilibration temperature: 80℃

Equilibration time: 40 min

Injection volume: 1ml.

Quantitative ring temperature: 100℃

Transmission line temperature: 110℃

1.2 Solution Preparation

Reference solution: Take approximately 0.075g to 100ml of methanol (accurate to 0.0002g) into a volumetric flask, weigh accurately, add water to the mark, shake well, accurately measure 1ml of reference stock solution into a 10ml volumetric flask, dilute with water to the mark, shake well, draw 2ml into a 20ml headspace flask, seal.

1.3 Operating procedure: After the chromatographic system is stable, take the reference solution, inject it 6 times continuously, and record the chromatogram number.

1.4 Acceptance criteria: The RSD% (n=6) of the measured value of the repeatable injection peak area shall not exceed 10.0%; the number of theoretical plates (N) ≥ 5000.

1.5Verification results

table 1 Confirmation of system suitability

Inspection date	Inspector	Sample weight, g	Times	Atlas number	Number of theoretical plates	Peak area	RSD%
2022.10.01	Liping Fan	0.0755	1	Reference substance 1-1	17842	52.278	0.9
			2	Reference substance 1-2	17842	52.137	
			3	Reference substance 1-3	17842	51.867	
			4	Reference substance 1-4	17842	53.190	

			5	Reference substance 1-5	17842	52.728	
			6	Reference substance 1-6	17842	52.290	

2 Exclusivity

2.1 Solution preparation

2.1.1 Blank: Precisely measure 2ml of water, put it in a headspace bottle, seal it, and use it as a blank solvent.

2.1.2 Test solution: take about 0.2g (accurate to 0.02g) of the test sample, accurately weigh it, put it in a headspace bottle, accurately measure 2ml of water to dissolve, and seal it.

2.1.3 Methanol positioning solution:Accurately measure 2ml of the reference solution and place it in a headspace bottle, seal it.

2.1.4 Sample spiking solution: Accurately weigh about 0.2g (accurate to 0.02g) of the sample into a headspace bottle, then accurately measure 2ml of the reference solution into the headspace bottle, dissolve it, and seal.

2.2 operating procedure

After the chromatographic system is stable, take the blank, the test solution, the methanol positioning solution, and the test solution for adding the standard, and inject the samples once respectively, and record the chromatogram number.

2.3 Acceptable standard

The blank solvent did not interfere with the methanol detection, and no other impurity peaks in the test solution interfered with the known impurity peaks.

2.4Verification results

table 2 Confirmation of exclusivity

Name	Methanol
Blank retention time (min)	--
Test solution retention time (min)	1.512
Methanol positioning solution retention time (min)	1.512
Retention time of test sample spiked solution (min)	1.512

3 The limit of quantitation

3.1 Operating procedures

Accurately weigh an appropriate amount of methanol reference substance, dilute it 50 times with water, shake well, and obtain the result. Prepare a total of 6 portions. After the chromatographic system is stable, take the quantitative limit solution, inject it 6 times continuously, and record the chromatogram number. Calculate the minimum concentration of methanol and the percentage relative to the test substance.

3.2 Acceptable standard

The corresponding concentration when the signal-to-noise ratio was about 10:1 was determined as the

limit of quantification, and the solution at the limit of quantification was taken for 6 consecutive repeated injections.
The RSD of the calculated peak area is ≤ 10.0%, and the RSD of the retention time is ≤ 10.0%.
3.3Verification results

table 3 Confirmation of limit of quantification

	Sample weight/g	Concentration /(μg/ml)	Peak area	Noise range	Signal to noise ratio	RSD，%	Retention time min	Retention time RSD，%
The limit of quantitation	0.0755	1.510	1.108	3.000-4.000	22.7	2.6	1.513	0
		1.510	1.154	3.000-4.000	23.3		1.513	
		1.510	1.099	3.000-4.000	25.7		1.513	
		1.510	1.075	3.000-4.000	25.7		1.513	
		1.510	1.102	3.000-4.000	22.2		1.513	
		1.510	1.137	3.000-4.000	21.8		1.513	
The limit of quantitation	1.510μg/ml		Percent content relative to the test product			0.0015%		

4 Detection limit

4.1 Operating procedures
Take 3ml of the quantitative limit solution and dilute it with water to 10ml to obtain the detection limit solution. After the chromatographic system is stable, inject once and record the chromatogram number. Calculate the concentration of methanol and the percentage relative to the test sample.
4.2 Acceptable standard
The detection limit was determined at the corresponding concentration when the signal-to-noise ratio was approximately 3:1.
4.3 Verification results

table 4 Confirmation of detection limit

Detection limit	Sample weight/g	Concentration /(μg/ml)	Peak area	Noise range	Signal to noise ratio
	0.0755	0.453	0.413	3.000-4.000	7.9
Detection limit	0.453μg/ml		Percent content relative to the test product		0.00045%

5 Linear

5.1 Preparation of storage solution:

Reference stock solution: Take approximately 0.075g to 100ml of methanol (accurate to 0.0002g) into volumetric flasks, weigh accurately, add water to the mark, shake well to obtain.

5.2 Linear solution preparation:

Linearity-20%:Precisely measure 2.0 ml of the reference stock solution into a 100ml volumetric flask, dilute with water to the mark, shake well, take 2ml into a 20ml headspace bottle, and seal.

Linearity-50%:Precisely measure 5.0ml of the reference stock solution into a 100ml volumetric flask, dilute with water to the mark, shake well, take 2ml into a 20ml headspace bottle, and seal.

Linearity-80%:Precisely measure 8.0ml of the reference stock solution into a 100ml volumetric flask, dilute with water to the mark, shake well, take 2ml into a 20ml headspace bottle, and seal.

Linearity-100%:Precisely measure 10.0ml of the reference stock solution into a 100ml volumetric flask, dilute with water to the mark, shake well, take 2ml into a 20ml headspace bottle, and seal.

Linearity-120%:Precisely measure 12.0ml of the reference stock solution into a 100ml volumetric flask, dilute with water to the mark, shake well, take 2ml into a 20ml headspace bottle, and seal.

Linearity-150%:Precisely measure 15.0ml of the reference stock solution into a 100ml volumetric flask, dilute with water to the mark, shake well, take 2ml into a 20ml headspace bottle, and seal.

Linearity-200%:Precisely measure 20.0ml of the reference stock solution into a 100ml volumetric flask, dilute with water to the mark, shake well, take 2ml into a 20ml headspace bottle, and seal.

Linearity-500%:Precisely measure 50.0ml of the reference stock solution into a 100ml volumetric flask, dilute with water to the mark, shake well, take 2ml into a 20ml headspace bottle, and seal.

5.3 Operating procedures

After the chromatographic system is stable, take the quantitative limit solution and each concentration reference solution and inject them once respectively. Take the concentration of methanol in the reference solution as the abscissa, the peak area as the ordinate, and draw a standard curve with at least 5 consecutive points (including at least the limit of quantification concentration). List its regression equation and calculate the correlation coefficient.

5.4 Acceptable standard： The correlation coefficient (R) should not be less than 0.990, the Y-axis intercept should account for less than 25% of the 100% response value, and the relative standard deviation of the response factor should not be greater than 10.0%.

5.5Verification results

table 5 Linear confirmation

Control substance name	Methanol

Weighing amount/g, concentration/(mg/ml)			0.1506g,0.753mg/ml			
Name	Take the volume of stock solution/ml	Dilution volume/ml	Concentration/(mg/ml)	Peak area	Response factor	RSD%
Limit of quantitation	--	--	0.00151	1.108	733.774	2.1
Linear 20%	2	100	0.01506	10.563	701.394	
Linear 50%	5	100	0.03765	25.995	690.438	
Linear 80%	8	100	0.06024	41.710	692.397	
Linear 100%	10	100	0.0753	52.723	700.172	
Linear 120%	12	100	0.09036	62.605	692.839	
Linear 150%	15	100	0.11295	77.566	686.728	
Linear 200%	20	100	0.1506	104.601	694.561	
Linear 500%	50	100	0.3756	258.156	685.673	
Y-axis intercept accounts for methanol 100% response value	0.91%					
Linear equation	Y=665.4x+0.4822					
Correlation Coefficient (R)	R=1					

6 Recovery rate

6.1 Reference substance stock solution: respectively take methanol (accurate to 0.0002g) in a volumetric flask of about 0.6g to 100ml, accurately weigh it, add water to the mark, and shake well. Triplicates were prepared as control stock solutions - 1, 2, 3.

6.2 50% reference solution: Precisely measure 5ml of the reference stock solution into a 100ml volumetric flask, dilute with water to the mark, draw 2ml into a 20ml headspace bottle, and seal.

6.3 100% reference solution: Precisely measure 10ml of the reference stock solution into a 100ml volumetric flask, dilute with water to the mark, draw 2ml into a 20ml headspace bottle, and seal.

6.4 150% reference solution: Precisely measure 15ml of the reference stock solution into a 100ml volumetric flask, dilute with water to the mark, draw 2ml into a 20ml headspace bottle, and seal.

6.5 Preparation of test solution: Take approximately 0.2g (accurate to 0.02g) of the test sample, weigh it accurately, and transfer it to a headspace bottle. Then, accurately measure 2ml of water into the headspace bottle, dissolve it, and seal it. (Prepare 2 copies)

6.6 Recovery Sample Preparation：

Recovery rate 50%-1: Take 0.2g of this product, accurately weigh it, and transfer it to a headspace bottle.

Then, precisely measure 2ml of 50% reference solution into the headspace bottle, dissolve it, and seal it. Prepare samples with a recovery rate of 50% -2 and a recovery rate of 50% -3 using the same method.
Recovery rate 100%-1: Take 0.2g of this product, accurately weigh it, and transfer it to a headspace bottle. Then, precisely measure 2ml of 100% reference solution into the headspace bottle, dissolve it, and seal it. Prepare samples with a recovery rate of 100% -2 and a recovery rate of 100% -3 using the same method.
Recovery rate 150%-1: Take 0.2g of this product, accurately weigh it, and transfer it to a headspace bottle. Then, precisely measure 2ml of 150% reference solution into the headspace bottle, dissolve it, and seal it. Prepare samples with a recovery rate of 150% -2 and a recovery rate of 150% -3 using the same method.
6.7 Operating procedure: After the chromatographic system is stable, inject the reference solution twice (once per reference solution), the test solution twice, and the recovery rate solution once each. Record the chromatogram number.
6.8 Acceptable standard： According to the external standard method, the detected amount and recovery rate of each impurity were calculated. The average recovery rate was between 80% and 120%, and the relative standard deviation should not exceed 10.0%.
6.9 Verification results

table 6-1 Confirmation of Recovery Rate

Reference substance	Name	Sample weight，g	Concentration of reference substance/(mg/ml)	Peak area of reference substance	F	The average value of F
	methanol	0.0755	0.0755	52.290	1.443×10^{-3}	1.422×10^{-3}
		0.0746	0.0746	53.196	1.402×10^{-3}	
Testing sample	Test sample weight，g	Test substance concentration(g/ml)	The peak area of the measured component in the test product	The amount of the measured component in the test product(mg/ml)		Average(mg/ml)
	0.1998	0.0999	8.575	0.0121		0.012
	0.2006	0.1003	8.519	0.0121		

table 6-2 Confirmation of Recovery Rate

Sample	Test sample weight/g	The amount of the measured component of the test	Add the weight of the reference substance/	Peak area	Actual measurement/mg	Recovery rate/%	The average recovery rate%	RSD%	Average recovery rate Total/%	RSD%

		product/mg	mg							
50%-1	0.2014	0.012	0.0377	37.114	0.0527	108	108	0	108	0.5
50%-2	0.2018	0.012		37.214	0.0528	108				
50%-3	0.2014	0.012		37.217	0.0528	108				
100%-1	0.2006	0.012	0.0755	65.787	0.0934	108	108	0.6		
100%-2	0.2003	0.012		65.378	0.0928	107				
100%-3	0.2006	0.012		65.709	0.0933	108				
150%-1	0.2015	0.012	0.114	94.581	0.134	108	108	0.6		
150%-2	0.2011	0.012		94.468	0.134	108				
150%-3	0.2011	0.012		94.233	0.133	107				

7 Repeatability

7.1 Reference solution: Accurately measure 5.0ml of reference stock solution into the same 50ml volumetric flask, dilute with water to the mark, shake well, take 2ml into a 20ml headspace bottle, and seal. (Prepare 6 reference solutions in parallel)

7.2 Test solution: Precisely weigh 6 parts of this product, each about 0.2g, put it in a headspace bottle, add 2ml of water to dissolve it, and seal it. The map is recorded as Lot-1, Lot-2, Lot-3, Lot-4, Lot-5, Lot-6.

7.3 Operating procedure: After the chromatographic system is stable, take the reference solution and the test solution and inject each sample once, recording the chromatogram number.

Take the last 2 injections of the reference solution and calculate the content of each impurity in the 6 test solutions.

7.4 Acceptable standard

The RSD of the peak area of the reference solution for 6 consecutive injections is ≤ 10.0%; the RSD of each impurity content in 6 samples of the test solution is ≤ 10.0%.

7.5 Verification results

table 7 Repeatability Confirmat

Reference substance						
Sample weight，g	Frequency	Atlas number	degree of separation	Peak area	Peak area average	RSD，%
0.0755	1	Reference substance -1	14.37	51.734	51.983	0.6
	2	Reference	14.45	52.215		

		substance -1				
	3	Reference substance -1	14.29	51.667		
	4	Reference substance -1	14.37	52.406		
	5	Reference substance -1	14.37	51.796		
	6	Reference substance -1	14.37	52.083		

Testing sample							
No.	Sample weight，g	Spectrum number	Peak area	Sample concentration g/ml	Content %	Average content，%	RSD，%
1	0.2015	MN2209002-1	8.680	0.10075	0.0125	0.012	1.0
2	0.2013	MN2209002-2	8.611	0.10065	0.0124		
3	0.2014	MN2209002-3	8.584	0.10070	0.0123		
4	0.2015	MN2209002-4	8.511	0.10075	0.0122		
5	0.2014	MN2209002-5	8.558	0.10070	0.0123		
6	0.2013	MN2209002-6	8.647	0.10065	0.0124		

8 Intermediate precision

8.1 Person 1: Use the test data of six samples in repeatability.

Person 2: Prepare six samples according to repeatability.

8.2 Operating procedure: After the chromatographic system is stable, take two injections of the reference solution and one injection of the test solution, and record the chromatogram number.

8.3 acceptable standard The RSD% (n=6) of each impurity in Person 2 is ≤10.0%, and the RSD% (n=12) is ≤15.0%.

8.4 Verification results

table 8-1 Confirmation of Intermediate Precision

Reference substance						
Sample weight，g	Frequency	Atlas number	Degree of separation	Peak area	Peak area average	RSD，%
0.0758	1	Reference substance -1	14.37	53.345	53.697	1.0

	2	Reference substance -2	14.37	54.050		
Testing sample						
No.	Sample weight，g	Atlas number	Peak area	Sample concentration g/ml	Content%	Average content，%	RSD，%
1	0.2020	MN2209002-1	8.653	0.10100	0.0120	0.012	0.7
2	0.2035	MN2209002-2	8.681	0.10175	0.0120		
3	0.2018	MN2209002-3	8.628	0.10090	0.0120		
4	0.2015	MN2209002-4	8.544	0.10075	0.0119		
5	0.2014	MN2209002-5	8.503	0.10070	0.0119		
6	0.2026	MN2209002-6	8.564	0.10130	0.0119		

table 8-2 Confirmation of Intermediate Precision

		Name	Content%	Average content，%	RSD%
Intermediate precision	Personnel-1	Sample-1	0.0125	0.012	2.0
		Sample-2	0.0124		
		Sample-3	0.0123		
		Sample-4	0.0122		
		Sample-5	0.0123		
		Sample-6	0.0124		
	Personnel-2	Sample-1	0.0120		
		Sample-2	0.0120		
		Sample-3	0.0120		
		Sample-4	0.0119		
		Sample-5	0.0119		
		Sample-6	0.0119		

9 Durability

9.1 Preparation of reference solution

Reference solution: Precisely measure 1ml of reference stock solution 1 and reference stock solution 2 into two 10ml volumetric flasks, dilute with water to the mark, shake well, take 2ml into a 20ml headspace bottle, and seal. (Prepare 2 copies)

9.2 Sample solution preparation

Test solution: Take about 0.2g of the test (accurate to 0.02g), accurately weigh it, and put it in a headspace bottle. Precisely measure 2ml of water to dissolve and seal. (Prepare 2 copies)

9.3 Operating procedure: After the chromatographic system is stable, take the reference solution and the test solution and inject them twice according to the following conditions, and record the chromatogram number.

parameter name	Standard conditions	variation range
flow rate	3ml/min	±0.2ml/min
Detector temperature	250℃	±5℃
Inlet temperature	150℃	±5℃
headspace equilibration time	40min	±5 min
headspace temperature	80℃	±5℃

9.4 Acceptable criteria: The RD of methanol content in the test solution under each condition shall not exceed 15.0%.

9.5 Verification results

table 9-1 Durability Confirmation

Detection conditions：Flow rate 2.8mL/min

Reference solution (without changing conditions)	Name	sample weight，g	Concentration of reference substance/mg/ml)	Peak area	F 值	F 均	RD,%
	Methanol	0.0755	0.0755	52.290	1.443×10^{-3}	1.422×10^{-3}	1.5
		0.0746	0.0746	53.196	1.402×10^{-3}		
Reference solution (change conditions)	Methanol	0.0755	0.0755	52.520	1.437×10^{-3}	1.433×10^{-3}	0.4
		0.0758	0.0758	53.070	1.428×10^{-3}		

Methanol					
Sample (unchanged conditions)	Sample weight/g	Concentration/ (mg/ml)	Peak area	content/%	RSD，%

	0.1998	99.90	8.575	0.012	0
	0.2006	100.30	8.519	0.012	
Sample (change conditions)	0.2017	100.85	8.460	0.012	
	0.2012	100.60	8.689	0.012	

table 9-2 Durability Confirmation

Detection conditions：Flow rate 3.2mL/min

Reference solution (without changing conditions)	Name	sample weight，g	Concentration of reference substance/mg/ml)	Peak area	F 值	F 均	RD,%
	Methanol	0.0755	0.0755	52.290	1.443×10^{-3}	1.422×10^{-3}	1.5
		0.0746	0.0746	53.196	1.402×10^{-3}		
Reference solution (change conditions)	Methanol	0.0755	0.0755	52.629	1.434×10^{-3}	1.424×10^{-3}	0.8
		0.0758	0.0758	53.629	1.413×10^{-3}		
Methanol							
Sample (unchanged conditions)		Sample weight/g	Concentration/ (mg/ml)	Peak area	content/%		RSD，%
		0.1998	99.90	8.575	0.012		0
		0.2006	100.30	8.519	0.012		
Sample (change conditions)		0.2012	100.60	8.476	0.012		
		0.2013	100.65	8.643	0.012		

table 9-3 Durability Confirmation

Detection conditions：Detector temperature 245 ℃

Reference solution (without changing conditions)	Name	sample weight，g	Concentration of reference substance/mg/ml)	Peak area	F 值	F 均	RD,%
	Methanol	0.0755	0.0755	52.290	1.443×10^{-3}	1.422×10^{-3}	1.5
		0.0746	0.0746	53.196	1.402×10^{-3}		
Reference solution (change conditions)	Methanol	0.0755	0.0755	52.452	1.439×10^{-3}	1.442×10^{-3}	0.3
		0.0758	0.0758	53.449	1.445×10^{-3}		

Methanol					
Sample (unchanged conditions)	Sample weight/g	Concentration/ (mg/ml)	Peak area	content/%	RSD，%
	0.1998	99.90	8.575	0.012	0
	0.2006	100.30	8.519	0.012	
Sample (change conditions)	0.2013	100.65	8.514	0.012	
	0.2010	100.50	8.340	0.012	

table 9-4 Durability Confirmation

Detection conditions：Detector temperature 255 ℃

Reference solution (without changing conditions)	Name	sample weight，g	Concentration of reference substance/mg/ml)	Peak area	F 值	F 均	RD,%
	Methanol	0.0755	0.0755	52.290	1.443×10^{-3}	1.422×10^{-3}	1.5
		0.0746	0.0746	53.196	1.402×10^{-3}		
Reference solution (change conditions)	Methanol	0.0755	0.0755	52.557	1.436×10^{-3}	1.436×10^{-3}	0.04
		0.0758	0.0758	52.800	1.435×10^{-3}		

Methanol					
Sample (unchanged conditions)	Sample weight/g	Concentration/ (mg/ml)	Peak area	content/%	RSD，%
	0.1998	99.90	8.575	0.012	0
	0.2006	100.30	8.519	0.012	
Sample (change conditions)	0.2013	100.65	8.680	0.012	
	0.2014	100.70	8.663	0.012	

table 9-5 Durability Confirmation

Detection conditions：Injection port temperature 145 ℃

Reference solution (without changing conditions)	Name	sample weight，g	Concentration of reference substance/mg/ml)	Peak area	F 值	F 均	RD,%
	Methanol	0.0755	0.0755	52.290	1.443×10^{-3}	1.422×10^{-3}	1.5
		0.0746	0.0746	53.196	1.402×10^{-3}		
Reference solution (change conditions)	Methanol	0.0755	0.0755	52.524	1.437×10^{-3}	1.426×10^{-3}	0.8
		0.0758	0.0758	53.550	1.415×10^{-3}		

Methanol					
Sample (unchanged conditions)	Sample weight/g	Concentration/ (mg/ml)	Peak area	content/%	RSD，%
	0.1998	99.90	8.575	0.012	0
	0.2006	100.30	8.519	0.012	
Sample (change conditions)	0.2016	100.80	8.417	0.012	
	0.2012	100.60	8.490	0.012	

table 9-6 Durability Confirmation

Detection conditions：Injection port temperature 155 ℃

Reference solution (without changing conditions)	Name	sample weight，g	Concentration of reference substance/mg/ml)	Peak area	F 值	F 均	RD,%
	Methanol	0.0755	0.0755	52.290	1.443×10^{-3}	1.422×10^{-3}	1.5
		0.0746	0.0746	53.196	1.402×10^{-3}		
Reference solution (change conditions)	Methanol	0.0755	0.0755	52.881	1.427×10^{-3}	1.423×10^{-3}	0.3
		0.0758	0.0758	53.389	1.419×10^{-3}		

Methanol					
Sample (unchanged conditions)	Sample weight/g	Concentration/ (mg/ml)	Peak area	content/%	RSD，%
	0.1998	99.90	8.575	0.012	0
	0.2006	100.30	8.519	0.012	
Sample (change conditions)	0.2013	100.65	8.612	0.012	
	0.2019	100.95	8.684	0.012	

table 9-7 Durability Confirmation

Detection conditions： headspace equilibration time 35 min

Reference solution (without changing conditions)	Name	sample weight，g	Concentration of reference substance/mg/ml)	Peak area	F 值	F 均	RD,%
	Methanol	0.0755	0.0755	52.290	1.443×10^{-3}	1.422×10^{-3}	1.5
		0.0746	0.0746	53.196	1.402×10^{-3}		
Reference solution (change conditions)	Methanol	0.0755	0.0755	53.588	1.408×10^{-3}	1.424×10^{-3}	1.2
		0.0758	0.0758	52.603	1.440×10^{-3}		

Methanol					
Sample (unchanged conditions)	Sample weight/g	Concentration/ (mg/ml)	Peak area	content/%	RSD，%
	0.1998	99.90	8.575	0.012	0
	0.2006	100.30	8.519	0.012	
Sample (change conditions)	0.2011	100.55	8.647	0.012	
	0.2018	100.90	8.606	0.012	

table 9-8 Durability Confirmation

Detection conditions： headspace equilibration time 45min

Reference solution (without changing conditions)	Name	sample weight，g	Concentration of reference substance/mg/ml)	Peak area	F 值	F 均	RD,%
	Methanol	0.0755	0.0755	52.290	1.443×10^{-3}	1.422×10^{-3}	1.5
		0.0746	0.0746	53.196	1.402×10^{-3}		
Reference solution (change conditions)	Methanol	0.0755	0.0755	53.403	1.413×10^{-3}	1.432×10^{-3}	1.4
		0.0758	0.0758	52.219	1.451×10^{-3}		

Methanol					
Sample (unchanged conditions)	Sample weight/g	Concentration/ (mg/ml)	Peak area	content/%	RSD，%
	0.1998	99.90	8.575	0.012	0
	0.2006	100.30	8.519	0.012	
Sample (change conditions)	0.2011	100.55	8.524	0.012	
	0.2011	100.55	8.394	0.012	

table 9-9 Durability Confirmation

Detection conditions：Headspace temperature 75℃

Reference solution (without changing conditions)	Name	sample weight，g	Concentration of reference substance/mg/ml)	Peak area	F 值	F 均	RD,%
	Methanol	0.0755	0.0755	52.290	1.443×10^{-3}	1.422×10^{-3}	1.5
		0.0746	0.0746	53.196	1.402×10^{-3}		
Reference solution (change conditions)	Methanol	0.0755	0.0755	43.179	1.748×10^{-3}	1.743×10^{-3}	0.3
		0.0758	0.0758	43.591	1.738×10^{-3}		

Methanol					
Sample (unchanged conditions)	Sample weight/g	Concentration/ (mg/ml)	Peak area	content/%	RSD，%
	0.1998	99.90	8.575	0.012	0
	0.2006	100.30	8.519	0.012	
Sample (change conditions)	0.2011	100.55	6.950	0.012	
	0.2009	100.45	6.829	0.012	

table 9-10 Durability Confirmation

Detection conditions：Headspace temperature 85℃

Reference solution (without changing conditions)	Name	sample weight，g	Concentration of reference substance/mg/ml)	Peak area	F 值	F 均	RD,%
	Methanol	0.0755	0.0755	52.290	1.443×10^{-3}	1.422×10^{-3}	1.5
		0.0746	0.0746	53.196	1.402×10^{-3}		
Reference solution (change conditions)	Methanol	0.0755	0.0755	63.818	1.183×10^{-3}	1.183×10^{-3}	0
		0.0758	0.0758	64.062	1.183×10^{-3}		

Methanol					
Sample (unchanged conditions)	Sample weight/g	Concentration/ (mg/ml)	Peak area	content/%	RSD，%
	0.1998	99.90	8.575	0.012	0
	0.2006	100.30	8.519	0.012	
Sample (change conditions)	0.2014	100.70	10.097	0.012	
	0.2014	100.70	10.246	0.012	