– *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) (0.05 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 (*2.4.4*): 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 SO_4) 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 (*2.4.8*): 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.

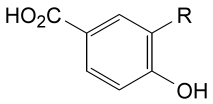
1 ml of *0.1 M sodium hydroxide* is equivalent to 13.81 mg of $C_7H_6O_3$.

STORAGE

Protected from light.

IMPURITIES

Specified impurities: A, B, C.



A. R = H: 4-hydroxybenzoic acid,

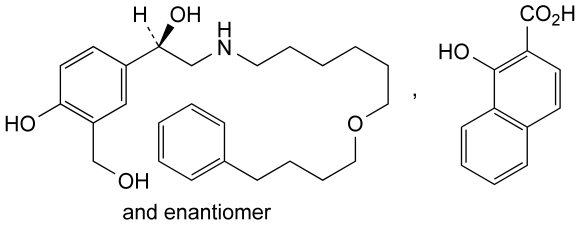
B. R = CO_2H: 4-hydroxyisophthalic acid,

C. phenol.

01/2008:1765

SALMETEROL XINAFOATE

Salmeteroli xinafoas



and enantiomer

$C_{36}H_{45}NO_7$ M_r 604
[94749-08-3]

DEFINITION

(1*RS*)-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.*

Solvent mixture: *acetonitrile R, water R* (50:50 *V/V*).

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*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 16	100	0
16 - 36	100 → 30	0 → 70
36 - 45	30	70
45 - 50	30 → 100	70 → 0

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*.

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.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm,
– *stationary phase*: *octadecylsilyl 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.

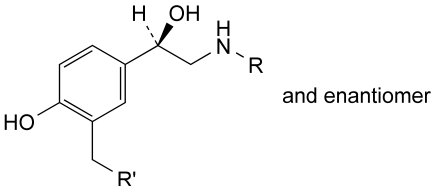
Calculate the percentage content of $C_{36}H_{45}NO_7$ using the chromatogram obtained with reference solution (a) and the declared content of $C_{36}H_{45}NO_7$ in *salmeterol xinafoate CRS*.

STORAGE

Protected from light.

IMPURITIES

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

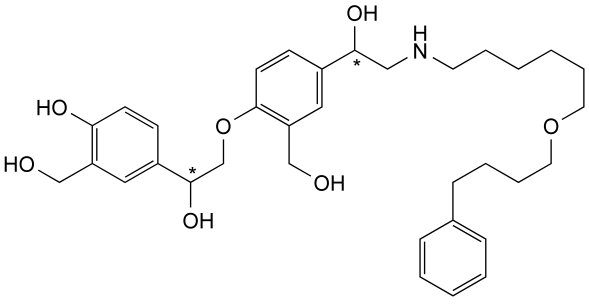


A. R = $[CH_2]_4$-C_6H_5, R′ = OH: (1*RS*)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]-2-[(4-phenylbutyl)amino]ethanol,

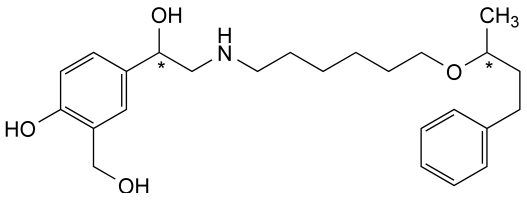
B. R = $[CH_2]_6$-O-$[CH_2]_2$-C_6H_5, R′ = OH: (1*RS*)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]-2-[[6-(2-phenylethoxy)hexyl]amino]ethanol,

C. R = $[CH_2]_6$-O-$[CH_2]_3$-C_6H_5, R′ = OH: (1*RS*)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]-2-[[6-(3-phenylpropoxy)hexyl]amino]ethanol,

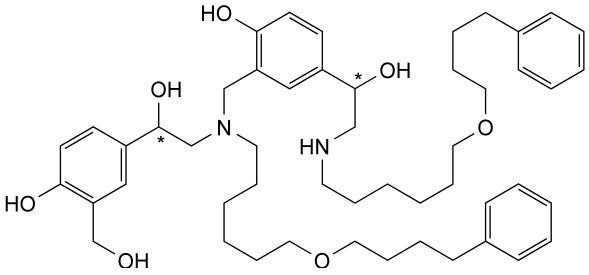
F. R = $[CH_2]_6$-O-$[CH_2]_4$-C_6H_5, R′ = H: (1*RS*)-1-(4-hydroxy-3-methylphenyl)-2-[[6-(4-phenylbutoxy)hexyl]amino]ethanol,



D. 1-[4-[2-hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethoxy]-3-(hydroxymethyl)phenyl]-2-[[6-(3-phenylbutoxy)hexyl]amino]ethanol,



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 ^{13}C 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.

TESTS

Absorbance (*2.2.25*): minimum 0.10, measured at the maximum between 470 nm and 480 nm.

Dissolve 5.0 ml in 5.0 ml of *trimethylpentane R*.

Acid value (*2.5.1*): maximum 2.0.

Anisidine value (*2.5.36*): maximum 10.0.

Peroxide value (*2.5.5, Method A*): maximum 5.0.

Unsaponifiable matter (*2.5.7*): maximum 1.5 per cent, determined on 5.0 g.

Linoleic acid (*2.4.29*): maximum 11.0 per cent.

Identify the peak due to linoleic acid using the chromatogram in Figure 1910.-1. Determine the percentage content by normalisation.

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 ^{13}C 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 $CDCl_3$ 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 1910.-1 – Shift values

Signal	Shift range (ppm)
β DHA	172.05 - 172.09
α DHA	172.43 - 172.47
β EPA	172.52 - 172.56
α EPA	172.90 - 172.94
β C18:4	172.56 - 172.60
α C18:4	172.95 - 172.99

System suitability: calculate the signal-to-noise ratio for the smallest relevant peak corresponding to α C18:4 signal (in the range δ 172.95-172.99 ppm). Measure the peak width at half-height for the central $CDCl_3$ signal (at δ 77.16 ppm). The system suitability criteria in Table 1910.-2 are fulfilled.