Determination of Residues of XDE-208 and its Major Metabolites in Soil and on Pads Using Online SPE HPLC/MS/MS

David Robaugh, Ph.D.

Pyxant Labs Inc.
4720 Forge Road, Suite 108
Colorado Springs, CO 80907
Phone: 719.593.1165
Fax: 719.593.1625
www.pyxant.com

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Signing Author:

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Dustin Barr
Chemist
Date

Approved By:

24 Jun 09

Jason Breaux
Group Leader
Pyxant Labs Inc.
Date

1.0 SCOPE

This method is applicable for the quantitative determination of residues of XDE-208 (Parent) and its major metabolites in soil. The target limit of quantitation (LOQ) is 0.001 µg/g for all four analytes. Validation experiments have been performed which have confirmed the LOQ and evaluated important method parameters including applicable concentration range, precision, accuracy, and ruggedness.

This method is also applicable for the quantitative determination of residues of XDE-208 (Parent) and its major metabolites on application pads. The target limit of quantification (LOQ) is 2.0 µg/pad for all four analytes. The method was specifically designed and tested for Gelman Laboratories Solvent Saturation Pads (13.7 x 22.0 cm).

2.0 PRINCIPLE

Soil Method - Residues of XDE-208 and its metabolites are extracted from the soil by shaking with an acetonitrile/1.0N HCl (90/10) solution. An aliquot of the extract is evaporated carefully to dryness after the addition of a methanol/glycerin (80/20) keeper. The sample is reconstituted in 1 mL of water/acetonitrile/acetic acid (95/5/0.1, v/v/v) solution and purified using on-line solid phase extraction (SPE) using a C18HD SPE. The SPE is washed with water and eluted onto a C18 analytical column and the residues are analyzed by high performance liquid chromatography with positive-ion electrospray (ESI) tandem mass spectrometry (LC/MS/MS).

Pads Method - Residues of XDE-208 and its metabolites are extracted from the pads by shaking with acetonitrile. A 1-mL aliquot of the extract is then diluted with water/acetonitrile/acetic acid (95/5/0.1, v/v/v) solution and directly analyzed using high performance liquid chromatography with positive-ion electrospray (ESI) tandem mass spectrometry (LC/MS/MS). The dilution of the 1-mL aliquot is varied depending on the concentration levels of the analytes, but typically would be at least a 1/10 dilution.

3.0 SAFETY

The analysts must also be acquainted with the potential hazards of the reagents and solvents employed in the laboratory. Such information may be obtained from the MSDS, literature, and other documents such as the laboratory chemical hygiene plan. Disposal of chemicals and reagents must comply with all federal, state, and local regulations.

Formic acid and acetic acid may cause burns to exposed skin and can be harmful if inhaled or ingested. Proper ventilation, eye protection, and personal protective equipment should be worn when handling this reagent.

4.0 GLASSWARE, HARDWARE, AND EQUIPMENT

4.1 Glassware and Hardware

4.1.1 Adjustable Pipettes, various sizes

4.1.2 11 dram glass vials and/or 50mL centrifuge tubes (VWR)

4.1.3 96 Deep Well Plate or 2mL autosampler vials

4.1.4 10 mL Volumetric Flask

4.1.5 50 mL Volumetric Flask

4.1.6 100 mL Volumetric Flask

4.1.7 HPLC Column: Phenomenex, Synergi hydro (75x4.6 mm id)

4.1.8 SPE plate: Spark-Holland, Hysphere C18HD

4.1.9 500 mL Nalgene Bottles

4.1.10 Gelman Laboratories Solvent Saturation Pads (13.7 X 22.0 cm) Part No. 51334 50/Pk.

4.2 Equipment

4.2.1 Analytical balance, to ± 0.1 µg, Model No. C35, Cahn Microbalance

4.2.2 Balance, Top-Loading, to ± 0.01g, Model No. BA 21005, Sartorius, Germany

4.2.3 Shaker, variable speed, Lab Line Instruments, Inc Model No. 3520

4.2.4 Centrifuge, Model TJ-6, Beckman Instruments

4.2.5 Ultra High Purity Water System, Model No. PL5232, ELGA

4.2.6 Sonicator, Bransonic or equivalent

4.2.7 Vortex mixer, single tube, Model Genie 2 or equivalent

4.2.8 Multi-tube evaporator, Zymark Turbovap LV or equivalent

5.0 ANALYTICAL INSTRUMENTATION

Instrumentation: Spark Holland Symbiosis Pharma Online SPE LC System
MDS SCIEX API 4000 LC/MS/MS System
MDS SCIEX Analyst 1.4.2 data system

5.1 Typical Online SPE Conditions

SPE Cartridge Tray: HySphere C18HD 7µm 10x2mm, Part Number 0822.609

SPE Solvation:	acetonitrile, 1 mL at 4 mL/min (SSM A)
SPE Equilibration:	water, 1 mL at 3 mL/min (SSM B)
Sample Extraction:	water, 1 mL at 2 mL/min (SSM B)
SPE Wash 1:	water, 1 mL at 2 mL/min (SSM B)
SPE Elution:	Focus mode using 300 µL of 80/20/0.1, v/v/v, acetonitrile/water/formic acid at 100µL/min
Clamp Flush:	water, 2 mL at 5 mL/min (HPD2)

5.2 Typical LC/MS Conditions

Column:	Phenomenex Synergi Hydro (75x4.6 mm id)
Column Temperature:	Ambient
Injection Volume:	5 to 50 µL depending on sensitivity and linearity of instrument
Injection Wash Program	1) 700 µL 80/20/0.1, v/v/v, acetonitrile/water/formic acid 2) 700 µL methanol 3) 700 µL water
Run Time:	14.0 minutes
Mobile Phase:	A – Water with 0.01% formic acid B – Acetonitrile with 0.01% formic acid
Flow Rate:	1.0 mL/min. The eluent may be split before entering the source. Whether to split and amount of the split is to be determined depending on sensitivity and linearity of instrument
Gradient:	Note: Gradient may be modified to obtain better separation or faster equilibration so long as separation is not compromised.

Time, min	A, %	B, %
0:00	100	0
3:01	100	0
3:05	90	10
8:15	0	100
10:00	0	100
10:15	100	0

	14:00	100	0

Flow Diverter Program (optional):
1) 0.0→3.0 min: flow to waste
2) 3.0→8.0 min: flow to source
3) 8.0→end of run: flow to waste

5.3 Typical Mass Spectrometry Conditions

Interface:	ESI
Polarity:	Positive
Scan Type:	MRM
Resolution:	Q1 – unit, Q3 – low
Collision Gas (CAD):	6.0
Curtain Gas (CUR):	20 psi
Ion Source Gas 1 (GS1):	50 or between 40 and 80 psi depending on the split ratio and instrumentation used.
Ion Source Gas 2 (GS2):	65 or between 60 and 80 psi depending on the split ratio and instrumentation used.
IS Temperature (TEM):	5000 600 °C or between 500°C and 700°C depending on the split ratio and instrumentation used.

Compound:	Ion, *m/z*		Time, ms	DP/CE/CXP
	Q1	Q3		
XDE-208 quant	278.287	174.000	75	36/15/14
XDE-208 conf	278.287	154.000	20	36/39/14
XDE-208-urea quant	296.333	174.000	75	36/17/12
XDE-208-urea conf	296.333	154.000	20	36/43/14
X11519540 sulfone quant	254.200	175.000	125	36/25/16
X11519540 sulfone conf	254.200	174.000	20	36/39/16
X11579457 sulfoximine quant	253.192	174.000	125	31/13/12
X11579457 sulfoximine conf	253.192	154.000	20	31.35.14
XDE-208-M+3 stable isotope (ISTD) Alternative Ions	281.233	177.000	75	41/13/12
XDE-208-urea-M+3 stable isotope (ISTD)	299.274	177.1	75	41/17/16

Note: The XDE-208-M+3 ISTD is used as IS for the XDE-208 quantitation. The Urea M+3 ISTD is used as an IS for the urea, sulfone and sulfoximine metabolites.

6.0 REAGENTS, STANDARDS, AND PREPARED SOLUTIONS

6.1 Materials and Reagents

6.1.1 Acetic Acid, HPLC grade, J.T. Baker or equivalent grade

6.1.2 Acetonitrile, HPLC grade, Burdick & Jackson or equivalent grade

6.1.3 Methanol, HPLC grade, Burdick & Jackson or equivalent grade

6.1.4 Water, Ultra High Purity (UHP), 18.2 MΩcm or equivalent grade

6.1.5 Hydrochloric Acid, HPLC grade, J.T. Baker or equivalent grade

6.1.6 Glycerol (Glycerin), ACS grade, Aldrich or equivalent grade

6.1.7 Formic Acid, HPLC grade, J.T. Baker or equivalent grade

Standards

Common and code names: XDE-208 (X11422208), 208-Urea (X11719474), 208-Sulfone (X11519540), 208-Sulfoximine (X11579457)

6.2.1 X11422208, DowAgro Sciences, Indianapolis, IN

6.2.2 X11719474, DowAgro Sciences, Indianapolis, IN

6.2.3 X11579457, DowAgro Sciences, Indianapolis, IN

6.2.4 X11519540, DowAgro Sciences, Indianapolis, IN

6.2.5 X11843864 (XDE-208-d_3-ISTD), DowAgro Sciences, Indianapolis, IN

6.2.6 X11817334 (XDE-208 Urea-d_3-ISTD), DowAgro Sciences, Indianapolis, IN

6.2 Prepared Solutions

Prepared solutions may be scaled appropriately.

6.3.1 water/acetonitrile/acetic acid ~(95/5/0.1) reconstitution solution containing 10 ng/mL urea D3 internal standard solution.

Combine 5-mL of acetonitrile, 0.1-mL of acetic acid, and 75 mL of water and mix thoroughly. Next add 1-mL of the 1000 ng/mL urea-D3 separate internal standard solution and mix. Bring to 100 mL final volume with water and mix thoroughly by inversion. Allow the solution to equilibrate to room temperature before final volume adjustment and use.

6.3.2 acetonitrile/1.0 N HCl (90/10) extraction solvent.

6.3.3 methanol/glycerin (80/20) "keeper".

6.3.4 acetonitrile/water/formic acid (80/20/0.1), injection wash solvent and SPE elution solvent.

Combine 200-mL of water, 800-mL of acetonitrile and 1-mL of acetic acid and mix thoroughly. Allow the solution to equilibrate to room temperature before use.

6.3.5 Mobile Phase A: Water with 0.01% formic acid. Add 100 µL of formic acid to 1000 mL of ultra high purity water.

6.3.6 Mobile Phase B: Acetonitrile with 0.01% formic acid. Add 100 µL of formic acid to 1000 mL of acetonitrile.

7.0 PREPARATION OF STANDARD SOLUTIONS

The following are recommended standard concentrations which may be adapted as appropriate; volumes of solutions may also be changed.

Stock solutions should be prepared in duplicate with two separate weighings of reference standards. One is to be utilized for the preparation of fortification standards and the other for calibration standards. Prior to any dilutions, an analytical comparison of the stocks should be made to assess their precision and presumed accuracy.

Alternative: One stock may be used to prepare standards and QC fortifications so long as the second separately weighed stock is used as a check of the preparation.

7.1 Preparation of Stock, Intermediate and Spiking (Fortification) Solutions

7.1.1. Weigh 0.0100 g of the XDE-208, the urea metabolite, the sulfone and sulfoximine standards and transfer to separate 10 ml volumetric flasks using acetonitrile. Dilute to volume to produce a 1000 µg/mL solution of each analyte (if larger amounts of compound is available, greater weights and volumes should be used to increase accuracy such as 0.0250 g into 25 mL).

7.1.2. Pipet 1.0 mL of each of the 1000-µg/mL solutions into a 10 mL volumetric flask and dilute to volume with acetonitrile to produce a 100 µg/mL mixed standard solution.

7.1.3. Pipet 1.0 mL of the 100-µg/mL solution into a 10 mL volumetric flask and dilute to volume with acetonitrile to produce a 10 µg/mL mixed standard solution.

7.1.4. Pipet 1.0 mL of the 10-µg/mL solution into a 10 mL volumetric flask and dilute to volume with acetonitrile to produce a 1.0 µg/mL mixed standard solution.

7.1.5. Pipet 1.0 mL of the 1.0-µg/mL solution into a 10 mL volumetric flask and dilute to volume with acetonitrile to produce a 0.1 µg/mL mixed standard solution.

7.1.6. Pipet 0.5 mL of the 1.0-µg/mL solution into a 10 mL volumetric flask and dilute to volume with acetonitrile to produce a 0.05 µg/mL mixed standard solution.

7.2 Preparation of Internal Standard Stocks and IS Fortification Solution

7.2.1. Weigh 0.005-g of XDE-208-M+3 stable isotope standard and XDE-208-urea-M+3 stable isotope standard in separate 50-ml volumetric flasks and dilute to volume with acetonitrile to produce a 100-µg/mL solution of each analyte.

7.2.3. Transfer 0.1 mL of the 100-µg/mL XDE-208-urea-M+3 stable isotope into a 1000 mL volumetric flask and dilute to volume with water/acetonitrile/acetic acid (95/5/0.1) to produce a 10 ng/mL ISTD. Use 1mL of this solution in step 8.1.10.

7.2.4. Transfer 0.1 mL of the 100-µg/mL XDE-208-M+3 stable isotope standard into a 100 mL volumetric flask and dilute to volume with water/acetonitrile/acetic acid (95/5/0.1) to produce a 100 ng/mL ISTD. Use 100uL of this solution to add IS to sample tubes in step 8.1.8.

7.3 Preparation of Calibration Standards

Dilute the 10, 1 and 0.1-µg/mL standard solutions with a water/acetonitrile/acetic acid (95/5/0.1) solution according to the following table. Add 1-mL of the 1-µg/mL mixed internal standard solution to each flask prior to volume adjustment in order to get solutions with 10-ng/mL of ISTD.

Original Standard Concentration	Aliquot of Spiking Standard	Final Standard Volume	Calibration Solution Final Conc.	Equivalent Sample Concentration in Soil after 2x extract concentration [a]
µg/mL	µL	mL	ng/mL	ng/g
0.1	75	100	0.075	0.3(LOD)
0.1	150	100	0.15	0.6 (1/2LOQ)
0.1	250	100	0.25	1(LOQ)
0.1	500	100	0.5	2
0.1	1000	100	1	4
1	500	100	5	20
1	1000	100	10	40
10	200	100	20	80
10	350	100	35	140
10	500	100	50	200

[a] Equivalent sample concentration is based on fortifying a 5.0g sample.

8.0 SOIL SAMPLE PREPARATION

8.1. Sample Extraction

8.1.1 Weigh 5.0 ± 0.05 g of the sample into a 50-mL polypropylene centrifuge tube (must fit in the centrifuge used in step 8.1.4). (Optionally, where the exact weights are used in the calculations, the weight tolerance may be increased to ± 0.3 g) For recovery samples, add appropriate aliquots of the spiking solutions to obtain concentrations including 0. 3ng/g (proposed LOD) and 1ng/g (proposed LOQ) and the proposed upper quality control level for validation of 0.150µg/g (150XLOQ, equivalent standard 37.5 ng/mL). Higher levels found in samples during a study may require additional higher level QC's be run during the study. The solvent for spiking solutions is acetonitrile which is allowed to evaporate for a few minutes before fortified samples are extracted.

8.1.2. After weighing and spiking add ~19 mL of acetonitrile/1.0 N HCl (90/10) extraction solvent to each sample. Shake by hand for a few minutes to

disperse the solid material.

8.1.3. Cap the tube and shake the sample for 60 minutes on a flat-bed shaker at approximately 180 excursions/minute. Place the tubes horizontally to achieve the best mixing.

8.1.4. Shake the tube by hand to remove soil from the sides of the container and then centrifuge the sample for 5 minutes at 3000 rpm and decant the extract into a clean 50-mL plastic centrifuge tube.

8.1.5. Add an additional ~19-mL of extraction solution to each sample and shake upside down for a few minutes to dislodge and disperse the soil plug. Shake 20 minutes on a flat bed shaker at approximately 180 excursions/minute.

8.1.6. Shake the tube by hand to remove soil from the sides of the container and then centrifuge the sample for 5 minutes at 3000 rpm and decant and combine the extract into the 50-mL plastic centrifuge tube. (Section 8.1.4.).

8.1.7. Adjust the volume in the centrifuge tube to the 40mL in the 50-mL tubes with acetonitrile/1.0 N HCl (90/10) extraction solution. (For improved accuracy, use a Class A 20mL pipet to prepare two centrifuge tubes with a pre-measured 40mL-amount of extraction solution. Use the level in these tubes instead of the printed 40mL mark to bring the unknown tubes to 40mL volume.) Cap the centrifuge tube and mix thoroughly.

8.1.8. Pipette a 2-mL aliquot into a culture tube containing 100 µL of a methanol/glycerin (80:20) solution and 100 µL of the 100 ng/mL XDE-208-M+3 internal standard solution.

8.1.9. Evaporate the sample to near dryness in a TurboVap evaporator set at 40°C and approximately 15 psig (approximately 20µL - 50µL of glycerin will remain in the vial and should take ~20 to 30 minutes). Longer evaporation times should be avoided.
Ensure that each nozzle is lined up on one side of the opening of each sample tube to allow vortexing nitrogen to expedite evaporation.

8.1.10. Reconstitute the samples in 1 mL of 95/5/0.1, v/v/v, water/acetonitrile/acetic acid containing the separate urea-D3 internal standard (10 ng/mL). Vortex mix for a few seconds and then sonicate the sample for 30 seconds.

8.1.11. If a sample needs to be diluted, use acetonitrile/1 N HCl (90/10) solution to dilute an aliquot of the extract. Next as before, measure 2-mL of the diluted extract and dry down as in step 8.1.9 above. It is strongly recommended to always evaporate the same volume (2-mL) for all samples even after dilution.

8.1.12 Transfer samples via disposable plastic Pasteur pipettes to either autosampler vials or directly into a 96-deep well plate. Agitate extracts with the pipette before transfer.

8.1.13 Analyze the samples and calibration standards by on-line SPE LC/MS with positive-ion electrospray tandem mass spectrometry as described above.

8.2 Analysis Time

An analytical batch may include up to 48 samples which can be prepared in approximately eight hours. Approximately 10 to 24 hours of LC/MS/MS analysis time may be required depending on the batch size.

9.0 PAD SAMPLE PREPARATION

9.1. Sample Extraction

9.1.1 For recovery samples, add appropriate aliquots of the spiking solutions to each pad to obtain concentrations including 2.0 µg/pad (proposed LOQ) and 100 µg/pad, the proposed upper quality control level for validation. Higher levels found in samples during a study may require additional higher level QC's be run during the study. The solvent for spiking solutions is acetonitrile which is allowed to evaporate for a few minutes before fortified pads are extracted. Note: It is best to fortify pads with Class A glass pipettes and volumes of about 1-mL. The solution should be spread evenly across the pad with no pooling in any one spot. Care should be exercised to make sure that liquid does not completely penetrate and drip off the backside of the pad. Allow sufficient time for the spiking solvent solution to evaporate before further processing.

9.1.2 Cut pads into strips (i.e. typically four) to easily fit into a 500-mL Nalgene bottle. Folding the strips lengthwise and alternating the strips in the bottle helps to keep them apart and solvent flowing over all strips evenly. (Note: Be careful of contamination at all times. Scissors used should be cleaned with acetone between pads and gloves changed frequently so as not to contaminate samples).

9.1.3 Add 500-mL of acetonitrile extraction solvent to each Nalgene bottle and cap tightly. Shake by hand for a few minutes to wet the pads and make sure that they stay separated. Solvent must easily flow between and around the pad strips when shaking.

9.1.4 Shake the sample for 60 minutes on a flat-bed shaker at approximately 180 excursions/minute. If needed, place the bottles horizontally to achieve the

best mixing.

9.1.5 Pipette a 1-mL aliquot into a culture tube.

9.1.6 For a 1/10 dilution, add 9 mL of 95/5/0.1, v/v/v, water/acetonitrile/acetic acid. Other dilutions may be used as needed.

9.1.7 Spike a 900 µL aliquot of each diluted sample including spiked reagent blanks, with 100 µL of the combined XDE-208 D3 and Urea internal standards (100ng/mL).

9.1.8 Vortex mix for a few seconds.

9.1.9 Transfer samples via disposable plastic Pasteur pipettes to either autosampler vials or directly into a 96-deep well plate. Agitate extracts with the pipette before transfer.

9.1.10 Analyze the samples and calibration standards by on-line SPE LC/MS with positive-ion electrospray tandem mass spectrometry as described above.

10.0 TYPICAL CALCULATIONS

10.1 Analyte Concentration

Generate a best fit regression calibration curve for instrument response ratio versus analyte concentration ratio in the calibration standards. The response may be linear or quadratic with or without weighting depending on the instrumentation and conditions used. Determine the analyte concentration in the final extracts of samples using the regression equation. These calculations should be performed using the Analyst software installed on the LC/MS/MS data system.

Soil Method - The following calculations may be used to determine the analyte concentration in the original commodity.

$$Gross\ Analyte\ (\mu g/g) = CF \times \frac{V_f}{V_a} \times \frac{V_i}{W} \times \frac{1\ \mu g}{1000\ ng}$$

Where:

CF = Analyte concentration in ng/mL
V_f = Volume of final extract (1 mL)
V_i = Volume of initial extract (40 mL)

V_a = Volume of aliquot (2 mL)
W = Weight (g)

Pad Method - The following calculations may be used to determine the analyte concentration on the pads:

$$Gross\ Analyte\ (\mu g/pad) = CF \times Df \times \frac{1\ \mu g}{1000\ ng} \times Vi$$

Where:

CF = Analyte concentration in ng/mL
D_f = Dilution factor of 1-mL aliquot
V_i = Volume of initial extract (500 mL)

Recovery of fortified controls is calculated by the following equation:

$$\%\ Recovery = \frac{AC}{FC} \times 100$$

Where:
AC = Analyte concentration - concentration found in UTC
FC = Concentration fortified

10.2 Percent Recovery

The percent recovery of the QC samples is determined by dividing the net concentration of analyte found by the fortified concentration.

$$\%\ Recovery = \frac{AF_{net}}{FC} \times 100 \quad (5)$$

Where:
AF_{net} = net concentration of analyte found
FC = fortified concentration

10.2 Statistical Treatment of Data

The Analyst® software program will be used to construct calibration curves and determine the best regression fit of the data. The program will also be used to calculate the concentration of the analyte based upon these curves and when applicable, convert

these concentrations into the appropriate units. Mean recoveries will be calculated using the "AVERAGE" function of the Microsoft Excel® spreadsheet computer program which divides the sum of the selected cells by the number of determinations. The standard deviations were calculated using the "STDEV" function of the same spreadsheet program which sums the squares of the individual deviations from the mean, divides by the number of degrees of freedom, and extracts the square root of the quotient. Percent relative standard deviation, % RSD, is calculated by dividing the standard deviation by the mean, then multiplying by 100.

11.0 NOTES

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 therefore, are not listed.

12.0 REFERENCES

Residue Chemistry Test Guidelines, OPPTS 860.1340, Residue Analytical Method, U.S. Environmental Protection Agency. U.S. Government Printing Office: Washington, DC, 1996; EPA-712-C-95-174.

Proposed Procedure for the Analysis of XDE-208 in Soil by LC/MS/MS (July 2008), Dow AgroSciences, USA

Analysis of 208 in Soil by LC/MS/MS, (HCK, DAS Brazil, Apr 16th, 2008), Dow AgroSciences, Brazil