Lactulose Solution

Lactulose Solution is solution of *4-O-β-D-galactopyranosyl-D-arabino-hex-2-ulofuranose* normally prepared by alkaline isomerisation of lactose. It may contain other sugars including lactose, epilactose, galactose, tagatose and fructose.

Lactulose Solution contains minimum 620 g per litre of lactulose and not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of lactulose, $C_{12}H_{22}O_{11}$. It may contain a suitable antimicrobial preservative.

Usual strength. 10 g per 15 ml.

Category. Osmotic laxative.

Identification

Test A may be omitted if tests B, C and D are carried out. Test B, may be omitted if tests A, C and D is carried out.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel*.

Mobile phase. A mixture of 10 volumes of *glacial acetic acid,* 15 volumes of a 5 per cent w/v solution of *boric acid,* 20 volumes of *methanol* and 55 volumes of *ethyl acetate.*

Test solution. Dilute 0.5 g of the substance under examination in *water* and dilute to 50.0 ml with the *water.*

Reference solution. A 0.6 per cent w/v solution of *lactulose IPRS* in *water.*

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate at 105 ° for 5 minutes and allow to cool. Spray with a 0.1 per cent w/v solution of *1,3dihydroxynaphthalene* in a mixture of 10 volumes of *sulphuric acid* and 90 volumes of *methanol.* Heat a 110 ° for 5 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (b).

C. Dissolve 100 mg in 10 ml of *water.* Add 3 ml of *cupri-tartaric solution* and heat. A red precipitate is formed.

D. Dissolve 250 mg in 5 ml of *water* and 5 ml of *ammonia*. Heat on a water-bath at 80° for 10 minutes. A red colour develops.

Tests

Solution A. 10 per cent w/v solution in *carbon dioxide-free water.*

Appearance of solution. Solution A is clear (2.4.1) and not more intensely coloured than reference solution BYS5 (2.4.1).

pH (2.4.24). 3.0 to 7.0, determined in 10 ml of solution A after addition of 0.1 ml of a saturated solution of *potassium chloride.*

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Mix 4.0 g of the substance under examination and 20 ml of *water.* Add 25.0 ml of *acetonitrile* with gentle heating and dilute to 50.0 ml with *water*.

Reference solution (a). Dilute 5.0 ml of the test solution with 47.5 ml of *acetonitrile* with gentle heating and dilute to 100.0 ml with *water*.

Reference solution (b). Dissolve 1.0 g of *lactulose IPRS* in 10 ml of *water.* Add 12.5 ml of *acetonitrile* with gentle heating and dilute to 25.0 ml with *water.*

Reference solution (c). Dissolve 65 mg of *fructose IPRS* (impurity D) in a mixture of equal volumes of *acetonitrile* and *water* and dilute to 100.0 ml with the mixture of equal volumes of *acetonitrile* and *water*.

Reference solution (d). A solution containing 0.5 per cent w/v, each of lactulose impurity *A, B, C, E, F, G and H* in reference solution (c).

Reference solution (e). Dilute 5.0 ml of reference solution (a) to 100.0 ml with a mixture of equal volumes of *acetonitrile* and *water.*

Chromatographic system

- a stainless steel column 5 cm x 4.6 mm, packed with aminopropylsilane bonded to porous silica (3 µm), with a series of another stainless steel column 15 cm x 4.6 mm, packed with aminopropylsilane bonded to porous silica (3 µm),
- column temperature: 38°,
- mobile phase: a mixture of 20 volumes of 0.0253 per cent w/v of *sodium dihydrogen phosphate* in *water*, and 80 volumes ml of *acetonitrile*,
- flow rate: 1 ml per minute,
- Differential refractometer maintained at a constant temperature 35°C.
- injection volume: 20 µl.

Name	Relative retention time
Lactulose Impurity F[1]	0.2
Lactulose Impurity E[2]	0.38
Lactulose Impurity D[3]	0.42
Lactulose Impurity B[4]	0.6
Lactulose Impurity G*	0.8
Lactulose Impurity A[5]	0.9
Lactulose (Retention time: about 18 minutes)	1.0
Lactulose Impurity C[6]	1.2
Lactulose Impurity H*	1.5

[1](2Ξ,4Ξ)-2-(hydroxymethyl) oxolane-2,4-diol,
[2]D-lyxo-hex-2-ulopyranose (tagatose),
[3]D-arabino-hex-2-ulopyranose (fructose),
[4]D-galactopyranose (galactose),
[5]4-O-β-D-galactopyranosyl-D-mannopyranose (epilactose),
[6]4-O-β-D-galactopyranosyl-D-glucopyranose (lactose),
*Unknown structure

Inject the reference solution (d). The test is not valid unless the peak-to-valley ratio is not less than 5.0, where Hp is the height above the baseline of the peak due to lactulose impurity A and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to Lactulose.

Inject reference solutions (a), (e) and the test solution. Run the chromatogram 2 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding to lactulose impurity B is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (15.0 per cent), the area of any peak corresponding to lactulose impurities A and C, each of, is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (10.0 per cent), the area of any peak corresponding to lactulose impurities E and F, each of, is not more than 0.8 times the area of the principal in the chromatogram obtained with reference solution (a) (4.0 per cent), the area of any peak corresponding to lactulose impurities G and H each of impurity is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent), the area of any peak corresponding to lactulose impurity D is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). The area of any other secondary peak is not more 0.1 times than the area of principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), and the sum of the areas of all the secondary peaks other than lactulose impurities B and C is not more than 2.4 times the area of principal peak in the chromatogram obtained with the reference solution (a) (12 per cent). Ignore any peak with an area less than the area of principal peak in the chromatogram obtained with reference solution (e) (0.25 per cent).

Methanol. Not more than 30 ppm.

Determine by Head-space gas chromatography (2.4.13).

Internal standard solution. Mix 0.5 ml of *propanol* with 100.0 ml of *water.* Dilute 1.0 ml of this solution to 100.0 ml with *water.* Dilute 5.0 ml of the solution to 50.0 ml with *water.*

Test solution. To 130 mg of the substance under examination in a 20 ml vial, add 1.0 ml of the internal standard solution and 5 µl of a 0.1 per cent v/v solution of *methanol.*

Reference solution. To 1.0 ml of the internal standard solution in a 20 ml vial, add 5 µl of a 0.1 per cent v/v solution of *methanol.*

Chromatographic system

- a glass column 2 m x 2 mm, packed with *ethylvinylbenzene-divinylbenzene copolymer* (180 mm),
- column temperature: 140°,
- inlet port at 200° and detector at 220°,

- flame ionization detector,
- flow rate: 30 ml per minute, helium as the carrier gas.

Head space conditions

- equilibration temperature: 60°,
- equilibration time 1 hour,
- pressurisation time: 1 minute.

Inject l ml of the reference solution and the test solution (gaseous phase).

Calculate the content of methanol, taking its density at 20° to be 0.79 g per ml and using the ratio of the area of the peak due to methanol to the area of the peak due to the internal standard in the chromatogram obtained with the reference solution and the ratio of the area of the peak due to methanol to the area of the peak due to the internal standard in the chromatogram obtained with the test solution.

Sulphites. Not more than 30 ppm.

Mix 5.0 g with 40 ml of *water*, add 2.0 ml of 0.1 M *sodium hydroxide* and dilute to 100 ml with *water*. To 10.0 ml of this solution, add 1.0 ml of *hydrochloric acid*, 2.0 ml of *decolorised fuchsin solution* and 2.0 ml of a 0.5 per cent v/v solution of *formaldehyde*. Allow to stand for 30 minutes and measure the absorbance (2.4.7) at 583 nm using as the compensation liquid a solution prepared at the same time and in the same manner with 10.0 ml of *water* instead of the solution of the substance under examination. The absorbance is not greater than that of a reference solution prepared at the same time and in the same manner using 10.0 ml of *sulphite reference solution (1.5 ppm SO_2)* instead of the solution of the substance to be examined.

Boron. Not more than 5 ppm.

NOTE-Avoid where possible the use of glassware.

Reference solution. Dissolve 56 mg of *boric acid* in 100.0 ml of *water.* Dilute 5.0 ml of this solution to 100.0 ml with *water.*

NOTE-Keep in a well closed polyethylene container.

Place separately 4 polyethylene 25 ml flasks. In first polyethylene 25-ml flask, mix 1.0 g of the substance under examination with 1.0 ml of *water* (solution A), in second polyethylene 25-ml flask, mix 1.0 g of the substance under examination with 1.0 ml of the reference solution (solution B), in third polyethylene 25 ml flask, mix 1.0 ml of the reference solution with 1.0 ml of *water* (solution C) and in fourth polyethylene 25 ml flask, take 2.0 ml of *water* (solution D).

To each flask, add 4.0 ml of *acetate edetate buffer solution pH 5.5.* Mix and add 4.0 ml of freshly prepared *azomethine H solution.* Mix and allow to stand for 1 hour. Measure the absorbance of solutions A, B and C at 420 nm (2.4.7), using solution D as the compensation liquid. The test is not valid unless the absorbance of solution C is not less than that 0.25. The absorbance of solution B is not less than twice that of solution A.

Sulphated ash (2.3.18). Not more than 0.2 per cent, determined on 1.5 g and calculated with reference to the declared content of lactulose.

Microbial Contamination (2.2.9). Total aerobic viable count not more than 100 CFU per g, total yeast and mold count not more than 10 CFU per g, 1 g is free from *Escherichia coli.*

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances with the following modifications.

Inject the reference solution (b). The test is not valid unless the tailing factor is not less than 0.6 and not more than 2.0 for the principal peak.

Injection reference solution (b) and Test solution.

Calculate the content of $C_{12}H_{22}O_{11}$ in Lactulose solution.

Storage. Store protected from moisture, preferably at a temperature between 2^0 and 30^0 avoid subfreezing temperatures.

Solubility (2.4.26). Miscible with *water*. It may be a supersaturated solution or may contain crystals that disappear on heating. A 10 per cent v/v solution is laevorotatory.