

01/2005:0187

**Bulk and tapped density (2.9.15).** Determine the bulk density and the tapped density. Calculate the Hausner index using the following expression:

$$\frac{V_0}{V_f}$$

$V_0$  = volume of bulk substance,

$V_f$  = volume of tapped substance.

**$\alpha$ -Lactose and  $\beta$ -lactose.** Gas chromatography (2.2.28).

**Silylation reagent.** Mix 28 volumes of *N*-trimethylsilylimidazole R and 72 volumes of pyridine R.

**Test solution.** Dissolve about 1 mg of the substance to be examined in 0.45 ml of dimethyl sulphoxide R. Add 1.8 ml of the silylation reagent. Mix gently and allow to stand for 20 min.

**Reference solution.** Prepare a mixture of  $\alpha$ -lactose monohydrate R and  $\beta$ -lactose R having an anomeric ratio of about 1:1 based on the labelled anomeric contents of the  $\alpha$ -lactose monohydrate and  $\beta$ -lactose. Dissolve about 1 mg of this mixture in 0.45 ml of dimethyl sulphoxide R. Add 1.8 ml of the silylation reagent. Mix gently and allow to stand for 20 min.

**Column:**

- material: glass,
- size:  $l = 0.9$  m,  $\varnothing = 4$  mm,
- stationary phase: silanised diatomaceous earth for gas chromatography R impregnated with 3 per cent *m/m* of poly[(cyanopropyl)(methyl)][(phenyl)(methyl)] siloxane R.

**Carrier gas:** helium for chromatography R.

**Flow rate:** 40 ml/min.

**Temperature:**

- column: 215 °C,
- injection port and detector: 275 °C.

**Detection:** flame ionisation.

**Injection:** 2  $\mu$ l.

**System suitability:** reference solution:

- relative retention with reference to  $\beta$ -lactose:  $\alpha$ -lactose = about 0.7,
- resolution: minimum 3.0 between the peaks due to  $\alpha$ -lactose and  $\beta$ -lactose.

Calculate the percentage content of  $\alpha$ -lactose from the expression:

$$\frac{100S_a}{S_a + S_b}$$

Calculate the percentage content of  $\beta$ -lactose from the expression:

$$\frac{100S_b}{S_a + S_b}$$

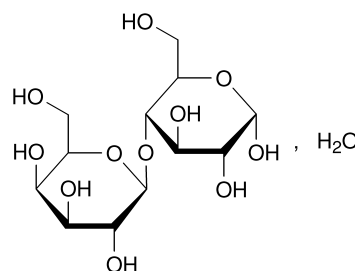
$S_a$  = area of the peak due to  $\alpha$ -lactose,

$S_b$  = area of the peak due to  $\beta$ -lactose.

**Loss on drying (2.2.32).** Determine on 1.000 g by drying in an oven at 80 °C for 2 h.

## LACTOSE MONOHYDRATE

Lactosum monohydricum



$C_{12}H_{22}O_{11} \cdot H_2O$

$M_r$  360.3

### DEFINITION

Lactose monohydrate is the monohydrate of *O*- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -D-glucopyranose.

### CHARACTERS

A white or almost white, crystalline powder, freely but slowly soluble in water, practically insoluble in ethanol (96 per cent).

### IDENTIFICATION

**First identification:** A, D.

**Second identification:** B, C, D.

A. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with lactose CRS.

B. Examine by thin-layer chromatography (2.2.27), using a TLC silica gel G plate R.

**Test solution.** Dissolve 10 mg of the substance to be examined in a mixture of 2 volumes of water R and 3 volumes of methanol R and dilute to 20 ml with the same mixture of solvents.

**Reference solution (a).** Dissolve 10 mg of lactose CRS in a mixture of 2 volumes of water R and 3 volumes of methanol R and dilute to 20 ml with the same mixture of solvents.

**Reference solution (b).** Dissolve 10 mg each of fructose CRS, glucose CRS, lactose CRS and sucrose CRS in a mixture of 2 volumes of water R and 3 volumes of methanol R and dilute to 20 ml with the same mixture of solvents.

Apply separately to the plate 2  $\mu$ l of each solution and thoroughly dry the starting points. Develop over a path of 15 cm using a mixture of 10 volumes of water R, 15 volumes of methanol R, 25 volumes of glacial acetic acid R and 50 volumes of ethylene chloride R, measured accurately since a slight excess of water produces cloudiness. Dry the plate in a current of warm air. Repeat the development immediately, after renewing the mobile phase. Dry the plate in a current of warm air and spray evenly with a solution of 0.5 g of thymol R in a mixture of 5 ml of sulphuric acid R and 95 ml of alcohol R. Heat at 130 °C for 10 min. 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). The test is not valid unless the chromatogram obtained with reference solution (b) shows 4 clearly separated spots.

C. Dissolve 0.25 g in 5 ml of *water R*. Add 5 ml of *ammonia R* and heat in a water-bath at 80 °C for 10 min. A red colour develops.

D. It complies with the test for water (see Tests).

#### TESTS

**Appearance of solution.** Dissolve 1.0 g in boiling *water R*, dilute to 10 ml with the same solvent. The solution is clear (2.2.1) and not more intensely coloured than reference solution BY<sub>7</sub> (2.2.2, *Method II*).

**Acidity or alkalinity.** Dissolve 6.0 g by heating in 25 ml of *carbon dioxide-free water R*, cool and add 0.3 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.4 ml of 0.1 M *sodium hydroxide* is required to change the colour of the indicator to pink.

**Specific optical rotation** (2.2.7). Dissolve 10.0 g in 80 ml of *water R*, heating to 50 °C. Allow to cool and add 0.2 ml of *dilute ammonia R1*. Allow to stand for 30 min and dilute to 100.0 ml with *water R*. The specific optical rotation is + 54.4 to + 55.9, calculated with reference to the anhydrous substance.

**Absorbance** (2.2.25). Dissolve 1.0 g in boiling *water R* and dilute to 10.0 ml with the same solvent (solution A). The absorbance of the solution measured at 400 nm is not greater than 0.04. Dilute 1.0 ml of solution A to 10.0 ml with *water R*. Examine the solution from 210 nm to 300 nm. At wavelengths from 210 nm to 220 nm, the absorbance is not greater than 0.25. At wavelengths from 270 nm to 300 nm, the absorbance is not greater than 0.07.

**Heavy metals** (2.4.8). Dissolve 4.0 g in *water R* with warming, add 1 ml of 0.1 M *hydrochloric acid* and dilute to 20 ml with *water R*. 12 ml of the solution complies with limit test A for heavy metals (5 ppm). Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

**Water** (2.5.12): 4.5 per cent to 5.5 per cent, determined on 0.50 g by the semi-micro determination of water, using a mixture of 1 volume of *formamide R* and 2 volumes of *methanol R* as the solvent.

**Sulphated ash.** Not more than 0.1 per cent. To 1.0 g add 1 ml of *sulphuric acid R*, evaporate to dryness on a water-bath and ignite to constant mass.

**Microbial contamination.** Total viable aerobic count (2.6.12) not more than 10<sup>2</sup> micro-organisms per gram, determined by plate-count. It complies with the test for *Escherichia coli* (2.6.13).

#### STORAGE

In an airtight container.

#### FUNCTIONALITY-RELATED CHARACTERISTICS

*The following test are not mandatory requirements but in view of their known importance for achieving consistency in manufacture, quality and performance of medicinal products, it is recommended that suppliers should verify these characteristics and provide information on the results and analytical method applied to users. The methods indicated below have been found suitable however, other methods may be used.*

*Lactose monohydrate is predominantly used as a filler/diluent in solid dosage forms (compressed and powder). The following characteristics are relevant for this type of application.*

**Particle size distribution.** Determine by laser diffraction or sieve analysis.

**Bulk and tapped density** (2.9.15). Determine the bulk density and the tapped density. Calculate the Hausner Index using the following expression:

$$\frac{V_0}{V_f}$$

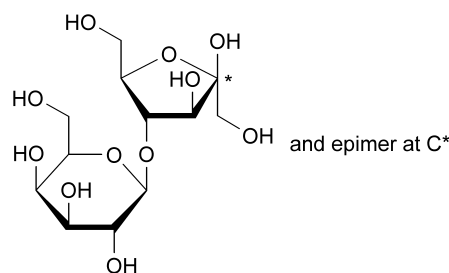
$V_0$  = volume of bulk substance,

$V_f$  = volume of tapped substance.

01/2005:1230

## LACTULOSE

### Lactulosum



C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>

$M_r$  342.3

#### DEFINITION

Lactulose contains not less than 95.0 per cent and not more than the equivalent of 102.0 per cent of 4-*O*-(β-D-galactopyranosyl)-D-arabino-hex-2-ulofuranose, calculated with reference to the anhydrous substance.

#### CHARACTERS

A white or almost white, crystalline powder, freely soluble in water, sparingly soluble in methanol, practically insoluble in toluene.

It melts at about 168 °C.

#### IDENTIFICATION

*First identification:* B, C, D, E.

*Second identification:* A, C, D, E.

A. Examine by thin-layer chromatography (2.2.27), using *silica gel G R* as the coating substance.

*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.

Apply separately to the plate 2 μl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *glacial acetic acid R*, 15 volumes of a 50 g/l solution of *boric acid R*, 20 volumes of *methanol R* and 55 volumes of *ethyl acetate R*. Dry the plate at 100-105 °C for 5 min and allow to cool. Spray the plate with a 1.0 g/l solution of *1,3-dihydroxynaphthalene R* in a mixture of 10 volumes of *sulphuric acid R* and 90 volumes of *methanol R*. Heat the plate at 110 °C for 5 min. 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.