Column:
- *size*: *l* = 0.250 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:
- *mobile phase A*: *phosphoric acid R, water R* (0.5:99.5 *V/V*);
- *mobile phase B*: *phosphoric acid R, acetonitrile R* (0.5:99.5 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	92	8
1 - 20	92 → 75	8 → 25
20 - 33	75	25
33 - 35	75 → 0	25 → 100

Flow rate: 1.2 ml/min.

Detection: spectrophotometer at 330 nm.

Injection: 25 µl.

System suitability: reference solution (b):
- *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak immediately after the peak due to chlorogenic acid and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to chlorogenic acid;
- the chromatogram obtained is similar to the chromatogram supplied with the *standardised artichoke leaf dry extract CRS*

Calculate the percentage content of chlorogenic acid using the following expression:

$$\frac{A_1 \times m_2 \times p \times 0.125}{A_2 \times m_1}$$

A_1 = area of the peak due to chlorogenic acid in the chromatogram obtained with the test solution;

A_2 = area of the peak due to chlorogenic acid in the chromatogram obtained with reference solution (a);

m_1 = mass of the extract to be examined used to prepare the test solution, in milligrams;

m_2 = mass of *chlorogenic acid CRS* used to prepare reference solution (a), in milligrams;

p = percentage content of chlorogenic acid in *chlorogenic acid CRS*.

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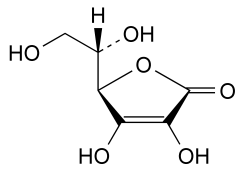
ASCORBIC ACID

Acidum ascorbicum



$C_6H_8O_6$ M_r 176.1
[50-81-7]

DEFINITION

(5*R*)-5-[(1*S*)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one.

Content: 99.0 per cent to 100.5 per cent.

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless crystals, becoming discoloured on exposure to air and moisture.

Solubility: freely soluble in water, soluble in ethanol (96 per cent).

mp: about 190 °C, with decomposition.

IDENTIFICATION

First identification: B, C.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dissolve 0.10 g in *water R* and dilute immediately to 100.0 ml with the same solvent. Add 1.0 ml of this solution to 10 ml of *0.1 M hydrochloric acid* and dilute to 100.0 ml with *water R*.

Absorption maximum: at 243 nm, determined immediately after dissolution.

Specific absorbance at the absorption maximum: 545 to 585.

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *ascorbic acid CRS*.

C. pH (*2.2.3*): 2.1 to 2.6 for solution S (see Tests).

D. To 1 ml of solution S add 0.2 ml of *dilute nitric acid R* and 0.2 ml of *silver nitrate solution R2*. A grey precipitate is formed.

TESTS

Solution S. Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*): + 20.5 to + 21.5.

Dissolve 2.50 g in *water R* and dilute to 25.0 ml with the same solvent.

Impurity E: maximum 0.2 per cent.

Test solution. Dissolve 0.25 g in 5 ml of *water R*. Neutralise to *red litmus paper R* using *dilute sodium hydroxide solution R* and add 1 ml of *dilute acetic acid R* and 0.5 ml of *calcium chloride solution R*.

Reference solution. Dissolve 70 mg of *oxalic acid R* in *water R* and dilute to 500 ml with the same solvent; to 5 ml of this solution add 1 ml of *dilute acetic acid R* and 0.5 ml of *calcium chloride solution R*.

Allow the solutions to stand for 1 h. Any opalescence in the test solution is not more intense than that in the reference solution.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Phosphate buffer solution. Dissolve 6.8 g of *potassium dihydrogen phosphate R* in *water R* and dilute to about 175 ml with the same solvent. Filter (porosity 0.45 µm) and dilute to 1000 ml with *water R*.

Test solution. Dissolve 0.500 g of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). Dissolve 10.0 mg of *ascorbic acid impurity C CRS* in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (b). Dilute 2.5 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase. Mix 1.0 ml of this solution with 1.0 ml of reference solution (a).

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (5 µm);

– *temperature*: 45 °C.

Mobile phase: phosphate buffer solution, *acetonitrile R1* (30:70 *V/V*).

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 210 nm.

Injection: 20 µl of the test solution and reference solutions (b) and (c).

Run time: twice the retention time of ascorbic acid.

Relative retention with reference to ascorbic acid (retention time = about 8 min): impurity C = about 1.4.

System suitability: reference solution (c):

– *resolution*: minimum 3.0 between the peaks due to ascorbic acid and impurity C.

Limits:

– *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent);

– *unspecified impurities*: for each impurity, not more than the area of the peak due to impurity C in the chromatogram obtained with reference solution (b) (0.10 per cent);

– *total*: not more than twice the area of the peak due to impurity C in the chromatogram obtained with reference solution (b) (0.2 per cent);

– *disregard limit*: 0.5 times the area of the peak due to impurity C in the chromatogram obtained with reference solution (b) (0.05 per cent).

Copper: maximum 5.0 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution. Dissolve 2.0 g in *0.1 M nitric acid* and dilute to 25.0 ml with the same acid.

Reference solutions. Prepare the reference solutions (0.2 ppm, 0.4 ppm and 0.6 ppm) by diluting *copper standard solution (10 ppm Cu) R* with *0.1 M nitric acid.*

Source: copper hollow-cathode lamp.

Wavelength: 324.8 nm.

Atomisation device: air-acetylene flame.

Adjust the zero of the apparatus using *0.1 M nitric acid.*

Iron: maximum 2.0 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution. Dissolve 5.0 g in *0.1 M nitric acid* and dilute to 25.0 ml with the same acid.

Reference solutions. Prepare the reference solutions (0.2 ppm, 0.4 ppm and 0.6 ppm) by diluting *iron standard solution (20 ppm Fe) R* with *0.1 M nitric acid.*

Source: iron hollow-cathode lamp.

Wavelength: 248.3 nm.

Atomisation device: air-acetylene flame.

Adjust the zero of the apparatus using *0.1 M nitric acid.*

Heavy metals (*2.4.8*): maximum 10 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in a mixture of 10 ml of *dilute sulphuric acid R* and 80 ml of *carbon dioxide-free water R*. Add 1 ml of *starch solution R*. Titrate with *0.05 M iodine* until a persistent violet-blue colour is obtained.

1 ml of *0.05 M iodine* is equivalent to 8.81 mg of $C_6H_8O_6$.

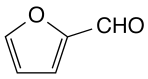
STORAGE

In a non-metallic container, protected from light.

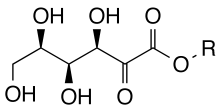
IMPURITIES

Specified impurities: *C, E.*

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, D.*



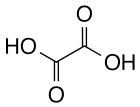
A. 2-furaldehyde,



B. R = $[CH_2]_3$-CH_3: butyl D-sorbosonate,

C. R = H: D-sorbosonic acid,

D. R = CH_3: methyl D-sorbosonate,



E. oxalic acid.