585. Fosthiazate

HPLC method

CIPAC Full Scale Collaborative Trial

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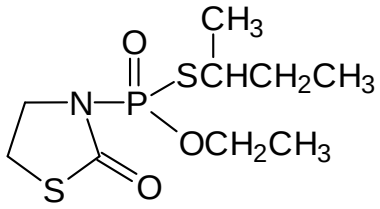
FOSTHIAZATE
585



ISO common name:	Fosthiazate
Chemical name:	*(RS)*-[*S*-(*RS*)-*sec*-butyl *O*-ethyl 2-oxo-1,3-thiazolidin-3-ylphosphonothioate] or (3*RS*)-3-[(*RS*)-*sec*-butylthio(ethoxy)phosphinoyl]-1,3-thiazolidin-2-one (IUPAC)
CAS-Number:	98886-44-3
RMM:	283.36
Empirical formula:	$C_9H_{18}NO_3PS_2$
m.p.	Not applicable; fosthiazate is a liquid.
V.p.	5.6 x 10^{-4} Pa at 25 °C (98.4 % purity)
Solubility	In water: at 25°C (99.3% purity): 9.88 g/l at pH 5, 9.00 g/l at pH 7 and 9.46 g/l at pH 9. In organic solvents: In n-hexane : not solubilised 15.1g/l at 20°C (92.2% purity) In xylene: solubilised at 20°C (92.2% purity) In N-methyl-2-pyrrolidone: solubilised at 20°C (92.2% purity) In isopropyl alcohol: solubilised at 20°C (92.2% purity)
Stability	Keep frozen (< -18 °C) when not in use.
Hydrolysis	DT_{50} at 25°C in the dark; calculated DT 50s for [^{14}C]-butyl and [^{14}C]-thiazolidinone labelled fosthiazate:

pH	B-label	T-label
5	191 days	163 days
7	102 days	107 days
9	3.2 days	3.3 days

Description	Form: clear liquid
Formulation	Granules (GR)

FOSTHIAZATE TECHNICAL
585/TC/M/-

1. Sampling. Take at least 20 g.

2. Identity test

2.1 HPLC. Use the reversed phase HPLC method 3 described below. The relative retention time of the fosthiazate peak in the sample solution should not deviate by more than 2% from that of the calibration solution. The UV spectrum measured from this peak should match that obtained from the calibration substance.

3. Fosthiazate

OUTLINE OF METHOD. Fosthiazate is determined by reversed phase high performance liquid chromatography using UV detection at 220 nm and internal standard calibration.

REAGENTS

Fosthiazate reference standard of known content
Dimethyl phthalate, *99.95 %* w/w, internal standard
Acetone, for analysis
Acetonitrile, HPLC grade

Internal standard solution. Dissolve (to the nearest 0.1 mg) 1200 mg of dimethyl phthalate with acetone in a volumetric flask (100 ml) using an ultrasonic bath. Ensure that a sufficient quantity of this solution is prepared for all samples and standards to be analysed.

Calibration solutions. Weigh in duplicate (to the nearest 0.1 mg) 100 mg of fosthiazate reference standard (*s* mg) into separate volumetric flasks (100 ml). Pipette internal standard solution (5.0 ml) into each flask, add acetone (about 60 ml) and sonicate until complete dissolution. Allow the solutions to cool to ambient temperature and dilute to the mark with acetone. Mix thoroughly. Pipette 5 ml of each solution into separate volumetric flasks (50 ml) and fill to the mark with acetonitrile. Mix well (calibration solutions C_A and C_B).

APPARATUS

High performance liquid chromatograph equipped with a detector suitable for operation at 220 nm (UV-detection) and an injection system capable of injecting 10 µl

Liquid chromatographic column stainless steel, 125 x 4 mm i.d., Agilent Hypersil ODS C_{18}, 5 µm, or equivalent with the same selectivity

Electronic integrator or data system
Ultrasonic bath

PROCEDURE

(a) Chromatographic conditions (typical)

Column temperature	25°C
Flow rate	1 ml/min
Detector wavelength	220 nm
Injection volume	10 µl
Mobile phases	A: acetonitrile – water (1 – 2 v/v) B: acetonitrile

Gradient program

Time [min]	Eluent A [%]	Eluent B [%]
0	100	0
8	100	0
8.5	0	100
14	0	100 (stop time)
(Post time) 3 min	100	0

Retention times dimethyl phthalate (internal standard) approximately 4.7 min
fosthiazate approximately 6.4 min

(b) Equilibration of the system. Pump sufficient mobile phase (use gradient programme) through the column to equilibrate the system. Inject 10 µl portions of the calibration solution C_A and repeat the injections until retention times and peak areas deviate by less than ± 0.5 % from the mean for three successive injections.

(c) Sample preparation. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about 100 mg of fosthiazate (*s* mg) into a volumetric flask (100 ml). Pipette internal standard solution (5.0 ml), add acetone (about 60 ml) and sonicate until complete dissolution. Allow the solution to cool to ambient temperature and dilute to the mark with acetone. Mix thoroughly. Pipette 5 ml of the solution into a volumetric flask (50 ml) and fill to the mark with acetonitrile. Mix thoroughly. Filter an aliquot of each prepared solution through a 0.45 µm PTFE filter prior to analysis (sample solutions S_1 and S_2).

(d) Determination. Inject 10 µl portions of the second calibration solution (C_B) for two successive injections. The mean response factor for this solution should deviate by no more than 1% from those for the first calibration solution (C_A) (see paragraph (b) Equilibration of the system), otherwise the calibration solutions should be prepared again.

Inject in duplicate 10 µl portions of each sample solution (S_1, S_2, …,etc.) bracketing them by single injections of calibration solutions (C_A and C_B) using the following sequence:

$C_A, S_1, S_1, S_2, S_2, C_B, S_3, S_3, S_4, S_4, C_A$…

(e) Calculation. Determine the peak area of fosthiazate and calculate the mean value of response factors from the calibration solutions bracketing the injections of the sample solutions and use this value for calculating the fosthiazate content of the bracketed sample solutions. The fosthiazate content is the mean value of two sample solutions.

$$f_i = \frac{I_r \times s \times \mathrm{P}}{H_s}$$

$$\text{fosthiazate content} = \frac{f \times H_w}{I_q \times w} \quad \text{[g/kg] } (M)$$

where:

f_i = individual response factor
f = mean response factor
H_s = peak area of fosthiazate in the calibration solution
H_w = peak area of fosthiazate in the sample solution
I_r = peak area of the internal standard in the calibration solution
I_q = peak area of the internal standard in the sample solution
s = mass of fosthiazate reference standard in the calibration solution (mg)
w = mass of sample taken (mg)
P = purity of fosthiazate reference standard (g/kg)

FOSTHIAZATE GRANULES
585/GR/M/-

1. Sampling. Take at least 100 g.

2. Identity test

2.1 HPLC. As for fosthiazate technical 585/TC/M/2.1

3. Fosthiazate
As for fosthiazate technical 585/TC/M/3 except

(c) Sample preparation. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about 100 mg of fosthiazate (*s* mg) into a volumetric flask (100 ml). Pipette internal standard solution (5.0 ml), add acetone (about 60 ml) and sonicate for minimum 10 minutes. Then shake manually vigorously. Repeat this sonication/shaking operation twice. Allow the solutions to cool to ambient temperature and fill to the mark with acetone. Mix thoroughly. Pipette 5 ml of the solution into a volumetric flask (50 ml) and fill to the mark with acetonitrile. Mix thoroughly. Filter an aliquot of each prepared solution through a 0.45 µm PTFE filter prior to analysis (sample solutions S_1 and S_2).

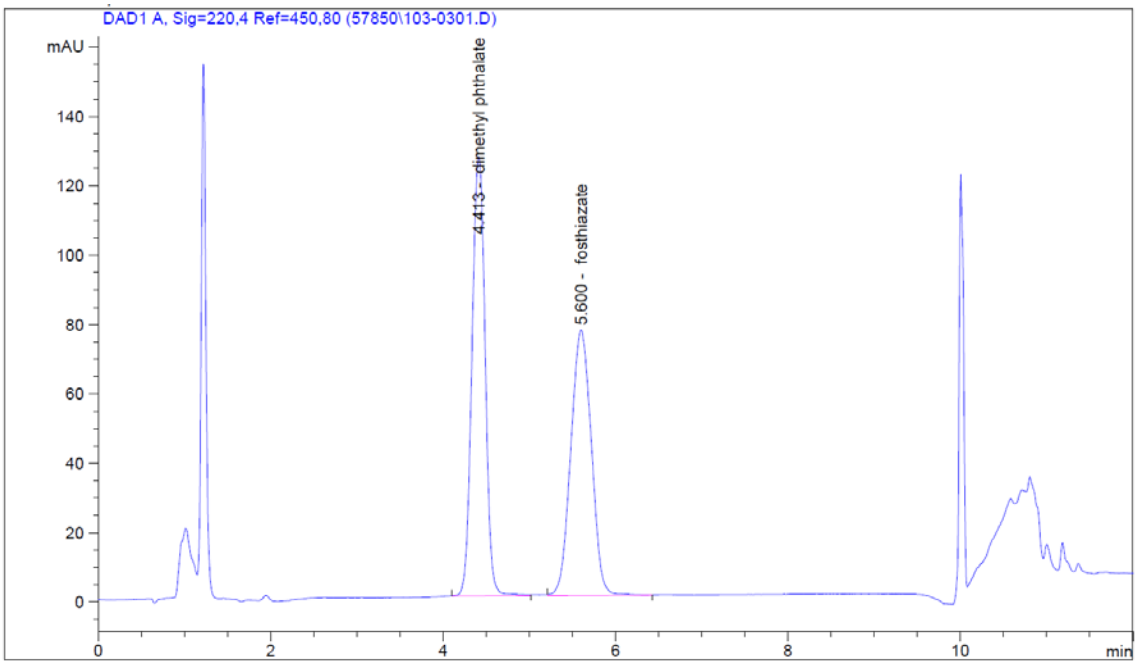


Fig 1 Typical HPLC-chromatogram of calibration solution

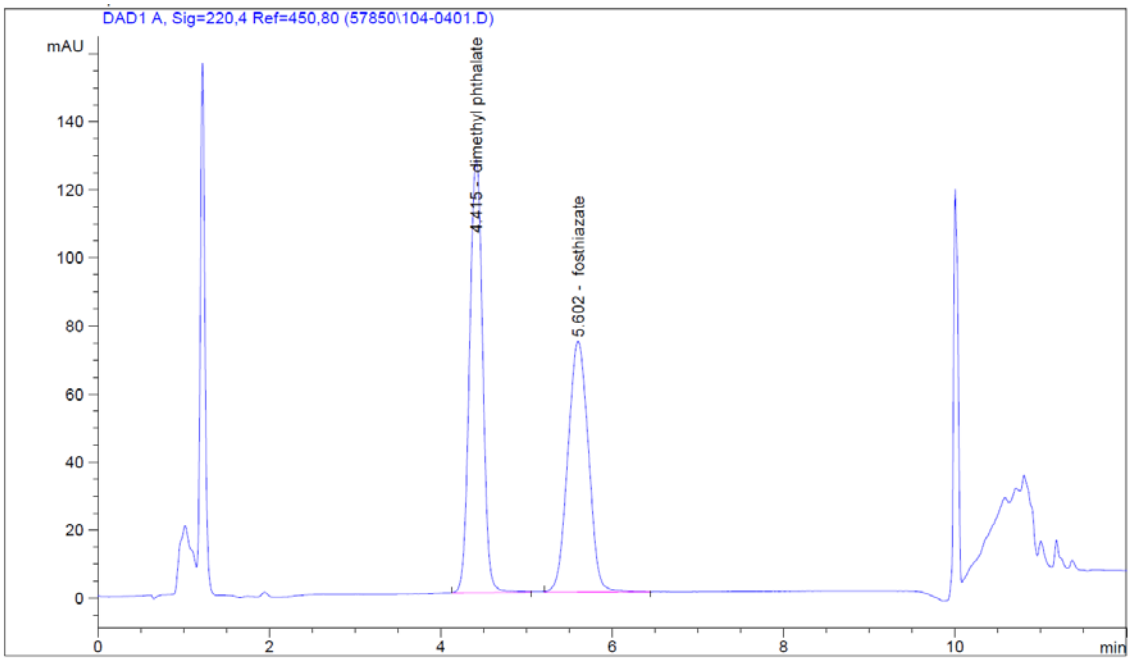


Fig 2 Typical HPLC-chromatogram of Fosthiazate technical material

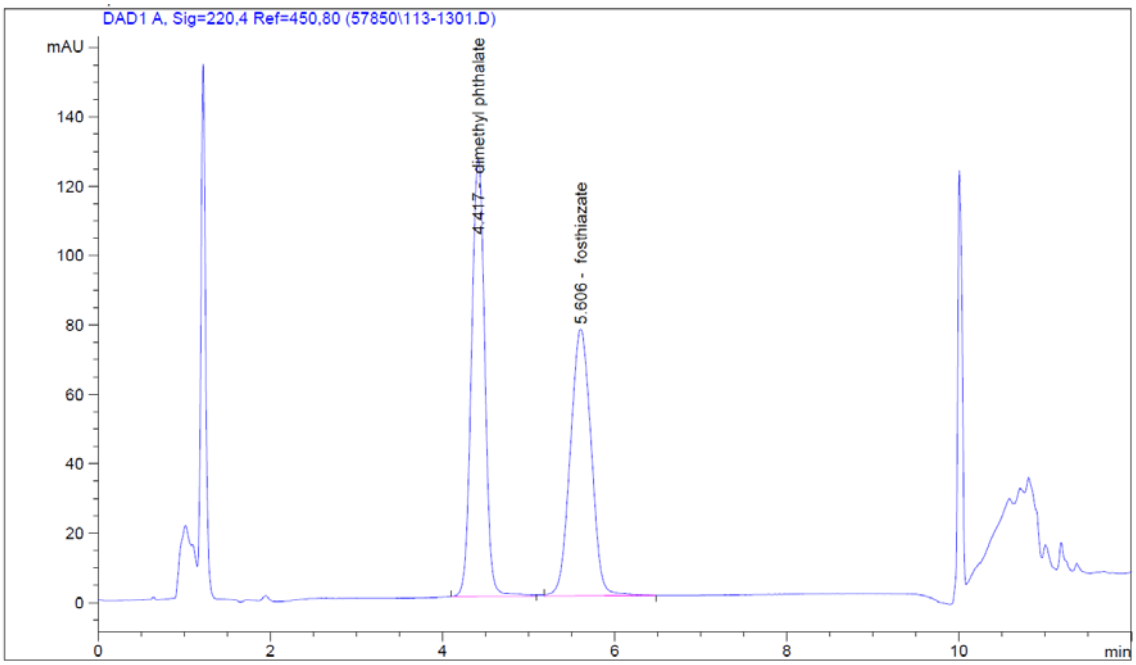


Fig 3 Typical HPLC-chromatogram of Fosthiazate 10 GR