PHARMACOPEIAL DISCUSSION GROUP

CORRECTION 1

E23: LACTOSE, ANHYDROUS

Correction to Rev. 4 signed on 26 October 2016

	Harmonized attributes		
	EP	JP	USP
Definition	+	+	+
Identification (IR)	+	+	+
Identification (TLC)	+(1)	-	+
Clarity and colour of solution	+	+(2)	+
Specific optical rotation	+	+	+
Acidity or alkalinity	+	+	+
Loss on drying	+(3)	+	+
Water	+	+	+
Content of alpha and beta anomers	+(4)	+	–
Residue on ignition	+	+	+
Protein and light-absorbing impurities	+	+	+
Microbial limits (TAMC, E.coli)	+	+	+
Microbial limits (TYMC)	–	+	+

(1) In EP, the identification by TLC is included in the second series of identification.
(2) In JP, reference suspension I is not used to evaluate the opalescence of the solution in the test for clarity and colour of solution. Each pharmacopeia has similar but minor difference in the acceptance criteria.
(3) & (4) In EP, "Contents of alpha and beta anomers" and "Loss on drying" are included in the non-mandatory FRC section. EP will not stipulate the specification for Loss on drying.

Legend: + will adopt and implement; – will not stipulate

Non-harmonised attributes
Characters/Description, Labeling, Packaging and storage

Local requirements

EP	JP	USP
Identification (water), Second identification (TLC, colour	Microbial limits: *Salmonella*; Heavy metals; Content of	Content of alpha and beta anomers (USP requires a limit

reaction, water); FRC (particle-size distribution, Bulk and tapped density, Alpha- and beta-lactose, Loss on drying)	alpha and beta anomers (System Repeatability)	to be stated on the label, where needed)

Reagents and reference materials
Each pharmacopeia will adapt the text to take account of local reference materials and reagent specifications.

European Pharmacopoeia

Signature: Date

2/10/19

Japanese Pharmacopoeia

Signature: Date

Oct 2nd, 2019

United States Pharmacopeia

Signature: Date

02-OCT-2019

E-23 ANHYDROUS LACTOSE

Correction 1 to Rev. 4, Stage 3B

Anhydrous Lactose is *O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose (β-lactose), or a mixture of *O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose and *O*-β-D-galactopyranosyl-(1→4)-α-D-glucopyranose (α-lactose).

Identification

A. Infrared absorption spectrophotometry

Record the infrared absorption spectrum of anhydrous lactose and compare with the Reference Spectrum or the spectrum obtained with the Reference Standard: the transmission minima correspond in position and relative size.

B. Thin-layer chromatography

Adsorbent: 0.25-mm layer of chromatographic silica gel

Diluent: Methanol and water (3:2)

Standard solution A: 0.5 mg/mL of Anhydrous Lactose RS in *Diluent*

Standard solution B: Contains 0.5 mg/mL of _Dextrose RS, 0.5 mg/mL of Anhydrous Lactose RS, 0.5 mg/mL of Fructose RS, and 0.5 mg/mL of Sucrose RS in *Diluent*

Sample solution: 0.5 mg/mL of Anhydrous Lactose in *Diluent*

Application volume: 2 µL

Developing solvent system: Ethylene dichloride, glacial acetic acid, methanol, and water (10:5:3:2)

Spray reagent: 5 mg/mL of thymol in a mixture of alcohol and sulfuric acid (19:1)

Analysis Samples:

Standard solution A, *Standard solution B*, and *Sample solution*

Allow the spots to dry, and develop the plate in a paper-lined chromatographic chamber equilibrated with the *Developing solvent system* for about 1 h prior to use. Allow the chromatogram to develop until the solvent front has moved about three-quarters of the length of the plate. Remove the plate from the chamber, dry in a current of warm air, and redevelop the plate in fresh *Developing solvent system*. Remove the plate from the chamber, mark the solvent front, and dry the plate in a current of warm air. Spray the plate evenly with *Spray reagent*. Heat the plate at 130° for 10 min.

System suitability:

The test is not valid unless *Standard solution B* shows four clearly discernible spots, disregarding any spots at the origin.

Acceptance criteria:

The principal spot from the *Sample solution* corresponds in appearance and *RF* value to that from *Standard solution A*.

Clarity and color of solution

Dissolve 1 g in 10.0 mL of boiling water. Allow to cool.

The solution is clear and nearly colorless: its clarity is the same as that of water or its opalescence is not more pronounced than that of reference suspension I, and it is not more coloured than the reference solution.

Primary solutions:

- Ferric chloride primary solution: a 45.0 g/L solution of ferric chloride ($FeCl_3$, $6H_2O$).
- Cobalt chloride primary solution: a 59.5 g/L solution of cobalt chloride ($CoCl_2$, $6H_2O$).
- Copper sulphate primary solution: a 62.4 g/L solution of copper sulphate ($CuSO_4$, $5H_2O$).

Reference solution:

To 2.5 mL of cobalt chloride primary solution, 6.0 mL of ferric chloride primary solution and 1.0 mL of copper sulphate primary solution, add hydrochloric acid (10 g/L HCl) to make 1000.0 mL.

Determine the absorbance of this solution at a wavelength of 400 nm. The absorbance divided

by the path length in centimeters is not more than 0.04.

Specific optical rotation - Dissolve 10 g by heating in 80 mL of water to 50 degrees. Allow to cool, and add 0.2 mL of 6 N ammonium hydroxide. Allow to stand for 30 minutes, and dilute with water to 100 mL: the specific rotation, calculated on the anhydrous basis, determined at 20 degrees, is between +54.4 degrees and +55.9 degrees.

Acidity or alkalinity - Dissolve 6 g by heating in 25 mL of carbon dioxide-free water, cool, and add 0.3 mL of a solution of phenolphthalein (1 g in 100 mL of alcohol): the solution is colorless, and not more than 0.4 mL of 0.1 N sodium hydroxide is required to produce a pink or red color.

Loss on drying - Dry it at 80° for 2 hours; it loses not more than 0.5% of its weight.

Residue on ignition - not more than 0.1%.

Water, Karl Fischer - not more than 1.0%, determined on a preparation containing anhydrous lactose in a mixture of methanol and formamide (2:1).

Protein and light-absorbing impurities - Measure the light absorption of a 1% (w/v) solution in the range of 210 to 300 nm. The absorbance divided by the path length in centimeters is not more than 0.25 in the range of 210 to 220 nm and is not more than 0.07 in the range of 270 to 300 nm.

Content of alpha and beta anomers - Gas chromatography.

Test solution. Introduce 10 mg of the substance to be examined in a vial with a screw cap. Add 4 ml of a mixture of 19.5 per cent of dimethyl sulfoxide, 22.0 per cent of trimethylsilylimidazole and 58.5 per cent of pyridine. Sonicate for 20 min at room temperature. Allow to cool. Transfer 400 µL to an injection vial. Add 1 mL of pyridine. Close the vial and mix well.

Reference solution. Prepare a mixture of alpha-lactose monohydrate and beta-lactose having an anomeric ratio of about 1:1 based on the labeled anomeric contents of the alpha-lactose monohydrate and the beta-lactose. Introduce 10 mg of this mixture in a vial with a screw cap. Add 4 ml of a mixture of 19.5 per cent of dimethyl sulfoxide, 22.0 per cent of

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trimethylsilylimidazole and 58.5 per cent of pyridine. Sonicate for 20 min at room temperature. Allow to cool. Transfer 400 µL to an injection vial. Add 1 mL of pyridine. Close the vial and mix well.

Pre-column:

— *material:* intermediate polarity deactivated fused-silica[1]

— *size:* l = 2 m, Ø = 0.53 mm,

[1] *Restek Guard column is suitable.*

Column:

— *material:* fused-silica,

— *size:* l = 15 m, Ø = 0.25 mm,

— *stationary phase: poly(dimethyl)(95)(diphenyl)(5)siloxane (film thickness = 0.25µm)*[2]

[2] *Varian CP Sil 8 CB is suitable*

Carrier gas: helium for chromatography.

Temperature:

— *temperature program* as follows:

	Time (min)	Temperature (°C)
Column	0-1	80
	1-3	80 ->150
	3-15.5	150 ->300
	15.5-17.5	300
Injection port		275 or use cold-on column injection

Detector		325

Flow rate: 2.8 ml/min

Detection: flame-ionization.

Injection: 0.5 µL splitless or by cold on-column injection.

Relative retention with reference to beta-lactose (retention time = about 12 min): alpha-lactose = about 0.9.

System suitability: reference solution:

— *resolution:* minimum 3.0 between the peaks due to alpha-lactose and beta-lactose.

Calculate the percentage content of alpha-lactose from the following expression:

$$100\ S_a / (S_a + S_b)$$

Calculate the percentage content of beta-lactose from the following expression:

$$100\ S_b / (S_a + S_b)$$

S_a= area of the peak due to alpha-lactose

S_b =area of the peak due to beta-lactose

Microbial Limits (internationally harmonized methods) -

The total aerobic microbial count is NMT 10^2 cfu/g and the total combined molds and yeasts count is NMT 50 cfu/g. It meets the requirements of the test for absence of *Escherichia coli*.

REAGENTS

Hydrazine sulphate solution. Dissolve 1.0 g of hydrazine sulphate in water and dilute to 100.0 mL with the same solvent. Allow to stand for 4-6 h.

Hexamethylenetetramine solution. In a 100 mL ground-glass-stoppered flask, dissolve 2.5 g of hexamethylenetetramine in 25.0 mL of water.

Primary opalescent suspension (formazin suspension). To the hexamethylenetetramine solution in the flask add 25.0 mL of the hydrazine sulphate solution. Mix and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Standard of opalescence. Dilute 15.0 mL of the primary opalescent suspension to 1000.0 mL with water. This suspension is freshly prepared and may be stored for up to 24 h.

Reference suspension I. To 5.0 ml of standard of opalescence add 95.0 ml of water. Mix and shake before use.