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# Determination of Residues of XDE-742 and its Metabolites in Drinking Water, Ground Water, and Surface Water by Liquid Chromatography with Tandem Mass Spectrometry

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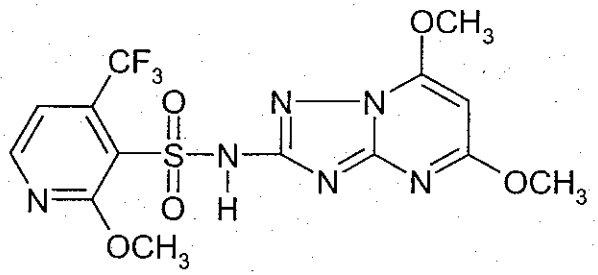
## 1. SCOPE

This method is applicable for the quantitative determination of residues of XDE-742 and its metabolites in drinking water, ground water, and surface water. The method was validated over the concentration range of 0.05-5.0 µg/L with a validated limit of quantitation of 0.05 µg/L.




XDE-742
CAS No. 422556-08-9

Common and chemical names, molecular formulas, and the nominal masses for XDE-742 and its metabolites are given in Table 1.

## 2. PRINCIPLE

Residues of XDE-742 and its metabolites are analyzed without need for prior extraction, concentration or cleanup. The parent compound, XDE-742 and its 7-OH-XDE-742, ADTP and ATSA metabolites are analyzed directly by liquid chromatography with positive-ion electrospray tandem mass spectrometry (LC/MS/MS). The XDE-742 sulfinic acid and sulfonic acid metabolites are analyzed by liquid chromatography with negative-ion electrospray tandem mass spectrometry.

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 should be used in well-ventilated areas away from ignition sources.

3.3. Formic acid is corrosive and can cause severe burns. Liquid nitrogen can also cause severe burns. It is imperative that proper eye and personal protection equipment be used when handling all chemicals.

4. EQUIPMENT (Note 12.1.)

4.1. Laboratory Equipment

4.1.1. Balance, analytical, Model AE100, Mettler-Toledo Inc., Columbus, OH 43240.

4.1.2. Pipetter, adjustable, Eppendorf, 10-100 µL, catalog number 05-402-48, Brinkmann Instruments, Inc., Westbury, NY 11590. (Note 12.2.)

4.1.3. Pipetter, adjustable, Eppendorf, 50-1000 µL, catalog number 21-378-83, Brinkmann Instruments, Inc. (Note 12.2.)

4.1.4. Pipetter, adjustable, Eppendorf, 1000-5000 µL, catalog number 22-46-134-6, Brinkmann Instruments, Inc. (Note 12.2.)

4.1.5. Pipet, positive-displacement, 10-100 µL capacity, model number M100, catalog number F148504, Gilson Inc., Middleton, WI 53562.

4.1.6. Pipet, positive-displacement, 100-1000 µL capacity, model number M1000, catalog number F148506, Gilson Inc.

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

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

4.2. Chromatographic System

4.2.1. Column, analytical, Synergi Hydro RP 80A, 2.0 x 50 mm, 4.0-µm, catalog number 008-4252-B0, Phenomenex, Torrance, CA 90501.

4.2.2. Liquid chromatograph, Symbiosis Pharma, Spark Holland Inc., Plainsboro, NJ 08536.

4.2.3. Mass spectrometer, Model API 4000, Applied Biosystems, Foster City, CA 94404.

4.2.4. Mass spectrometer data system, Analyst 1.4.1, Applied Biosystems.

5. GLASSWARE AND MATERIALS (Note 12.1.)

5.1. Bottle, 1.0-L, media bottle, catalog number 06-423-3D, Fisher Scientific, Pittsburgh, PA 15275.

5.2. Collection plate, 96-well, 2-mL, catalog number 121-5203, Argonaut Technologies, Inc., Redwood City, CA 94063.

5.3. Collection plate sealing cap, catalog number 121-5205, Argonaut Technologies, Inc.

5.4. Flask, volumetric, 100-mL, catalog number 5640-100, Corning Inc., Acton, MA 01720.

5.5. Flask, volumetric, 200-mL, catalog number 5640-200, Corning Inc.

5.6. Cylinder, graduated, 100-mL, catalog number 20024-100, Kimble/Kontes, Vineland, NJ 08360.

5.7. Cylinder, graduated, 500-mL, catalog number 20024-500, Kimble/Kontes.

5.8. Cylinder, graduated, 1000-mL, catalog number 20024-1000, Kimble/Kontes.

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

5.10. Pipet, volumetric, 3.0-mL, catalog number 13-650-2D, Fisher Scientific Company.

5.11. Pipet, volumetric, 5.0-mL, catalog number 13-650-2F, Fisher Scientific Company.

5.12. Pipet, volumetric, 9.0-mL, catalog number 13-650-2K, Fisher Scientific Company.

5.13. Pipet, volumetric, 10.0-mL, catalog number 13-650-2L, Fisher Scientific Company.

5.14. Pipet, volumetric, 20.0-mL, catalog number 13-650-2N, Fisher Scientific Company.

5.15. Pipet, volumetric, 30.0-mL, catalog number 13-650-2Q, Fisher Scientific Company.

5.16. Pipetter tips, Brinkmann Eppendorf, 1-200-µL tip, catalog number 22351371, Brinkmann Instruments, Inc.

5.17. Pipetter tips, Brinkmann Eppendorf, 300-µL tip, catalog number 22351419, Brinkmann Instruments, Inc.

5.18. Pipetter tips, Brinkmann Eppendorf, 1000-µL tip, catalog number 22350901, Brinkmann Instruments, Inc.

5.19. Pipet tip, positive-displacement, 100-µL capacity, capillary piston number CP100, catalog number F148414, Gilson Inc.

5.20. Pipet tip, positive-displacement, 1000-µL capacity, capillary piston number CP1000, catalog number F148560, Gilson Inc.

5.21. Vial, autosampler, 2-mL, catalog number C4000-1W, National Scientific Company, Duluth, GA 30097.

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

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

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

6.1. Reagents

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

6.1.2. AmQuel+Plus®, instant water detoxifier, item number 33411 (1 oz.) or 33444 (4 oz.), Kordon® (Division of Novalek, Inc.), Hayward, CA 94545-1114. (Note 12.3.)

6.1.3. Formic acid, ACS reagent grade, 96% purity, catalog number 25,136-4, Sigma-Aldrich Inc., St. Louis, MO 63103.

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

6.1.5. Nitrogen, refrigerated liquid, catalog number LQNI, BOC Gases, New Providence, NJ 07974.

6.1.6. Water, OmniSolv grade, catalog number WX-0004-1, EMD Chemicals Inc., Gibbstown, NJ 08027.

6.2. Standards

6.2.1. XDE-742, *N*-(5,7-dimethoxy[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide

6.2.2. 7-OH-XDE-742, *N*-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide

6.2.3. ADTP metabolite of XDE-742, 5,7-dimethoxy[1,2,4]triazolo[1,5-*a*]pyrimidin-2-amine

6.2.4. ATSA metabolite of XDE-742, *N*-(5-amino-1H-1,2,4-triazol-3-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide

6.2.5. sulfinic acid metabolite of XDE-742, 2-methoxy-4-(trifluoromethyl)pyridine-3-sulfinic acid

6.2.6. sulfonic acid metabolite of XDE-742, 2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonic acid

Obtain all of the above from the Test Substance Coordinator, Dow AgroSciences LLC, 9330 Zionsville Road, Building 304, Indianapolis, IN 46268.

6.3. Prepared Solutions

6.3.1. acetonitrile + 0.3% formic acid (initial sample diluent)

Measure 1000 mL of acetonitrile using a 1-L graduated cylinder and transfer to a 1-L bottle. Pipet 3.0 mL of formic acid (96%) into the same 1-L bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.2. acetonitrile + 0.01% formic acid (mobile phase B)

Measure 1000 mL of acetonitrile using a 1-L graduated cylinder and transfer to a 1-L bottle. Pipet 0.1 mL of formic acid (96%) into the same 1-L bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.3. acetonitrile/water (80:20) + 0.01% formic acid (autosampler wash solution)

Measure 800 mL of acetonitrile using a 1-L graduated cylinder and transfer to a 1-L bottle. Measure 200 mL of HPLC-grade water using a 500-mL graduated cylinder and transfer to the same 1-L bottle. Pipet 0.1 mL of formic acid (96%) into the 1-L bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.4. AmQuel+Plus, 12.5-µl/mL aqueous dilution (instant water detoxifier)

Pipet 10 mL of HPLC-grade water into a 40-mL vial. Pipet 125 µL of the AmQuel+Plus product into the same vial. Cap the sample vial with a PTFE-lined cap and vortex mix for 5-10 seconds.

6.3.5. water + 0.01% formic acid (mobile phase A)

Measure 1000 mL of HPLC-grade water using a 1-L graduated cylinder and transfer to a 1-L bottle. Pipet 0.1 mL of formic acid (96%) into the same 1-L bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.6. water + 0.03% formic acid (final diluent for the calibration standards)

Measure 1000 mL of HPLC-grade water using a 1-L graduated cylinder and transfer to a 1-L bottle. Pipet 0.3 mL of formic acid (96%) into the same 1-L bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

6.3.7. water/acetonitrile (90:10) + 0.03% formic acid (used for final sample dilution when further dilution is needed) (Note: This solution will only need to be prepared for sample dilution if the analyte concentration is found to be outside the range of the calibration curve.)

Measure 900 mL of HPLC-grade water using a 1-L graduated cylinder and transfer to a 1-L bottle. Measure 100 mL of acetonitrile using a 100-mL graduated cylinder and transfer to the same 1-L bottle. Pipet 0.3 mL of formic acid (96%) into a 1-L bottle. Cap the bottle and mix. Allow the solution to equilibrate to room temperature before use.

7. PREPARATION OF STANDARDS (Note 12.4. and 12.5.)

7.1. Preparation of Fortification Solutions

7.1.1. Weigh 0.0100 g of XDE-742 analytical standard and quantitatively transfer to a 100-mL volumetric flask with methanol. Dilute to volume with methanol to obtain a 100-µg/mL stock solution.

7.1.2. Weigh 0.0100 g of 7-OH-XDE-742 analytical standard and quantitatively transfer to a 100-mL volumetric flask with methanol. Dilute to volume with methanol to obtain a 100-µg/mL stock solution.

7.1.3. Weigh 0.0100 g of ADTP metabolite of XDE-742 analytical standard and quantitatively transfer to a 100-mL volumetric flask with methanol. Dilute to volume with methanol to obtain a 100-µg/mL stock solution.

7.1.4. Weigh 0.0100 g of ATSA metabolite of XDE-742 analytical standard and quantitatively transfer to a 100-mL volumetric flask with methanol. Dilute to volume with methanol to obtain a 100-µg/mL stock solution.

7.1.5. Weigh 0.0102 g (corrected for the molecular weight as the lithium salt) of sulfinic acid metabolite of XDE-742, lithium salt analytical standard and quantitatively transfer to a 100-mL volumetric flask with methanol. Dilute to volume with methanol to obtain a 100-µg/mL stock solution.

7.1.6. Weigh 0.0100 g of sulfonic acid metabolite of XDE-742 analytical standard and quantitatively transfer to a 100-mL volumetric flask with methanol. Dilute to volume with methanol to obtain a 100-µg/mL stock solution.

7.1.7. Pipet 10.0 mL of each of the 100-µg/mL solutions prepared in Sections 7.1.1 through 7.1.6 into a single 200-mL volumetric flask and dilute to volume with acetonitrile to obtain a mixed stock solution containing 5.0 µg/mL of each compound.

7.1.8. Pipet 10.0 mL of the 5.0-µg/mL solution prepared in Section 7.1.7 into a 100-mL volumetric flask and dilute to volume with acetonitrile to obtain a mixed fortification solution containing 0.50 µg/mL of each compound.

7.1.9. Pipet 10.0 mL of the 0.50-µg/mL mixed solution in Section 7.1.8 into a 100-mL volumetric flask and dilute to volume with acetonitrile to obtain a 0.050-µg/mL mixed fortification solution.

7.1.10. Pipet 10.0 mL of the 0.05-µg/mL solution in Section 7.1.9 into a 100-mL volumetric flask and dilute to volume with acetonitrile to obtain a 0.005-µg/mL mixed fortification solution.

7.1.11. Prepare fortification solutions by diluting the above stock solutions from Sections 7.1.8-7.1.10 with acetonitrile as follows:

| Concentration of Stock Soln. | Aliquot of Stock Soln. | Final Soln. Volume | Spiking Soln. Final Conc. | Equivalent Sample Conc.[a] |
|---|---|---|---|---|
| µg/mL | mL | mL | µg/mL | µg/L |
| 0.005 | 30.0 | 100 | 0.0015 | 0.015 |
| 0.005 | - - - | - - - | 0.005 | 0.050 |
| 0.050 | - - - | - - - | 0.050 | 0.500 |
| 0.500 | - - - | - - - | 0.500 | 5.00 |

[a] The equivalent sample concentration is based on fortifying a 9.0-mL control water sample with 90 µL of spiking solution.

7.2. Preparation of Calibration Standards for Quantitation (Note 12.6.)

7.2.1. Prepare mixed calibration solution 10X concentrates by pipetting the specified volume of the mixed fortification solutions (Sections 7.1.8 - 7.1.10) and diluting each to a final volume of 100 mL with acetonitrile. The concentrations of the mixed stock calibration standard concentrates are as follows:

| Concentration of Stock Calibration Soln. | Aliquot of Stock Calibration Soln. | Final Soln. Volume | Calibration Soln. Concentrate Final Conc.[a] | Equivalent Sample Conc.[b] |
|---|---|---|---|---|
| µg/mL | mL | mL | ng/mL | µg/L |
| 0.005 | 3.0 | 100.0 | 0.15 | 0.0135 |
| 0.005 | 10.0 | 100.0 | 0.50 | 0.045 |
| 0.050 | 5.0 | 100.0 | 2.50 | 0.225 |
| 0.050 | 10.0 | 100.0 | 5.00 | 0.45 |
| 0.050 | 20.0 | 100.0 | 10.0 | 0.90 |
| 0.50 | 5.0 | 100.0 | 25.0 | 2.25 |
| 0.50 | 10.0 | 100.0 | 50.0 | 4.5 |
| 0.50 | 20.0 | 100.0 | 100.0 | 9.0 |

[a] Note that the mixed calibration solution concentrates are 10X the concentration of the final calibration standards. A 1.0-mL aliquot of each of these concentrates is diluted with 9.0 mL of water containing 0.03% formic acid in order to prepare the final calibration standards. This dilution to prepare the final calibration standards should be performed daily or with each new sample set.

[b] The equivalent sample concentration is based on taking a 9.0-mL water sample which will be diluted to a final volume of 10.0 mL.

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

### 8.1. Typical Instrumental Conditions for XDE-742 and its 7-OH-XDE-742, ADTP and ATSA Metabolites using Positive-ion Electrospray (Note 12.7.)

| | |
|---|---|
| Instrumentation: | Spark Holland Symbiosis Pharma<br>MDS/Sciex API 4000 LC/MS/MS System<br>MDS/Sciex Analyst® 1.4.1 data system |
| Column: | Phenomenex Synergi Hydro-RP 80A<br>50 x 2.00 mm, 4-µm |
| Column Temperature: | Ambient |
| Injection Volume: | 100 µL |
| Autosampler Wash Program: | Autosampler loop and needle washed with:<br>1) 500 µL of acetonitrile/water (80:20) containing 0.1% formic acid<br>2) 500 µL of acetonitrile/water (80:20) containing 0.1% formic acid with valve wash<br>3) 500 µL of methanol with valve wash<br>4) 500 µL of water with valve wash<br>5) 500 µL of water |
| Run Time: | Approximately 15.0 minutes |
| Mobile Phase: | A – water containing 0.01% formic acid<br><br>B – acetonitrile containing 0.01% formic acid |
| Flow Rate: | 300 µL/min |

Gradient:

| Time, min | Solvent A, % | Solvent B, % |
|---|---|---|
| 0.01 | 100 | 0 |
| 1.01 | 100 | 0 |
| 7.01 | 50 | 50 |
| 8.00 | 10 | 90 |
| 10.00 | 10 | 90 |
| 11.00 | 100 | 0 |
| 15.00 | 100 | 0 |

| Flow Diverter: | Time, min | Direction |
|---|---|---|
| | 0.0→3.9 | waste |
| | 3.9→8.0 | source |
| | 8.0→end of run | waste |

| | |
|---|---|
| Ionization Mode: | electrospray |
| Polarity: | positive |
| Scan Type: | MRM |
| Resolution: | Q1 – unit, Q3 – low |
| Curtain Gas (CUR): | 30 psi |
| Collision Gas (CAD): | 4 psi |
| Temperature (TEM): | 450 °C |
| Ion Source Gas 1 (GS1): | 45 psi |
| Ion Source Gas 2 (GS2): | 75 psi |
| Acquisition Time Delay: | 4.0 minutes |
| Period Duration | 4.0 minutes |
| IonSpray Voltage (IS): | +5000 volts |

| Analytes: | Precursor Ion Q1 | Product Ion Q3 | Dwell Time, ms | Collision Energy, v |
|---|---|---|---|---|
| XDE-742 (quantitation) | 435.1 | 195.1 | 100 | 37 |
| XDE-742 (confirmation) | 435.1 | 82.0 | 100 | 70 |
| 7-OH-XDE-742 (quantitation) | 420.9 | 181.0 | 100 | 37 |
| 7-OH-XDE-742 (confirmation) | 420.9 | 148.1 | 100 | 59 |
| ADTP (quantitation) | 196.2 | 115.1 | 100 | 29 |
| ADTP (confirmation) | 196.2 | 163.9 | 100 | 29 |
| ATSA (quantitation) | 339.0 | 99.1 | 100 | 33 |
| ATSA (confirmation) | 339.0 | 57.2 | 100 | 81 |

8.2. Typical Instrumental Conditions for the XDE-742 Sulfinic Acid and Sulfonic Acid Metabolites using Negative-ion Electrospray (Note 12.7.)

| | |
|---|---|
| Instrumentation: | Spark Holland Symbiosis Pharma<br>MDS/Sciex API 4000 LC/MS/MS System<br>MDS/Sciex Analyst® 1.4.1 data system |
| Column: | Phenomenex Synergi Hydro-RP 80A<br>50 x 2.00 mm, 4-µm |
| Column Temperature: | Ambient |
| Injection Volume:<br>Autosampler Wash Program: | 25 µL<br>Autosampler loop and needle washed with:<br>1) 500 µL of acetonitrile/water (80:20) containing 0.1% formic acid<br>2) 2 x 500 µL of acetonitrile/water (80:20) containing 0.1% formic acid with valve wash<br>3) 500 µL of methanol with valve wash<br>4) 500 µL of water with valve wash |
| Run Time: | Approximately 10.0 minutes |
| Mobile Phase: | A – water containing 0.01% formic acid<br><br>B – acetonitrile containing 0.01% formic acid |
| Flow Rate: | 300 µL/min |

Gradient:

| Time, min | Solvent A, % | Solvent B, % |
|---|---|---|
| 0.01 | 100 | 0 |
| 5.01 | 10 | 90 |
| 6.01 | 10 | 90 |
| 6.15 | 100 | 0 |
| 10.15 | 100 | 0 |

Flow Diverter Program:

| Time, min | Direction |
|---|---|
| 0.0→1.9 | waste |
| 1.9→6.0 | source |
| 6.0→end of run | waste |

| | |
|---|---|
| Ionization Mode: | electrospray |
| Polarity: | negative |
| Scan Type: | MRM |
| Resolution: | Q1 – unit, Q3 – unit |
| Curtain Gas (CUR): | 30 psi |
| Collision Gas (CAD): | 4 psi |
| Temperature (TEM): | 450 °C |
| Ion Source Gas 1 (GS1): | 45 psi |
| Ion Source Gas 2 (GS2): | 75 psi |
| | |
| Acquisition Time Delay: | 2.0 minutes |
| Period Duration | 4.0 minutes |
| IonSpray Voltage (IS): | -4200 volts |

| Analytes: | Precursor Ion Q1 | Product Ion Q3 | Dwell Time, ms | Collision Energy, v |
|---|---|---|---|---|
| Sulfinic acid (quantitation) | 239.9 | 175.8 | 150 | -12 |
| Sulfinic acid (confirmation) | 239.9 | 155.7 | 150 | -20 |
| | | | | |
| Sulfonic acid (quantitation) | 255.7 | 149.0 | 150 | -40 |
| Sulfonic acid (confirmation) | 255.7 | 79.7 | 150 | -54 |

8.3. Mass Spectra

Full-scan and product-ion mass spectra of XDE-742 and its metabolites are shown in Figures 1-6.

8.4. Typical Calibration Curves

Typical calibration curves for the determination of XDE-742 and its metabolites in water are shown in Figures 7-18.

8.5. Typical Chromatograms

Typical chromatograms of a standard, a control sample, and a 0.05-µg/L (LOQ) recovery sample for the determination of XDE-742 and each of its metabolites in drinking water are illustrated in Figures 19-24. (Chromatograms for ground water and surface water samples were similar to those for drinking water.)

## 9. DETERMINATION OF RECOVERY OF XDE-742 AND ITS METABOLITES FROM WATER

### 9.1. Method Validation

Validate the analytical procedure given in Section 9.3 by analyzing the following with each sample set:

At least one reagent blank.
At least one unfortified control.
At least one control fortified at the limit of detection.
At least two controls fortified at the limit of quantitation.
At least two controls fortified at the expected residue concentration in the samples.

### 9.2. Sample Preparation

It is recommended that field samples be treated with AmQuel+Plus, as per the instructions on the bottle, at the time of collection in order to stabilize residues prior to analysis. The manufacturer's standard treatment dose is 5 mL of AmQuel+Plus per each 10 gallons (40 liters) of water. No other prior preparation is needed for water samples. Samples may be stored refrigerated or frozen prior to analysis.

### 9.3. Sample Analysis

9.3.1. Pipet 9.0-mL portions of a well-mixed water sample into a series of 40-mL vials. (Critical Step: Proceed to Step 9.3.5 for field samples that have been previously treated with AmQuel+Plus, as per the instructions on the bottle, at the time of collection.)

9.3.2. For preparing unfortified or fortified control samples (and/or for samples that have not been previously treated with AmQuel+Plus), add 100 µL of a diluted solution of AmQuel+Plus (Section 6.3.4). (Critical Step: For fortified samples, it is critical that the diluted solution of AmQuel+Plus be added prior to fortification.)

9.3.3. Cap the sample vial with a PTFE-lined cap and vortex mix the sample for 5-10 seconds.

9.3.4. Additionally, for preparing fortified control samples, add a 90-µL aliquot of the appropriate spiking solution (Section 7.1.11) to control water samples to obtain concentrations ranging from 0.015 to 5.0 µg/L.

9.3.5. Add 1.0 mL of acetonitrile containing 0.3% formic acid to each sample.

9.3.6. Cap the sample vial with a PTFE-lined cap and then vortex mix the sample for 5-10 seconds.

9.3.7. Prepare the calibration standards by pipetting a 1.0-mL aliquot of each of the mixed calibration solution concentrates (Section 7.2.1) into a series of 40-mL vials.

9.3.8. Pipet 9.0 mL of HPLC-grade water containing 0.03% formic acid (as prepared in Section 6.3.6.) into each of the vials (from Step 9.3.7) in order to prepare the final mixed calibration standards from the concentrates. (Critical Step: This dilution step for preparation of the final calibration standards should be performed with each new sample set or at least daily.)

9.3.9. Cap the vials (Step 9.3.8) with PTFE-lined caps and then vortex mix the final calibration standards for 5-10 seconds.

9.3.10. Transfer a portion of the sample (Step 9.3.6) and portions of the final calibration standards (Step 9.3.9) to 2-mL autosampler vials and seal the vials with a cap, or transfer portions to a 96-well plate and cap the plate.

9.3.11. For the determination of XDE-742, and its 7-OH-XDE-742, ADTP and ATSA metabolites, analyze the calibration standards and samples by HPLC with positive-ion electrospray tandem mass spectrometry as described in Sections 8.1. For the determination of the XDE-742 sulfinic acid and sulfonic acid metabolites, analyze the calibration standards and samples a second time by HPLC with negative-ion electrospray tandem mass spectrometry as described in Section 8.2. Determine the suitability of the chromatographic system using the following performance criteria:

a. Standard curve linearity: Determine that the coefficient of determination ($r^2$) equals or exceeds 0.995 for the least squares equation which describes the detector response as a function of standard curve concentration.

b. Peak resolution: Visually determine that sufficient resolution has been achieved for the analyte relative to background interferences.

c. Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figures 19-24 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 each analyte in the 0.05-ng/mL calibration standard.

9.3.12. If any sample concentrations exceed the range of the standard calibration curve, use a water/acetonitrile solution (90:10) containing 0.03% formic acid to proportionally dilute those samples in order to obtain responses within the range of the calibration curve.

## 10. CALCULATIONS

### 10.1. Calculation of Standard Calibration Curves for XDE-742 and its Metabolites in Water

10.1.1. Inject a series of calibration standards (Section 7.2.1) using the conditions described in Section 8 and determine the peak areas for XDE-742 and its metabolites as indicated below:

| | |
|---|---|
| XDE-742 | *m/z* Q1/Q3 435/195 (quantitation)<br>*m/z* Q1/Q3 435/82 (confirmation) |
| 7-OH-XDE-742 | *m/z* Q1/Q3 421/181 (quantitation)<br>*m/z* Q1/Q3 421/148 (confirmation) |
| ADTP metabolite of XDE-742 | *m/z* Q1/Q3 196/115 (quantitation)<br>*m/z* Q1/Q3 196/164 (confirmation) |
| ATSA metabolite of XDE-742 | *m/z* Q1/Q3 339/99 (quantitation)<br>*m/z* Q1/Q3 339/57 (confirmation) |
| sulfinic acid metabolite of XDE-742 | *m/z* Q1/Q3 240/176 (quantitation)<br>*m/z* Q1/Q3 240/156 (confirmation) |
| sulfonic acid metabolite of XDE-742 | *m/z* Q1/Q3 256/149 (quantitation)<br>*m/z* Q1/Q3 256/80 (confirmation) |

10.1.2. Prepare a standard curve by plotting the concentration of XDE-742 and its metabolites on the abscissa (x-axis), and the respective peak area on the ordinate (y-axis), as shown in Figures 7-18. 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 12.8.)

For example, using XDE-742 data from Figure 7:

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

$$\text{XDE - 742 conc. (ng/mL)} = \left(\frac{\text{XDE - 742 peak area} - \text{intercept}}{\text{slope}}\right)$$

$$\text{XDE - 742 conc. (ng/mL)} = \left(\frac{\text{XDE - 742 peak area} - (30225.8)}{2804644}\right)$$

10.2. Calculation of Percent Recovery for XDE-742 and its Metabolites

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-742 from the injection of the drinking water sample fortified at 0.050 µg/L, Figure 19 (c):

$$\text{XDE - 742 conc. (ng/mL)} = \left(\frac{\text{XDE - 742 peak area} - (30225.8087)}{2804644}\right)$$

$$\text{XDE - 742 conc. (gross ng/mL)} = \left(\frac{146523 - (30225.8087)}{2804644}\right)$$

$$\text{XDE - 742 conc. (gross)} = 0.04147 \text{ ng/mL}$$

Correct the concentration of XDE-742 found in the final sample prepared for analysis diluted to a volume of 10.0 mL by accounting for the original water sample aliquot volume of 9.0 mL as follows:

$$\text{XDE - 742 conc. (gross ng/mL)} = 0.04147 \text{ ng/mL} \times \frac{\text{Final sample vol. (mL)}}{\text{Initial sample vol. (mL)}} \text{ x 1 (dil)}$$

$$\text{XDE - 742 conc. (gross ng/mL)} = 0.04147 \text{ ng/mL} \times \frac{10.0 \text{ mL}}{9.0 \text{ mL}} \text{ x 1 (dil)}$$

$$\text{XDE - 742 conc. (gross)} = 0.0460 \text{ ng/mL (or 0.0460 µg/L)}$$

where:

Aliquot taken (initial) = 9.0 mL
Final volume of sample = 10.0 mL
Dilution (dil) factor (of final sample volume, if needed) = 1

10.2.2. Determine the net concentration of XDE-742 and its metabolites in each recovery sample by subtracting any contribution found at the retention time of the analyte in the unfortified control sample from that of the gross analyte concentration found in the recovery sample.

For example, using the data for XDE-742 from Figure 19:

$$\begin{array}{lcl} \text{XDE-742 conc. (net µg/L)} & = & \text{XDE-742 conc. (gross µg/L)} - \text{XDE-742 conc. (control µg/L)} \\ \text{XDE-742 conc. (net µg/L)} & = & 0.0460\ \mu g/L - 0.0000\ \mu g/L \\ \text{XDE-742 conc. (net)} & = & 0.0460\ \mu g/L \end{array}$$

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

$$\text{Recovery} = \frac{\text{conc. found}}{\text{conc. added}} \times 100\%$$

$$\text{Recovery} = \frac{0.0460\,\mu g/L}{0.050\,\mu g/L} \times 100\%$$

$$\text{Recovery} = 92\%$$

10.3. Determination of XDE-742 and its Metabolites in Water Samples

10.3.1. Determine the gross concentration of XDE-742 and its metabolites in each water sample by substituting the respective peak area obtained into the equation for the standard calibration curve and calculating the uncorrected residue result as described in Section 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, continuing with the data from Figure 19 and the average recovery from Table 2 for the samples analyzed on 20-Jan-2006:

$$\text{XDE - 742 conc. (corrected µg/L)} = \text{XDE - 742 conc. (gross µg/L)} \times \left( \frac{100}{\text{Average \% Recovery}} \right)$$

$$\text{XDE - 742 conc. (corrected µg/L)} = 0.0460\ \mu g/L \times \frac{100}{95}$$

$$\text{XDE - 742 conc. (corrected)} = 0.0484\ \mu g/L$$

11.2. Specificity of Method and Confirmation of Residue Identity

The method is specific for the determination of XDE-742 and its metabolites by virtue of the chromatographic separation and selective detection system used. Further confirmation should not be necessary due to the highly specific nature of the MS/MS transitions. Further confirmation can be achieved, if necessary, by monitoring additional MS/MS transitions as described in Sections 8.1 and 8.2.

11.2.1. Inject the series of calibration standards described in Section 7.2.1 and determine the peak areas for the analytes as indicated below.

| | |
|---|---|
| XDE-742 | *m/z* Q1/Q3 435/195 (quantitation)<br>*m/z* Q1/Q3 435/82 (confirmation) |
| 7-OH-XDE-742 | *m/z* Q1/Q3 421/181 (quantitation)<br>*m/z* Q1/Q3 421/148 (confirmation) |
| ADTP metabolite of XDE-742 | *m/z* Q1/Q3 196/115 (quantitation)<br>*m/z* Q1/Q3 196/164 (confirmation) |
| ATSA metabolite of XDE-742 | *m/z* Q1/Q3 339/99 (quantitation)<br>*m/z* Q1/Q3 339/57 (confirmation) |
| sulfinic acid metabolite of XDE-742 | *m/z* Q1/Q3 240/176 (quantitation)<br>*m/z* Q1/Q3 240/156 (confirmation) |
| sulfonic acid metabolite of XDE-742 | *m/z* Q1/Q3 256/149 (quantitation)<br>*m/z* Q1/Q3 256/80 (confirmation) |

11.2.2. For each standard, calculate each analyte's confirmation ratio. Use the average confirmation ratio for each analyte to confirm the presence of the analyte in the water samples.

$$\text{confirmation ratio} = \frac{\text{peak area of confirmation transition}}{\text{peak area of quantitation transition}}$$

For example, using the data for XDE-742 from Figure 19a:

$$\text{confirmation ratio (}m/z\text{ 435.1/82.0 / }m/z\text{ 435.1/195.1)} = \frac{\text{peak area at }m/z\text{ 435.1/82.0}}{\text{peak area at }m/z\text{ 435.1/195.1}}$$

$$\text{confirmation ratio (}m/z\text{ 435.1/82.0 / }m/z\text{ 435.1/195.1)} = \frac{17430}{168747} = 0.1033$$

Confirmation of the presence of analytes is indicated when the retention time of the samples matches that of the standards and the confirmation ratio for the sample is within the range of ±20% of the average found for the standards.

11.3. Assay Time

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 25 samples. This typical analytical run can be prepared in approximately 3 hours, followed by the chromatographic analysis.

Since the preparation of samples for analysis is relatively short, there are no acceptable "stopping points" described in the method, where sample preparation (Section 9) may be suspended, upon completion of a step, without deleterious effects on the sample analysis.

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. Electronic pipets are used only for pipetting aqueous solutions. If they are used for pipetting non-aqueous solutions, the pipets should be calibrated following the manufacturer's instruction manual and Standard Operating Procedures (13.4).

12.3. The product AmQuel+Plus, is a proprietary solution containing sodium hydroxymethanesulfonate according to the manufacturer.

12.4. Section 7 provides suggested concentrations for the preparation of fortification and calibration standards. Other dilution schemes may be followed.

12.5. Sonication is required to ensure that some of the analytical standards thoroughly dissolve in the initial methanol solution.

12.6. The dilution of the calibration solutions for use should be made fresh daily or with each sample set as the sufinic acid is known to slowly degrade to the sulfonic acid in an aqueous environment.

12.7. The instrumental conditions may be modified to obtain optimal chromatographic separation and sensitivity.

12.8. Other regression techniques may also be used based on detector response.

## 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.

13.4. *Standard Operating Procedure for Pipettes*; Brinkmann/Eppendorf SOP 5101-C20, Brinkmann Instruments, Inc., Westbury, NY, 2001.

Table 1. Identity and Structures of XDE-742 and Its Metabolites

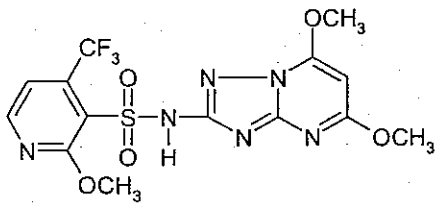
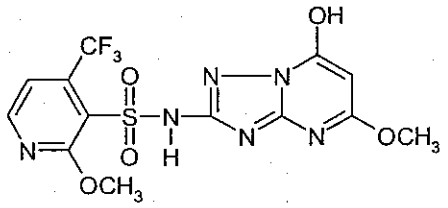
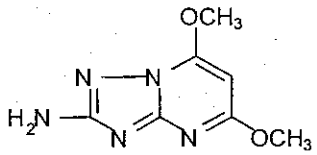
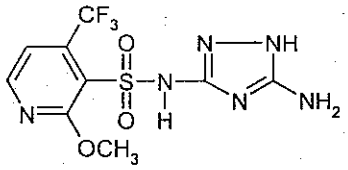
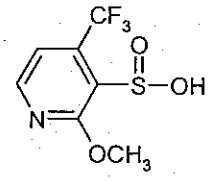
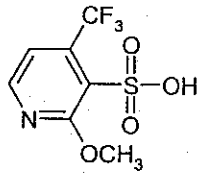
| Common Name of Compound | Structural Formula and Chemical Name (IUPAC) |
|---|---|
| XDE-742<br><br>Molecular Formula: $C_{14}H_{13}F_3N_6O_5S$<br>Formula Weight: 434.4 g/mole<br>Nominal Mass: 434<br><br>CAS Number 422556-08-9 |  <br>*N*-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide |
| 7-OH-XDE-742<br><br>Molecular Formula: $C_{13}H_{11}F_3N_6O_5S$<br>Formula Weight: 420.3 g/mole<br>Nominal Mass: 420<br><br>CAS Number NA |  <br>*N*-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide |
| ADTP metabolite of XDE-742<br><br>Molecular Formula: $C_7H_9N_5O_2$<br>Formula Weight: 195.2 g/mole<br>Nominal Mass: 195<br><br>CAS Number NA |  <br>5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-amine |
| ATSA metabolite of XDE-742<br><br>Molecular Formula: $C_9H_9F_3N_6O_3S$<br>Formula Weight: 338.27 g/mol<br>Nominal Mass: 338<br><br>CAS Number NA |  <br>*N*-(5-amino-1H-1,2,4-triazol-3-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide |

Table 1. (Cont.) Identity and Structures of XDE-742 and Its Metabolites

| | |
|---|---|
| sulfinic acid metabolite of XDE-742<br><br>Molecular Formula: $C_7H_6F_3NO_3S$<br>Formula Weight: 241.19 g/mol<br>Nominal Mass: 241<br><br>CAS Number NA |  <br>2-methoxy-4-(trifluoromethyl)pyridine-3-sulfinic acid |
| sulfonic acid metabolite of XDE-742<br><br>Molecular Formula: $C_7H_6F_3NO_4S$<br>Formula Weight: 257.19 g/mol<br>Nominal Mass: 257<br><br>CAS Number NA |  <br>2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonic acid |