L-arginine quality standard

Item	Internal control standard
Properties	White crystal or crystalline powder
Specific rotation(°)	+26.9 ~ +27.9
Identification	The infrared absorption spectrum of the sample should be in consistent with the control one (spectrum set 1075).
	Positive reaction
pH	10.5 ~ 12.0
Chloride (%)	≤ 0.02
Sulfate (%)	≤ 0.02
Residue on ignition (%)	≤ 0.3
Loss on drying (%)	≤ 0.5
Arsenic salt (ppm)	≤ 1
Iron salt (ppm)	≤ 10
Heavy metal (ppm)	≤ 10
Transmittance (%)	≥ 98.0
Other Amino Acids (%)	≤ 0.4
*Bacterial endotoxins（EU/mg)）	＜0.01
Assay (%)	98.0-101.0%

“ *” :Periodic inspection, inspecting 3 batches annually

1.1.2) Testing methods of arginine

1. Properties

Spread the arginine sample on clean A4 paper and observe it by eyes. The sample should be white, odorless, crystal or crystalline powder.

2. Specific rotation

Weigh two samples of 4.0g with a scale accurate to one ten-thousandth gram; place one of the sample in a 50ml volumetric flask, to which 6mol/L hydrochloric acid is added to dissolve the sample and make up to the volume; keep the flask in water bath for 20min at a temperature of 20°C, and conduct the measure according to "Standard Operating Procedures for WZZ-2B Digital Automatic Polarimeters" and a blank test is performed

Calculation formula:

$$[a]^{D}_{20}=\frac{(\alpha-\alpha_0)\times 100}{l\times m\times(1-X)\times 100/50}$$

Where:

α—measured optical rotation of the sample under test

α0—blank optical rotation

m —sample mass, g

l— length of the optical tube, dm

X—loss on drying of the sample, %

Judging criteria: +26.9° ~ +27.9°

3. Identification

3.1 According to General Rule 0402 (from the 4th volume of Chinese Pharmacopoeia (2015 edition), the potassium bromide pellet method should be adopted to peform the test. The infrared absorption spectrum of the sample should be in consistent with the control one (spectrum set 1075).

3.2 Take about 50mg of this product, dissolve it in 1ml of water, add 0.5ml of alpha naphthol solution (0.5g of alpha naphthol dissolved in 10ml of 10% sodium hydroxide solution) and 0.5ml of sodium hypobromite test solution (0.2ml of bromine dissolved in 20ml of 5% sodium hydroxide solution), and the color will turn red.

4. pH

Weigh 2.5g of the sample, and add 25ml of water to dissolve it; then measure the solution according to the "PB-10 Standard Operating Procedures of Precision pH Meter". Judging criteria: 10.5 ~ 12.0

5. Chloride

Sample tube: Weigh 0.3g of sample and place it in 50ml Nessler colorimetric tube; add water into the tube to make the solution up to 25ml; and add 10ml of dilute nitric acid into it; then add more water to the solution to make it up to 40ml; Shake it up, and add 1.0 ml of silver nitrate test solution into it, then dilute it with water to make 50 ml, and shake well.

Standard tube: absorb 6.0ml of standard sodium chloride solution, and place it in 50ml natron colorimetric tube; add water to dissolve it into 25ml; then add 10ml of dilute nitric acid and water to make it into 40ml, shake it well; add 1.0ml of silver nitrate test solution, and dilute it with water to make it into 50ml, then shake it well.

Place the two tubes in the dark for 5 min, then, under the clarifier, place them on a black background and look down from above the colorimetric tube to compare the resulting turbidity. The turbidity of the sample tube must be lower than that of the standard tube.

Judging criteria: ≤ 0.02%

6. Sulfate

Sample tube: Weigh 1.0g of sample, and place it in 50ml Nessler colorimetric tube; add water into the tube to make it up to 40ml and dissolve the sample with ultrasonic waves. add 2ml of dilute nitric acid into it, then shake it well; add 5ml of 25% cesium chloride into the solution and shake it well; add purified water into the solution to make it up to 50ml and shake it well. Leave it for 10 minutes and shake it well.

Standard tube: Pipette 2.0m of standard potassium sulfate solution into seven 50ml Nessler colorimetric tubes respectively; add water into the tubes to make them to 40ml; add 2ml of dilute hydrochloric acid into the tubes and shake them well; then add 5ml of 25% of cerium chloride into them respectively and shake them well; add purified water into the solutions to make them to 50ml; shake them well; stand it for 10 minutes, then repeat the shake.

Under the clarifier, place the sample tube and the standard tube against the same black background; observed from the top of the colorimetric tube to perform the colorimetric analysis. the milky white turbidity produced by the sample tube should not be deeper than the standard tube.

Judging criteria: ≤ 0.02%

7. Residue on ignition

Precisely weigh 1.0g of the sample into a constant-weight crucible, add 1ml of sulfuric acid to wet the sample, burn it on an electric furnace until the sulfuric acid vapor is completely removed, then move to a high-temperature furnace and burn at 700°C to completely ashed to constant weight.

Judging criteria: ≤ 0.3%.

8. Loss on drying

Weigh accurately 1.0g of sample into the constant-weight weighing bottle, and place it openly in the blast drying oven.

When the temperature rises to 105°C, start timing and dry it for 3h; then cover the weighing bottle, move it to a desiccator, and let it cool to the room temperature. Weigh the weighing bottle containing the sample precisely until a constant weight is achieved.

Calculation formula:

$$X = \frac{m-(m_1-m_0)}{m} \times 100\%$$

Where：

m_0-mass of the empty weighing bottle at a constant weight, g;

m-sample mass, g;

m_1-mass of the weighing bottle and sample at a constant weight, g;

9. Arsenic salt

(1) Sample bottle: Take 2.0g of sample, add 23ml of water to dissolve, and then add 5ml of hydrochloric acid.

(2) Standard bottle: Precisely measure 2.0ml of standard arsenic solution, add 5ml of hydrochloric acid and 21ml of water.

(3) Add 5 ml of potassium iodide test solution and 5 drops of acidic stannous chloride test solution to the two bottles respectively. After standing at room temperature for 10 minutes, add 2g of zinc particles. Immediately seal the installed airway C (with 60 mg of lead acetate cotton) on the bottle A, place the bottle A in a water bath at 25-40 °C, react for 45 minutes, and take out the

mercury bromide test paper. Spots in sample vials should not be deeper than those in standard vials.

standard one. Judging criteria: ≤ 1ppm

10. Iron salt

Sample tube: Weigh 1.0g of sample, and place it in 50ml Nessler colorimetric tube; add water into the tube to make the solution up to 25ml; add 4 ml of dilute hydrochloric acid and 50 mg of ammonium persulfate into it, then dilute with water to make 35 ml; then add 3 ml of 30% ammonium thiocyanate solution, and add water to make 50 ml, then shake it well.

Standard tube: absorb 1.0ml of standard iron solution, and place it in 50ml natron colorimetric tube and add water to make it to 25ml; then add 4ml of dilute hydrochloric acid and 50mg of ammonium persulfate and water to make it into 35ml; then add 3ml of 30% ammonium thiocyanate solution and water to make it into 50ml, then shake it well.

Place the two tubes against a white background immediately and look down from above the colorimetric tube to compare the resulting color. The color of sample tube must not be deeper than that of the standard tube.

Judging criteria: ≤ 10ppm

11. Heavy metals

Standard tube A: Take 2.0ml of standard lead solution into 25ml Nessler colorimetric tube, and add 2ml of acetate buffer (pH=3.5) into it; dilute with water to 25ml, and shake it up.

Sample tube B: Take 2.0g of sample into a 25ml Nessler colorimetric tube, and add 23ml of water to dissolve it; then add 2ml of acetate buffer (pH=3.5) and water to make it t25ml, then shake it well.

Control tube C: Take 2.0g of the sample into a 25ml Nessler colorimetric tube, and add water to dissolve it; then add 2ml of standard lead solution and 2ml of acetate buffer (pH=3.5) into it, and dilute with water to make 25ml, then shake it up.

Add 2ml of thioacetamide test solution to tube A, B and C respectively and shake them well. Leave the tubes for 2min, then place them against a white background. Observe from the top of the colorimetric tube and compare the colors displayed

by tube A, B and C. The color of B shall not be deeper than that of A. The color of the C should be lighter than that of the A.

Judging criteria: ≤ 10ppm

12. Transmittance

Weigh 2.5g of the sample into a 25ml volumetric flask; dissolve it with water and dilute to the mark. Measure the light transmittance according to spectrophotometry by 1cm cuvette at 430 nm.

Judging criteria: ≥ 98.0%

Allowable deviation: Absolute difference of parallel samples ≤ 0.3%

13.Assay

Weigh accurately 0.08g of sample, and add 3ml of anhydrous formic acid to dissolve it; then add 50ml of glacial acetic acid, and titrate it with 0.1mol/L perchloric acid volumetric solution according to the Standard Operating Procedure of Metrohm 877 Titrino Plus, and correct the titration results with a blank test. Each 1 ml of perchloric acid titration solution (0.1mol/L) is equivalent to 8.710 mg of arginine.

Calculation formula:

$$Y = \frac{C \times (V - V_0) \times 8.710}{0.1 \times m \times (1 - X) \times 1000} \times 100\%$$

Where:

C-concentration of perchloric acid titration solution, mol / L;

V-volume of the perchloric acid titration solution consumed by the sample, ml;

V0- volume of the perchloric acid titration solution consumed by the blank, ml;

m- mass of the sample taken, g;

X-Loss on drying of the sample, % Judging criteria: based on dry product, content is 98.0~101.0%。

14.Other Amino Acids

Solvent: 0.1mol/L hydrochloric acid solution.

Test solution

Take an appropriate amount of this product, dissolve it in a solvent and dilute it to make a solution containing approximately 10mg per 1ml.

Reference solution

Accurately measure 1ml of the test solution, place it in a 250ml volumetric flask, dilute with solvent to the mark, and shake well.

System suitability solution

Take appropriate amounts of arginine reference substance and lysine hydrochloride reference substance, place them in the same volumetric flask, dissolve them in solvent, and dilute them to obtain solutions containing approximately 10mg of arginine and 0.4mg of lysine hydrochloride per 1ml, respectively.

Chromatographic conditions

Using silica gel G thin-layer plate and n-propanol concentrated ammonia solution (6:3) as the developing agent.

Measurement method

Take 5ul samples of the test solution、 control solution, and system suitability solution . Point on the same thin layer plate separately, unfold about 20cm, let it dry, dry at 90 ℃ for about 10 minutes, let it cool, spray with 1% ninhydrin n-propanol solution, heat at 90 ℃ until spots appear, and immediately inspect.

System suitability requirements

The control solution should show a clear spot, and the system suitability solution should show two completely separated spots.

Limit

If there are impurity spots in the test solution, it should not exceed one, and its color should not be darker (0.4%) compared to the main spot in the control solution.

15. Bacterial endotoxins

Take 0.5g of this product and check according to General Rule 1143 of the Chinese Pharmacopoeia 2020 edition, the endotoxin content in 1mg of arginine should be less than 0.01EU.