**Sulphated ash (2.6.14)**: the residue until the pink colour is discharged.

**Bacterial endotoxins (2.6.14)**: less than 5 IU/g if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins. Before use, neutralise the test solution to pH 7.0–7.5 with strong sodium hydroxide solution R and shake vigorously.

**ASSAY**

Place 1.000 g in a ground-glass-stoppered flask and add 10 ml of water R and 20.0 ml of 1 M sodium hydroxide. Close the flask and allow to stand for 30 min. Using 0.5 ml of phenolphthalein solution R as indicator, titrate with 1 M hydrochloric acid until the pink colour is discharged. 1 ml of 1 M sodium hydroxide is equivalent to 90.1 mg of C₃H₆O₃.

**LACTITOL MONOHYDRATE**

**Lactitolum monohydricum**

C₅H₁₁O₃·H₂O

**DEFINITION**

4-O-(β-D-galactopyranosyl)-D-glucitol.

**Content**: 96.5 per cent to 102.0 per cent (anhydrous substance).

**CHARACTERS**

**Appearance**: white or almost white, crystalline powder.
Solubility: very soluble in water, slightly soluble in alcohol, practically insoluble in methylene chloride

IDENTIFICATION
First identification: B.
Second identification: A, C.
A. Specific optical rotation (see Tests).
B. Infrared absorption spectrophotometry (2.2.24).
Comparison: lactitol monohydrate CRS.
C. Thin-layer chromatography (2.2.27).
Test solution. Dissolve 50 mg of the substance to be examined in methanol R and dilute to 20 ml with the same solvent.
Reference solution (a). Dissolve 5 mg of lactitol monohydrate CRS in methanol R and dilute to 2 ml with the same solvent.
Reference solution (b). Dissolve 5 mg of sorbitol CRS in 2 ml of reference solution (a) and dilute to 20 ml with methanol R.
Plate: TLC silica gel G plate R.
Application: 2 µl.
Development: over 2/3 of the plate.
Drying: in air.
Detection: spray with 4-aminobenzoic acid solution R.
Dry the plate in a current of cold air until the solvent is removed. Heat at 100 °C for 15 min. Allow to cool and spray with a 2 g/l solution of phenolphthalein solution R. Dry the plate in a current of cold air. Heat at 100 °C for 15 min.
System suitability: the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.
Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS
Solution S. Dissolve 5.000 g in carbon dioxide-free water R and dilute to 50.0 ml with the same solvent.
Appearance of solution. Solution S is clear (2.2.1) and not more intensely coloured than reference solution BY7 (2.2.2, Method II).
Acidity or alkalinity. To 10 ml of solution S add 10 ml of carbon dioxide-free water R. To 10 ml of this solution add 0.05 ml of phenolphthalein solution R. Not more than 0.2 ml of 0.01 M sodium hydroxide is required to change the colour of the indicator to pink. To a further 10 ml of the solution add 0.05 ml of methyl red solution R. Not more than 0.3 ml of 0.01 M hydrochloric acid R is required to change the colour of the indicator to red.
Specific optical rotation (2.2.7): + 13.5 to + 15.5 (anhydrous substance), determined on solution S.
Related substances. Liquid chromatography (2.2.29).
Test solution (a). Dissolve 50.0 mg of the substance to be examined in water R and dilute to 10.0 ml with the same solvent.
Test solution (b). Dilute 2.0 ml of test solution (a) to 50.0 ml with water R.
Reference solution (a). Dissolve 5.0 mg of lactitol monohydrate CRS and 5 mg of glycerol R in water R and dilute to 25.0 ml with the same solvent.
Reference solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with water R. Dilute 5.0 ml of this solution to 100.0 ml with water R.
Reference solution (c). Dilute 2.5 ml of reference solution (a) to 10.0 ml with water R.
Column:
- size: l = 0.30 m, Φ = 7.8 mm,
- stationary phase: strong cation exchange resin (calcium form) R,
- temperature: 60 °C.
Mobile phase: water R.
Flow rate: 0.6 ml/min.
Detection: refractive index detector maintained at a constant temperature.
Injection: 100 µl; inject test solution (a) and reference solutions (b) and (c).
Run time: 2.5 times the retention time of lactitol.
Relative retention with reference to lactitol (retention time = about 13 min): impurity A = about 0.7; impurity B = about 0.8; glycerol = about 1.3; impurity C = about 1.5; impurity D = about 1.8; impurity E = about 1.9.
System suitability: reference solution (c):
- resolution: minimum 5 between the peaks due to lactitol and glycerol.
Limits:
- impurity B: not more than the area of the peak due to lactitol in the chromatogram obtained with reference solution (c) (1.0 per cent),
- total of other impurities: not more than the area of the peak due to lactitol in the chromatogram obtained with reference solution (c) (1.0 per cent),
- disregard limit: the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard any peak due to the solvent.
Reducing sugars: maximum 0.2 per cent.
Dissolve 5.0 g in 3 ml of water R with gentle heating. Cool and add 20 ml of cupri-citric solution R and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent V/V solution of glacial acetic acid R and 20.0 ml of 0.025 M iodine. With continuous shaking, add 25 ml of a mixture of 6 volumes of hydrochloric acid R and 94 volumes of water R. When the precipitate has dissolved, titrate the excess of iodine with 0.05 M sodium thiosulphate using 1 ml of starch solution R added towards the end of the titration, as indicator. Not less than 12.8 ml of 0.05 M sodium thiosulphate is required.
Lead (2.4.10): maximum 0.5 ppm.
Nickel (2.4.15): maximum 1 ppm.
Water (2.5.12): 4.5 per cent to 5.5 per cent, determined on 0.30 g.
Sulphated ash (2.4.14): maximum 0.1 per cent, determined on 1.0 g.
Microbial contamination. Total viable aerobic count (2.6.12) not more than 10^2 micro-organisms per gram. It complies with the tests for Escherichia coli, Salmonella and Pseudomonas aeruginosa (2.6.13).
ASSAY
Liquid chromatography (2.2.29) as described in the test for related substances with the following modification.
Injection: test solution (b) and reference solution (a).
Calculate the percentage content of C_{12}H_{24}O_{11} using the chromatograms obtained with test solution (b) and reference solution (a) and the declared content of lactitol monohydrate CRS.
IMPURITIES

Specified impurities: A, B, C, D, E.

A. lactose,

B. lactulitol,

C. mannitol,

D. dulcitol (galactitol),

E. sorbitol.

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LACTOBIONIC ACID

Acidum lactobionicum

\[ \text{C}_{12}\text{H}_{22}\text{O}_{12} \text{ (acid form)} \]

\[ M, 358.3 \]

\[ \text{C}_{12}\text{H}_{20}\text{O}_{11} \text{ (δ-lactone)} \]

\[ M, 340.3 \]

DEFINITION

Mixture in variable proportions of 4-O-β-D-galactopyranosyl-D-gluconic acid and 4-O-β-D-galactopyranosyl-D-glucono-1,5-lactone.

Content: 98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: freely soluble in water, slightly soluble in glacial acetic acid, in anhydrous ethanol and in methanol.

mp: about 125 °C with decomposition.

IDENTIFICATION

A. Infrared absorption spectrophotometry (2.2.24).

Comparison: lactobionic acid CRS.

If the spectra obtained show differences, dissolve the substance to be examined and the reference substance separately in water \( R \), dry at 105 °C and record new spectra using the residues.

B. Thin-layer chromatography (2.2.27).

Test solution. Dissolve 10 mg of the substance to be examined in water \( R \) and dilute to 1 ml with the same solvent.

Reference solution. Dissolve 10 mg of lactobionic acid CRS in water \( R \) and dilute to 1 ml with the same solvent.

Plate: TLC silica gel plate \( R \).

Mobile phase: concentrated ammonia \( R1 \), ethyl acetate \( R \), water \( R \), methanol \( R \) (2:2:2:4 V/V/V/V).

Application: 5 µl.

Development: over 3/4 of the plate.

Detection: spray 3 times with ammonium molybdate solution \( R6 \) and heat in an oven at 110 °C for 15 min.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and colour to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution. The solution is clear (2.2.1) and not more intensely coloured than reference solution \( Y5 \) (2.2.2, Method II).

Dissolve 3.0 g in 25 ml of water \( R \).

Specific optical rotation (2.2.7): +23.0 to +29.0 (anhydrous substance).

Dissolve 1.0 g in 80 ml of water \( R \) and dilute to 100.0 ml with the same solvent. Allow to stand for 24 h.

Reducing sugars: maximum 0.2 per cent, calculated as glucose.

Dissolve 5.0 g in 25 ml of water \( R \) with the aid of gentle heat. Cool and add 20 ml of cupric citric solution \( R \) and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent \( V/V \) solution of glacial acetic acid \( R \) and 94 volumes of water \( R \) and, when the precipitate has dissolved, titrate the excess of iodine with 0.05 M sodium thiosulphate using 1 ml of starch solution \( R \), added towards the end of the titration, as indicator. Not less than 12.8 ml of 0.05 M sodium thiosulphate is required.

Heavy metals (2.4.8): maximum 20 ppm.

1.0 g complies with limit test E. Prepare the reference solution using 2 ml of lead standard solution (10 ppm Pb) \( R \).

Water (2.5.12): maximum 5.0 per cent, determined on 0.50 g. Use a mixture of 1 volume of formamide \( R \) and 2 volumes of methanol \( R \) as solvent.

Total ash (2.4.16): maximum 0.2 per cent.

ASSAY

Dissolve 0.350 g in 50 ml of carbon dioxide-free water \( R \), previously heated to 30 °C. Immediately titrate with 0.1 M sodium hydroxide and determine the 2 equivalence points potentiometrically (2.2.20).