L －**Aspartate** Quality Standard

1） Quality Standard

Item	Internal control standard
Properties	White or off-white crystalline powder
Specific rotation (°)	+24.0 ~ +26.0
Identification	Consistent with standard infrared patterns
	The position and color of the main spots on the test solution should be the same as those on the control solution.
Acidity	2.5 ~ 3.5
Light transmittance (%)	≥ 98.0
Chloride (%)	≤ 0.02
Sulfate (%)	≤ 0.02
Ammonium salt (%)	≤ 0.04
Loss on drying (%)	≤ 0.2
Residue on ignition (%)	≤ 0.1
Iron salt (%)	≤ 0.001
Heavy metal (%)	≤ 0.001
Arsenic salt (%)	≤ 0.0001
Other Amino Acids （%）	≤0.5
Content (%)	99.0 ~ 100.5

2) Inspection method

1. Properties

Spread the aspartic acid sample on clean A4 paper and observe it by eyes. The sample Should be white or off-white 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±0.5°C, and conduct the measure according to “Standard Operating Procedures for SGWzz-2 Thermostatic 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: +24.0o ~ +26.0o

3.Identify

3.1)Take an appropriate amount of this product and tablet with potassium bromide. Check the "Standard Operating Procedure of Infrared Spectrophotometer" according to law, and the infrared absorption spectrum of the sample is consistent with the spectrum set 913.

3.2) Take 10mg of this product and 10mg of aspartic acid reference substance, place them in 25ml volumetric flasks, add 2ml of ammonia test solution to dissolve, dilute with water to the mark, shake well, and use as the test solution and reference solution. According to the methods under other amino acid categories, the position and color of the main spots on the test solution should be the same as those on the control solution.

4. Acidity

Weigh 0.1g of the sample, and add 20ml of freshly boiled and purified water (within 4h) into it; stir it with a glass rod, then conduct measure according to “Standard Operating Procedure of PB-10 Precision pH Meter”.

Judging criteria: 2.5 ~ 3.5

5. Light transmittance

Weigh2.5g of the sample into a 25ml volumetric flask; dissolve it with 1 mol/L hydrochloric acid and dilute it to the mark. Measure the light transmittance

according to spectrophotometry by 1cm cuvette at 430 nm. Judging criteria: ≥ 98.0%

6. 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%

7. Sulfate

Sample tube: Weigh 1.0g of sample, and place it in 50ml Nessler colorimetric tube; add 4ml of dilute hydrochloric acid to it and shake it well. Should the solution be not clear, filter it with 0.22μm water membrane, and add water to make it 40ml; then add 5ml of 25% barium chloride and shake it well; then 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: Take 2.0ml of standard potassium sulfate solution into 50ml Nessler colorimetric tube, and dilute with water to 40ml, then add 5ml of 25% barium chloride solution and shake it well. Then add purified water to make it to 50ml and shake it well. Repeat the shake after 10minutes' of standing.

Under the clarifier, place the sample tube and the standard tube against the same black background; observe from the top of the colorimetric tube to perform the colorimetric analysis. It is normal when the solution is cloudy and muddy; it is

abnormal when the solution is silver white and a redetermination is needed. The color of the sample tube shouldn't be deeper than that of the standard one.

Judging criteria: ≤ 0.02%

8. Ammonium salt

(1) Sample tube: Weigh 0.10g of sample, add 200ml of ammonia-free distilled water, and add 1g of magnesium oxide. Heating and distillation, the distillate was introduced into a 50ml Nessler colorimetric tube with 1 drop of dilute hydrochloric acid and 5ml of ammonia-free distilled water. The distillation was stopped when the distillate reached 40 ml. Add 5 drops of sodium hydroxide test solution, add ammonia-free distilled water to 50ml and shake well.

(2) Standard tube: add 4.0ml of standard ammonium chloride solution, 200ml of ammonia-free distilled water, and 1g of magnesium oxide. Heating and distillation, the distillate was introduced into a 50ml Nessler colorimetric tube with 1 drop of dilute hydrochloric acid and 5ml of ammonia-free distilled water. When the distillate reaches 40ml, stop the distillation, add 5 drops of sodium hydroxide test solution, add ammonia-free distilled water to 50ml and shake well.

(3) Add 2ml of alkaline mercury potassium iodide test solution to each of the sample tube and the standard tube, shake well, let stand for 15 minutes, put it on a white background, observe from top to bottom, compare the color, and it should not be darker.

Judging criteria: ≤ 0.04%

9. Loss on drying

Weigh accurately 1.0g of sample and place it in a weighing bottle at a constant weight; keep the bottle open and put it in a 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：

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

m-sample mass, g;

m1-mass of the weighing bottle and sample at a constant weight, g;

Judging criteria: ≤ 0.2%

10. Residue on ignition

Weigh precisely 1.0g of sample, and place it in a constant-weight crucible; place the crucible on the resistance furnace in a fume hood; slowly burn the crucible containing the sample (to avoid the sudden explosion or burning of the sample because of heat) until the sample is completely carbonized and no longer smoking. Allow it to cool to the room temperature. Add 1 ml of sulfuric acid to make the carbide completely wet and continue to heat it on the electric furnace until the removal of sulfuric acid vapor and the complete disappearance of the white smoke. Place the crucible in a box-type resistance furnace and ignite it at a temperature of 550 ° C until the full ashing of the sample. Stop the heating when the sample achieve a constant weight. Remove the crucible from the box, and cool it in the air for 1 ~ 2min, then place it in a suitable desiccator to cool it to room temperature, then accurately weigh the crucible.

Calculation formula:

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

Where：

m-mass of the sample before ignition, g;

m0- mass of the crucible before ignition, g;

m1-mass of the crucible and residue after ignition, g;

Judging criteria: ≤ 0.1%

11.Heavy metals

(1) Tube A: Take the residue left under the residue on ignition of this product, add 0.5ml of nitric acid, evaporate to dryness, and let it cool. Add 2 ml of hydrochloric acid, evaporate to dryness on a water bath, and add 15 ml of water. Add ammonia test solution dropwise until it is neutral to the phenolphthalein indicator solution, add 2ml of acetate buffer (pH=3.5), dissolve with slight heat,

transfer it to a Nessler colorimetric tube, add water to dilute to 25ml, and shake well.

(2) Tube B: Take the reagent for preparing the test solution. After evaporating to dryness in a porcelain dish, add 2ml of acetate buffer (pH=3.5) and 15ml of water. After dissolving with slight heat, transfer it into a Nessler colorimetric tube, add 1ml of standard lead solution, and then dilute it with an appropriate amount of water to make 25ml, and shake well.

(3) Add 2ml of thioacetamide test solution to tubes A and B respectively, shake well, and let stand for 2 minutes. Put it on the same white paper and see through it from top to bottom. The color displayed in tube A should not be darker compared to tube B.

Judging criteria: ≤ 0.001%

12. Arsenic salt

Sample bottle: Take 2.0g of sample to arsenic-containing Bottle A , and add 5ml of hydrochloric acid and 23ml of water to dissolve it; then add 5ml of potassium iodide test solution and 5 drops of acid stannous chloride test solution into it , then leave it at room temperature for 10min; add 2g of zinc particles into it , then densely plug the well-prepared airway tube C (containing 60 mg of lead acetate cotton) to Bottle A; place the bottle in a 25~40 ° C water bath for 45 minutes; then take the mercury bromide test paper out.

Standard bottle: Take 2.0ml of standard arsenic solution arsenic to Bottle A, then add 5ml of hydrochloric acid and 21ml of water to dissolve it; then add 5ml of potassium iodide test solution and 5 drops of acidic stannous chloride test solution into it, and leave it at room temperature for 10min; add 2g of zinc granules and densely plug the well-prepared airway tube C (containing 60 mg of lead acetate cotton) to Bottle A immediately. Place the bottle in a 25~40 ° Cwater bath for 45 minutes, then take the mercury bromide test paper out.

The arsenic spots produced by the sample should not be deeper than those of the standard one.

Judging criteria: ≤ 0.0001%

13.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

14 Other Amino Acids

Test solution

Take 0.10g of this product and place it in a 10ml volumetric flask. Add 2ml of concentrated ammonia solution to dissolve, dilute with water to the mark, and shake well.

Reference solution

Accurately measure 1ml of the test solution, place it in a 200ml volumetric flask, dilute with water to the mark, and shake well.

System suitability solution

Take 10mg of aspartic acid reference substance and 10mg of glutamic acid reference substance, place them in the same 25ml volumetric flask, add 2ml of ammonia test solution to dissolve, dilute with water to the mark, and shake well.

Chromatographic conditions

Using silica gel G thin-layer plate and acetic acid water n-butanol (1:1: 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 for at least 15cm, air dry, spray with a mixed solution of 0.2% ninhydrin n-butanol-2mol/L acetic

acid solution (95:5), heat at 120 ℃ for about 15 minutes 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 clearly separated spots.

Limit

If there are impurity spots in the test solution, the color should not be darker (0.5%) compared to the main spot in the control solution.

15. Content

Accurately weigh 80 mg of the sample, add 5 ml of anhydrous formic acid, and 30 ml of glacial acetic acid, and titrate with 0.1 mol/L perchloric acid according to the Standard Operating Procedure of Automatic Potentiometric Titrator, and correct the titration result with a blank test. Each 1ml of perchloric acid titration solution (0.1mol/L) is equivalent to 13.31mg of aspartic acid.

Calculation formula:

$$Y = \frac{C \times (V - V_0) \times 13.31}{0.1 \times m \times (1 - X) \times 1000} \times 100\%$$

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 99.0 ~ 100.5%