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Determination of Residues of XDE-570 and 5-hydroxy XDE-570 in Soil Using Organic Extraction

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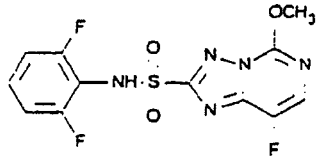
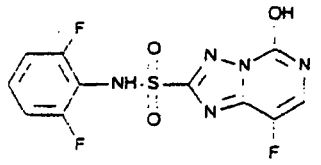
1. SCOPE

This method is applicable to the quantitative determination of XDE-570* and its 5-hydroxy metabolite** (hereafter known as 5-hydroxy XDE-570) in soil over the range 0.05 to 50.0 µg/kg. The method has been validated by the analysis of untreated samples and samples fortified with XDE-570 and 5-hydroxy XDE-570 down to a lowest validated level of 0.05 µg/kg.



XDE-570



5-hydroxy XDE-570

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

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

ERC 96.21
10 February 1997

2. PRINCIPLE

XDE-570 and 5-hydroxy XDE-570 are extracted twice from soil by shaking with acetone/1% aqueous acetic acid (9:1 v/v). The combined extracts are evaporated to a small volume. Following the addition of water, the analytes are first purified from the co-extractive material by a non polar (C18) solid phase extraction column. Further purification is achieved using a strong anion exchange (SAX) Bond Elut solid-phase extraction column followed by partitioning into ethyl acetate. Aqueous acetic acid is added and the organic phase is evaporated. The remaining aqueous solution is made to volume in further aqueous acetic acid. Quantification is carried out by Electrospray Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (ESP LC/MS/MS) which allows the formation of MH^+ ions of XDE-570 and 5-hydroxy XDE-570 at m/z 360 and 346, respectively. Mass spectral analysis is carried out using a triple sector quadrupole mass spectrometer. The second sector is operated as a collision chamber facilitating the fragmentation of the MH^+ ions. Quantitation is performed by the third quadrupole monitoring the m/z 129 fragment ion common to both XDE-570 and 5-hydroxy XDE-570.

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 Techne sample concentrator - Fisons Scientific Equipment Ltd.

4.1.2 MSE Mistral 3000 Centrifuge - Measuring and Scientific Equipment Ltd.

4.1.3 Edmund Bühler Swip SM 25 reciprocating shaker - Fisons Scientific Equipment Ltd.

4.1.4 SPE vacuum manifold - Supelco UK.

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

4.2 Chromatographic System

4.2.1 Liquid chromatograph : Jasco PU 980 pump connected to a Jasco LG 980-02 gradient system (Jasco UK) and Gilson 213 auto sampler (Anachem Ltd).

4.2.2 LC column : 25 cm x 0.46 cm id, 5 µm, Spherisorb ODS B (Fisons Scientific Equipment Ltd).

4.3 Mass Spectrometry System

4.3.1 Mass spectrometer : VG Quattro triple sector quadrupole with electrospray interface

4.3.2 Integration system : VG Masslynx software

Above equipment supplied by Fisons VG Biotech.

4.4 Laboratory Glassware and Plasticware

4.4.1 1 mL to 25 mL graduated pipettes.

4.4.2 100 mL measuring cylinders.

4.4.3 8 dram (28 mL) screw cap vials.

4.4.4 5 mL to 200 mL volumetric flasks.

4.4.5 5 mL and 10 mL disposable pipettes.

4.4.6 Glass funnels.

4.4.7 250 mL round bottomed flasks

Items in 4.4.1 to 4.4.7 available from BDH.

4.4.8 2 mL gas chromatography vials and aluminium/red rubber vial caps- Owen Polyscience Ltd.

4.4.9 2 mL plastic disposable transfer pipettes - Fisons Scientific Equipment Ltd.

4.4.10 100 mL polypropylene jars - Technical Treatments.

4.4.11 250 mL separating funnels - York Glassware Services Ltd.

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

5. MATERIALS AND REAGENTS*

5.1 Materials

5.1.1 Water - HPLC grade - BDH.

5.1.2 Acetic acid - AnalaR grade - BDH.

5.1.3 Ethyl acetate - HPLC grade - Rathburn.

5.1.4 Acetonitrile - HPLC grade - Rathburn.

5.1.5 Hydrochloric acid - AnalaR grade - BDH.

5.1.6 Acetone - glass distilled grade - Rathburn.

5.1.7 Methanol - HPLC grade - Rathburn.

5.1.8 Sodium hydrogen carbonate - GPR grade - BDH.

5.1.9 Dichlorodimethylsilane- Aldrich Chemical Co Ltd.

5.1.10 Glass wool - Fisons Scientific Equipment Ltd.

5.1.11 Bond Elut cartridges - SAX 1 g - Jones Chromatography Ltd.

5.1.12 PolarPlus cartridges - C18 - 2 g - J T Baker

5.1.13 Analytical standards of XDE-570 and 5-hydroxy XDE-570 available from Analytical Standards Co-ordinator, Research and Development Centre, DowElanco Europe.

5.1.14 Nitrogen - 99.998% purity - BOC Ltd.

5.1.15 Argon - 5.0 grade - Air Products.

5.2 Reagents

5.2.1 Extractant solution - acetone/1% aqueous acetic acid (9:1 v/v).

5.2.2 1% acetic acid in HPLC grade water.

5.2.3 1N Hydrochloric Acid.

5.2.4 0.1M Hydrochloric Acid.

5.2.5 0.1M sodium hydrogen carbonate solution.

5.2.6 0.01M sodium hydrogen carbonate solution

5.2.7 Methanol/0.1M hydrochloric acid (1:9 v/v).

5.2.8 Acetonitrile/HPLC water (1:1 v/v).

5.2.9 Dichlorodimethylsilane.

5.2.10 HPLC mobile phase - A: water/acetonitrile/acetic acid (60/40/1 v/v/v)
B: 1% acetic acid in acetonitrile.

5.2.11 CID gas - Argon.

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

6. PROCEDURE

6.1 Fortification Stock Solution

6.1.1 Weigh accurately XDE-570 and 5-hydroxy XDE-570. Dissolve in acetonitrile/water (1:1 v/v) and make up to volume to give two separate stock solutions (1 mg/mL). Store refrigerated in glassware.

6.1.2 Pipette 1 mL aliquots of each of the 1 mg/mL standards prepared above into a 100 mL volumetric flask and make to the mark with acetonitrile/water (1:1 v/v), to give a 10 µg/mL mixed standard. Pipette 1 mL of the mixed standard into a 100 mL volumetric flask and made to the mark with acetonitrile/water (1:1 v/v) to give a 100 ng/mL mixed standard. Pipette 10 mL of the 100 ng/mL mixed standard into a 100 mL volumetric flask and made to the mark with acetonitrile/water (1:1 v/v) to give a 10 ng/mL mixed standard. Use these standards for fortifying purposes and store refrigerated.

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

6.2.1 Pipette 0.03, 0.05, 0.10, 0.25, 0.5, 1.0, 2.0 and 3.0 mL aliquots of the 100 ng/mL mixed standard prepared in section 6.1.2 into separate silanised 10 mL volumetric flasks. Make up to the mark with water containing 1% acetic acid, and use to obtain a calibration curve in the range 0.30 to 30 ng/mL. Store these refrigerated.

6.2.2 Plot mass spectrometer response against concentration to establish detector linearity using conditions given in section 6.3. Typical calibration curves are shown in Figures 1a and 1b.

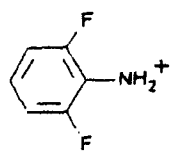
ERC 96.21
10 February 1997

6.3 Liquid Chromatography-Mass Spectrometry Conditions

Instrument	:	VG Quattro triple sector quadrupole mass spectrometer with electrospray interface
Column	:	25 cm x 0.46 cm id, 5 μm, Spherisorb ODS B
Mode	:	Positive ion electrospray
Flow rate	:	1.0 mL/min (20:1 split into MS)
Mobile phase	A :	water/acetonitrile/acetic acid (60:40 1 v/v/v)
	B :	1% acetic acid in acetonitrile

Time (mins)	%A	%B
0	100	0
10	100	0
11	0	100
15	0	100
16	100	0
21	100	0

Injection volume	:	100 μL
Source temperature	:	150°C
Multiple Reaction Monitoring (MRM) parameters	:	XDE-570: 360.0 to 129.0 Da 5-hydroxy XDE-570: 346.0 to 129.0 Da

Ion (m/z)	Description
360	MH^+ of XDE-570
346	MH^+ of 5-hydroxy XDE-570
129	

The following conditions were used for multiple reaction monitoring :

Characteristic ions (m/z)	Dwell time (sec)	Cone voltage (V)	Collision energy (eV)
346 > 129	0.5	20	20
360 > 129	0.5	20	20

Typical Retention time : XDE-570 approximately 9 minutes
5-hydroxy XDE-570 approximately 5 minutes

CID gas: Argon

Collision gas cell pressure gave suppression of reference acetonitrile solvent ion m/z 42 down to 30% on MS2.

6.4 Sample Preparation

Moist or air dried soils are mixed thoroughly by hand and then passed through a 4 mm sieve. The sieved soils are then again thoroughly mixed by hand prior to analysis.

6.5 Method Validation

Validate the analytical procedure given in Section 6.6 by analysing the following:
At least three untreated samples (each in duplicate).

At least three untreated samples (each in duplicate) after fortification at the lowest validation level (equivalent to LOQ). The lowest validation level is defined as "at least 4 times the average control value".

Further untreated samples (each in duplicate) fortified at intermediate levels and at a level exceeding the maximum residue found.

6.6 Sample and Fortified Sample Analysis

Include a control sample and a minimum of one procedural recovery in each analytical batch. Ensure care is taken when handling test articles and samples. All glassware used for evaporation stages and LC vials should be silanised.

6.6.1 Weigh 25 ± 0.1 g of sample into a polypropylene jar (100 mL capacity). Add fortification solutions at this stage if appropriate.

6.6.2 Add 75 mL acetone/1% aqueous acetic acid (9:1 v/v) and place on a reciprocating shaker for 60 minutes. Centrifuge at approximately 3000 rpm for 10 minutes. Decant supernatant through a glass wool plug into a 250 mL round bottom flask. Repeat shake with a further 25 mL acetone/1% aqueous acetic acid (9:1 v/v) for 60 minutes, combining supernatant phases in the round bottom flask.

6.6.3 Remove the acetone by rotary evaporation until only the aqueous phase remains. Dilute sample to 100 mL with water. Condition a PolarPlus C18 cartridge (2 g, 6cc) with 10 mL methanol followed by 10 mL 0.1M HCl not allowing the column to run dry. Prior to application of sample to C18 add 2 mL 1M HCl and mix thoroughly (ensure pH is <3, add further 1M HCl to adjust if necessary). Apply sample allowing the eluent to drain to the top of the column packing. Wash the column with 10 mL water (discarding the rinse and allowing the column to go dry). Elute with 3 mL acetonitrile, dilute to 30 mL with 0.01 M sodium hydrogen carbonate.

6.6.4 Condition a SAX Bond Elut column (1 g, 6cc) with 10 mL methanol followed by 10 mL of 0.01 M sodium hydrogen carbonate not allowing the column to run dry. Apply sample allowing eluent to drain to the top of the column packing. Rinse the measuring cylinder with 10 mL 0.01 M sodium hydrogen carbonate followed by 10 mL acetone and apply to column allowing the column to fully drain after each application (discarding the rinsates). Elute with 15 mL of 0.1M HCl/methanol (9:1 v/v) collecting eluent in a 28 mL silanised glass vial.

6.6.5 Partition with 10 mL ethyl acetate, transferring the organic phase into a silanised 28 mL glass vial. Repeat the partition with 5 mL ethyl acetate, combining the organic phases in a silanised glass tube (see note 9.1).

6.6.6 Add 2.0 mL of 1% aqueous acetic acid and evaporate under nitrogen at room temperature until no ethyl acetate is present. Make sample up to volume in a volumetric flask (2.0 mL) with 1% aqueous acetic acid ready for analysis by LC-MS/MS.

7. CALCULATIONS

7.1 Calculation of XDE-570 and 5-hydroxy XDE-570 Residues :

The residues of XDE-570 and 5-hydroxy XDE-570 are determined on the basis of peak area using a calibration curve, Figures 1a and 1b, respectively. All peak measurements and calculations are performed on VG Masslynx software.

The analytical specifications are as follows :

Number of calibration standards to be used ≥ 5

Standard working range $C_{min} \leq$ LOQ
$C_{max} >$ highest concentration found

Correlation coefficient of the calibration ≥ 0.995

The response from calibration solutions (R) is plotted against the amount of test article injected (A) to generate a straight line graph (R = BO + B1A) where B1 is the gradient and BO is the intercept.

Concentrations of test article (A) in samples are calculated from their response using the equation :

$$A\ (ng/mL) = \frac{(R - BO)}{B1}$$

The residues of XDE-570 and 5-hydroxy XDE-570 in test samples are calculated as follows :

$$\text{Residue}\ (\mu g/kg) = \frac{\text{extract concentration (ng/mL) x final volume (mL)}}{\text{sample weight (g)}}$$

where the final volume includes dilution steps if applicable.

Recovery data from fortified samples are calculated using the following equation :

$$\text{Recovery}\ (\%) = \frac{A - C}{S} \times 100$$

Where

A = concentration found in fortified control sample (μg/kg)
C = concentration found in control sample (μg/kg)
S = concentration added to the fortified control sample (μg/kg)

ERC 96.21
10 February 1997

9. NOTES

9.1 Silanising reagent - a 10% solution of Dichlorodimethysilane in hexane (glassware soaked for 15 min).

9.2 Peak area was used for quantitation during the validation of this method although peak height could also be used.

9.3 A correction factor is made for moisture content of the soil when applicable. The moisture content can be determined by heating pre-weighed samples at 105°C for 16 hours using the following formula:

$$\% \text{ moisture content} = \frac{\text{wet weight - dry weight}}{\text{(wet weight)}} \times 100$$

$$\text{corrected result (µg/kg dry weight)} = \frac{\text{(uncorrected residue x 100)}}{\text{(100 - moisture content)}}$$

9.4 Elution profiles given in Sections 6.6.3 and 6.6.4 are given only as a guideline and may vary between batches.