**TRIBENOSIDE**

*Tribenosidum*

C₉₄H₇₂O₆<br>[(10310-32-4)]

*M, 478.6*

**DEFINITION**

Mixture of α- and β-anomers of ethyl 3,5,6-tri-O-benzyl-β-glucosuranoside.

**Content:** 96.0 per cent to 102.0 per cent.

**CHARACTERS**

**Appearance:** yellowish to pale yellow, clear, viscous liquid.

**Solubility:** practically insoluble in water, very soluble in acetone, in methanol and in methylene chloride.

**IDENTIFICATION**

Infrared absorption spectrophotometry (2.2.24).

**Preparation:** discs.

**Comparison:** tribenoside CRS.

**TESTS**

**Solution S.** Dissolve 4.00 g in methanol R and dilute to 20 ml with the same solvent.

**Appearance of solution.** Solution S is clear (2.2.1) and its absorbance (2.2.25) at 420 nm has a maximum of 0.10.

**Specific optical rotation (2.2.7):** –31.0 to –40.0.

Dilute 2.0 ml of solution S to 20.0 ml with methanol R.

**Related substances.** Liquid chromatography (2.2.29).

**Test solution (a).** Dissolve 1.000 g of the substance to be examined in a mixture of 5 volumes of water R and 95 volumes of acetonitrile R and dilute to 25.0 ml with the same mixture of solvents.

**Test solution (b).** Dissolve 50.0 mg of the substance to be examined in a mixture of 5 volumes of water R and 95 volumes of acetonitrile R and dilute to 50.0 ml with the same mixture of solvents.

**Reference solution (a).** Dissolve 25.0 mg of benzaldehyde R and 30.0 mg of tribenoside impurity A CRS to 100.0 ml with acetonitrile R. Introduce 20.0 ml of this solution into a 50 ml volumetric flask, add 2.5 ml of water R and dilute to 50.0 ml with acetonitrile R.

**Reference solution (b).** Dissolve 50.0 mg of tribenoside CRS in a mixture of 5 volumes of water R and 95 volumes of acetonitrile R and dilute to 50.0 ml with the same mixture of solvents.

**Reference solution (c).** Dissolve 12.0 mg of benzyl ether R in a mixture of 5 volumes of water R and 95 volumes of acetonitrile R and dilute to 100.0 ml with the same mixture of solvents.

**Column:**

- *size:* l = 0.15 m, Ø = 4.6 mm,
- *stationary phase:* octadecylsilyl silica gel for chromatography R (3 µm).

**Mobile phase:**

- mobile phase A: 0.1 per cent V/V solution of phosphoric acid R,
- mobile phase B: acetonitrile R,

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent V/V)</th>
<th>Mobile phase B (per cent V/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 40</td>
<td>55 → 10</td>
<td>45 → 90</td>
</tr>
<tr>
<td>40 - 55</td>
<td>10</td>
<td>90</td>
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<tr>
<td>55 - 56</td>
<td>10 → 55</td>
<td>90 → 45</td>
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<td>56 - 60</td>
<td>55</td>
<td>45</td>
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</tbody>
</table>

**Flow rate:** 1.3 ml/min.

**Detection:** spectrophotometer at 254 nm.

**Injection:** 20 µl; inject test solution (a) and reference solutions (a), (b) and (c).

**Relative retentions** with reference to the β-anomer of tribenoside (retention time = about 18 min):

- α-anomer = about 1.1; impurity C = about 0.2;
- impurity B = about 0.6; impurity D = about 0.8;
- impurity A = about 1.4.

**System suitability:** reference solution (b):

- resolution: minimum 3.0 between the peaks due to the α-anomer and to the β-anomer of tribenoside.
**Limits:**

- **impurity A:** not more than 1.7 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
- **impurity C:** not more than twice the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent); if the area of the peak due to impurity C in the chromatogram obtained with the test solution is greater than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.25 per cent), dilute the test solution to obtain an area equal to or smaller than the area of the peak in the chromatogram obtained with reference solution (a); calculate the content of impurity C taking into account the dilution factor;
- **impurity D:** not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent),
- **any other impurity:** not more than the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) (0.3 per cent),
- **total:** not more than 6.7 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) (2.0 per cent),
- **disregard limit:** 0.17 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Heavy metals** (2.4.8): maximum 20 ppm.

Dilute 5.0 ml of solution S to 20.0 ml with methanol R. 12 ml of the solution complies with limit test B. Prepare the standard using lead standard solution (1 ppm Pb) obtained by diluting lead standard solution (100 ppm Pb) R with methanol R.

**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 (b). Calculate the sum of the percentage contents of the α-anomer and the β-anomer of tribenoside.

**STORAGE**

Under nitrogen, in an airtight container.

**IMPURITIES**

A. \( R = \text{CH}_2\text{C}_6\text{H}_5 \): 3,5,6-tri-O-benzyl-1,2-O-(1-methylethylidene)-α-D-glucopyranose,

B. \( R = \text{H} \): 3,5-di-O-benzyl-1,2-O-(1-methylethylidene)-α-D-glucopyranose,

C. \( \text{C}_6\text{H}_5\text{CHO} \): benzaldehyde,

D. \( \text{C}_6\text{H}_5\text{CH}_2\text{O}\text{CH}_2\text{C}_6\text{H}_5 \): dibenzyl ether.

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**TRIBUTYL ACETYLCITRATE**

Tributyl acetylcitrate

\[
\text{C}_{20}\text{H}_{34}\text{O}_8
\]

\( M_r = 402.5 \)

[77-90-7]

**DEFINITION**

Tributyl 2-(acetyloxy)propane-1,2,3-tricarboxylate.

**Content:** 99.0 per cent to 101.0 per cent (anhydrous substance).

**CHARACTERS**

**Appearance:** clear, oily liquid.

**Solubility:** not miscible with water, miscible with alcohol and with methylene chloride.

**IDENTIFICATION**

Infrared absorption spectrophotometry (2.2.24).

**Comparison:** Ph. Eur. reference spectrum of tributyl acetylcitrate.

**TESTS**

**Appearance.** The substance to be examined is clear (2.2.1) and not more intensely coloured than reference solution BY (2.2.2, Method II).

**Acidity.** Dilute 10 g with 10 ml of previously neutralised alcohol R, add 0.5 ml of bromothymol blue solution R2. Not more than 0.3 ml of 0.1 M sodium hydroxide is required to change the colour of the indicator to blue.

**Refractive index** (2.2.6): 1.442 to 1.445.

**Related substances.** Gas chromatography (2.2.28).

**Test solution.** Dissolve 1.0 g of the substance to be examined in methylene chloride R and dilute to 20.0 ml with the same solvent.

**Reference solution (a).** Dissolve 50 mg of the substance to be examined and 50 mg of tributyl citrate R in methylene chloride R and dilute to 20.0 ml with the same solvent.

**Reference solution (b).** Dilute 1.0 ml of the test solution to 20.0 ml with methylene chloride R. Dilute 1.0 ml of this solution to 25.0 ml with methylene chloride R.

**Column:**

- **material:** fused silica,
- **size:** \( l = 30 \text{ m}, \theta = 0.53 \text{ mm} \),
- **stationary phase:** poly(cyanopropyl)(methyl)(phenyl)(methyl)siloxane R (film thickness 1.0 µm).

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

**Linear velocity:** 36 cm/s.

**Split ratio:** 1:20.

**Temperature:**

- **column:** 200 °C,
- **injection port and detector:** 250 °C.

**Detection:** flame ionisation.

**Injection:** 1 µl.

**Run time:** twice the retention time of tributyl acetylcitrate.