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3-O-Ethyl-L-ascorbic acid

Enterprise quality standards and Test Methods

(2018-12-4)

1. Quality standards

- **Appearance**: white or off-white crystalline powder
- **Moisture content**: <=1.0%
- **pH, 3%aqu.**: 3.5 – 5.5
- **Ignition residue content**: <=0.1%
- **Purity**: >=99.5%
- **Related substances**: A<=0.1%

  B<=0.2%

  C<=0.2%

  Total impurity content: <=0.5%
- **Residual solvent content**: 1-butanol <=500ppm

  Toluene <=500ppm

  Ethyl acetate <=500ppm
- **Content**: 99.0% - 101.0%
- **Period of validity**: tentatively 3 years
- **Package**: The inner package is polyethylene bag; the outer package is aluminum plastic bag or fiber drum.
- **Storage**: store below 20℃, sealed and protected from light.

2. Analytical method

2.1 Appearance

The product appears as white or off-white crystalline powder to naked eyes.

2.2 Moisture content

Accurately weigh 0.2g of sample and measure with K.F. reagent.

Result: <=1.0%

2.3 pH

Weigh 1.5g of sample, dissolve it in 50ml of purified water and measure the pH with a pH meter.

Result: 3.5 – 5.5

2.4 Ignition residue

Take a constant-weight crucible, weigh accurately the crucible (weight M1), add about 1g of sample, and weigh accurately the crucible again (weight M2). Then place the crucible in a muffle furnace and burn it slowly until the sample is completely carbonized. Let it cool, add 0.5 ml of sulfuric acid to make it moist, and heat it at low temperature (about 300℃) until the sulfuric acid evaporates

completely. Heat it to 700-800℃ until the sample becomes completely ashed and the weight remains invariable. At last weigh accurately the crucible (weight M3).

Calculation:

Ignition residue content = (M2 – M3) / (M2 – M1) * 100%

M1: crucible mass, g;

M2: weight of crucible + weight of sample (before ignition), g;

M3: weight of crucible + weight of sample (after ignition), g.

Result: <=0.1%

2.5 Purity

Preparation of solutions

Solution: weigh 1g of H_3PO_4 and dissolve it in 1000ml of purified water.

Test liquid: weigh precisely 50 mg of sample, add it to a 100 ml volumetric flask, dilute to the scale line with 1% phosphoric acid aqueous solution, and shake well.

HPLC system and test conditions

Chromatographic column: stainless steel 18 alkyl silylated silicone gel column, 250*4.6mm 5um.

Mobile phase: 0.1% H_3PO_4 aqueous solution: acetonitrile = 70 : 30 (v/v).

Flow rate: 1.0ml/min

Detection wavelength: 254nm

Sample introducing amounts: 10μ1

Operating procedures: introduce 10ul of sample and record the chromatogram. Calculate the main peak area ratio by normalization method.

Calculation: calculate the purity of sample by normalization method.

Result: purity >= 99.5%

2.6 Related substances

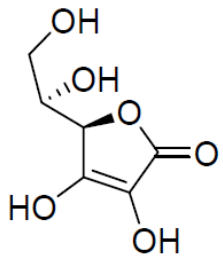
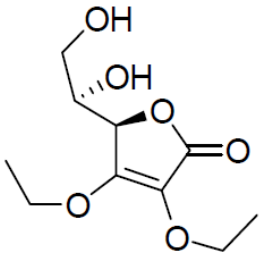
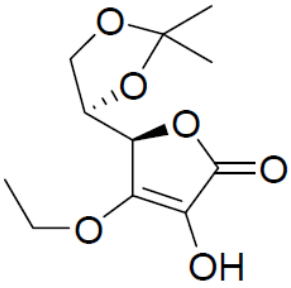
The same test method as that adopted in purity testing.

Calculation: calculate content of the related substances by normalization method.

Result: single impurity content: <=0.2%

total impurity content: <=0.5%

Related substances	Structure	Retention time	Note

Impurity A		1.6min	Vitamin C
Impurity B		2.8min	
Impurity C		6.6min	

2.7 Residual solvent content

Preparation of reference solution

Weigh accurately: butanol (0.05g), toluene (0.05g), ethyl acetate (0.05g); then dissolve them in 1000ml of purified water (the solution contains butanol 0.05mg/ml, toluene 0.05mg/ml and ethyl acetate 0.05mg/ml).

Preparation of sample solution

Weigh accurately: 1.0g of sample (weight M) and dissolve it in 10ml of water, shake well.

Apparatus:

Chromatographic column: DB-624

Detector: FID

INJ: 200℃

DET: 250℃

Amount of sample introduced: 1μl

Apparatus parameters are set based on the conditions of above apparatus. After the instruments are stable, measure the reference solution and sample solution twice, and calculate the contents of butanol, toluene and ethyl acetate in the sample according to the external standard method.

Result: all kinds of solvent do not exceed 500ppm (0.05%).

2.8 Product content

Preparation of solutions

Solution: weigh 1g of H_3PO_4 and dissolve it in 1000ml of purified water.

Reference solution: weigh precisely 50 mg of reference, add it to a 100 ml volumetric flask, dilute to the scale line with 1% phosphoric acid solution.

Test solution: weigh precisely 50 mg of sample, add it to a 100 ml volumetric flask, dilute to the scale line with 1% phosphoric acid solution.

HPLC system and test conditions

Chromatographic column: stainless steel 18 alkyl silylated silicone gel column, 250*4.6mm 5um.

Mobile phase: 0.1% H_3PO_4 aqueous solution: acetonitrile = 70 : 30 (v/v).

Flow rate: 1.0ml/min

Detection wavelength: 254nm

Sample introducing amounts: 10μ1

Operating procedures: introduce 10ul of sample and record the chromatogram. Calculate the main peak area ratio by external standard method.

Calculation:

Content = (VIm * WST) / [VIST * Wm * (1 - Hm)] * AST * 100%

VCE: 3-O-Ethyl-L-ascorbic acid

VIST: peak area of VCE reference

VIm: peak area of VCE sample

WST: weight of VCE in the reference solution, mg

Hm: moisture content of VCE sample, %

Wm: weight of sample in the test solution, mg

AST: content of VCE reference, %

Result: content = 99.0% - 101%