impurity B: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.05 per cent);

impurity C: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.02 per cent);

any other impurity: for each impurity, not more than the area of the peak due to impurity B in the chromatogram obtained with reference solution (f) (0.2 per cent);

total: not more than twice the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (0.2 per cent);

disregard limit: 0.01 times the area of the principal peak in the chromatogram obtained with reference solution (f).

Chlorides (2.4.A): maximum 100 ppm.

Dilute 10 ml of solution S to 15 ml with water R.

Sulphates: maximum 200 ppm.

Dissolve 1.0 g in 5 ml of dimethylformamide R and add 4 ml of water R. Mix thoroughly. Add 0.2 ml of dilute hydrochloric acid R and 0.5 ml of a 25 per cent m/m solution of barium chloride R. After 15 min any opalescence in the solution is not more intense than that in a standard prepared as follows: to 2 ml of sulphate standard solution (100 ppm SO4)R add 0.2 ml of dilute hydrochloric acid R, 0.5 ml of a 25 per cent m/m solution of barium chloride R, 3 ml of water R and 5 ml of dimethylformamide R.

Heavy metals (2.4.B): maximum 20 ppm.

Dissolve 2.0 g in 15 ml of ethanol (96 per cent) R and add 5 ml of water R. 12 ml of the solution complies with test B. Prepare the reference solution using lead standard solution (2 ppm Pb) prepared by diluting lead standard solution (100 ppm Pb) R with a mixture of 5 volumes of water R and 15 volumes of ethanol (96 per cent) R.

Loss on drying (2.2.32): maximum 0.5 per cent, determined on 1.000 g by drying in a desiccator.

Sulphated ash (2.4.14): maximum 0.1 per cent, determined on 2.0 g.

ASSAY

Dissolve 0.120 g in 30 ml of ethanol (96 per cent) R and add 20 ml of water R. Titrate with 0.1 M sodium hydroxide, using 0.1 ml of phenol red solution R as indicator.

1 ml of 0.1 M sodium hydroxide is equivalent to 13.81 mg of C23H23NO7.

STORAGE

Protected from light.

IMPURITIES

Specified impurities: A, B, C.

HO2C
R
OH

A. R = H: 4-hydroxybenzoic acid,

B. R = CO2H: 4-hydroxysiphaslic acid,

C. phenol.

SALMETEROL XINAFOATE

Salmeteroli xinafoas

C23H23NO7
M, 604

[94749-08-3]

DEFINITION

(1RS)-1-[4-Hydroxy-3-(Hydroxymethyl)phenyl]-2-[6-(4-Phenylbutoxy)hexyl]amino]ethanol 1-hydroxynaphthalene-2-carboxylate.

Content: 97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: practically insoluble in water, soluble in methanol, slightly soluble in anhydrous ethanol.

IDENTIFICATION

Infrared absorption spectrophotometry (2.2.24).

Comparison: salmeterol xinafoate CRS.

TESTS

Related substances. Liquid chromatography (2.2.29).

Protect the solutions from light.


Test solution. Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a). Dissolve 11 mg of salmeterol xinafoate for system suitability CRS (salmeterol containing impurities E and G) in the solvent mixture and dilute to 2 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Column:

- size: l = 0.15 m, Ø = 4.6 mm,
- stationary phase: octadecylsilyl silica gel for chromatography R (5 µm).

Mobile phase:

- mobile phase A: mix 24 volumes of a 7.71 g/l solution of ammonium acetate R with 24 volumes of a 28.84 g/l solution of sodium dodecyl sulphate R and adjust to pH 2.7 with glacial acetic acid R; mix with 52 volumes of acetonitrile R;
- mobile phase B: acetonitrile R.

See the information section on general monographs (cover pages)
impurity D
— Limits:
— the chromatogram obtained is similar to the chromatogram supplied with salmeterol xinafoate for system suitability CRS.

Limits:
— impurity D: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent),
— impurities A, F, G: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),
— impurities B, C, E: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
— any other impurity: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),
— total: not more than 9 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.9 per cent),
— disregard limit: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard the peak due to xinafoic acid and any peaks due to the blank.

Water (2.5.12): maximum 0.5 per cent, determined on 1.000 g.
Dissolve the sample with 30 ml of anhydrous methanol R.

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

ASSAY
Liquid chromatography (2.2.29).
Test solution. Dissolve 12.50 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.
Reference solution (a). Dissolve 12.50 mg of salmeterol xinafoate CRS in the mobile phase and dilute to 50.0 ml with the mobile phase.
Reference solution (b). Dilute 1 ml of reference solution (a) described in the test for related substances to 20 ml with the mobile phase.

Flow rate: 2 ml/min.
Detection: spectrophotometer at 278 nm.

Injection: 20 µl; inject the solvent mixture as a blank solution.

Relative retention with reference to salmeterol (retention time = about 13 min): xinafoic acid = about 0.2; impurity A = about 0.3; impurity B = about 0.5; impurity C = about 0.7; impurity D = about 0.8; impurity E = about 0.9; impurity F = about 1.6; impurity G = about 2.7.

System suitability: reference solution (a):
— peak-to-valley ratio: minimum 10, where \( H_p \) = height above the baseline of the peak due to impurity E and \( H_v \) = height above the baseline of the lowest point of the curve separating this peak from the peak due to salmeterol,
— the chromatogram obtained is similar to the chromatogram supplied with salmeterol xinafoate for system suitability CRS.

Column:
— size: \( l = 0.15 \) m, \( \phi = 4.6 \) mm,
— stationary phase: octadecylsilil silica gel for chromatography R (5 µm).

Mobile phase: mix 24 volumes of a 7.71 g/l solution of ammonium acetate R with 24 volumes of a 28.84 g/l solution of sodium dodecyl sulphate R and adjust to pH 2.7 with glacial acetic acid R. Mix with 52 volumes of acetonitrile R.

Flow rate: 2 ml/min.
Detection: spectrophotometer at 278 nm.

Injection: 20 µl.
Run time: until complete elution of the peak due to salmeterol (about 16 min).

System suitability: reference solution (b):
— peak-to-valley ratio: minimum 10, where \( H_p \) = height above the baseline of the peak due to impurity E and \( H_v \) = height above the baseline of the lowest point of the curve separating this peak from the peak due to salmeterol.

The stationary phase may be regenerated using the gradient described under the test for related substances.
Calculate the percentage content of C₃₆H₄₅NO₇ in the chromatogram obtained with reference solution (a) and the declared content of C₃₆H₄₅NO₇ in salmeterol xinafoate CRS.

STORAGE
Protected from light.

IMPURITIES
Specified impurities: A, B, C, D, E, F, G.

A. \( R = [\text{CH}_{2}]_4-C_6H_5, R' = \text{OH} : (1RS)-1-[4\text{-hydroxy-3-(hydroxymethyl)phenyl}]-2\{4\text{-phenylbutyl}amino\}ethanol,
B. \( R = [\text{CH}_{3}O][\text{CH}_{2}]_2-C_6H_5, R' = \text{OH} : (1RS)-1-[4\text{-hydroxy-3-(hydroxymethyl)phenyl}]-2\{6\text{-2-phenylethoxy}hexyl\}amino\}ethanol,
C. \( R = [\text{CH}_3O][\text{CH}_2][C_6H_5], R' = \text{OH} : (1RS)-1-[4\text{-hydroxy-3-(hydroxymethyl)phenyl}]-2\{6\text{-3-phenylpropoxy}hexyl\}amino\}ethanol,
D. 1-[4\text{-2-hydroxy-2-[4\text{-hydroxy-3-(hydroxymethyl)phenyl}]-ethoxy}]-3\{4\text{-phenylbutoxy}hexyl\}amino\}ethanol,
Salmon oil, farmed

**EUROPEAN PHARMACOPOEIA 6.0**

**E. 1-[4-hydroxy-3-(hydroxymethyl)phenyl]-2-[6-(1-methyl-3-phenylpropoxy)hexyl]amino]ethanol,**

**G. 1-[4-hydroxy-3-[[2-hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl] [6-(4-phenylbutoxy)hexyl]amino]methyl]phenyl]-2-[6-(4-phenylbutoxy)hexyl]amino]ethanol.**

01/2008:1910

**SALMON OIL, FARMED**

Salmonis domestici oleum

**DEFINITION**

Purified fatty oil obtained from fresh farmed *Salmo salar*. The positional distribution (β(2)-acyl) is 60-70 per cent for cervonic (docosahexaenoic) acid (C22:6 n-3; DHA), 25-35 per cent for timnodonic (eicosapentaenoic) acid (C20:5 n-3; EPA) and 40-55 per cent for moroctic acid (C18:4 n-3).

**Content:**

— sum of the contents of EPA and DHA (expressed as triglycerides): 10.0 per cent to 28.0 per cent.

Authorised antioxidants in concentrations not exceeding the levels specified by the competent authority may be added.

**PRODUCTION**

The fish shall only be given feed with a composition that is in accordance with the relevant EU or other applicable regulations.

The oil is produced by mechanical expression of fresh raw materials, either from the whole fish, or fish where the fillets have been removed, at a temperature not exceeding 100 °C, and without using solvents. After centrifugation, solid substances may be removed from the oil by cooling and filtering (winterisation).

**CHARACTERS**

**Appearance:** pale pink liquid.

**Solubility:** practically insoluble in water, very soluble in acetone and in heptane, slightly soluble in anhydrous ethanol.

**IDENTIFICATION**

Examine the 13C NMR spectra obtained in the assay for positional distribution (β(2)-acyl) of fatty acids. The spectra contain peaks between 172 ppm and 173 ppm with shifts similar to those in the type spectrum (Figure 1910.-2). The oil to be examined complies with the limits of this assay.

**ASSAY**

**Positional distribution (β(2)-acyl) of fatty acids (2.2.33)**

Use a high resolution FT-NMR spectrometer operating at minimum 300 MHz.

*Test solution.* Dissolve 190-210 mg of fresh salmon oil in 500 µl of deuterated chloroform R. Prepare at least 3 samples and examine within 3 days.

*Acquisition of 13C NMR spectra.* The following parameters may be used:

- sweep width: 200 ppm (−5 to 195 ppm),
- irradiation frequency offset: 95 ppm,
- time domain: 64 K,
- pulse delay: 2 s,
- pulse program: zgig 30 (inverse gated, 30° excitation pulse),
- dummy scans: 4,
- number of scans: 4096.

*Processing and plotting.* The following parameters may be used:

- size: 64 K (zero-filling),
- window multiplication: exponential,
- Lorentzian broadening factor: 0.2 Hz.

Use the CDCl3 signal for shift referencing. The shift of the central peak of the 1:1:1 triplet is set to 77.16 ppm. Plot the spectral region δ 171.5-173.5 ppm. Compare the spectrum with the reference spectrum in Figure 1910.-2. The shift values lie within the ranges given in Table 1910.-1.

<table>
<thead>
<tr>
<th>Signal</th>
<th>Shift range (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β DHA</td>
<td>172.05 - 172.09</td>
</tr>
<tr>
<td>α DHA</td>
<td>172.43 - 172.47</td>
</tr>
<tr>
<td>β EPA</td>
<td>172.52 - 172.56</td>
</tr>
<tr>
<td>α EPA</td>
<td>172.90 - 172.94</td>
</tr>
<tr>
<td>β C18:4</td>
<td>172.56 - 172.60</td>
</tr>
<tr>
<td>α C18:4</td>
<td>172.95 - 172.99</td>
</tr>
</tbody>
</table>

**System suitability:** calculate the signal-to-noise ratio for the smallest relevant peak corresponding to α C18:4 signal (in the range 8 172.95-172.99 ppm). Measure the peak width at half-height for the central CDCl3 signal (at 8 77.16 ppm). The system suitability criteria in Table 1910.-2 are fulfilled.