Plate: TLC silica gel plate R.
Mobile phase: glacial acetic acid R, hydrochloric acid R, acetonitrile R (1:3.5:45 V/V/V).
Application: 10 µl as bands.
Development: over 2/3 of the plate.
Drying: in a current of warm air until the odour of acetic acid is no longer perceptible.
Detection: spray with potassium iodobismuthate solution R and subsequently with a 5 g/l solution of sodium nitrite R.
System suitability: reference solution (b):
– the chromatogram shows 2 clearly visible and separated zones.
Limit: 
– impurity C: any zone due to impurity C is not more intense than the zone in the chromatogram obtained with reference solution (a) (0.5 per cent).

Related substances. Liquid chromatography (2.2.29).
Test solution. Dissolve 30.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.
Reference solution (a). Dissolve 6.0 mg of trospium impurity A CRS in the mobile phase and dilute to 20.0 ml with the mobile phase.
Reference solution (b). Dissolve 7.5 mg of trospium impurity B CRS in the mobile phase and dilute to 5.0 ml with the mobile phase.
Reference solution (c). Dilute a mixture of 0.3 ml of the test solution, 1.0 ml of reference solution (a) and 1.0 ml of reference solution (b) to 100.0 ml with the mobile phase.
Column:
– size: l = 0.25 m, Ø = 4.6 mm,
– stationary phase: end-capped octylsilyl silica gel for chromatography R (5 µm),
– temperature: 40 °C.
Mobile phase: mix 1 volume of triethylamine R and 3 volumes of phosphoric acid R with 700 volumes of water R and add 300 volumes of acetonitrile R.
Flow rate: 1 ml/min.
Detection: spectrophotometer at 215 nm.
Injection: 20 µl.
Run time: 3 times the retention time of trospium.
Relative retention with reference to trospium (retention time = about 10 min): impurity B = about 0.7; impurity A = about 1.9.
System suitability: reference solution (c):
– resolution: minimum 3 between the peaks due to impurity B and trospium.
Limits:
– impurity A: not more than 3 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent),
– impurity B: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent),
– any other impurity: for each impurity, not more than 0.2 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (c) (0.1 per cent),
– total: not more than twice the area of the peak due to impurity B in the chromatogram obtained with reference solution (c) (1.0 per cent),

Disregard limit: 0.1 times the area of the peak due to impurity B in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (2.2.32): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

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

ASSAY
Dissolve 0.300 g in 50 ml of water R. Titrate with 0.1 M silver nitrate, determining the end-point potentiometrically (2.2.20).
1 ml of 0.1 M silver nitrate is equivalent to 42.80 mg of C_{25}H_{30}ClNO_{3}.

STORAGE
Protected from light.

IMPURITIES
Specified impurities: A, B, C.

A. hydroxydiphenylacetic acid (benzilic acid),

B. (1R,3R,5S)-8-azabicyclo[3.2.1]oct-3-yl hydroxydiphenylacetate,

C. (1R,3R,5S)-3-hydroxyspiro[8-azoniabicyclo[3.2.1]octane-8,1′-pyrrolidinium].

01/2008:2133 corrected 6.0

TROXERUTIN

Troxerutinum

C_{33}H_{42}O_{19} M_r 743
**DEFINITION**
Mixture of O-hydroxyethylated derivatives of rutin containing minimum 80 per cent of 2-[3,4-bis(2-hydroxyethoxy)phenyl]-3-[[6-O-6-deoxy-α-L-mannopyranosyl]-13-O-glucopyranosyl(oxy)-5-hydroxy-7-(2-hydroxyethoxy)-4H-1-benzopyran-4-one (tris(hydroxyethyl)rutin).

**Content:** 95.0 per cent to 105.0 per cent (dried substance).

**CHARACTERS**
**Appearance:** yellowish-green, crystalline powder, hygroscopic.

**Solubility:** freely soluble in water, slightly soluble in ethanol (96 per cent) and practically insoluble in methylene chloride.

**IDENTIFICATION**
A. Infrared absorption spectrophotometry (2.2.24).

**Comparison:** troxerutin CRS.

B. Examine the chromatograms obtained in the test for composition.

**Results:** the principal peak in the chromatogram obtained with the test solution is similar in position and size to the principal peak in the chromatogram obtained with the reference solution (a).

**TESTS**
**Composition.** Liquid chromatography (2.2.29): use the normalisation procedure.

**Test solution.** Dissolve 10.0 mg of the substance to be examined in the mobile phase, if necessary using an ultrasonic bath and dilute to 10.0 ml with the mobile phase.

**Reference solution (a).** Dissolve 10.0 mg of troxerutin CRS in the mobile phase, if necessary using an ultrasonic bath and dilute to 10.0 ml with the mobile phase.

**Reference solution (b).** Dilute 1 ml of reference solution (a) to 10 ml with the mobile phase. Dilute 1 ml of this solution to 100 ml with the mobile phase.

**Column:**
- **size:** l = 0.25 m, Ø = 4.6 mm,
- **stationary phase:** end-capped octadecylsilyl silica gel for chromatography R (5 μm).

**Mobile phase:** mix 20 volumes of acetonitrile R and 80 volumes of a 15.6 g/l solution of sodium dihydrogen phosphate R adjusted to pH 4.4 with dilute phosphoric acid R or dilute sodium hydroxide solution R.

**Flow rate:** 0.5 ml/min.

**Detection:** spectrophotometer at 350 nm.

**Injection:** 10 μl.

**Run time:** twice the retention time of the main compound of troxerutin (tris(hydroxyethyl)rutin).

**Relative retention** with reference to tris(hydroxyethyl)rutin (retention time = about 25 min): tetrakis(hydroxyethyl)rutin = about 0.5; mono(hydroxyethyl)rutin = about 0.8; bis(hydroxyethyl)rutin = about 1.1.

**System suitability:** reference solution (a):
- **peak-to-valley ratio:** minimum 2.0, where \( H_v = \) height above the baseline of the peak due to bis(hydroxyethyl)rutin and \( H_l = \) height above the baseline of the lowest point of the curve separating this peak from the peak due to tris(hydroxyethyl)rutin;
- **signal-to-noise ratio:** minimum 10 for the principal peak in the chromatogram obtained with reference solution (b).

**Limits:**
- **principal peak:** minimum 80 per cent,
- **any other peak:** for each peak, maximum 5 per cent, except for 1 peak which can be maximum 10 per cent.

**Ethylene oxide.** Head-space gas chromatography (2.2.28).

**Test solution.** To 1.0 g of the substance to be examined in a vial, add 1.0 ml of water R. Mix to obtain a homogeneous solution. Heat at 70 °C for 45 min.

**Reference solution.** To 1.0 g of the substance to be examined in a vial, add 50 μl of ethylene oxide solution R4 and 950 μl of water R and close tightly. Mix to obtain a homogeneous solution. Heat at 70 °C for 45 min.

**Column:**
- **material:** fused silica,
- **size:** l = 30 m, Ø = 0.32 mm,
- **stationary phase:** poly(cyanopropyl)[7](phenyl)[7]methyl[86]siloxane R (film thickness 1 μm).

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

**Flow rate:** 1.1 ml/min.

**Static head-space conditions which may be used:**
- **equilibration temperature:** 80 °C,
- **equilibration time:** 45 min,
- **transfer line temperature:** 110 °C,
- **pressurisation time:** 2 min,
- **injection time:** 12 s.

**Temperature:**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>0 - 5</td>
</tr>
<tr>
<td>5 - 18</td>
<td>40 → 200</td>
</tr>
<tr>
<td>Injection port</td>
<td>150</td>
</tr>
<tr>
<td>Detector</td>
<td>250</td>
</tr>
</tbody>
</table>

**Detection:** flame ionisation.

**Injection:** 1.0 ml.

The peak due to ethylene oxide is identified by injecting solutions of ethylene oxide of increasing concentration.

Determine the content of ethylene oxide (ppm) in the substance to be examined using the following expression:

\[
\frac{A_1 \times m_1}{A_2 \times m_2} - \frac{A_1 \times m_3}{A_1 \times m_3}
\]

- **A_1** = area of the peak due to ethylene oxide in the chromatogram obtained with the test solution,
- **A_2** = area of the peak due to ethylene oxide in the chromatogram obtained with the reference solution,
- **m_1** = mass of ethylene oxide in the reference solution, in micrograms,
- **m_2** = mass of the substance to be examined in the test solution, in grams,
- **m_3** = mass of the substance to be examined in the reference solution, in grams.

**Limit:**
- **ethylene oxide:** maximum 1 ppm.

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

1.0 g complies with test F. Prepare the reference solution using 2 ml of lead standard solution (10 ppm Pb) R.

**Loss on drying (2.2.32):** maximum 5.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.
Sulphated ash (2.4.14): maximum 0.4 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.200 g in 100.0 ml of water R. Dilute 10.0 ml of this solution to 100.0 ml with water R. Dilute 0.5 ml of solution S to 10.0 ml with water R. Measure the absorbance (2.2.25) at the absorption maximum at 350 nm.

Calculate the percentage content of C_{33}H_{42}O_{19} taking the specific absorbance to be 250.

STORAGE
In an airtight container, protected from light.

01/2008:0694

TRYPSIN
Trypsinum

[9002-07-7]

DEFINITION
Trypsin is a proteolytic enzyme obtained by the activation of trypsinogen extracted from the pancreas of healthy mammals. It has an activity of not less than 0.5 microkatal per milligram, calculated with reference to the dried substance. In solution, it has maximum enzymic activity at pH 8; the activity is reversibly inhibited at pH 3, at which pH it is most stable.

PRODUCTION
The animals from which trypsin is derived must fulfil the requirements for the health of animals suitable for human consumption.

The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Histamine (2.6.10). Not more than 1 μg of histamine base per 0.2 microkatal of trypsin activity. Use a 10 g/l solution of the substance to be examined in 0.0015 M borate buffer solution pH 8.0 R inactivated by heating on a bath for 30 min. Carry out dilutions with a 9 g/l solution of sodium hydroxide R.

CHARACTERS
A white or almost white, crystalline or amorphous powder, sparingly soluble in water. The amorphous form is hygroscopic.

IDENTIFICATION
A. Dilute 1 ml of solution S (see Tests) to 100 ml with water R. In a depression in a white spot-plate, mix 0.1 ml of this solution with 0.2 ml of tosylarginine methyl ester hydrochloride solution R. A reddish-violet colour develops within 3 min.

B. Dilute 0.5 ml of solution S to 5 ml with water R. Add 0.1 ml of a 20 g/l solution of tosyl-lysyl-chloromethane hydrochloride R. Adjust to pH 7.0, shake for 2 h and dilute to 50 ml with water R. In one of the depressions of a white spot-plate, mix 0.1 ml of this solution with 0.2 ml of tosylarginine methyl ester hydrochloride solution R.

No reddish-violet colour develops within 3 min.

TESTS
Solution S. Dissolve 0.10 g in carbon dioxide-free water R and dilute to 10.0 ml with the same solvent.

Appearance of solution. Solution S is not more opalescent than reference suspension III (2.2.1).

pH (2.2.3). The pH of solution S is 3.0 to 6.0.

Absorbance (2.2.25). Dissolve 30.0 mg in 0.001 M hydrochloric acid and dilute to 100.0 ml with the same acid. The solution shows an absorption maximum at 280 nm and a minimum at 250 nm. The specific absorbance at the absorption maximum is 13.5 to 16.5 and at the absorption minimum is not greater than 7.0.

Chymotrypsin. To 1.8 ml of buffer solution pH 8.0 R add 7.4 ml of water R and 0.5 ml of 0.2 M acetyl-L-lysine ethyl ester R. While shaking the solution, add 0.5 ml of solution S and start a timer. After exactly 5 min, measure the pH (2.2.3) (test solution). Prepare a reference solution in the same manner, replacing solution S by 0.5 ml of a 0.5 g/l solution of chymotrypsin BRP and measure the pH (2.2.3) exactly 5 min after adding the chymotrypsin. The pH of the test solution is higher than that of the reference solution.

Loss on drying (2.2.32). Not more than 5.0 per cent, determined on 0.500 g by drying at 60 °C at a pressure not exceeding 670 Pa for 2 h.

Microbial contamination. Total viable aerobic count (2.6.12) not more than 10⁶ micro-organisms per gram, determined by plate count. It complies with the tests for Escherichia coli and Salmonella (2.6.13).

ASSAY
The activity of trypsin is determined by comparing the rate at which it hydrolyses benzoylarginine ethyl ester hydrochloride R with the rate at which trypsin BRP hydrolyses the same substrate in the same conditions.

Apparatus. Use a reaction vessel of about 30 ml capacity provided with:
- a device that will maintain a temperature of 25.0 ± 0.1 °C;
- a stirring device (for example, a magnetic stirrer);
- a lid with holes for the insertion of electrodes, the tip of a burette, a tube for the admission of nitrogen and the introduction of reagents.

An automatic or manual titration device may be used. For the latter, the burette is graduated in 0.005 ml and the pH meter is provided with a wide-range scale and glass-calomel or glass-silver-silver chloride electrodes.

Test solution. Dissolve sufficient of the substance to be examined in 0.001 M hydrochloric acid and dilute to 25.0 ml with the same acid in order to obtain a solution containing approximately 700 nanokatals per millilitre.

Reference solution. Dissolve 25.0 mg of trypsin BRP in 0.001 M hydrochloric acid and dilute to 25.0 ml with the same acid.

Store the solutions at 0–5 °C. Warm 1 ml of each solution to about 25 °C over 15 min and use 50 μl of each solution for each titration. Carry out the titration in an atmosphere of nitrogen. Transfer 10.0 ml of 0.0015 M borate buffer solution pH 8.0 R to the reaction vessel and, while stirring, add 1.0 ml of a freshly prepared 6.86 g/l solution of benzoylarginine ethyl ester hydrochloride R. When the temperature is steady at 25.0 ± 0.1 °C (after about 5 min) adjust the pH to exactly 8.0 with 0.1 M sodium hydroxide. Add 50 μl of the test solution and start a timer. Maintain the pH at 8.0 by the addition of 0.1 M sodium hydroxide, the tip of the microburette being immersed in the solution; note the volume added every 30 s. Follow the reaction for 8 min. Calculate the volume of 0.1 M sodium hydroxide used per second. Carry out a titration in the same manner using the reference solution and calculate the volume of 0.1 M sodium hydroxide used per second.