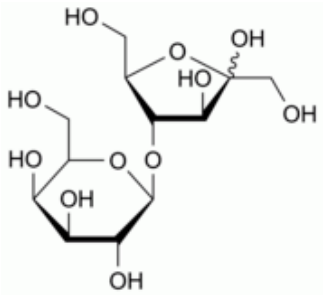
Edition: BP 2025 (Ph. Eur. 11.6 update)

Lactulose

General Notices

(Ph. Eur. monograph 1230)



$C_{12}H_{22}O_{11}$ 342.3 *4618-18-2*

Action and use

Osmotic laxative.

Preparation

Lactulose Oral Powder

Ph Eur

DEFINITION

4-*O*-β-D-Galactopyranosyl-D-*arabino*-hex-2-ulofuranose.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, sparingly soluble in methanol, practically insoluble in toluene.

mp

About 168 °C.

IDENTIFICATION

First identification: B, C, D, E.

Second identification: A, C, D, E.

A. Thin-layer chromatography (2.2.27).

Test solution Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 50.0 mg of *lactulose CRS* in *water R* and dilute to 10.0 mL with the same solvent.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R*, 50 g/L solution of *boric acid R*, *methanol R*, *ethyl acetate R* (10:15:20:55 *V/V/V/V*).

Application 2 µL.

Development Over 3/4 of the plate.

Drying At 100-105 °C for 5 min; allow to cool.

Detection Spray with a 1.0 g/L solution of *1,3-dihydroxynaphthalene R* in a mixture of 10 volumes of *sulfuric acid R* and 90 volumes of *methanol R*; heat at 110 °C for 5 min.

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 the reference solution.

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (b).

C. Dissolve 50 mg in 10 mL of *water R*. Add 3 mL of *cupri-tartaric solution R* and heat. A red precipitate is formed.
D. Dissolve 0.125 g in 5 mL of *water R*. Add 5 mL of *ammonia R*. Heat in a water-bath at 80 °C for 10 min. A red colour develops.
E. Specific optical rotation (see Tests).

TESTS

Solution S

Dissolve 3.0 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution BY_5 (*2.2.2, Method II*).

pH (*2.2.3*)

3.0 to 7.0.

To 10 mL of solution S add 0.1 mL of a saturated solution of *potassium chloride R*.

Specific optical rotation (*2.2.7*)

-50.0 to -46.0 (anhydrous substance).

Dissolve 1.25 g in *water R*, add 0.2 mL of *concentrated ammonia R* and dilute to 25.0 mL with *water R*.

Related substances

Liquid chromatography (*2.2.29*).

Test solution Dissolve 1.00 g of the substance to be examined in 10 mL of *water R*. Add 12.5 mL of *acetonitrile R* with gentle heating and dilute to 25.0 mL with *water R*.

Reference solution (a) To 3.0 mL of the test solution add 48.5 mL of *acetonitrile R* with gentle heating and dilute to 100.0 mL with *water R*.

Reference solution (b) Dissolve 1.00 g of *lactulose CRS* in 10 mL of *water R*. Add 12.5 mL of *acetonitrile R* with gentle heating and dilute to 25.0 mL with *water R*.

Reference solution (c) Dissolve 10 mg of *lactulose R*, 10 mg of *epilactose R* (impurity A) and 10 mg of *lactose monohydrate R* (impurity C) in 2 mL of *water R*. Add 2.5 mL of *acetonitrile R* with gentle heating and dilute to 5 mL with *water R*.

Reference solution (d) To 5.0 mL of the test solution add 47.5 mL of *acetonitrile R* with gentle heating and dilute to 100.0 mL with *water R*. Dilute 5.0 mL of this solution to 100.0 mL with a mixture of equal volumes of *acetonitrile R* and *water R*.

Column 1:

— *size*: *l* = 0.05 m, Ø = 4.6 mm;

— *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 38 ± 1 °C.

Column 2:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 38 ± 1 °C.

Columns 1 and 2 are coupled in series.

Mobile phase Dissolve 0.253 g of *sodium dihydrogen phosphate R* in 200 mL of *water for chromatography R* and dilute to 1000 mL with *acetonitrile R*.

Flow rate 1.0 mL/min.

Detection Differential refractometer maintained at a constant temperature (e.g. 35 °C).

Injection 20 µL of the test solution and reference solutions (a), (c) and (d).

Run time Twice the retention time of lactulose.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and C.

Relative retention With reference to lactulose (retention time = about 18 min): impurity A = about 0.9; impurity C = about 1.2.

System suitability Reference solution (c):

— *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to lactulose.

Limits:

— *impurity C*: not more than the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (3.0 per cent);

— *unspecified impurities*: for each impurity, not more than twice the area of the peak due to lactulose in the chromatogram obtained with reference solution (d) (0.5 per cent);

— *total*: not more than the area of the peak due to lactulose in the chromatogram obtained with reference solution (a) (3.0 per cent);

— *disregard limit*: the area of the peak due to lactulose in the chromatogram obtained with reference solution (d) (0.25 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Methanol

Head-space gas chromatography (*2.2.28*).

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

Test solution To 79 mg of the substance to be examined 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 R*.

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 R*.

Column:

— *size*: *l* = 2 m, Ø = 2 mm;

— *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (180 µm).

Carrier gas *helium for chromatography R*.

Flow rate 30 mL/min.

Static head-space conditions that may be used:

— *equilibration temperature*: 60 °C;

— *equilibration time*: 1 h;

— *pressurisation time*: 1 min.

Temperature:

— *column*: 140 °C;

— *injection port*: 200 °C;

— *detector*: 220 °C.

Detection Flame ionisation.

Injection 1 mL of the gaseous phase.

Calculate the content of methanol, taking its density (*2.2.5*) at 20 °C to be 0.79 g/mL.

Limit:

— *methanol*: calculate the ratio (*R*) 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; calculate 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: this ratio is not greater than 2*R* (50 ppm).

Boron

Maximum 9 ppm.

Avoid where possible the use of glassware.

Reference solution Dissolve 50.0 mg of *boric acid R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with *water R*. *Keep in a well-closed polyethylene container*.

In 4 polyethylene 25 mL flasks, place separately:

— 0.50 g of the substance to be examined dissolved in 2.0 mL of *water R* (solution A);

— 0.50 g of the substance to be examined dissolved in 1.0 mL of the reference solution and 1.0 mL of *water R* (solution B);

— 1.0 mL of the reference solution and 1.0 mL of *water R* (solution C);

— 2.0 mL of *water R* (solution D).

To each flask add 4.0 mL of *acetate-edetate buffer solution pH 5.5 R*. Mix and add 4.0 mL of freshly prepared *azomethine H solution R*. Mix and allow to stand for 1 h. Measure the absorbance (*2.2.25*) of solutions A, B and C at 420 nm, using solution D as the compensation liquid. The test is not valid unless the absorbance of solution C is at least 0.25. The absorbance of solution B is not less than twice that of solution A.

Water (*2.5.12*)

Maximum 2.5 per cent, determined on 0.500 g.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

Microbial contamination

TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (2.6.13).

ASSAY

Liquid chromatography (2.2.29) as described in the test for related substances with the following modifications.

Injection Test solution and reference solution (b).

System suitability Reference solution (b):

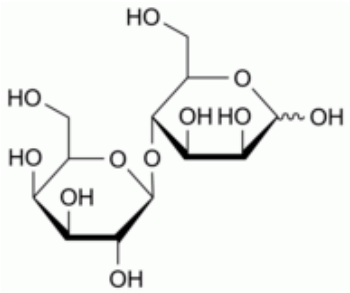
— *symmetry factor*: 0.6 to 2.0 for the principal peak.

Calculate the percentage content of $C_{12}H_{22}O_{11}$ taking into account the assigned content of *lactulose CRS*.

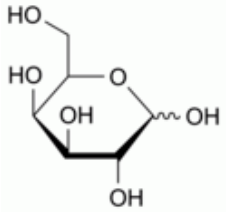
IMPURITIES

Specified impurities *C.*

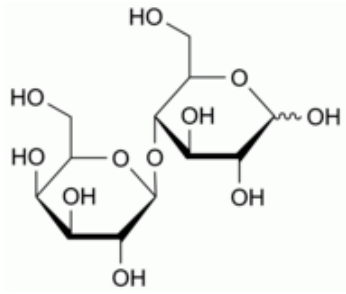
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for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



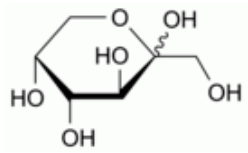
A. 4-*O*-β-D-galactopyranosyl-D-mannopyranose (epilactose),



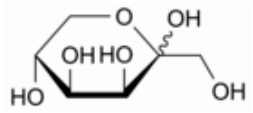
B. D-galactopyranose (galactose),



C. 4-*O*-β-D-galactopyranosyl-D-glucopyranose (lactose),



D. D-*arabino*-hex-2-ulopyranose (fructose),



E. D-*lyxo*-hex-2-ulopyranose (tagatose).

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