Cover Sheet for

Environmental Chemistry Method

Pesticide Name: Penoxsulam

MRID#: 458308-10

Matrix: Water

Analysis: LC/MS/MS

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STUDY TITLE

Validation Report for Method GRM 01.30 - Determination of Residues of XDE-638 in Water by Liquid Chromatography with Tandem Mass Spectrometry

DATA REQUIREMENTS

Commission Directive 96/46/EC 4.2.1
OPPTS 850.7100

AUTHOR

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STUDY COMPLETED ON

August 28, 2002

PERFORMING LABORATORY

Regulatory Laboratories—Indianapolis Lab
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, Indiana 46268-1054

LABORATORY STUDY ID

020010

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compounds: XDE-638

Title: Validation Report for Method GRM 01.30 - Determination of Residues of XDE-638 in Water by Liquid Chromatography with Tandem Mass Spectrometry

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d)(1)(A)(B), or (C).*

Company: Dow AgroSciences LLC

Company Agent: R. L. Herrera

Title: Global Regulatory Manager

Signature:

Date: 08/14/2002

*In the United States, the above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.

THIS DATA MAY BE CONSIDERED CONFIDENTIAL IN COUNTRIES OUTSIDE THE UNITED STATES.

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Title: Validation Report for Method GRM 01.30 - Determination of Residues of XDE-638 in Water by Liquid Chromatography with Tandem Mass Spectrometry

Study Initiation Date:	29-Jan-2002	Study Completion Date:	28-Aug-2002
Experimental Start Date:	31-Jan-2002	Experiment Termination Date:	25-Jul-2002

This report represents data generated after the effective date of the EPA FIFRA Good Laboratory Practice Standards.

United States Environmental Protection Agency
Title 40 Code of Federal Regulations Part 160
FEDERAL REGISTER, August 17, 1989

Organisation for Economic Co-Operation and Development
ENV/MC/CHEM(98)17, Paris January 26, 1998

All aspects of this study were conducted in accordance with the requirements for Good Laboratory Practice Standards, 40 CFR 160 with no exceptions.

08/14/2002

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Sponsor
Dow AgroSciences LLC

Date

08/14/2002.

R. L. Herrera
Submitter
Dow AgroSciences LLC

Date

28 Aug 2002

M. J. Hastings
Study Director/Author
Dow AgroSciences LLC

Date

Dow AgroSciences Quality Assurance Unit
Good Laboratory Practice Statement Page

Compound: XDE-638 **Study ID:** 020010

Title: Validation Report for Method GRM 01.30 – Determination of Residues of XDE-638 in Water by Liquid Chromatography with Tandem Mass Spectrometry

Study Initiation Date: January 29, 2002 **Study Completion Date:** August 28, 2002

GLP Quality Assurance Inspections

Date of GLP Inspection(s)	Date Reported to the Study Director and to Management	Phases of the Study which received a GLP Inspection by the Quality Assurance Unit
January 29, 2002	January 29, 2002	Protocol Review
January 31, 2002	January 31, 2002	Sample Preparation, Analysis and Maintenance Logs
August 21 – 23, 2002	August 23, 2002	Report and Raw Data, Test and Reference Material Verification

QUALITY ASSURANCE STATEMENT:

The Quality Assurance Unit has reviewed the final study report and has determined that the report reflects the raw data generated during the conduct of this study.

Lynn M Kennard
Lynn M. Kennard
Dow AgroSciences, Quality Assurance

August 28, 2002
Date

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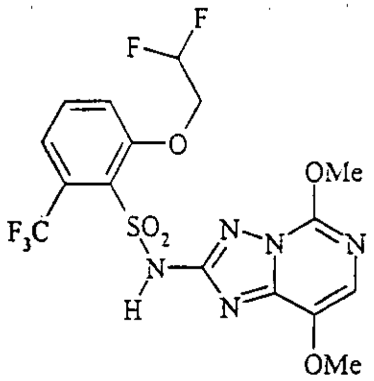
Validation Report for Method GRM 01.30 - Determination of Residues of XDE-638 in Water by Liquid Chromatography with Tandem Mass Spectrometry

ABSTRACT

This report contains validation data for Dow AgroSciences LLC analytical residue method GRM 01.30, “Determination of Residues of XDE-638 in Water by Liquid Chromatography with Tandem Mass Spectrometry.” 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)-benzenesulfonamide in drinking water, ground water and surface water. The method was validated over the concentration range of 0.05-100 µg/L with a validated limit of quantitation of 0.05 µg/L. The average recovery for XDE-638 in water was 99% with a standard deviation of 4.9%.

INTRODUCTION

XDE-638 (2-(2,2-difluoroethoxy)-*N*-(5,8-dimethoxy [1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)-benzenesulfonamide) is the active ingredient in a broad spectrum sulfonamide herbicide being developed by Dow AgroSciences LLC for control of grass, broadleaf, and sedge weeds in water- and direct-seeded rice.



XDE-638
CAS Number: 219714-96-2

Dow AgroSciences LLC analytical residue method GRM 01.30, "Determination of Residues of XDE-638 in Water by Liquid Chromatography with Tandem Mass Spectrometry" (Appendix A) was developed in order to support future registrations. This report describes the data that were generated during the validation study and defines the accuracy, precision, specificity, and ruggedness of the method.

EXPERIMENTAL

Sample Origin, Numbering, Preparation and Storage

The untreated control drinking water and pond water sample were obtained from the drinking water supply and pond within the Dow AgroSciences Research and Development Center, Indianapolis, Indiana. The ground water sample was obtained from a residential well in Cicero, Indiana.

Following collection, samples were assigned unique sample numbers that were used to track the samples throughout sampling, storage, and analysis. These samples were given unique tracking numbers by logging them into the computerized Dow AgroSciences Sample Tracking and Reporting (DSTAR) system.

All samples were stored in temperature-monitored refrigerators, only being removed for analysis. All samples were stored refrigerated prior to analysis.

Test Substance/Analytical Standards

The analytical standards used for fortification of the untreated water samples and for the preparation of the calibration standards are listed below.

Test Substance/ Analytical Standard	Identification Number	Percent Purity	Recertification Date	Reference
XDE-638	TSN101649	99.1	23-Jan-2003	FA&PC 013015

Preparation of Fortification Solutions and Calibration Standard Solutions

Fortification solutions and calibration standard solutions were prepared as described in Dow AgroSciences Laboratory Notebooks C1300 and C1382 and are cross-referenced in Section 7 of the final method, GRM 01.30 (Appendix A).

Fortification of Recovery Samples

The method used for the determination of XDE-638 in water is described in the draft analytical method referenced in Dow AgroSciences Laboratory Notebook C1382 on pages 1-7 and was cross-referenced in Section 9 of Dow AgroSciences LLC analytical residue method GRM 01.30 (Appendix A).

Method performance, in terms of analyte recovery, was determined on control water samples that were freshly fortified on the day of analysis with appropriate concentrations of XDE-638. Recovery samples were fortified with XDE-638 over the concentration range of 0.05-100 µg/mL. Reagent blank and control samples which received no fortification were also prepared for analysis along with the recovery samples to monitor for background interferences (except for two re-analysis sets which contained no control samples).

Sample Extraction and Analysis

Samples were analyzed for residues of XDE-638 using the draft analytical method found in Dow AgroSciences Laboratory Notebook C1382 on pages 1-7, which was later cross-referenced in Section 9 of Dow AgroSciences LLC analytical residue method GRM 01.30 (Appendix A).

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) using a Zorbax SB C8 column installed in a PE SCIEX API 3000 LC/MS/MS system as described in Section 8 of the final method GRM 01.30 (Appendix A).

Chromatographic data were collected and integrated using a PE SCIEX Analyst 1.1 Data System.

All analyses were performed at the Dow AgroSciences Regulatory Laboratories in Indianapolis, Indiana.

Calculation of Percent Recovery

The calculation of the percent recovery for samples fortified with XDE-638 were performed as described in Section 10 of the method, GRM 01.30 (Appendix A).

Statistical Treatment of Data

Statistical treatment of the data included the calculation of the linear regression equations, correlation coefficients for describing the linearity of the calibration curves, means, standard deviations, and the relative standard deviations of the results for the fortified recovery samples.

Study Personnel

Method development work and method validation was performed by M. J. Hastings of Dow AgroSciences and C. D. McAfee of Kelly Scientific Resources. The method and the validation report were written by M. J. Hastings.

RESULTS AND DISCUSSION

Analytical Recovery Data

The method validation study was conducted under Dow AgroSciences LLC study protocol number 020010, "Residue Method Validation for the Determination XDE-638 in Water." The results are summarized in the table below and are also listed individually in method GRM.01.30, Appendix A, Table 1.

Compound	Validation Range (µg/L)	Number Analyzed	Average Recovery %	Standard Deviation %
XDE-638	0.05 - 100	54	99	4.9

Calculated Limits of Quantitation and Detection

Following established guidelines (1), the limits of quantitation (LOQ) and detection (LOD) were calculated for XDE-638 using the standard deviation from the 0.05-µg/mL results for water. The LOQ was calculated as ten times the standard deviation (10*s*), and the LOD was calculated as three times the standard deviation (3*s*) of the results of the analysis of nine samples fortified with XDE-638. The results are summarized below and listed individually in Appendix A, Page 12.

Compound	Average Recovery µg/L	Standard Deviation (*s*)	Limit of Detection (3*s*)	Limit of Quantitation (10*s*)
XDE-638	0.0495	0.0017	0.005	0.017

The calculated LOQ supports the validated method LOQ of 0.05 µg/L for XDE-638.

Confirmation of Residue Identity

The presence of XDE-638 was confirmed by comparison of the analyte peak retention time (liquid chromatography) resulting from selected reaction monitoring (MS/MS) of the samples and standard solutions. Due to the specific nature of the MS/MS transformation (tandem mass spectrometry) monitored during detection, no further confirmation was required. Tandem mass spectrometry conditions for confirmation analysis are listed in Section 8.2 of method GRM 01.30 (Appendix A).

Representative Full-Scan Spectra

A typical full-scan mass spectra and a typical product-ion mass spectra of XDE-638 is shown in Appendix A, Figure 1.

Representative Calibration Curves

A typical calibration curve for the determination of XDE-638 is shown in Appendix A, Figure2.

Representative Chromatograms

A typical chromatogram of a 0.0005-µg/mL XDE-638 calibration standard solution is shown in Figure 3a of method GRM 01.30. A typical chromatogram of a control drinking water sample is shown in Figure 3b of method GRM 01.30. A typical chromatogram of a control drinking water sample fortified at 0.05 µg/mL is shown in Figure 3c of method GRM 01.30. A typical chromatogram of a control drinking water sample fortified at 100 µg/mL is shown in Figure 3d of method GRM 01.30.

CONCLUSION

Dow AgroSciences LLC analytical residue method, GRM 01.30, "Determination of Residues of XDE-638 in Water by Liquid Chromatography with Tandem Mass Spectrometry" (Appendix A) has been demonstrated to be suitable for its intended purpose. The method was validated over the concentration range of 0.05-100 µg/mL for drinking water, ground water and surface water. The individual mean recovery values for XDE-638 in fortified water samples are listed below.

Compound	Validation Range (µg/L)	Number Analyzed	Recovery Range %	Average Recovery %	Standard Deviation %
XDE-638	0.05 - 100	54	86-108	99	4.9

Recoveries were within the EPA acceptance range of 70% to 120% for all of the samples (2).

ARCHIVING

The protocol, raw data, and the original version of the final report are all filed in the Dow AgroSciences LLC archives at 9330 Zionsville Road in Indianapolis, IN 46268-1054.

REFERENCES

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

2. *Ecological Effects Test Guidelines, OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods*; U.S. Environmental Protection Agency: U.S. Government Printing Office: Washington, DC, 1996; EPA-712-C-96-348.

APPENDIX A—Dow AgroSciences LLC Method GRM 01.30

Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, Indiana 46268-1054

GRM: 01.30
EFFECTIVE: August 7, 2002
SUPERSEDES: New

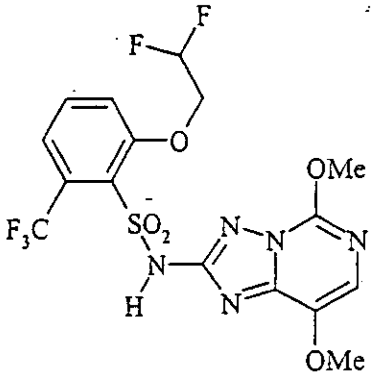
Dow AgroSciences

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

M. J. Hastings

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 µg/mL	Aliquot of Original Standard mL	Final Soln. Volume mL	Calib Soln. Final Conc. µg/mL	Equivalent Sample Conc. 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:

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

$$\underset{(\mu\text{g/mL})}{\text{XDE - 638 Conc.}} = \left(\frac{\text{XDE - 638 peak area } - \text{intercept}}{1.89\text{e}7}\right)$$

$$\underset{(\mu\text{g/mL})}{\text{XDE - 638 Conc.}} = \left(\frac{\text{XDE - 638 peak area} - 855}{1.89\text{e}7}\right)$$

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:

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

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

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

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:

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

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

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:

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

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

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

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

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

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

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

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:

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

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

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

11. RESULTS AND DISCUSSION

11.1. Method Validation

11.1.1. Recovery Levels and Precision

A method validation study was conducted to determine the recovery levels and the precision of the method for the determination of XDE-638 in water. The results are summarized below and listed individually in Table 1.

Compound	Matrix	Average Recovery %	Recovery Range %	Standard Deviation %
XDE-638	Water	99	86-108	4.9

For XDE-638 analysis, all of the individual recovery samples at each fortification level were between 70 and 110% with standard deviations less than 7%.

11.1.2. Standard Curve Linearity

For the linear regression analysis, the correlation coefficients (r) were greater than 0.999 for all of the calibration curve determinations during the method validation. The results indicate linearity of the detector response as a function of the standard calibration curve.

11.1.3. Calculated Limits of Quantitation and Detection

Following established guidelines (13.3), the limits of quantitation (LOQ) and detection (LOD) were calculated for XDE-638 using the standard deviation from the 0.05-µg/L recovery results. The LOQ was calculated as ten times the standard deviation (10*s*), and the LOD was calculated as three times the standard deviation (3*s*) of the results of the analysis of 9 samples. The results are summarized below and individual values are presented in Table1.

Compound	Matrix	Average Recovery µg/L	Standard Deviation (*s*)	Limit of Detection (3*s*)	Limit of Quantitation (10*s*)
XDE-638	Water	0.0496	0.0017	0.005	0.017

The calculated LOQ supported the validated LOQ of 0.05 µg/L. Since the lowest level of fortification for recovery samples was 0.05 µg/L, the method LOQ is considered to be 0.05 µg/L.

The calculated LOD was 0.005 µg/L. In actual residue samples, numerical results should be reported as less than the LOQ (<0.05 µg/L) for residues that are greater or equal to the LOD but less than the validated LOQ. For results less than the LOD, numerical results should be reported as not detected.

11.2. Confirmation of Residue Identity

The presence of XDE-638 is confirmed by comparing the liquid chromatography retention times of the analyte in the calibration standards with those found in the samples as well as by the MS/MS transitions monitored. Due to the highly specific nature of the MS/MS transition monitored during detection, no further confirmation technique is required.

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.

Table 1. Recovery of XDE-638 from Drinking water, Ground Water and Surface Water

Sample Number	Matrix	Date of Analysis	XDE-638, ug/L		Recovery[a] %
			Added	Found	
37129701	Drinking Water	31-Jan-02	0.00	0.0000	--
36969101	Pond Water	07-Feb-02	0.00	0.0000	--
36966701	Well Water	07-Feb-02	0.00	0.0000	--
37129701	Drinking Water	31-Jan-02	0.01	0.0073	NA
36969101	Pond Water	07-Feb-02	0.01	0.0084	NA
36966701	Well Water	07-Feb-02	0.01	0.0078	NA
37129701	Drinking Water	31-Jan-02	0.05	0.0480	96
37129701	Drinking Water	31-Jan-02	0.05	0.0496	99
37129701	Drinking Water	31-Jan-02	0.05	0.0486	97
36969101	Pond Water	07-Feb-02	0.05	0.0493	99
36969101	Pond Water	07-Feb-02	0.05	0.0493	99
36969101	Pond Water	07-Feb-02	0.05	0.0539	108
36966701	Well Water	07-Feb-02	0.05	0.0487	97
36966701	Well Water	07-Feb-02	0.05	0.0492	98
36966701	Well Water	07-Feb-02	0.05	0.0496	99
37129701	Drinking Water	31-Jan-02	0.5	0.509	102
37129701	Drinking Water	28-Feb-02	0.5	0.505	101
37129701	Drinking Water	31-Jan-02	0.5	0.522	104
36969101	Pond Water	07-Feb-02	0.5	0.506	101
36969101	Pond Water	07-Feb-02	0.5	0.526	105
36969101	Pond Water	07-Feb-02	0.5	0.506	101
36966701	Well Water	07-Feb-02	0.5	0.527	105
36966701	Well Water	07-Feb-02	0.5	0.507	101
36966701	Well Water	07-Feb-02	0.5	0.510	102
37129701	Drinking Water	08-Feb-02	5.0	4.68	94
37129701	Drinking Water	08-Feb-02	5.0	4.30	86
37129701	Drinking Water	08-Feb-02	5.0	4.96	99
36969101	Pond Water	07-Feb-02	5.0	4.91	98
36969101	Pond Water	07-Feb-02	5.0	4.87	98
36969101	Pond Water	07-Feb-02	5.0	5.02	100
36966701	Well Water	07-Feb-02	5.0	4.51	90
36966701	Well Water	07-Feb-02	5.0	4.66	93
36966701	Well Water	07-Feb-02	5.0	4.60	92

Table 1. (Cont.) Recovery of XDE-638 from Drinking water, Ground Water and Surface Water

Sample Number	Matrix	Date of Analysis	XDE-638, µg/L Added	XDE-638, µg/L Found	Recovery[a] %
37129701	Drinking Water	08-Feb-02	10.0	9.98	100
37129701	Drinking Water	08-Feb-02	10.0	9.94	99
37129701	Drinking Water	08-Feb-02	10.0	9.93	99
36969101	Pond Water	07-Feb-02	10.0	10.05	101
36969101	Pond Water	07-Feb-02	10.0	10.59	106
36969101	Pond Water	07-Feb-02	10.0	10.20	102
36966701	Well Water	07-Feb-02	10.0	9.15	92
36966701	Well Water	07-Feb-02	10.0	9.29	93
36966701	Well Water	07-Feb-02	10.0	9.36	94
37129701	Drinking Water	31-Jan-02	50.0	50.37	101
37129701	Drinking Water	31-Jan-02	50.0	52.77	106
37129701	Drinking Water	31-Jan-02	50.0	52.90	106
36969101	Pond Water	08-Feb-02	50.0	47.85	96
36969101	Pond Water	08-Feb-02	50.0	46.55	93
36969101	Pond Water	28-Feb-02	50.0	51.93	104
36966701	Well Water	08-Feb-02	50.0	42.41	85
36966701	Well Water	08-Feb-02	50.0	49.58	99
36966701	Well Water	08-Feb-02	50.0	49.57	99
37129701	Drinking Water	31-Jan-02	100.0	101.9	102
37129701	Drinking Water	31-Jan-02	100.0	104.3	104
37129701	Drinking Water	31-Jan-02	100.0	105.8	106
36969101	Pond Water	08-Feb-02	100.0	99.1	99
36969101	Pond Water	08-Feb-02	100.0	97.7	98
36969101	Pond Water	08-Feb-02	100.0	96.5	97
36966701	Well Water	08-Feb-02	100.0	92.5	93
36966701	Well Water	08-Feb-02	100.0	94.4	94
36966701	Well Water	28-Feb-02	100.0	100.4	100

Fortification Rate µg/L	Average Recovery (%)	Recovery Range (%)	SD (%)	RSD (%)	n
0.05	99	96-108	3.4	3.4	9
0.5	103	101-105	1.9	1.8	9
5.0	95	86-100	4.7	5.0	9
10.0	98	92-106	4.7	4.8	9
50.0	99	85-106	6.7	6.8	9
100.0	99	93-106	4.4	4.5	9
0.05-100.0	99	85-106	4.9	5.0	54

[a] All calculations were performed in Microsoft Excel 97 with full precision.

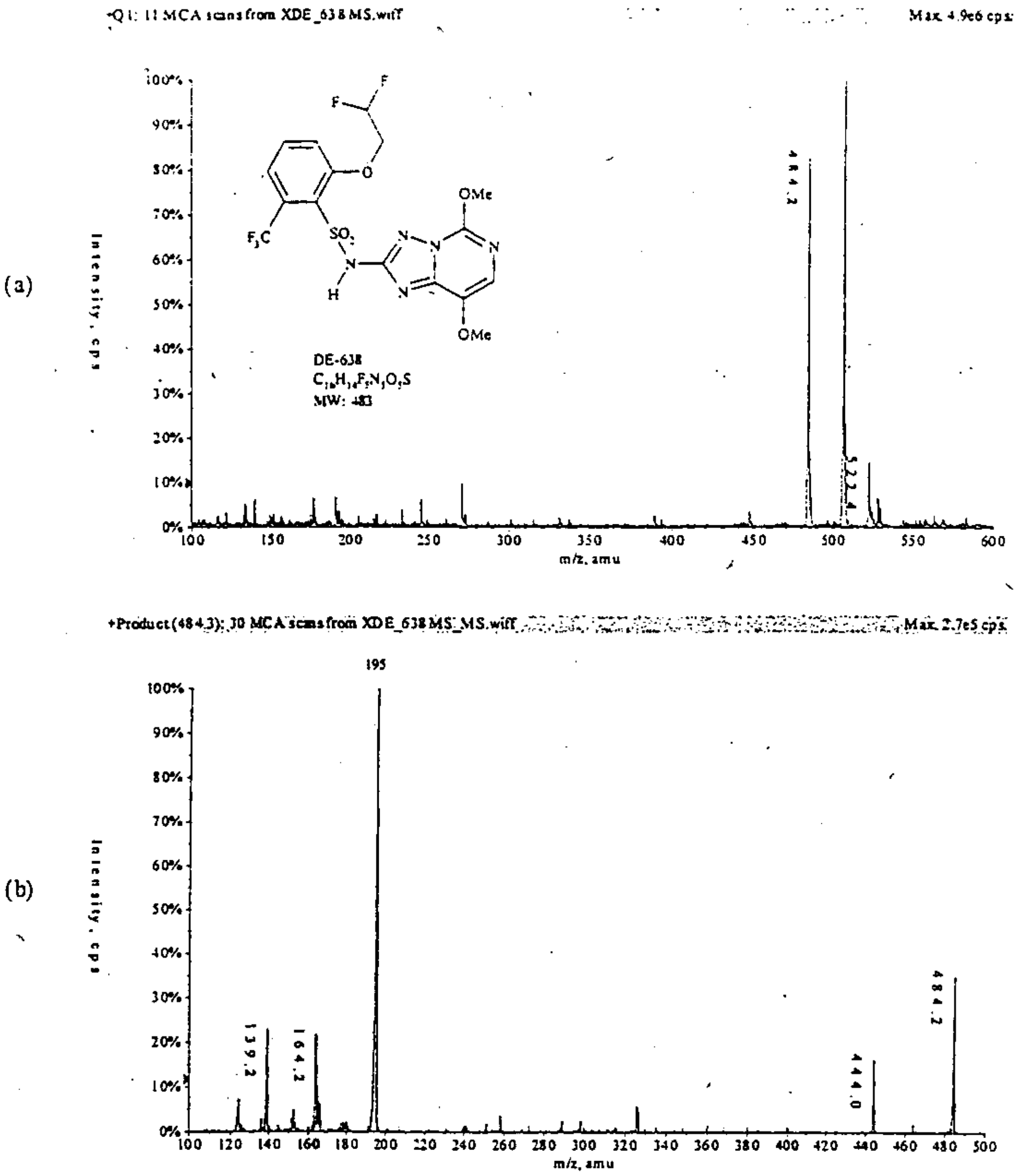
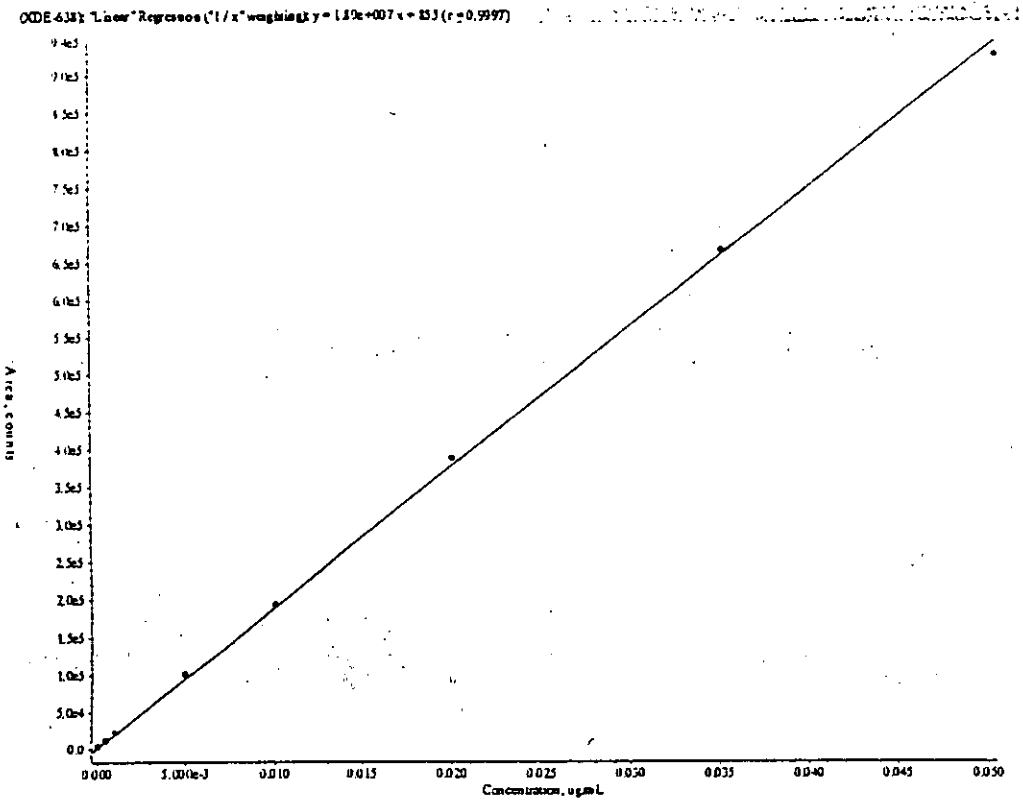


Figure 1. Mass Spectra for XDE-638: (a) Mass Spectrum for Q1 Scan using Electrospray Positive Ionization $(M+H)^+$ at *m/z* 484 (b) Product-Ion Mass Spectrum of XDE-638 Showing Fragment Ion at *m/z* 195



Linear Equation:	Y= 1.89e7 X + 855	
Correlation Coefficient (r):	0.9997	
Weighting:	1/x	

XDE-638 Conc. µg/mL	Equivalent Sample Conc. µg/L	XDE-638 Peak Area
0.0001	0.01	2.39e3
0.0005	0.05	1.06e4
0.001	0.1	2.13e4
0.005	0.5	1.00e5
0.01	1.0	1.92e5
0.02	2.0	3.86e5
0.035	3.5	6.64e5
0.05	5.0	9.24e5

Figure 2. Typical Calibration Curve for the Determination of XDE-638 in Water

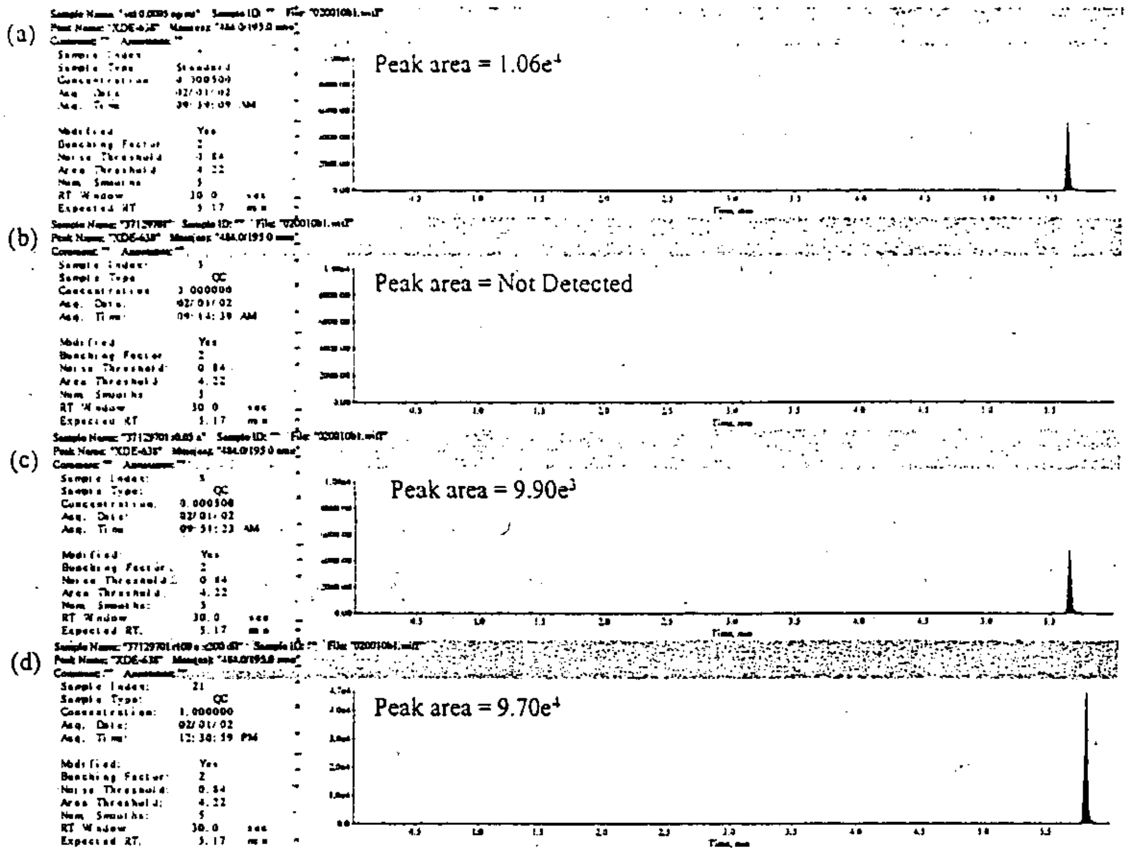


Figure 3. Typical MRM Chromatograms for the Determination of XDE-638 in Drinking Water

(a) 0.0005-µg/mL Standard. Equivalent to a sample concentration of 0.05 µg/L.
(b) Control drinking water sample 37129701. No Detectable (ND) Residue.
(c) Control drinking water sample 37129701 A. Fortified at 0.05 µg/L.
96% Recovery.
(d) Control drinking water sample 37129701 A. Fortified at 100 µg/L.
Diluted x 200. 102% Recovery.

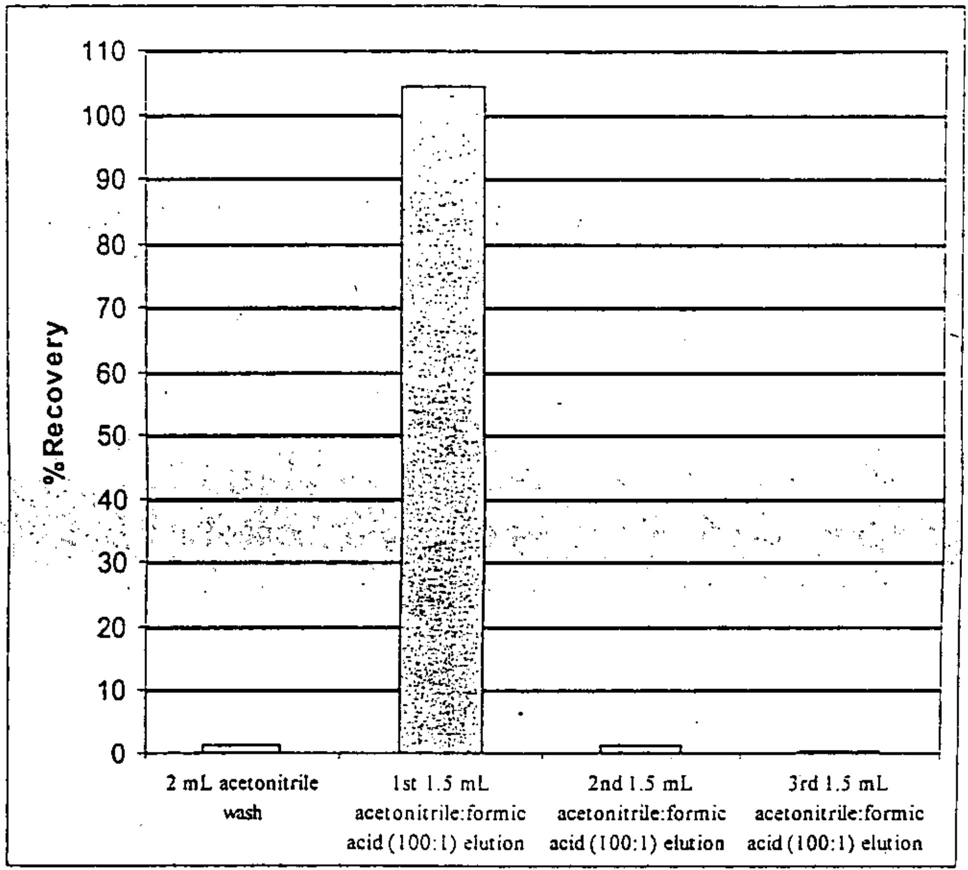


Figure 4. Typical Waters MAX SPE Cartridge Profile for XDE-638