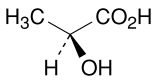
**01/2008:1771**

# (*S*)-LACTIC ACID

## Acidum (*S*)-lacticum



$C_3H_6O_3$ $M_r$ 90.1

DEFINITION

Mixture of (*S*)-2-hydroxypropanoic acid, its condensation products, such as lactoyl-lactic acid and polylactic acids, and water. The equilibrium between lactic acid and polylactic acids depends on the concentration and temperature.

*Content*: 88.0 per cent *m/m* to 92.0 per cent *m/m* of $C_3H_6O_3$, not less than 95.0 per cent of which is the (*S*)-enantiomer.

CHARACTERS

*Appearance*: colourless or slightly yellow, syrupy liquid.

*Solubility*: miscible with water and with ethanol (96 per cent).

IDENTIFICATION

A. Dissolve 1 g in 10 ml of *water R*. The solution is strongly acidic (*2.2.4*).

B. Relative density (*2.2.5*): 1.20 to 1.21.

C. It gives the reaction of lactates (*2.3.1*).

D. It complies with the limits of the assay.

TESTS

**Solution S**. Dissolve 5.0 g in 42 ml of *1 M sodium hydroxide* and dilute to 50 ml with *distilled water R*.

**Appearance**. The substance to be examined is not more intensely coloured than reference solution $Y_6$ (*2.2.2, Method II*).

**Ether-insoluble substances**. Dissolve 1.0 g in 25 ml of *ether R*. The solution is not more opalescent than the solvent used for the test.

**Sugars and other reducing substances**. To 1 ml of solution S add 1 ml of *1 M hydrochloric acid*, heat to boiling, allow to cool and add 1.5 ml of *1 M sodium hydroxide* and 2 ml of *cupri-tartaric solution R*. Heat to boiling. No red or greenish precipitate is formed.

**Methanol** (*2.4.24*): maximum 50 ppm, if intended for use in the manufacture of parenteral dosage forms.

**Citric, oxalic and phosphoric acids**. To 5 ml of solution S add *dilute ammonia R1* until slightly alkaline (*2.2.4*). Add 1 ml of *calcium chloride solution R*. Heat on a water-bath for 5 min. Both before and after heating, any opalescence in the solution is not more intense than that in a mixture of 1 ml of *water R* and 5 ml of solution S.

**Sulphates** (*2.4.13*): maximum 200 ppm.

Dilute 7.5 ml of solution S to 15 ml with *distilled water R*.

**Calcium** (*2.4.3*): maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

**Heavy metals** (*2.4.8*): maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

**Sulphated ash** (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

**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 $C_3H_6O_3$.

**(*S*)-enantiomer**

Transfer an amount of the substance to be examined equivalent to 2.00 g of lactic acid into a round-bottomed flask, add 25 ml of *1 M sodium hydroxide* and boil gently for 15 min. Cool down and adjust to pH 7.0 using *1 M hydrochloric acid*. Add 5.0 g of *ammonium molybdate R*, dissolve and dilute to 50.0 ml with *water R*. Filter and measure the angle of optical rotation (*2.2.7*). Calculate the percentage content of (*S*)-enantiomer using the expression:

$$50 + \left(24.18 \times \alpha \times \frac{2.222}{m} \times \frac{90}{c}\right)$$

$\alpha$ = angle of optical rotation (absolute value),

$m$ = mass of the substance to be examined, in grams,

$c$ = percentage content of $C_3H_6O_3$ in the substance to be examined.

The complex of (*S*)-lactic acid formed under these test conditions is laevorotatory.

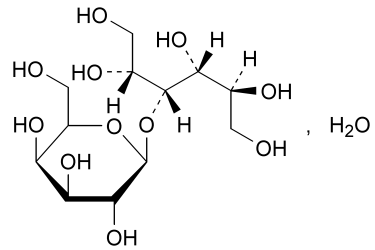
LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

**01/2008:1337**
**corrected 6.0**

# LACTITOL MONOHYDRATE

## Lactitolum monohydricum



$C_{12}H_{24}O_{11},H_2O$ $M_r$ 362.3
[81025-04-9]

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.*

*Mobile phase*: *water R, acetonitrile R* (25:75 *V/V*).

*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 *sodium periodate 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 $BY_7$ (*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* 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, Ø = 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 $10^3$ 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.

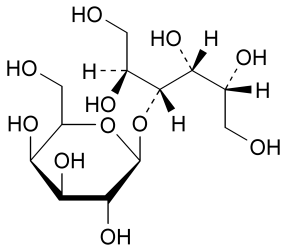
*Injection*: test solution (b) and reference solution (a).

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

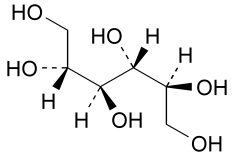
*Specified impurities: A, B, C, D, E.*

A. lactose,



B. lactulitol,

C. mannitol,



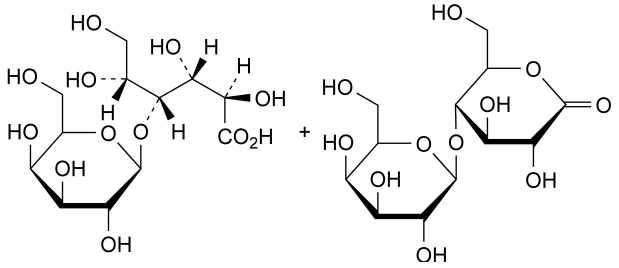
D. dulcitol (galactitol),

E. sorbitol.

**01/2008:1647**

# LACTOBIONIC ACID

## Acidum lactobionicum



$C_{12}H_{22}O_{12}$ (acid form) $M_r$ 358.3
[96-82-2]

$C_{12}H_{20}O_{11}$ (δ-lactone) $M_r$ 340.3
[5965-65-1]

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 $Y_5$ (*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 *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* 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*).