VALENT U.S.A. CORPORATION
VALENT TECHNICAL CENTER
DUBLIN, CALIFORNIA

DETERMINATION OF S-2188 AND S-2188-DC IN Soil
METHOD RM-45S **DATE: July 7, 2008**

INTRODUCTION

This method determines residues of S-2188 and metabolite S-2188-DC in soil.

Briefly, S-2188 and S-2188-DC residues are extracted from soil using methanol and water. The methanol is evaporated and sample is cleaned-up using an Oasis HLB column. Residues are quantitated by triple quadrupole HPLC/MS/MS.

REAGENTS

Acetic acid – glacial, reagent grade (or equivalent).

Acetone – Pesticide quality (or equivalent).

Acetonitrile - Pesticide quality (or equivalent).

Oasis HBL Cartridges– 12cc/500mg, Waters Cat # 186000116 or equivalent.

Sodium Ascorbate – Sigma-Aldrich Cat # EC 205-126-1 or equivalent.

Water – HPLC grade.

REAGENT SOLUTIONS

0.05% acetic acid – Add 0.5 mL of acetic acid, glacial to 1 liter of HPLC grade water. Store at room temperature.

Methanol/HPLC grade water (5/1, v/v) – combine 5 parts methanol with one part water. For example, add 500 mL of methanol and 100 mL of HPLC water sequentially to a reagent bottle and mix. Store at room temperature.

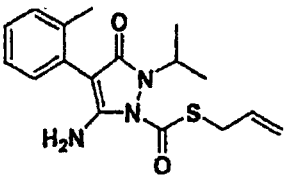
Acetonitrile/HPLC Grade Water, 1/1 (v/v) - Combine 1 part acetonitrile with 1 part HPLC grade water. For example, add 100 mL of acetonitrile and 100 mL of HPLC grade water sequentially to a reagent bottle. Store at room temperature.

Acetonitrile/HPLC Grade Water, 1/10 (v/v) - Combine 1 part acetonitrile with 10 parts HPLC grade water. For example, add 10 mL of acetonitrile and 100 mL of HPLC grade water sequentially to a reagent bottle. Store at room temperature.

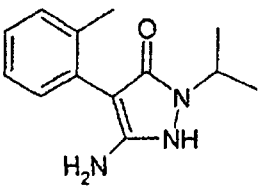
1M Sodium Ascorbate – Add 198 g of sodium ascorbate to 1 liter volumetric flask and bring up to volume in HPLC water. Store at room temperature.

REFERENCE STANDARDS

S-2188 (aka V-10135), [2-propenyl 5-amino-2-(1-methylethyl)-4-(2-methylphenyl)-3-oxo-2,3-dihydro-1H-1-pyrazolecarbothioate]- analytical standard of a known purity.



S-2188-DC, analytical standard of a known purity.



STANDARD SOLUTIONS (See Note 1)

Prepare a S-2188 stock solution containing 1.0 mg/mL in acetonitrile. Store in a freezer when not in use. Discard and prepare this standard every 12 months.

Prepare a S-2188-DC stock solution containing 1.0 mg/mL in acetonitrile. Store in a freezer when not in use. Discard and prepare this standard every 12 months.

10.0 µg/mL Mixed Fortifying Solution: Transfer 1.0 mL of each 1.0 mg/mL Stock Solution (S-2188 and S-2188-DC) into a single 100 mL volumetric flask and dilute to volume with acetonitrile. Store in a freezer when not in use. Discard and prepare this standard dilution every 12 months.

1.0 µg/mL Mixed Fortifying Solution: Transfer 10.0 mL of the 10.0 µg/mL Mixed Fortifying Solution into a 100 mL volumetric flask and dilute to volume with acetonitrile. All solutions should be stored in a freezer when not in use. Discard and prepare this standard dilution every four months from the 10.0 µg/mL Mixed Fortifying Solution.

Mixed Intermediate Standard Solutions: 0.10 µg/mL to 0.0020 µg/mL. Prepare a minimum of five intermediate linearity standards by diluting the mixed 1.0 µg/mL Mixed Fortifying Solution with acetonitrile. All solutions should be kept in the freezer when not in use. Discard and prepare these standard dilutions every two weeks.

Linearity Standard Solutions – 0.05µg/mL to 0.001µg/mL – Dilute the above Mixed Intermediate Standard Solutions 1:1 with HPLC water in an autosampler vial just prior to analyzing samples.

Calibrating Standard Solution – the calibrating standard solution will be the 0.005 µg/mL Linearity Standard Solution.

EQUIPMENT

Büchner funnels - 9 cm diameter.

Filter flasks - 500 mL.

Filter paper - Whatman #1 (or equivalent), 9 cm diameter.

Glass vials – 60 mL Fisher# 03-339-5E or equivalent.

Glass wool - Pyrex® (or equivalent).

Graduated cylinders – various sizes for making reagents.

High Pressure Liquid Chromatograph with MS/MS detector – Hewlett Packard 1100 Quatenary Pump HPLC system with an autosampler coupled to a Applied Biosystems API 2000 MS/MS triple quadrupole mass spectrometer with an electrospray ionization interface or equivalent system.

Reciprocating shaker – Eberbach or equivalent.

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

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

Ultrasonic bath – Branson 3200 (or equivalent).

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

Volumetric flasks –100 mL.

ANALYTICAL PROCEDURE

1. Extraction

Weigh 10 grams (± 0.1 grams) of soil sample into a 60 mL glass vial. At this point, if required by the testing facility, fortify control samples for method recovery with S-2188 and S-2188-DC (See Note 2). Add 5 mL of 1M sodium ascorbate solution and let stand for 10 minutes. Add 40 mL of methanol/HPLC grade water, (5/1,v/v) to the sample and place the tube on its side in a reciprocating shaker and shake for 1 hour.

Filter the sample into a 500 mL filter flask through a Büchner funnel containing a Whatman #1 filter paper. Roll the filter paper and return to the extraction tube. Re-extract the sample with an additional 40 mL portion of methanol/water, (5/1,v/v) as described above and filter as described previously using a new piece of filter paper. Rinse the extraction vial with 20 mL of methanol/water, (5/1,v/v) and filter. All filtrations are collected in the same filter flask. Transfer the combined filtrates into a 500 mL round bottom flask.

3. Oasis HLB Cartridge Cleanup (See Note 3)

Evaporate the methanol to aqueous mixture using a rotary-evaporator and water bath set to < 40°C.

Place a glass wool plug in an Oasis HLB cartridge. Using gravity flow, pre-condition the column by sequentially washing with 5 ml of acetonitrile followed by 10 ml of HPLC grade water. Note: insure elution of acetonitrile is complete before addition of water.

Transfer the extract to the cartridge and allow to elute by gravity flow. Rinse the round bottom flask with 10 mL HPLC grade water followed by 5 mL acetontrile/ HPLC grade water (1/10, v/v). Transfer each rinse to the cartridge, allowing each portion to elute through the cartridge before adding the next rinse. Discard all eluates.

Place a 40 mL vial under the cartridge and elute the residues with 25 mL of acetonitrile/HPLC water (1/1, v/v). Dilute samples 1:4 in an autosampler vial by taking 250 µL of sample and adding 750 µL of acetonitrile/HPLC water (1/1, v/v). NOTE: Use only HPLC grade water to elute residues from HLB column and for final dilution. (See Note 1)

4. LC/MS/MS Conditions

Condition the instrument with at least five injections of sample extract. Analyze a range of linearity standards within the analytical sequence. Each sequence must begin and end with a calibrating standard (the mid-range linearity standard 0.005 µg/mL). The recommended sequence of samples and standards for analysis is: calibrating standard, linearity standard, sample, sample, sample, linearity standard, etc.

HPLC Conditions:

Column:	YMS ODS, 3µm, 100mm x 3.0mm (Waters Part # AM125031003WT)
Column Oven Temperature:	20 ± 1°C
Mobile Phase:	A = 0.05% acetic acid in HPLC water B= Acetonitrile
Gradient Program:	T = 0 min, 80% A + 20% B T = 2.0 min, 80% A + 20% B T = 4.0 min, 20% A + 80% B T = 15.00 min, 20% A + 80% B T = 15.5 min, 80% A + 20% B T = 21.00 min, 80% A + 20% B
Flow Rate:	0.4 mL/minute
Injection:	
Drawing Speed:	200 µL/minute
Injection Volume:	50 µL
Ejecting Speed:	200 µL/minute

LC/MS Interface Conditions:

Interface:	Electrospray Ionization

Typical Values Depending Upon Instrument Tune:

Mass Spectrometer Method Properties	S-2188	S-2188-DC
Scan Type	MRM	MRM
Polarity:	Positive	Positive
Resolution Q1:	Unit	Low

Resolution Q3:	Unit	Unit
Precursor Ion (amu):	331.8	231.80
Product Ion (amu):	229.9, 188.9	189.5, 145.2
Dwell time (msec):	150	150

Probe/Source: Turb Ion Spray (Electrospray)

MS/Parameters:

Ion Source voltage:	4000	4000
Temperature:	450	450
Declustering Potential:	26	26
Focusing Potential:	370	290
Collision Energy:	25.0	25.0

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, or to utilize other types of LC/MS instruments. Each set of chromatograms must be clearly labeled with the LC/MS/MS parameters used.

5. Calculations

The concentration of S-2188 or S-2188-DC in each sample extract is calculated on the basis of peak area using a second order polynomial equation. The equation is automatically generated through the use of the graphing functions of an Excel spreadsheet.(See Note 4). The data is presented graphically as concentration of the linearity standards verses the peak areas of the linearity standards which results in the following equation:

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

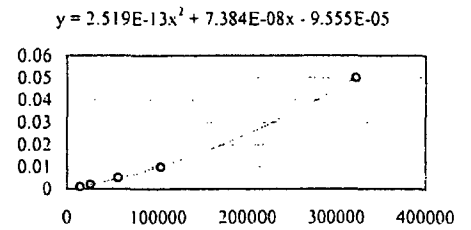
The data is weighted inversely proportional to the concentration of each standard. The weighting is accomplished by incorporating each data point into the graph with a frequency equal to (1/concentration). For example, a data point for a linearity standard with a concentration of 0.005 µg/mL would be entered into the graph 200 times while a data point for a linearity standard with a concentration of 0.05 µg/mL would be entered into the same graph 20 times.

Example:

For a linearity area response of:

µg/mL	Area
0.05	322623
0.01	103187
0.005	55983
0.002	25944
0.001	14405

The resulting graph from the Excel spreadsheet is as follows:



$Y = Ax^2 + Bx + C$
A = 2.519 E-13
B = 7.384 E-08
C = -9.555 E-05

To ensure that the equation is appropriate, the areas of the linearity standards are entered into the equation of the line and the concentrations are calculated. Each calculated standard concentration must be within 15% of it's known concentration. An example of this from the above data is the 0.01 µg/mL standard, which has an area of 103187. The calculated concentration would be 0.0102 µg/mL, which is 102% of the known concentration.

A sample extract with an area response of 23081 would have a concentration as follows:

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

$$\mu g/mL = (2.519\text{ E-}13 \times 103187 \times 103187) + (7.384\text{ E-}08 \times 103187) - 9.555\text{ E-}05$$
$$\mu g/mL = 0.0017$$

The amount of S-2188 or S-2188-DC found in each sample is calculated using the following formula:

$$ppm = \frac{CxFVxDF}{W}$$

Where:

C = concentration of extract.(µg/mL from equation)

FV = final volume of extract.(25 mL)

DF = dilution factor. (4 if diluted as per method)

W = sample weight analyzed.(10 g)

Example: From the above example, a sample with a calculated concentration of 0.0019 µg/mL would be calculated as follows:

$$ppm = \frac{(0.0017ug/mL)x(25mL)x(4)}{10g}$$

ppm = 0.017

LIMITS OF DETECTION AND QUANTITATION

The validated limit of quantitation (LOQ) of S-2188 or S-2188-DC in soil analyzed by this method is 0.02 ppm. The estimated limit of detection (LOD) is 0.01 ppm. This LOD is calculated by dividing the lowest analyte concentration from the validated linear range (0.001 µg/mL) of the measurement system by matrix concentration in the sample extracts (0.1 g/mL):

LOD = [0.001 µg/mL] ÷ [0.1 g/mL] = 0.01 ppm

ANALYSIS TIME

A trained analyst, familiar with this method, can complete the analysis of a set of twelve samples for S-2188 and S-2188-DC in approximately 8 hours. The results are available within 24 hours of initiating the analysis.

NOTES

1. Preliminary work has indicated that S-2188-DC at low solution concentrations in acetonitrile (≤ 1.0 µg/mL) are unstable even when stored under frozen conditions, therefore, standard solutions at this concentration or lower require preparation on a two week basis. Additionally, stability of S-2188-DC in aqueous mixtures were also found to be unstable

except in mixtures using HPLC grade water. Therefore HPLC grade water is specified for all steps in the method.

2. At Valent, a standard operating procedure 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 LOQ of the method and/or five times the LOQ. If residues higher than five times LOQ are anticipated than fortifications should be at that concentration. Method recoveries must be 70% to 120% to be acceptable unless approved by the chemist responsible for the analysis.

3. Each lot of Oasis HBL must be checked for recovery of S-2188 and S-2188-DC as follows: Transfer 1.0 mL of the 1.0 µg/mL S-2188 and S-2188-DC fortifying standard solution to a 250 mL round-bottom flask containing 60 mL of acetone/HPLC grade water (4/1,v/v) and evaporate the acetone using a rotary-evaporator with a water temperature bath set to ≤40°C. Elute and dilute the S-2188 and S-2188-DC as described under Step 3, **Oasis HLB Cartridge Cleanup.**

   Analyze the sample with the 0.01 µg/mL linearity standard as described under Step 3, **LC/MS/MS Conditions**. If the S-2188 and S-2188-DC peaks for the eluate are less than 90% of the linearity standard, then the elution profile of S-2188 and S-2188-DC must be determined.

4. There are other programs that can calculate a weighted regression graph such as Curve Expert 1.3 (Hyams Development, Starkville,MS).