Column 1:
- *size*: *l* = 0.3 m, Ø = 7.8 mm,
- *stationary phase*: *styrene-divinylbenzene copolymer R* (7 µm) with a pore size of 10 nm.

Columns 2 and 3, placed closest to the injector:
- *size*: *l* = 0.3 m, Ø = 7.8 mm,
- *stationary phase*: *styrene-divinylbenzene copolymer R* (7 µm) with a pore size of 50 nm.

Mobile phase: *tetrahydrofuran R*.

Flow rate: 0.8 ml/min.

Detection: differential refractometer.

Injection: 40 µl.

System suitability: reference solution:
- *elution order*: tridocosahexaenoin, didocosahexaenoin, monodocosahexaenoin.
- *resolution*: minimum of 2.0 between the peaks due to monodocosahexaenoin and to didocosahexaenoin and minimum of 1.0 between the peaks due to didocosahexaenoin and to tridocosahexaenoin.

Identify the peaks from the chromatogram (Figure 1912.-1). Calculate the percentage content of oligomers using the following expression:

$$\frac{B}{A} \times 100$$

A = sum of the areas of all the peaks in the chromatogram,

B = area of the peak with a retention time less than the retention time of the triglyceride peak.

Limit:
- *oligomers*: maximum 1.5 per cent.

ASSAY

EPA and DHA (*2.4.29*). See Figure 1912.-2.

Total omega-3-acids (*2.4.29*). See Figure 1912.-2.

STORAGE

In an airtight, well-filled container, protected from light, under inert gas.

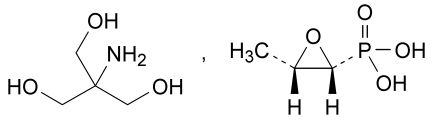
LABELLING

The label states:
- the concentration of EPA, DHA and total omega-3-acids, expressed as triglycerides,
- the name and the concentration of any added antioxidant.

04/2006:1425

FOSFOMYCIN TROMETAMOL

Fosfomycinum trometamolum



$C_7H_{18}NO_7P$ M_r 259.2

DEFINITION

2-Amino-2-(hydroxymethyl)propane-1,3-diol hydrogen (2*R*,3*S*)-(3-methyloxiran-2-yl)phosphonate.

Content: 98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, hygroscopic powder.

Solubility: very soluble in water, slightly soluble in ethanol (96 per cent) and in methanol, practically insoluble in acetone.

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *fosfomycin trometamol CRS*.

B. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 50 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution. Dissolve 50 mg of *fosfomycin trometamol CRS* in *water R* and dilute to 10 ml with the same solvent.

Plate: *cellulose for chromatography R* as the coating substance.

Mobile phase: *concentrated ammonia R, water R, 2-propanol R* (10:20:70 *V/V/V*).

Application: 10 µl.

Development: over 3/4 of the plate.

Drying: in a current of warm air.

Detection: expose to iodine vapour until the spots appear.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. To about 15 mg add 2 ml of *water R*, 1 ml of *perchloric acid R* and 2 ml of *0.1 M sodium periodate*. Heat on a water-bath for 10 min and add, without cooling, 1 ml of *ammonium molybdate solution R5* and 1 ml of *aminohydroxynaphthalenesulphonic acid solution R*. Allow to stand for 30 min. A blue colour develops.

TESTS

Solution S. Dissolve 1.00 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

pH (*2.2.3*): 3.5 to 5.5 for solution S.

Specific optical rotation (*2.2.7*): – 13.5 to – 12.5 (anhydrous substance), determined on solution S at 365 nm using a mercury lamp.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 0.600 g of the substance to be examined in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (a). Dissolve 0.600 g of *fosfomycin trometamol CRS* in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 3.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (c). Wet 0.3 g of the substance to be examined with 60 µl of *water R* and heat in an oven at 60 °C for 24 h. Dissolve the residue in the mobile phase and dilute to 20.0 ml with the mobile phase (solution A). Dissolve 0.6 g

of the substance to be examined in solution A and dilute to 5.0 ml with the same solution (*in situ* degradation to obtain impurities A, B, C and D).

Blank solution. The mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: 10.89 g/l solution of *potassium dihydrogen phosphate R* in *water for chromatography R*.

Flow rate: 1.0 ml/min.

Detection: differential refractometer at 35 °C.

Injection: 10 µl of the blank solution, the test solution and reference solutions (b) and (c).

Run time: twice the retention time of fosfomycin.

Relative retention with reference to fosfomycin (retention time = about 9 min): trometamol (2 peaks) = about 0.3; impurity B = about 0.48; impurity C = about 0.54; impurity A = about 0.88; impurity D = about 1.27.

System suitability: reference solution (c):

– *resolution*: minimum 1.5 between the peaks due to impurity A and fosfomycin,
– *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B.

Limits:

– *impurities A, B*: for each impurity, not more than the area of the peak due to fosfomycin in the chromatogram obtained with reference solution (b) (0.3 per cent),
– *impurities C, D*: for each impurity, not more than 0.33 times the area of the peak due to fosfomycin in the chromatogram obtained with reference solution (b) (0.1 per cent),
– *unspecified impurities*: for each impurity, not more than 0.33 times the area of the peak due to fosfomycin in the chromatogram obtained with reference solution (b) (0.1 per cent),
– *total*: not more than 1.67 times the area of the peak due to fosfomycin in the chromatogram obtained with reference solution (b) (0.5 per cent);
– *disregard limit*: 0.17 times the area of the peak due to fosfomycin in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard the 2 peaks due to trometamol and any peak due to the blank.

Phosphates: maximum 500 ppm.

Dissolve 0.1 g in 3 ml of *dilute nitric acid R* and dilute to 10 ml with *water R*. To 5 ml of this solution add 5 ml of *water R* and 5 ml of *molybdovanadic reagent R*. Shake vigorously. After 5 min, any colour in the test solution is not more intense than that in a standard prepared at the same time in the same manner, using 5 ml of *phosphate standard solution (5 ppm PO_4) R*.

Heavy metals (*2.4.8*): maximum 10 ppm.

Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Water (*2.5.12*): maximum 0.5 per cent, determined on 0.500 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: 5 µl of the test solution and reference solution (a).

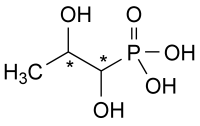
Calculate the percentage content of $C_7H_{18}NO_7P$ from the areas of the peaks due to fosfomycin and the declared content of *fosfomycin trometamol CRS*.

STORAGE

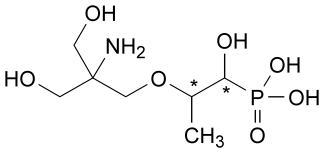
In an airtight container.

IMPURITIES

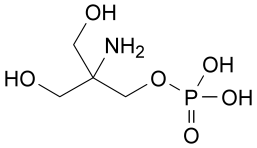
Specified impurities: A, B, C, D.



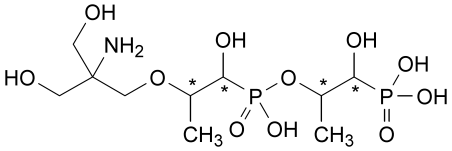
A. (1,2-dihydroxypropyl)phosphonic acid,



B. [2-[2-amino-3-hydroxy-2-(hydroxymethyl)propoxy]-1-hydroxypropyl]phosphonic acid,



C. 2-amino-3-hydroxy-2-(hydroxymethyl)propyl dihydrogen phosphate (trometamol phosphoric acid monoester),



D. [2-[[[2-[2-amino-3-hydroxy-2-(hydroxymethyl)propoxy]-1-hydroxypropyl]hydroxyphosphoryl]oxy]-1-hydroxypropyl]phosphonic acid (trometamoyloxy fosfomycin dimer).

Monographs D-K