ASCORBIC ACID
Acidum ascorbicum

C₆H₈O₆  \( M \_176.1 \)

DEFINITION
Ascorbic acid contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of (5/2)-5-(1S)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2-(5H)-one.

CHARACTERS
A white or almost white, crystalline powder or colourless crystals, becoming discoloured on exposure to air and moisture, freely soluble in water, soluble in alcohol.
It melts at about 190 °C, with decomposition.

IDENTIFICATION
First identification: B, C.
Second identification: A, C, D.
A. Dissolve 0.10 g in water R and dilute immediately to 100.0 ml with the same solvent. To 10 ml of 0.1 M hydrochloric acid, add 1.0 ml of the solution and dilute to 100.0 ml with water R. Measure the absorbance (2.2.25) at the maximum at 243 nm immediately after dissolution. The specific absorbance at the maximum is 545 to 585.
B. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with ascorbic acid CRS. Examine the substance prepared as discs containing 1 mg.
C. The pH (2.2.3) of solution S (see Tests) is 2.1 to 2.6.
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; (2.2.2, Method II).

Specific optical rotation (2.2.7). Dissolve 2.50 g in water R and dilute to 25.0 ml with the same solvent. The specific optical rotation is +20.5 to +21.5.

Oxalic acid. 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 (test solution). Prepare a reference solution as follows: 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 (reference solution). Allow the solutions to stand for 1 h. Any opalescence in the test solution is not more intense than that in the reference solution (0.2 per cent).

Copper. Not more than 5 ppm of Cu, determined by atomic absorption spectrometry (2.2.23, Method I).

Test solution. Dissolve 2.0 g of the substance to be examined in 0.1 M nitric acid R and dilute to 25.0 ml with the same acid.

Reference solutions. Prepare reference solutions containing 0.2 ppm, 0.4 ppm and 0.6 ppm of Cu by diluting copper standard solution (10 ppm Cu) R with 0.1 M nitric acid R. Measure the absorbance at 324.8 nm using a copper hollow-cathode lamp as a source of radiation and an air-acetylene flame. Adjust the zero of the apparatus using 0.1 M nitric acid R.

Iron. Not more than 2 ppm of Fe, determined by atomic absorption spectrometry (2.2.23, Method I).

Test solution. Dissolve 5.0 g of the substance to be examined in 0.1 M nitric acid R and dilute to 25.0 ml with the same acid.

Reference solutions. Prepare reference solutions containing 0.2 ppm, 0.4 ppm and 0.6 ppm of Fe by diluting iron standard solution (20 ppm Fe) R with 0.1 M nitric acid R. Measure the absorbance at 248.3 nm using an iron hollow-cathode lamp as a source of radiation and an air-acetylene flame. Adjust the zero of the apparatus using 0.1 M nitric acid R.

Heavy metals (2.4.8). Dissolve 2.0 g in water R and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using lead standard solution (1 ppm Pb) R.
**ASCORBYL PALMITATE**

Ascorbylis palmitas

\[ \text{C}_{22}\text{H}_{38}\text{O}_{7} \]  \[ M \_ \_ 414.5 \]

**DEFINITION**

Ascorbyl palmitate contains not less than 98.0 per cent and not more than the equivalent of 100.5 per cent of (2S)-2-(5R)-3,4-dihydroxy-5-oxo-2,5-dihydrofuran-2-yl)-2-hydroxyethyl hexadecanoate, calculated with reference to the dried substance.

**CHARACTERS**

A white or yellowish-white powder, practically insoluble in water, freely soluble in alcohol and in methanol, practically insoluble in methylene chloride and in fatty oils.

**IDENTIFICATION**

A. It complies with the test for specific optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the Ph. Eur. reference spectrum of ascorbyl palmitate.

C. Dissolve about 10 mg in 5 ml of methanol \( \text{R} \). The solution decolourises dichlorophenolindicophenol standard solution \( \text{R} \).

**TESTS**

**Solution S.** Dissolve 2.50 g in methanol \( \text{R} \) and dilute to 25.0 ml with the same solvent.

**Appearance of solution.** Solution S is clear (2.2.1) and not more intensely coloured than reference solution BY 1 (2.2.2, Method I).

**Specific optical rotation** (2.2.7): +21 to +24, determined on solution S and calculated with reference to the dried substance.

**Heavy metals** (2.4.8). 2.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 2 ml of lead standard solution (10 ppm Pb) \( \text{R} \).

**Loss on drying** (2.2.32). Not more than 1.0 per cent, determined on 1.000 g by drying in vacuo at 60 °C for 5 h.

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**Sulphated ash** (2.4.14). Not more than 0.1 per cent, determined on 1.0 g.

**ASSAY**

Dissolve 0.150 g in a mixture of 10 ml of dilute sulphuric acid \( \text{R} \) and 80 ml of carbon dioxide-free water \( \text{R} \). Add 1 ml of starch solution \( \text{R} \). 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 \( \text{C}_{22}\text{H}_{38}\text{O}_{7} \).

**STORAGE**

Store in a non-metallic container, protected from light.

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**ASH LEAF**

Fraxini folium

**DEFINITION**

Dried leaf of Fraxinus excelsior L. or Fraxinus oxyphylla M. Bieb.

**Content:** minimum 2.5 per cent of total hydroxycinnamic acid derivatives expressed as chlorogenic acid (\( \text{C}_{16}\text{H}_{18}\text{O}_{9} \); \( M \_ \_ 354.3 \)) (dried drug).

**CHARACTERS**

Macroscopic and microscopic characters described under identification tests A and B.

**IDENTIFICATION**

A. The leaf consists of leaflets which are sometimes detached and separated from the rachis. The leaflet is about 6 cm long and 3 cm wide. Each leaflet is subsessile or shortly petiolate, oblong, lanceolate, somewhat unequal at the base, acuminate at the apex, with fine acute teeth on the margins, the upper surface is dark green and the lower surface is greyish-green. The midrib and secondary veins are whitish and prominent on the lower surface.

B. Reduce to a powder (355). The powder is greyish-green. Examined under a microscope, using chloral hydrate solution \( \text{R} \). The powder shows fragments of the lamina in surface view, with the lower epidermis showing numerous anomocytic stomata (2.8.3) and some of the cells of the upper epidermis with cuticular striations; occasional uniseriate, conical covering trichomes composed of 1 or 2 cells with thick walls and a striated cuticle; rare peltate glands with a unicellular stalk and a shield-like glandular head composed of 8 radiating cells; groups of fibres and fragments of vascular tissue from the veins.

C. Examine the chromatograms obtained in the test for Fraxinus ornus L.

**Results:** see below the sequence of the zones present in the chromatograms obtained with the reference and test solutions. Furthermore, other fluorescent zones are present in the chromatogram obtained with the test solution.

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**Top of the plate**

<table>
<thead>
<tr>
<th>Reference solution</th>
<th>Test solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid: a blue fluorescent zone</td>
<td>An intense blue fluorescent zone (acteoside)</td>
</tr>
<tr>
<td>Rutin: an orange fluorescent zone</td>
<td>A blue fluorescent zone (chlorogenic acid)</td>
</tr>
<tr>
<td></td>
<td>An orange fluorescent zone (rutin)</td>
</tr>
</tbody>
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See the information section on general monographs (cover pages)