VALENT U.S.A. CORPORATION
Valent Technical Center
Dublin, California

DETERMINATION OF S-2200 AND METABOLITES IN SOIL
METHOD RM-48S-3 **DATE: October 11, 2012**

INTRODUCTION

This method determines residues of S-2200 and it's metabolites DX-CA-S-2200, 2-COOH-S-2200, 2-CONH2-S-2200, 5-COOH-S-2200, and 5- CONH2-S-2200 in soil.

Briefly, S-2200 residues are extracted from soil using acetone and 0.05 M HCl. The sample is partitioned into dichloromethane. The dichloromethane is evaporated, the residues are dissolved in acidified methanol/water solution, and the residues are analyzed by LC/MS-MS.

REAGENTS

Acetone – pesticide quality (or equivalent).

Dichloromethane - pesticide quality (or equivalent).

Formic acid - pesticide quality (or equivalent).

Hydrochloric acid - 36.5-38.0%, Baker-Analyzed, JT Baker Cat.#9530-00 (or equivalent).

Methanol – pesticide quality (or equivalent).

Sodium Chloride – reagent grade (or equivalent).

Water – deionized water and HPLC quality water.

REAGENT SOLUTIONS

Acetone/0.05 M HCl (80/20, v/v) - Combine 4 parts acetone with 1 part 0.05 M HCl. For example, add 800 mL of acetone and 200 mL of 0.05 M HCl sequentially to a reagent bottle. Stopper and mix. Store at room temperature.

0.05% Formic acid in methanol – Add 0.50 mL of formic acid to 1000 mL of methanol in a reagent bottle. Store at room temperature. (These amounts may be scaled as necessary.)

0.05% Formic acid in HPLC water – Add 0.50 mL of formic acid to 1000 mL of HPLC water in a reagent bottle. Mix and then store at room temperature. (These amounts may be scaled as necessary.)

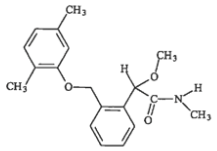
0.05% Formic acid in methanol/0.05% Formic acid in HPLC water (1/1, v/v) - Combine 1 part 0.05% formic acid in methanol with 1 part 0.05% formic acid in HPLC water. For example, add 500 mL of 0.05% formic acid in methanol and 500 mL of 0.05% formic acid in HPLC water sequentially to a reagent bottle. Mix and then store at room temperature.

Hydrochloric acid, 0.05 M - Carefully add 4.17 ml of concentrated hydrochloric acid to 1 liter of deionized water. Stopper and mix. Store at room temperature.

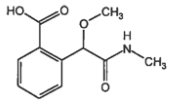
Sodium chloride, 5% solution (w/v) - Add 50 grams of sodium chloride to 1 L of deionized water and shake until dissolved. Store at room temperature.

REFERENCE STANDARDS

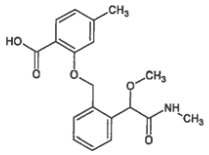
S-2200 (*RS)* 2-[(2,5-dimethylphenoxy)methyl]-α-methoxy-*N*-methyl-benzeneacetamide - analytical standard of a known purity. Prepare a stock solution containing 1.0 mg/mL in acetone. All solutions should be kept in the refrigerator when not in use.



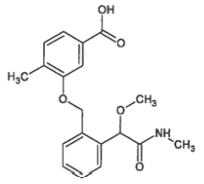
DX-CA-S-2200 (*RS*)-2-(*N*-methylcarbamoyl-methoxymethyl)benzoic acid - analytical standard of a known purity. Prepare a stock solution containing 1.0 mg/mL in acetone. All solutions should be kept in the refrigerator when not in use.



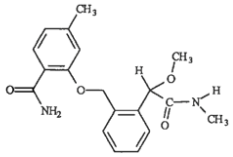
2-COOH-S-2200 (*RS*)-2-{2-[1-methoxy-1-(*N*-ethylcarbamoyl)methyl]benzyloxy}-4-methyl-benzoic acid - analytical standard of a known purity. Prepare a stock solution containing 1.0 mg/mL in acetone. All solutions should be kept in the refrigerator when not in use.



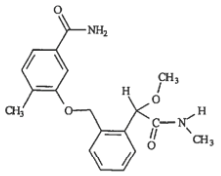
5-COOH-S-2200 (*RS*)-3-{2-[1-methoxy-1-(*N*-methylcarbamoyl)methyl]benzyloxy}-4-methylbenzoic acid - analytical standard of a known purity. Prepare a stock solution containing 1.0 mg/mL in acetone. All solutions should be kept in the refrigerator when not in use.



2-CONH2-S-2200 (*RS*)-2-{2-[1-methoxy-1-(*N*-methylcarbamoyl)methyl]benzyloxy}-4-methylbenzamide - analytical standard of a known purity. Prepare a stock solution containing 1.0 mg/mL in acetone. All solutions should be kept in the refrigerator when not in use.



5-CONH2-S-2200 (*RS*)-3-{2-[1-methoxy-1-(*N*-methylcarbamoyl)methyl]benzyloxy}-4-methylbenzamide - analytical standard of a known purity. Prepare a stock solution containing 1.0 mg/mL in acetone. All solutions should be kept in the refrigerator when not in use.



STANDARD SOLUTIONS

S-2200 + metabolites (S-2200, DX-CA-S-2200, 2-COOH-S-2200, 5-COOH-S-2200, 2-CONH2-S-2200, and 5-CONH2-S-2200)

Fortifying Solution - 10 µg/mL – Transfer 1.0 mL of the 1.0 mg/mL of the S-2200 stock solution and 1.0 mL of each of the 1.0 mg/mL metabolite stock solutions into a 100 mL volumetric flask. Dilute to volume with acetone, and mix. Store in the refrigerator when not in use.

Fortifying Solution - 1 µg/mL – Transfer 10 mL of the 10.0 µg/mL S-2200 + metabolites fortifying solution into a 100 mL volumetric flask. Dilute to volume with acetone and mix. Store in the refrigerator when not in use.

Calibration Standard Solutions – Prepare a minimum of five calibration standards by diluting the 1 µg/mL S-2200 + metabolites fortifying solution with 0.05% formic acid in methanol/0.05% formic acid in HPLC water, 1/1 (v/v) to concentrations ranging from 1.25 µg/L to 50 µg/L. Calibration standard solutions should be kept refrigerated when not in use.

Continuing Calibration Standard Solution – the 10 µg/L calibration standard S-2200 + metabolites will be used for the continuing calibration standard (see Note 1).

EQUIPMENT

Centrifuge – Sorvall Evolution RC (or equivalent).

Graduated cylinders – various sizes for making reagents.

LC/MS-MS – Hewlett Packard 1200 Binary Pump HPLC system with an autosampler coupled to an Applied Biosystems API 2000 mass spectrometer with an electrospray ionization interface (or an equivalent system).

Polypropylene centrifuge tubes – 30 x 115mm, VWR part # 82018-052 (or equivalent).

Reciprocating shaker – Eberbach or equivalent.

Rotary evaporator – Büchi (or equivalent), equipped with a heated water bath.

Round-bottom flasks – 250 mL with 24/40 ground-glass joints.

Ultrasonic bath – Branson 3200 (or equivalent).

Vials – #6 screw cap, 40 mL (or equivalent).

Volumetric flasks –100 mL.

Separatory funnels – 250 or 500 mL.

ANALYTICAL PROCEDURE

1. Extraction

For each of the soil samples, weigh 2.5 grams (± 0.1 grams) of soil into a centrifuge tube. At this point, if required by the testing facility, fortify control samples for method recovery with the S-2200 + metabolites fortifying standard solution (see Note 2). Add 25 mL of the acetone/0.05 M HCl (80/20, v/v) solution to each of the samples, place the tubes horizontally in a reciprocating shaker, and shake for one hour.

Centrifuge the samples for 5 minutes (at about 2000 rpm, or as needed to pelletize the solids). Decant the liquid phase for each sample into a 250 or 500-mL separatory funnel. Add 25 mL of the acetone/0.05 M HCl (80/20, v/v) solution to each centrifuge tube. Break up the solids in the centrifuge tubes, if needed, and then cap, shake the samples for one hour, and centrifuge as before. Decant the liquid phase for each sample into the separatory funnel, combining the second extract with the first extract.

2. Dichloromethane Partition

Add 50 mL of 5% sodium chloride solution and 50 mL of dichloromethane to each of the separatory funnels containing the sample extracts. Shake for 1 minute, and then allow the layers to separate. Drain the dichloromethane (the lower layer) into a 250-mL round-bottom flask. Re-extract the aqueous phase with a second 50-mL portion of dichloromethane as before. Drain the dichloromethane layer into the 250-mL round-bottom flask, combining it with the first dichloromethane extract. Discard the aqueous layer.

3. LC/MS-MS Analysis

Evaporate the dichloromethane to dryness using a rotary-evaporator (with a water bath set to ≤40°C). Add 20 mL of 0.05% formic acid in methanol/0.05% formic acid in HPLC water (1/1, v/v) to the round-bottom flask and sonicate briefly to dissolve the residues. Samples may be stored in a freezer prior to analysis.

Transfer a portion of sample extract into an autosampler vial for analysis.

Condition the instrument with at least three injections of sample extract. Analyze a range of calibration standards within the analytical sequence. The calibration standards should be interspersed with the samples in the analytical sequence. Each sequence must begin and end with a continuing calibrating standard (the mid-range calibration standard, 10 µg/L). The recommended sequence of samples and standards for analysis is: continuing calibration standard, matrix condition, calibration standard, sample, sample, sample, calibration standard, etc.

HPLC Conditions:

Column:	Eclipse XDB-C18, 5µm, 150mm x 4.6mm (Agilent Part # 993967-902)
Column Oven Temperature:	20 ± 1°C
Mobile Phase:	A = 0.05% formic acid in HPLC water B = 0.05% formic acid in methanol
Gradient Program:	T = 0 min, 50% A + 50% B T = 2.0 min, 50% A + 50% B T = 8.0 min, 10% A + 90% B T = 9.0 min, 10% A + 90% B T = 9.5 min, 10% A + 90% B T = 13.0 min, 10% A + 90% B T = 13.5 min, 10% A + 90% B T = 20.0 min, 10% A + 90% B T = 22.5 min, 50% A + 50% B T = 30.0 min, 50% A + 50% B
Flow Rate Program:	T = 0 min, 500 mL/min T = 9.0 min, 500 mL/min T = 9.5 min, 250 mL/min T = 13.0 min, 250 mL/min T = 13.5 min, 500 mL/min T = 30.0 min, 500 mL/min
Injection,	
Drawing Speed:	200 µL/minute
Injection Volume:	10 µL
Ejecting Speed:	200 µL/minute

LC/MS Interface: Electrospray Ionization

Typical Mass Spectrometer Parameters:

	DX-CA-S-2200	2-CONH2-S-2200	2-COOH-S-2200	5-CONH2-S-2200	5-COOH-S-2200	S-2200
Scan type	MRM	MRM	MRM	MRM	MRM	MRM
Polarity	Positive	Positive	Positive	Positive	Positive	Positive
Resolution Q1	Unit	Unit	Unit	Unit	Unit	Unit
Resolution Q3	Unit	Unit	Unit	Unit	Unit	Unit
Precursor ion	224	343	344	343	344	314
Product ion	146	192	192	192	192	192
Dwell time (msec)	150	150	150	150	150	150

	DX-CA-S-2200	2-CONH2-S-2200	2-COOH-S-2200	5-CONH2-S-2200	5-COOH-S-2200	S-2200
Ion Source Voltage	3500	3000	4000	4000	5000	3500
Temperature	450	450	450	450	450	450
Declustering Potential	25	25	25	10	25	20
Focusing Potential	400	400	300	300	300	400
Collision Energy	40	20	20	30	30	20

The instrument parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography, to resolve matrix interferences (if observed), or to utilize other types of LC/MS-MS instruments. Each set of chromatograms must be clearly labeled with the LC/MS-MS parameters used.

4. Calculations

The concentration of the analytes (S-2200, DX-CA-S-2200, 2-COOH-S-2200, 5-COOH-S-2200, 2-CONH2-S-2200, and 5-CONH2-S-2200) in each sample extract is calculated on the basis of peak area using a second-order polynomial equation. The equation may be generated through the use of the graphing function in an Excel spreadsheet (see Note 3). The data are presented graphically as concentration of the calibration standards verses their peak areas, to calculate a curve expressed as the following equation:

$$Y = Ax^2 + Bx + C$$

The data are weighted relative (i.e., proportional) to the concentration of the highest standard concentration. For example, relative weighting may be done with multiple data entries:

Standard Concentration	Number of Entries in Data Set
50 µg/L	1
25 µg/L	2
10 µg/L	5
5 µg/L	10
2.5 µg/L	20
1.25 µg/L	40

For example, a set of calibration standards might gives peak areas as follows:

µg/L	Area
50	113,556
25	61,993
10	26,096
5	13,032
2.5	6,496
1.25	3,310

The resulting equation from the Excel spreadsheet is as follows:

$$Y = Ax^2 + Bx + C$$

A = 6.651 E-10
B = 3.634 E-04
C = 7.222 E-02

To ensure that the equation is appropriate, the areas of the calibration standards are entered into this equation and the standard concentrations are calculated. For each standard, the calculated concentration must each be within 15% of the actual concentration. In addition, the coefficient of determination (r^2) must be greater than 0.99 For example (from the above data), the 5µg/L standard has an area of 13,032 and the calculated concentration (using the equation) is 4.92 µg/L. This is acceptable as this is 98% of the known concentration. The criteria listed above for recalculated concentrations and the coefficient of determination must be met for acceptance of the each analytical set, unless approved by the chemist responsible for the analysis.

Sample extract concentration are also calculated using the equation from the calibration standards. For example, a sample extract with an area response of 6,330 would have a concentration as follows:

$$\mu g/mL = Ax^2 + Bx + C$$

$$\mu g/L = (6.651\text{ E-}10 \times 6{,}330 \times 6{,}330) + (3.634\text{ E-}04 \times 6{,}330) + 7.222\text{ E-}02 = 2.40$$

Concentrations of each analyte (S-2200, DX-CA-S-2200, 2-COOH-S-2200, 5-COOH-S-2200, 2-CONH2-S-2200, and 5-CONH2-S-2200) in the soil sample is calculated as using the following formula:

$$ppm = \frac{C \times (1/1000) \times FV \times DF}{W}$$

where:

C = concentration of extract (in µg/L, from equation)

FV = final volume of extract (20 mL).

DF = dilution factor (if diluted after final volume).

W = sample weight analyzed (2.5 g).

For example, the concentration in a soil sample (with a calculated extract concentration of 2.40 µg/L) would be calculated as follows:

$$ppm = \frac{(2.40\,\mu g/L) \times (1\,L/1000\,mL) \times (20mL)}{(2.5g)} = 0.0192$$

The recoveries for fortified samples are calculated using the formula:

$$\text{Percent recovery (\%)} = \frac{\text{ppm in fortified sample - ppm in control sample}}{\text{fortification level, ppm}} \times 100\%$$

For the above fortification sample fortified at 0.02 ppm, the following values were utilize to calculate the amount of 2-CONH2-S-2200 in the sample:

ppm found in fortified sample = 0.0192
ppm found in untreated control sample = 0.0000

$$\text{Percent recovery (\%)} = \frac{0.0192 - 0.0000 \text{ ppm}}{0.02 \text{ ppm}} \times 100\% = 96\%$$

LIMITS OF DETECTION AND QUANTITATION

The validated limit of quantitation (LOQ) for S-2200 and for each of its metabolites (DX-CA-S-2200, 2-COOH-S-2200, 5-COOH-S-2200, 2-CONH2-S-2200, and 5-CONH2-S-2200) in soil is 0.02 ppm. The estimated limit of detection (LOD) for each of these analytes is 0.01 ppm. This LOD is calculated by dividing the lowest calibration standard concentration (1.25 µg/L = 0.00125 µg/mL) by the effective matrix concentration in the sample extracts (2.5 g/20 mL = 0.125 g/mL):

$$\text{LOD} = [0.00125 \mu g/mL] \div [0.125 \text{ g/mL}] = 0.01 \text{ ppm}$$

ANALYSIS TIME

A trained analyst, familiar with this method, can complete the analysis of a set of twelve samples for S-2200, DX-CA-S-2200, 2-COOH-S-2200, 5-COOH-S-2200, 2-CONH2-S-2200, and 5-CONH2-S-2200 in approximately 8 hours. The results are available within 24 hours of initiating the analysis.

NOTES

1. At Valent, reproducibility of an analytical run is determined by calculating the coefficient of variation (CV) from the peak units obtained from the continuing calibration standards analyzed in the analytical sequence (set). For the analytical set to be acceptable, these CV's must be 15% or less unless approved by the chemist responsible for the analysis.

2 The standard operating procedure at Valent requires that a fortified control sample be analyzed with each set of samples. If the testing facility does not require concurrent analysis of fortified control samples, or if a UTC sample is not available, this method requirement may be waived.

The level of fortification is generally at the method LOQ (0.02 ppm) and/or at five times the LOQ (0.1 ppm). If residues higher than five times LOQ are anticipated, then fortifications should be made at a higher concentration (typically slightly above the highest residues). Method recoveries must be 70% to 120% to be acceptable unless approved by the chemist responsible for the analysis.

3. Other programs can be used to calculate the equation of a polynomial curve with relative weighting, such as Curve Expert 1.3 (Hyams Development, Starkville,MS).

METHOD APPROVAL

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