Analytical procedure of L-Carnitine L-Tartrate

【Characteristics】white crystalline powder

【Identification】

(1) Weigh about 50mg of sample to a test tube，add one drop carbon bisulfide solution (contain 2％ sulphur)，mix well. Heat for a moment，covered a lead acetate test paper on mouth of the test tube, then put the test tube into a about 170℃ preheated glycerin bath, 3-4minutes later, black spots should be appeared in the test paper.

(2) Examine by infrared absorption spectrophotometry, spectrum of sample should be in accordance with the standard spectrum.

【Assay】

(1). Assay of levocarnitine

Accurately weigh about 0.1g of the sample, add 20ml of glacial acetic acid to dissolve it, add one drop of crystal violet solution indicator solution, titrate with 0.1M perchloric acid untill the color changes to blue. Calibrate the result with blank test. 1ml of 0.1M perchloric acid is equivalent to 16.12mg of levocarnitine.

Calculate:

$$\text{Assay} = \frac{(V_1 - V_0) \times C_1 \times 0.01612}{W \times 0.1 \times (1\text{-loss on drying\%})} \times 100\%$$

Where:

V_1—volume of perchloric acid volumetric solution consumed by the test sample, ml；

V_0—volume of perchloric acid volumetric solution consumed by the blank，ml；

C_1—t_1(temperature while testing) concentration of perchloric acid，mol/L；

W —Weight of test sample，g.

Crystal violet indicator solution preparation: dissolve 0.5g of crystal violet into 100ml of glacial acetic acid.

(2).Assay of tartaric acid

Accurately weigh 0.3g of the sample, dilute with 50ml of water, add two drop of phenolphthalein indicator solution，titrate with 0.1M sodium hydroxide until the color changes from colourless to pink. 1ml 0.1M sodium hydroxide is equivalent to 0.007504g of tartaric acid.

Calculation:

$$\text{Assay} = \frac{V \times C \times 0.007504}{W \times 0.1 \times (1\text{-loss on drying\%})} \times 100\%$$

Where:

V— volume of sodium hydroxide volumetric solution consumed by sample, ml；

C— concentration of sodium hydroxide volumetric solution；
W — Weight of test sample， g.
Phenolphthalein indicator solution preparation: dissolve 1g of phenolphthalein into 100ml of ethanol, the test solution is obtained.

【Specific rotation】accurately weigh 2.5g of sample to a 25ml volumetric flask, dissolve and dilute with water to volume(1ml contain 100mg substance), determine it. The specific rotation should be between -9.5 °—-11.0 °.

【Acidity】（pH value）weigh 0.5g of sample, dissolve it with 20ml of water, determine it. pH value should be between 3.0~4.5.

【Loss on drying】 weigh 1g of sample, drying to constant weight at 105 ℃. It should be not more than 0.5%.

【Heavy metals】
Tube B: weigh 5.0 g of sample, put in crucible, adding suitable amount of sulfuric acid to moisten it, after it is charred while heat gently, add 2 ml of nitric acid and 5 drops of sulfuric acid, heat gently until the white smoke is wiped off, transfer it in the high temperature furnace, incinerate completely at 550 ℃, removed it out after cooling, add 2 ml of 6mol /L hydrochloric acid to moisten the residue, evaporate to dryness slowly on the water bath. Add one drop of thick hydrochloric acid to moisten the residue, and add 10 ml of water, heat again on the water bath for 2 min, transfer the solution into a 50 ml volumetric flask, filter if necessary, use a small amount of water to wash crucible and filter, transfer the washing liquid into the volumetric flask, mix well, it is the test stock solution. Take 10 ml of the solution (equivalent to 1.0 g of sample) as the test solution.
Tube A: while the sample is incinerated, conduct blank test in another crucible according to the above methods. Take 10 ml of reagent blank solution with 1.0 ml of lead standard solution.
Tube C: add 1.0 ml of lead standard solution to 10 ml of the test stock solution.
Put the above three solution in 50 ml nessler tubes, add water to 25 ml, mix well, add 1 drop of phenolphthalein indicator, adjust pH to neutral with 6 mol/L dilute hydrochloric acid or 1 mol/L dilute ammonia (phenolphthalein red is just faded), add 5 ml of pH3.5 acetate buffer solution, shake well and set aside.
Add 2 ml of thioacetamide TS in tube A, B, C respectively, shake well, allow to stand for 2 minutes, place the tubes on white paper, and view downward: while the color of the tube C is not lighter than that of the tube A， the color of the tube B is not darker than that of the tube A（10ppm）.

【Arsenic】weigh 1.0g of sample in a conical flask, dissolve it with 10ml of water,determine it. Compared with the reference solution prepared by 1ml of the arsenic standard solution (by same method), the arsenic spot should not be more intense (1ppm).

【Cyanide】 weigh 1.0g of sample, determine it (the details see the Analysis Method for

Cyanide). The cyanide content should not be more than 0.0005%.

【Residue on ignition】take 1.0g of sample, determine it. It should not be more than 0.5%.

【Melting point】 The melting point should be at 169－175 ℃. Raising temperature rate is at 1.5 ℃/minute.

【Residual solvents】

Instruments:

- Gas chromatograph HP 6890
- Headspace injection HP 7694
- Chromatographic workstation HP

Headspace conditions:

- Injection vial: 20 ml;
- Headspace temperature: 85 ℃
- Transfer-line temperature: 100 ℃
- Equilibration time: 30 min
- GC circulation time: 6 min

Chromatographic conditions:

- Column: DB-624, 30 m×0.53 mm (length × inner diameter), thickness of liquid membrane: 3.0 μm;
- Carrier gas: Nitrogen gas (Pressure: constant flow; Flow rate: 5.0 ml/min);
- Model of injection: Automatic headspace split injection (split ratio: 4.3:1)
- Temperature:

  Injection: 200 ℃;

  Column: 60 ℃, maintain for 5 minutes

  Detector: 250 ℃;

- Detector: FID

  H_2, 30 ml/min; Air, 300 ml/min; make-up gas (N_2), 30 ml/min;

- Injection: 1.0 ml

Preparation of the solutions:

Test solution: Accurately weigh 0.5 g of the substance to be examined into a 20 ml headspace vial, add 5.0 ml of purified water and seal immediately.

Reference solution: Accurately weigh 1.0 g of acetone (analytical reagent), 1.5 g of anhydrous ethanol (analytical reagent) and 1.0 g of methanol (Chromatogram reagent) to a 100 ml volumetric flask. Dilute to volume with water and mix well. Consider the solution as the stock solution. Accurately measure 1.0 ml of the stock solution to a 100 ml volumetric flask, dilute to volume with water. Transfer 5.0 ml of the solution to a 20 ml headspace vial and seal.

System suitability:

Inject the reference solution to the system, carry out 6 replicates. Record the chromatograms and calculate the resolution: resolution between two adjacent solvent peak should be more than 1.5; the relative standard deviations (RSDs) of the peak areas of single solvent peak should all be less than 10%.

Procedure:

Inject the test solution to the system and determine. Carry out a parallel determination. Calculate the content of residual solvents by the following expression:

$$\text{Residual solvent} = \frac{A_s \times W_r \times 5 \times P}{W \times A_r \times 100 \times 100} \times 100\%$$

Where:

A_r is the average peak area of a single solvent obtained with the reference solution in the system suitability test (6 injections);

A_s is the peak area of a single residual solvent obtained with the test solution;

W is the weight of the substance to be examined, g;

P is the purity of single reference substance;

Wr is the weight of single reference substance, g.