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Determination of Residues of XDE-638 in Water by Liquid Chromatography with Tandem Mass Spectrometry

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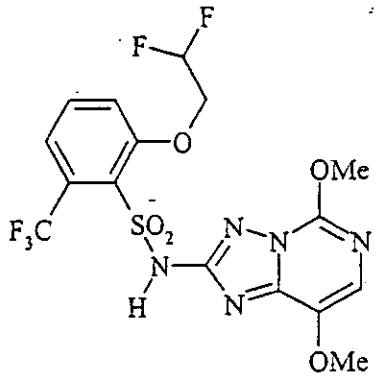
1. SCOPE

This method is applicable for the quantitative determination of XDE-638 (2-(2,2-difluoroethoxy)-N-(5,8-dimethoxy [1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)-6-(trifluoromethyl)-benzensulfonamide) in drinking water, ground water, and surface water. The method was validated over the concentration range 0.05-100 µg/L with a validated limit of quantitation of 0.05 µg/L.



XDE-638
CAS No: 219714-96-2

2. PRINCIPLE

An aliquot of the water sample is purified using a polymeric-anion exchange solid phase extraction cartridge (SPE). The SPE cartridge is washed with acetonitrile and eluted with an acetonitrile:formic acid solution (100:0.1). The eluate is evaporated to dryness and the residues reconstituted in an acetonitrile:methanol:water:acetic acid mobile phase (15:15:70:0.1). The final solution is analyzed by liquid chromatography with positive-ion electrospray tandem mass spectrometry (LC/MS/MS).

A calibration curve resulting from the injection of eight standards demonstrated linearity with a correlation coefficient of at least 0.999. LC/MS/MS affords a highly specific method for quantitation and confirmation of XDE-638 by retention time matching in conjunction with monitoring a compound specific precursor-ion/product-ion transition (*m/z* 484/195).

3. SAFETY PRECAUTIONS

3.1. Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information on non Dow AgroSciences LLC products should be obtained from the container label or from the supplier. Disposal of reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.

3.2. Acetonitrile and methanol are flammable and volatile and should be used in well-ventilated areas away from ignition sources.

3.3. Acetic acid and formic acid are corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be worn when handling these chemicals.

4. EQUIPMENT (Note 12.1)

4.1. Laboratory Equipment

4.1.1. Balance, analytical, Model AE100, Mettler Instrument Corporation, Hightstown, NJ 08520.

4.1.2. Balance, analytical, Model P-1200, Mettler Instrument Corporation.

4.1.3. Evaporator, TurboVap LV, Zymark Corporation, Hopkinton, MA 01748.

4.1.4. Pipetter, adjustable, Eppendorf, 10-100 µL, catalog number 05-402-48, Fisher Scientific, Pittsburgh, PA 15275.

4.1.5. Pipetter, adjustable, Eppendorf, 50-1000 µL, catalog number 21-378-83, Fisher Scientific.

4.1.6. Ultrasonic cleaner, Model 1200, Branson Cleaning Equipment Company, Shelton, CT 06484.

4.1.7. Vacuum manifold, VacMaster-20, catalog number 121-2027, International Sorbent Technology Ltd, Hengoed, Mid Glamorgan UK and distributed by Jones Chromatography USA, Inc., Lakewood, CO 80228.

4.1.8. Vortex mixer, Model G-560, Scientific Industries, Inc., Bohemia, NY 11716.

4.2. Chromatographic Equipment (Note 12.1.)

4.2.1. Column, analytical, ZORBAX SB-C8, 4.6 x 75 mm, 3.5-µm, catalog number 866953-906, Agilent Technologies, Wilmington, DE 19808.

4.2.2. Liquid chromatograph autosampler, Model 1100, Agilent Technologies.

4.2.3. Liquid chromatograph binary pump, Model 1100, Agilent Technologies.

4.2.4. Liquid chromatograph degasser, Model 1100, Agilent Technologies.

4.2.5. Mass spectrometer, Model API 3000, Applied Biosystems, Foster City, CA 94404.

4.2.6. Mass spectrometer data system, Analyst 1.1, Applied Biosystems.

5. GLASSWARE AND MATERIALS (Note 12.1)

5.1. Bottle, 1.0-L PyrexPlus media bottle, catalog number 06-423-3D, Fisher Scientific.

5.2. Cylinder, graduated mixing, 100-mL, catalog number 20039-0100, Kimble/Kontes, Vineland, NJ 08360.

5.3. Cylinder, graduated, 500-mL, catalog number C7000-500, National Scientific Company, Lawrenceville, GA 30243.

5.4. Cylinder, graduated, 1000-mL, catalog number C7000-1L, National Scientific Company.

5.5. Flask, volumetric, 10-mL, catalog number 161-8986, National Scientific Company.

5.6. Flask, volumetric, 100-mL, catalog number 161-8987, National Scientific.

5.7. Pipet, 10-mL disposable seriological, catalog number, 13-666-7E, Fisher Scientific.

5.8. Pipet, 3-mL disposable transfer, catalog number, 13-711-7, Fisher Scientific.

5.9. Pipet, volumetric, 0.5-mL, catalog number 261-6010, National Scientific Company.

5.10. Pipet, volumetric, 1.0-mL, catalog number 261-6011, National Scientific Company.

5.11. Pipet, volumetric, 2.0-mL, catalog number 261-6012, National Scientific Company.

5.12. Pipet, volumetric, 3.0-mL, catalog number 261-6013, National Scientific Company.

5.13. Pipet, volumetric, 5.0-mL, catalog number 261-6015, National Scientific Company.

5.14. Pipet, volumetric, 10.0-mL, catalog number 261-6020, National Scientific Company.

5.15. Vial, autosampler, 2-mL, catalog number C4000-1, National Scientific Company.

5.16. Vial cap, for autosampler vial, catalog number C4000-54B, National Scientific Company.

5.17. Vial, 40-mL, with PTFE-lined screw cap, catalog number B7800-6, National Scientific Company.

5.18. Waters MAX SPE cartridge, 60-mg packing, catalog number 186000367, Waters, Milford, MA 01757.

6. REAGENTS, STANDARDS, AND PREPARED SOLUTIONS (Note 12.1.)

6.1. Reagents

6.1.1. Acetic acid, Certified ACS Plus, catalog number A38S-500, Fisher Scientific.

6.1.2. Acetonitrile, HPLC grade, catalog number 2856, Mallinckrodt Baker, Inc., Paris, KY 40361.

6.1.3. Formic acid, 98% GR grade, catalog number FX0440-7, EM Science, Gibbstown, NJ 08027.

6.1.4. Methanol, HPLC grade, catalog number 3041, Mallinckrodt Baker Inc.

6.1.5. Water, HPLC grade, catalog number WX0004-1, EM Science.

6.2. Standards

6.2.1. XDE-638: 2-(2,2-difluoroethoxy)-*N*-(5,8-dimethoxy[1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)-6-(trifluoromethyl)-benzenesulfonamide.

Compounds can be obtained from Test Substance Coordinator, Dow AgroSciences LLC, 9330 Zionsville Road, Building 304, Indianapolis, IN 46268-1054.

6.3. Prepared Solutions

6.3.1. acetonitrile:formic acid (100:0.1)

Pipette 0.1 mL of formic acid into 100 mL of HPLC grade acetonitrile and mix. This reagent must be prepared on the day of sample analysis.

6.3.2. acetonitrile:methanol:acetic acid (50:50:0.1)

Measure 500 mL of HPLC grade methanol with a graduated cylinder (500-mL) and transfer to a 1-L bottle. Measure 500 mL of HPLC grade acetonitrile with a graduated cylinder (500-mL) and transfer to the 1-L bottle. Add 1.0 mL of acetic acid and cap the bottle. Shake to mix and allow the solution to equilibrate to room temperature before use.

6.3.3. acetonitrile:methanol:water:acetic acid (15:15:70:0.1)

Measure 15 mL of HPLC grade methanol with a graduated cylinder (25-mL) and transfer to a clean 100-mL bottle. Measure 15 mL of HPLC grade acetonitrile with a graduated cylinder (25-mL) and transfer to the 100-mL bottle. Measure 70 mL of HPLC grade water with a graduated cylinder (100-mL) and transfer to the 100-mL bottle. Add 100 µL of acetic acid and cap the bottle. Shake to mix and allow the solution to equilibrate to room temperature before use.

6.3.4. water:acetic acid (100:0.1)

Measure 1000 mL of HPLC grade water with a graduated cylinder (1-L) and transfer to a clean 1-L bottle. Add 1.0 mL of acetic acid and cap the bottle. Shake to mix and allow the solution to equilibrate to room temperature before use.

7. PREPARATION OF STANDARD SOLUTIONS (Note 12.2.)

7.1. Preparation of XDE-638 Spiking Solutions

7.1.1. Weigh 0.0100 g of XDE-638 analytical standard and quantitatively transfer to a 10-mL volumetric flask. Dilute to volume with acetonitrile to obtain a 1000-µg/mL stock solution of XDE-638.

7.1.2. Pipet 1.0 mL of the 1000-µg/mL XDE-638 solution (Section 7.1.1) into a 100-mL volumetric flask. Adjust to volume with acetonitrile to obtain a 10.0-µg/mL XDE-638 spiking solution.

7.1.3. Pipet 10.0 mL of the 10.0-µg/mL solution in Section 7.1.2 into a 100-mL volumetric flask and adjust to volume with acetonitrile to obtain a 1.0-µg/mL XDE-638 spiking solution.

7.1.4. Pipet 10.0 mL of the 1.0-µg/mL solution in Section 7.1.3 into a 100-mL volumetric flask and adjust to volume with acetonitrile to obtain a 0.1-µg/mL XDE-638 spiking solution.

7.1.5. Pipet 10.0 mL of the 0.1-µg/mL solution in Section 7.1.4 into a 100-mL volumetric flask and adjust to volume with acetonitrile to obtain a 0.01-µg/mL XDE-638 spiking solution.

7.2. Preparation of XDE-638 Calibration Solutions

Prepare calibration standard solutions in acetonitrile:methanol:water:acetic acid (15:15:70:0.1) from the 10.0-µg/mL mixed standard (Section 7.1.2) over the range 0.0001–0.05 µg/mL as described below.

Original Standard Concentration	Aliquot of Original Standard	Final Soln. Volume	Calib Soln. Final Conc.	Equivalent Sample Conc.
µg/mL	mL	mL	µg/mL	µg/L
10.00	10.00	100	1.00	N/A
1.00	10.00	100	0.10	N/A
1.00	5.0	100	0.05	5.00
1.00	3.5	100	0.035	3.50
1.00	2.0	100	0.02	2.00
1.00	1.0	100	0.01	1.00
0.10	5.0	100	0.005	0.50
0.10	1.0	100	0.001	0.10
0.01	5.0	100	0.0005	0.05
0.01	1.0	100	0.0001	0.01

8. LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

8.1. Typical Liquid Chromatography Operating Conditions (Note 12.3.)

Instrumentation: Agilent Model 1100 autosampler
Agilent Model 1100 binary pump
Agilent Model 1100 degasser
PE SCIEX API 3000 LC/MS/MS System
PE SCIEX Analyst 1.1 data system

Column: Zorbax SB C8
4.6 x 75 mm, 3.5-µm

Column Temperature: 35 °C

Injection Volume: 50 µL

Run Time: 8 minutes

Mobile Phase: A –methanol:acetonitrile (1:1) with 0.1% acetic acid
B –water with 0.1% acetic acid

Flow Rate: 900 µL/min, 150 µL/min split to ESI source

Gradient:	Time, min	A, %	B, %
	0.0	30	70
	4.0	100	0
	6.0	100	0
	6.1	30	70
	8.0	30	70

8.2. Typical Mass Spectrometry Operating Conditions

API 3000:

Interface:	TurboIonSpray
Scan Type:	MRM
Resolution:	Q1 – unit, Q3 – low
Curtain Gas (CUR):	14
Collision Gas (CAD):	4.0
Temperature (TEM):	425 °C
Ion Source Gas 1 (GS1):	10
Ion Source Gas 2 (GS2):	6000
Period 1	
Time:	6.0 minutes
Polarity:	Positive
IonSpray Voltage (IS)	5500

Compound	Ion, *m/z*		Time, *ms*	CE, *v*
	Q1	Q3		
XDE-638	484.0	195.0	150	37

8.3. Typical Mass Spectra

Typical mass spectra and product ion spectra of XDE-638 are presented in Figure 1.

8.4. Typical Calibration Curve

A typical calibration curve for the determination of XDE-638 in water is shown in Figure 2.

8.5. Typical Chromatograms

Typical chromatograms for a calibration standard, control drinking water sample, and control drinking water samples fortified at 0.05 µg/L (limit of quantitation) and the highest fortified level are presented in Figure 3.

9. DETERMINATION OF RECOVERY OF XDE-638 IN WATER

9.1. Method Validation

Unless otherwise specified, a sample set should contain the following samples:

At least one reagent blank.
At least one control.
At least one control fortified at the limit of detection.
At least two controls fortified at the limit of quantitation.
At least one control fortified at a higher concentration.

9.2. Sample Analysis for XDE-638 in Water

9.2.1. Pipette 10.0 mL of the sample into a 40-mL vial. For recovery samples, add appropriate aliquots of the spiking solutions (Section 7.1.) to obtain concentrations ranging from 0.05 to 100 µg/L.

9.2.2. Cap the vials and shake by hand to mix.

9.2.3. Purify the samples using the following SPE procedure (Note 12.4.):

a. Condition a Waters MAX SPE cartridge (60 mg) with 2.0 mL of acetonitrile followed by 2.0 mL of HPLC grade water. Dry the cartridge under full vacuum for 10 seconds between solvents.

b. Attach a 20-mL reservoir to the SPE cartridge and transfer the sample from Step 9.2.2. to the reservoir. Draw the sample through the cartridge at approximately 2 mL/min, discarding the eluate. Dry the cartridge under full vacuum for 10 seconds after the solvent has eluted.

c. Wash the SPE cartridge with 2 mL of acetonitrile, discarding the eluate. Dry the cartridge for 2 minutes under full vaccum.

d. Elute the XDE-638 from the cartridge at approximately 1 mL/min with two 1.50-mL aliquots of an acetonitrile:formic acid solution (100:0.1), collecting the eluate in a 40-mL vial. (Allow the solvent to soak on the cartridge for 30 seconds before eluting.)

9.2.4. Evaporate the eluate to dryness using a TurboVap evaporator set at 40°C and 10 psi of nitrogen pressure.

9.2.5. Reconstitute the samples in 1.0 mL of acetonitrile:methanol:water:acetic acid (15:15:70:0.1). Vortex mix and sonicate the vials for approximately 30 seconds.

9.2.6. Dilute samples which contain XDE-638 concentrations >0.05 µg/mL with acetonitrile:methanol:water:acetic acid (15:15:70:0.1) to give a concentration within the calibration range.

9.2.7. Analyze the samples along with the calibration standards using the LC/MS/MS conditions listed in Section 8. Determine the suitability of the chromatographic system using the following criteria:

a. Standard curve linearity: Determine that the correlation coefficient equals or exceeds 0.995 for the least squares equation which describes the detector response as a function of standard curve concentration.

b. Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figures 3a-3d with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 10:1 has been attained for the 0.0005-µg/mL calibration standard (equivalent to 0.05 µg/L of XDE-638 in the water sample).

10. CALCULATIONS

10.1. Calculation of Percent Recovery for XDE-638

10.1.1. Inject a series of calibration standards (Section 7.2.) as described in Section 8 and determine the peak areas for XDE-638 as indicated below.

XDE-638 *m/z* Q1/Q3 484/195

10.1.2. Prepare a standard curve by plotting the concentration of XDE-638 on the abscissa (x-axis) and the respective peak area on the ordinate (y-axis) as shown in Figure 2. Using linear regression analysis (13.1) with a 1/x weighting (13.2.), determine the equation for the curve with respect to the abscissa (Note 12.5.).

For example, using linear regression with the XDE-638 data from Figure 2:

$$X = \left(\frac{Y - \text{intercept}}{\text{slope}}\right)$$

$$\text{XDE - 638 Conc. } (\mu g/mL) = \left(\frac{\text{XDE - 638 peak area} - \text{intercept}}{1.89e7}\right)$$

$$\text{XDE - 638 Conc. } (\mu g/mL) = \left(\frac{\text{XDE - 638 peak area} - 855}{1.89e7}\right)$$

10.2. Calculation of Percent Recovery for XDE-638

10.2.1. Determine the gross concentration in each recovery sample by substituting the peak area obtained into the above equation and solving for the concentration.

For example, using the data for XDE-638 from Figure 3c:

$$\text{XDE - 638 Conc. (gross µg/mL)} = \left(\frac{\text{XDE - 638 peak area} - 855}{1.89e7}\right)$$

$$\text{XDE - 638 Conc. (gross µg/mL)} = \left(\frac{9.90e3 - 855}{1.89e7}\right)$$

$$\text{XDE - 638 Conc. (gross)} = 0.00048 \text{ µg/mL}$$

Convert the concentration of µg/mL of XDE-638 found in the final sample prepared for analysis to µg/L of XDE-638 in the original water sample aliquot as follows:

$$\text{XDE - 638 Conc. (gross µg/L)} = 0.00048 \text{ µg/mL} \times \frac{1000 \text{ mL}}{10 \text{ mL}}$$

$$\text{XDE - 638 Conc. (gross)} = 0.048 \text{ µg/L}$$

10.2.2. Determine the net concentration of XDE-638 in each recovery sample by subtracting any XDE-638 concentration found at the retention time of XDE-638 in the untreated control sample from that of the gross XDE-638 concentration in the recovery sample.

For example, using the data from Figures 3b and 3c:

$$\text{XDE-638 Conc. (net µg/L)} = \text{XDE-638 conc. (gross µg/L)} - \text{XDE-638 conc. (control µg/L)}$$

$$\text{XDE-638 Conc. (net µg/L)} = 0.048 \text{ µg/L} - 0.0000 \text{ µg/L}$$

$$\text{XDE-638 Conc. (net)} = 0.048 \text{ µg/L}$$

10.2.3. Determine the percent recovery by dividing the net concentration of each recovery sample by the theoretical concentration added.

$$\text{Recovery} = \frac{\text{Conc. Found}}{\text{Conc. Added}} \times 100\%$$

$$\text{Recovery} = \frac{0.048\ \mu g/L}{0.050\ \mu g/L} \times 100\%$$

$$\text{Recovery} = 96\%$$

10.3. Determination of XDE-638 in Water Samples

10.3.1. Determine the gross concentration of XDE-638 in each water sample by substituting the peak area obtained into the equation for the standard calibration curve, and calculating the uncorrected residue result as described in Sections 10.2.1.

10.3.2. For those samples that require correction for method recovery, use the average recovery of all the recovery samples from a given sample set to correct for method efficiency. For example, using the XDE-638 data from Figure 3c and the average recovery from Table 1 for the sample analyzed on 31-Jan-2002:

$$\text{XDE - 638 Conc. (corrected } \mu g/L) = \text{XDE - 638 Conc. (gross } \mu g/L) \times \left(\frac{100}{\text{Average \% Recovery}}\right)$$

$$\text{XDE - 638 Conc. (corrected } \mu g/L) = 0.048\ \mu g/L \times \frac{100}{102}$$

$$\text{XDE - 638 Conc. (corrected)} = 0.047\ \mu g/L$$

11.3. Assay Time and Stopping Points

A typical analytical run would consist of a minimum of eight standards encompassing the expected range of sample concentrations, a reagent blank, a control (a non-fortified sample), a minimum of three fortified controls (two of which must be at the LOQ), and 30 samples. This typical analytical set can be prepared in approximately 3 hours followed by the chromatographic analysis.

Acceptable "stopping points" are after Sections 9.2.3d, 9.2.4 and 9.2.5. Samples should be stored in a refrigerator during these stopping points.

11.4. Standardization of MAX SPE Cartridges

11.4.1. Pipette 10.0 mL of HPLC water into a 11-dram vial. Add 100-µL of a 0.1-µg/mL XDE-638 standard solution. Cap the vial and shake to mix.

11.4.2. Profile the SPE cartridge using the following procedure:

a. Condition a Waters MAX SPE cartridge (60-mg) with 2.0 mL of acetonitrile followed by 2.0 mL of HPLC grade water. Dry the cartridge under full vacuum for 10 seconds between solvents.

b. Attach a 20-mL reservoir to the SPE cartridge and transfer the sample from Step 11.4.1. to the cartridge. Draw the sample through the cartridge at approximately 2 mL/min, discarding the eluate. Dry the cartridge under full vacuum for 10 seconds after the sample has eluted.

c. Wash the cartridge with 2 mL of acetonitrile, collecting the eluate in a 40-mL vial. Dry the cartridge under full vacuum for 2 minutes.

d. Elute the XDE-638 from the cartridge at approximately 1 mL/min with three 1.50-mL aliquots of acetonitrile:formic acid solution (100:0.1), collecting the eluate in separate 40-mL vials. (Allow the solvent to soak on the cartridge for 30 seconds before eluting.)

11.4.3. Evaporate the eluates from steps 11.4.2c and 11.4.2d to dryness using a TurboVap evaporator set at 40°C and 10 psi of nitrogen pressure.

11.4.4. Reconstitute the samples in 1.0 mL of acetonitrile:methanol:water:acetic acid (15:15:70:0.1). Vortex mix and sonicate the vials for approximately 30 seconds.

11.4.5. Transfer the samples and standards to 2-mL autosampler vials and cap. Analyze the calibration standards and samples using the LC/MS/MS conditions listed in Section 8. Calculate the percent recovery as described in Section 10.

A typical elution profile is illustrated in Figure 4. If the elution profile differs from that shown, adjust the volume of acetonitrile:formic acid (100:0.1) to be collected in Step 9.2.3c and 9.2.3d.

12. NOTES

12.1. Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory supplies are assumed to be readily available and are, therefore, not listed.

12.2. Section 7 provides suggested concentrations for calibration standard preparation. Other dilution schemes may be followed.

12.3. The data presented in this method were generated using a Sciex 3000 API 3000 in optimal condition. Operating conditions may be modified to obtain optimal separation or sensitivity. However, method performance may be compromised by increasing injection volume to compensate for low instrument sensitivity.

12.4. Before using each lot of MAX SPE cartridges, determine the elution profile as described in section 11.4.

12.5. Linear Regression analysis using a quadratic curve fit may also be used.

13. REFERENCES

13.1. Freund, J. E.; Williams, F. J. *Dictionary/Outline of Basic Statistics*; Dover: New York, 1991; p 170.

13.2. Neter, J.; Kutner, M. H.; Nachtssheim, C. J. ; Wasserman, W. *Applied Linear Regression Models*; The McGraw-Hill Company: New York, 1996; p 409.

13.3. Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983; *55*, 2210-2218.