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Determination of the Residues of XDE-570 and its 5-Hydroxy Metabolite in Surface Water

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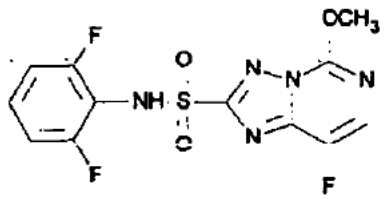
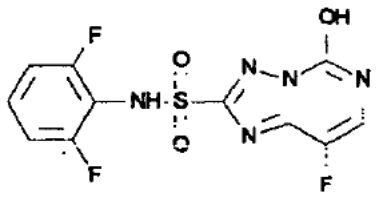
1. SCOPE

This method is applicable to the quantitative determination of the residues of XDE-570* (0.10-1.00 µg/L) and its 5-hydroxy metabolite** (0.20-2.00 µg/L) in surface water. The method has been validated by the analysis of untreated samples and samples fortified with XDE-570 down to a lowest validated level of 0.10 µg/L and with 5-hydroxy XDE-570 down to a lowest validated level of 0.20 µg/L.



XDE-570



5-hydroxy XDE-570

* Research name for: N-(2,6-difluorophenyl)-8-fluoro-5-methoxy-(1,2,4)-triazolo-(1,5 c)-pyrimidine-2-sulfonamide (IUPAC).

** Research name for: N-(2,6-difluorophenyl)-8-fluoro-5-hydroxy-(1,2,4)-triazolo-(1,5 c)-pyrimidine-2-sulfonamide (IUPAC).

2. PRINCIPLE

XDE-570 and the metabolite are extracted from surface water using a polystyrene divinylbenzene solid phase extraction cartridge, eluting both analytes from the cartridge with 50:50 (v/v) acetonitrile / aqueous acid. The eluate is partitioned with methyl-tertiary-butyl ether (MTBE). The ether extract is purified using an aminopropyl solid phase extraction cartridge eluting the analytes with a formic acid / acetonitrile / MTBE mixture. The eluate is evaporated to dryness. The residuum is further purified by using a silica solid phase extraction cartridge eluting the analytes with a formic acid / acetonitrile / toluene mixture. The eluate is evaporated to dryness and the residuum reconstituted in 20:80:1 (v/v/v) acetonitrile / water / acetic acid. XDE-570 and the metabolite are quantified by HPLC using a UV absorbance detector set at 260 nm.

3. SAFETY PRECAUTIONS

Each analyst should be acquainted with the potential hazards of the reagents, products and solvents before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, LITERATURE AND OTHER INTERNALLY GENERATED DATA. Safety information on non-DowElanco products should be requested from the supplier. Disposal of reagents, reactants and solvents must be in compliance with the appropriate government regulations.

4. EQUIPMENT*

4.1 Laboratory Equipment

4.1.1 MSE 2000 centrifuge fitted with a six place rotor and adaptors to take 8 dram vials.

4.1.2 Techne sample concentrator model SC3.

4.1.3 Ultrasonic bath - Decon FS100.

Items 4.1.1 - 4.1.3 are available from Fisher Scientific UK.

4.1.4 Gerhardt Shaker Model LS 20 - Gerhardt UK Ltd.

4.1.5 Vacuum manifold apparatus for solid-phase extraction - Jones Chromatography Ltd.

4.1.6 Gilson 'Microman' 50 µL, 100 µL, 200 µL and 250 µL fixed volume micropipettors - Anachem Ltd.

4.1.7 Mettler AT201 5 place balance with LCP45 printer or equivalent - Fisher Scientific UK.

4.1.8 Mettler PM460 2 figure balance with GA44 printer or equivalent - Fisher Scientific UK.

4.2 Chromatographic System

4.2.1 HPLC, consisting of :

Milton Roy spectroMonitor 3100 UV detector - LDC Analytical.
9010 solvent delivery system - Varian UK Ltd.
ISS100 autosampler - Perkin Elmer Ltd.

4.2.2 Integration system capable of giving peak area or peak height information *e.g.* Hewlett Packard 3350A Laboratory Data System.

4.2.3 HPLC column : 250 mm x 2.1 mm i.d. Kromasil 100 C_{18} 5 µm - Hichrom Ltd.

4.3 Laboratory Glassware and Plasticware

4.3.1 1.0 mL - 25.0 mL graduated pipettes.

4.3.2 8 dram (30 mL) screw cap vials.

4.3.3 50 mL - 100 mL volumetric flasks.

4.3.4 100 mL and 1 litre measuring cylinders.

4.3.5 5 litre vacuum flask.

Items 4.3.1 - 4.3.5 are available from Fisher Scientific UK.

4.3.6 1 litre glass screw cap bottles - Bristol Bottle Co. Ltd.

4.3.7 Reservoir adapters to fit isolute cartridges - Jones Chromatography Ltd.

4.3.8 2 mL gas chromatography vials - Owen Polyscience Ltd.

4.3.9 Septa : silicone elastomer PTFE lined - Jones Chromatography Ltd.

4.3.10 2 mL plastic disposable transfer pipettes - Fisher Scientific UK.

4.3.11 Unicaps R3 plastic screw top to fit 8 dram vials - Bristol Bottle Co. Ltd.

4.3.12 Caps to fit 1 litre bottles - Bristol Bottle Co. Ltd.

4.3.13 5 litre plastic (jerrican) containers with screw caps - Fisher Scientific UK.

4.3.14 PTFE tubing 1/8 inch O.D. - Jones Chromatography Ltd.

* The full address of all suppliers named above is included in Appendix 1.

5. REAGENTS AND MATERIALS*

5.1 Toluene - Distol grade.

5.2 Acetonitrile - HPLC grade.

5.3 Methyl-tertiary-butyl ether (MTBE) - HPLC grade.

5.4 Concentrated sulphuric acid, SG 1.84 - AnalaR grade.

5.5 Sulphuric acid - 1 M volumetric grade.

5.6 Water - HPLC grade.

5.7 Acetic acid - HPLC grade.

5.8 Formic Acid 98/100% - AnalaR grade.

5.9 Sodium chloride - AnalaR grade.

5.10 Acetone.

Items 5.1 - 5.10 are available from Fisher Scientific UK.

5.11 Isolute aminopropyl solid phase extraction cartridges (500 mg / 10 mL) - Jones Chromatography Ltd.

5.12 Isolute ENV+ solid phase extraction cartridges (1 g / 6 mL) - Jones Chromatography Ltd.

5.13 Isolute silica solid phase extraction cartridges (500 mg / 10 mL) - Jones Chromatography Ltd.

5.14 Elution solvent 1 - 250:250:1 (v/v/v) acetonitrile / water / concentrated sulphuric acid.

5.15 Elution solvent 2 - 3:25:72 (v/v/v) formic acid / acetonitrile / MTBE.

5.16 Elution solvent 3 - 25:75 (v/v) 2% formic acid in acetonitrile / toluene.

5.17 LC solvent - 20:80:1 (v/v/v) acetonitrile / water / glacial acetic acid.

5.18 Nitrogen for sample concentrator - available from BOC Ltd.

5.19 Analytical standard of XDE-570 - available from the Analytical Standards Coordinator, Research and Development Centre, DowElanco Europe.

5.20 Analytical standard of 5-hydroxy XDE-570 - available from the Analytical Standards Coordinator, Research and Development Centre, DowElanco Europe.

* The full address of all suppliers named above is included in Appendix 1.

6. PROCEDURE

6.1 Stock and Fortification Solutions

Dissolve 100 mg of analytical standard of XDE-570 in 100 mL acetone to give a 1000 µg/ml stock solution. Dissolve 100 mg of analytical standard of 5-hydroxy XDE-570 in 100 mL acetone to give a 1000 µg/ml stock solution. Dilute each stock solution to give 100, 10, 1.0 and 0.1 µg/mL fortification solutions in acetone for recovery determinations. Make the dilutions according to Table 1.

TABLE 1

Parent Std (µg/mL)	Volume Taken (mL)	Final Volume (mL)	New Fortification Solution (µg/mL)
1000	10	100	100
100	10	100	10
10	10	100	1
1	10	100	0.1

6.2 Calibration Solutions (XDE-570 and 5-hydroxy XDE-570)

Pipette 1.0 mL of the 1000 µg/mL XDE-570 stock solution (Section 6.1) into an 8 dram vial. Pipette 1.0 mL of the1000 µg/mL 5-hydroxy XDE-570 stock solution (Section 6.1) into the same 8 dram vial. Evaporate the acetone to dryness using a gentle stream of nitrogen at 40°C. Add 10 mL of LC solvent (Section 5.17) to the vial and sonicate for 2 minutes. Transfer this solution to a 100 mL volumetric flask. Rinse the vial with further LC solvent, adding the rinsings to the volumetric flask and make to the mark with LC solvent. This gives a stock solution containing both XDE-570 and the metabolite at a concentration of 10.0 µg/mL.

Prepare dilutions of this stock solution in LC solvent according to Table 2 to give calibration standards over the range 0.025 - 1.00 µg/mL. Chromatograph using the conditions described in Section 6.3.

Plot response (Note 9.1) against concentration to establish detector linearity. Typical calibration plots are shown in Figures 1 and 2.

TABLE 2

Parent Std (µg/mL)	Volume Taken (mL)	Final Volume (mL)	New Calibration Solution (µg/mL)
10.00	10	100	1.00
10.00	5	100	0.50
0.50	25	50	0.25
1.00	5	50	0.10
0.50	5	50	0.05
0.25	5	50	0.025

6.3 Chromatographic Conditions

Column : 250 mm x 2.1 mm i.d., Kromasil 100 C_{18} (5 µm)

Solvent Programme :

Time/min	acetonitrile (%) (+ 1% acetic acid)	water (%) (+ 1% acetic acid)
0	20	80
4.0	20	80
10.0	40	60
20.0	40	60
22.0	90	10
27.0	90	10
30.0	20	80

Injection Volume : 50 µL

Flow rate : 0.3 mL/min

Wavelength : 260 nm

Retention time:

5-hydroxy XDE-570 : *ca.* 15 minutes
XDE-570 : *ca.* 22 minutes

Quantitation : Peak height (Note 9.1)

Total analysis time : 40 minutes.

6.4 Sample Preparation

The surface water samples are stored, refrigerated, in 5 litre plastic containers.

6.5 Method Validation

Validate the analytical procedure given in Section 6.6 for each substrate by analysing the following (Note 9.2):

At least four untreated samples (in duplicate).

At least four untreated samples after fortification at the lowest validation level (in duplicate). The lowest validation level is defined as "at least 4 times the average untreated value". At least one sample (in duplicate) fortified at two intermediate levels and one sample at a level exceeding the expected maximum residue found (in duplicate).

6.6 Sample and Fortified Sample Analysis

Include a reagent blank and procedural recovery in each analytical batch. Analyse all treated and untreated samples in duplicate.

6.6.1 Measure 500 mL of surface water into a glass bottle. Add the required volume of the appropriate fortification solution to the recovery samples, then 250 µL of concentrated sulphuric acid. Cap the bottle and mix well by shaking.

6.6.2 Condition an ENV+ isolute cartridge with 5 mL of 250:250:1 (v/v/v) acetonitrile / water / concentrated sulphuric acid followed by 10 mL of HPLC water.

6.6.3 Pass the 500 mL water sample through the cartridge at a rate of 20-30 mL / minute by means of a length of PTFE tubing fitted to the top of the cartridge through a reservoir adapter.

6.6.4 Elute the cartridge with 12 mL of 250:250:1 (v/v/v) acetonitrile / water / concentrated sulphuric acid collecting the eluate in an 8 dram vial.

6.6.5 Add 7 mL of 1 M sulphuric acid, approximately 3 g sodium chloride and 7 mL of methyl-tertiary-butyl ether (MTBE). Shake for 5 minutes, then centrifuge for 1 minute at 1500 rpm.

6.6.6 Condition an aminopropyl Isolute cartridge with 10 mL MTBE.

6.6.7 Add the ether layer from step 6.6.5 to the cartridge; discard the eluate. Re-extract the aqueous phase with a further 5 mL of MTBE and again add this to the cartridge; discard the eluate.

6.6.8 Elute the cartridge with 6 mL of 3:25:72 (v/v/v) formic acid / acetonitrile / MTBE, collecting the eluate in an 8 dram vial.

6.6.9 Evaporate the eluate to dryness under a stream of nitrogen on a heating block at 40°C.

6.6.10 Add 500 µL of 2% formic acid in acetonitrile and sonicate for 5 minutes. Add 2 mL of toluene and mix thoroughly.

6.6.11 Condition a silica Isolute cartridge with 5 mL of 2% formic acid in acetonitrile followed by 7 mL of toluene.

6.6.12 Add the extract from step 6.6.10 to the cartridge. **Keep the eluent.** Add a further 500 µL of 2% formic acid in acetonitrile to the vial and sonicate for 5 minutes. Again add 2 ml of toluene and mix thoroughly; add this to the cartridge. **Collect and combine the eluents.**

6.6.13 Elute the cartridge with 4 mL of 25:75 (v/v) 2% formic acid in acetonitrile / toluene. **Combine the eluent solutions.**

6.6.14 Evaporate the organic sample to dryness and reconstitute in 500 µL 20:80:1 (v/v/v) acetonitrile / water / acetic acid.

6.6.15 During the analysis of samples and recoveries inject calibration standards containing both XDE-570 and 5-hydroxy XDE-570 (0.025 - 1.0 µg/mL) to check detector linearity and inject a 0.25 µg/mL standard (containing both parent and metabolite) between every two sample injections. For sample extracts which contain concentrations of >1.0 µg/mL of either XDE-570 or the metabolite, dilute with LC solvent to give a concentration which is <1.0 µg/mL after re-injection.

7. CALCULATIONS

7.1 Calculation of Residues of XDE-570 and the metabolite

$$\mu g/L = \frac{\text{Sample Response}}{\text{Average Standard Response}} \times \frac{A \times B \times C \times D}{E}$$

where:

A = concentration of LC standard (µg/mL), 0.25

B = final volume (mL), 0.5

C = procedural dilution factor, 1

D = additional dilution factor (if applicable)

E = sample volume (L), 0.5

7.2 Calculation of % Recovery

$$\% \text{ Recovery} = \frac{[\mu g/L \text{ found} - \mu g/L \text{ control}]}{\text{fortification level, } \mu g/L} \times 100$$

Appendix 1

Suppliers Addresses

Aldrich Chemical Co. Ltd., The Old Brickyard, New Road, Gillingham, UK.

Anachem, 20 Charles Street, Luton, Bedfordshire, UK.

BOC Ltd., Chimnor Road, Thame, Oxfordshire, UK.

Bristol Bottle Co. Ltd., Unit 1, Ashmead Trading Estate, Keynsham, UK.

Fisher Scientific UK, Bishop Meadow Road, Loughborough Road, Leicestershire, UK.

Gerhardt UK Ltd., Underwood Lane, Crewe, Cheshire, UK.

Hewlett Packard Ltd., King Street, Wokingham, Berkshire, UK.

Hichrom Ltd., The Markham Centre, Station Road, Theale, Reading, Berkshire, UK.

Jones Chromatography Ltd., New Road, Hengoed, Mid Glamorgan, UK.

LDC Analytical, Diamond Way, Stone Business Park, Stone, Staffordshire, UK.

Owen Polyscience Ltd, 34 Chester Road, Macclesfield, Cheshire, UK.

Perkin-Elmer Ltd., Post Office Lane, Beaconsfield, Buckinghamshire, UK.

Varian UK Ltd., 28 Manor Road, Walton-on-Thames, Surrey, UK.