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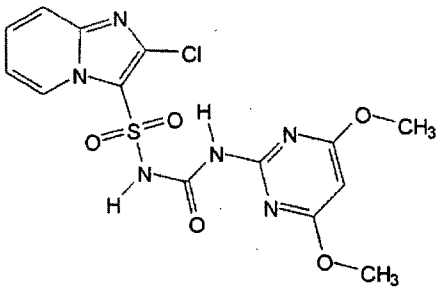
DETERMINATION OF IMAZOSULFURON, ADPM, HMS, AND IPSN IN SOIL
Method: **RM-42S-1-1** Date: April 23, 2007

I. INTRODUCTION

This method describes the determination of imazosulfuron, 1-(2-chloroimidazo[1,2-*a*]pyridin-3-ylsulfonyl)-3-(4,6-dimethoxypyrimidin-2-yl)urea, and its degradates (ADPM, HMS, and IPSN) in soil. Briefly, the method involves extraction with an aqueous acetonitrile mixture containing sodium bicarbonate (80:20 acetonitrile/water), centrifugation and filtration to remove solids, and rotary evaporation of the acetonitrile in the filtrate to obtain an aqueous residue. The residues are cleaned up by transfer onto an SPE cartridge, washing with deionized water to remove salts, and elution of the residues with aqueous methanol. The column eluant is rotary evaporated and the residues are redissolved in aqueous methanol/water (3:7 methanol/water) for analysis by LC/MS-MS for ADPM, HMS, IPSN, and imazosulfuron.

The method was modified from RM-42S-1 for analysis of sediment from ponds. This material is saturated with water and often contains some excess water. For these sediments, the amount of aqueous sodium bicarbonate added to the acetonitrile was reduced. The method was also modified to specify the use of Oasis HLB cartridges instead of C18 SPE cartridges.

II. ANALYTICAL STANDARDS



Imazosulfuron reference standard - Valent U.S.A. Corporation

Imazosulfuron Standard, 1.0 mg/mL Stock solution.

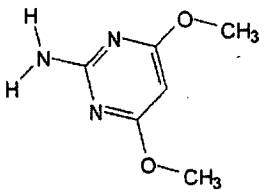
Weigh 0.100 grams into 100 mL volumetric flask (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard). Dilute to volume with acetone, and store in a freezer.

Imazosulfuron Standard, 100 µg/mL solution (in acetone).

Pipet 10.0 mL of the 1.0 mg/mL Imazosulfuron Stock solution (in acetone) into a 100 mL volumetric flask. Dilute to volume with acetone. Store in a freezer.

Imazosulfuron Standard, 25 µg/mL solution (in acetone).

Pipet 25.0 mL of the 100 µg/mL Imazosulfuron Standard (in acetone) into a 100 mL volumetric flask. Dilute to volume with acetone. Store in a freezer.



ADPM reference standard - Valent U.S.A. Corporation

ADPM Standard, 1.0 mg/mL Stock solution.

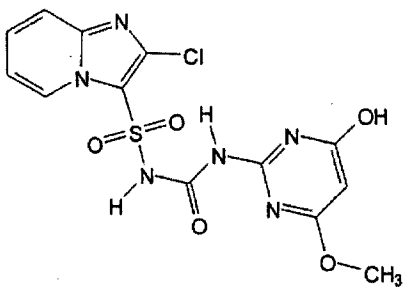
Weigh 0.100 grams into 100 mL volumetric flask (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard). Dilute to volume with acetone, and store refrigerated.

ADPM Standard, 100 µg/mL solution (in acetone).

Pipet 10.0 mL of the 1.0 mg/mL ADPM Stock solution (in acetone) into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

ADPM Standard, 25 µg/mL solution (in acetone).

Pipet 25.0 mL of the 100 µg/mL ADPM Standard (in acetone) into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.



HMS reference standard - Valent U.S.A. Corporation

HMS Standard, 1.0 mg/mL Stock solution.

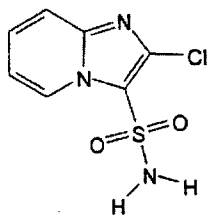
Weigh 0.100 grams into 100 mL volumetric flask (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard). *Add 25 mL water*, dilute to volume with acetone, and store refrigerated.

HMS Standard, 100 µg/mL solution (in methanol).

Pipet 10.0 mL of the 1.0 mg/mL HMS Stock solution (in acetone/water) into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.

HMS Standard, 25 µg/mL solution (in methanol).

Pipet 25.0 mL of the 100 µg/mL HMS Standard (in acetone) into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.



IPSN reference standard - Valent U.S.A. Corporation

IPSN Standard, 1.0 mg/mL Stock solution.

Weigh 0.100 grams into 100 mL volumetric flask (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard). Dilute to volume with acetone, and store refrigerated.

IPSN Standard, 100 µg/mL solution (in acetone).

Pipet 10.0 mL of the 1.0 mg/mL IPSN Stock solution (in acetone) into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

IPSN Standard, 25 µg/mL solution (in acetone).

Pipet 25.0 mL of the 100 µg/mL IPSN Standard (in acetone) into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

Fortification Mix, 1.0 µg/mL (in acetone)

Pipet 4.0 mL of each 25.0 µg/mL Standard (ADPM, HMS, IPSN, and imazosulfuron) into a 100 mL volumetric flask. Dilute to volume with acetone. Store in a freezer. *This standard should be prepared bi-weekly as imazosulfuron will degrade due to the trace amount of water present.*

Mixed Calibration Standard, 250 µg/L

Pipet 1.0 mL of each of the 25 µg/mL Standards (ADPM, HMS, IPSN, and imazosulfuron) into a 100 mL volumetric flask. Dilute to volume with 3:7 methanol/water (v/v). Store refrigerated. *This standard should be prepared weekly as imazosulfuron will degrade due to the water present.*

Mixed Calibration Standard, 100 µg/L

Pipet 4.0 mL of the Mixed 250 µg/L Calibration Standard into a 10.0 mL volumetric flask. Dilute to volume with 3:7 methanol/water (v/v). Store refrigerated. *This standard should be prepared weekly as imazosulfuron will degrade due to the water present.*

Mixed Calibration Standard, 50 µg/L

Pipet 5.0 mL of the Mixed 100 µg/L Calibration Standard into a 10.0 mL volumetric flask. Dilute to volume with 3:7 methanol/water (v/v). Store refrigerated. *This standard should be prepared weekly as imazosulfuron will degrade due to the water present.*

Mixed Calibration Standard, 25 µg/L

Pipet 5.0 mL of the Mixed 50 µg/L Calibration Standard into a 10.0 mL volumetric flask. Dilute to volume with 3:7 methanol/water (v/v). Store refrigerated. *This standard should be prepared weekly as imazosulfuron will degrade due to the water present.*

Mixed Calibration Standard, 5 µg/L

Pipet 2.0 mL of the Mixed 25 µg/L Calibration Standard into a 10.0 mL volumetric flask. Dilute to volume with 3:7 methanol/water (v/v). Store refrigerated. *This standard should be prepared weekly as imazosulfuron will degrade due to the water present.*

Note: Similar dilutions may also be performed to generate appropriate standards.

III. REAGENTS

Acetone - Pesticide quality

Acetonitrile - Pesticide quality

Ethyl Acetate - Pesticide quality

Formic Acid, 96% - Reagent grade

Methanol - Pesticide quality

Sodium Bicarbonate – Reagent grade

Water – Deionized

Water – HPLC grade

IV. REAGENT SOLUTIONS

Acetonitrile, with 0.05% Formic acid
Add 0.5 mL of formic acid per liter of acetonitrile. Store at room temperature.

Methanol/Water, 3:7 (v/v).
Combine 3 parts methanol with 7 parts water. For example, add 150 mL of methanol and 350 mL of water sequentially to a reagent bottle. Store at room temperature.

Sodium Bicarbonate Solution (0.02 M).
Dissolve 1.7 g sodium bicarbonate in 1 liter of water. [This preparation may be scaled as necessary.] Store at room temperature.

Sodium Bicarbonate Solution (0.04 M).
Dissolve 3.4 g sodium bicarbonate in 1 liter of water. [This preparation may be scaled as necessary.] Store at room temperature.

Water, with 0.05% Formic acid
Add 0.5 mL of formic acid per liter of HPLC grade water. Store at room temperature.

V. EQUIPMENT

Autosampler vials, screw-top with Teflon-coated septums

Balances, Analytical and Top Loading

Centrifuge, Sorval Evolution RC (or equivalent)

Cork Rings (80 mm and 110 mm diameter)

Filter Paper, 12.5 cm diameter (Whatman No. 1 or equivalent)

Glass Centrifuge Tubes, Graduated - 15 mL (or equivalent)

Graduated Cylinders (1000, 250, 100, 50, 10 mL)

Gravity Filter Funnels (approximately 100 mm diameter)

Heated Water Bath (temperature <40°C)

Pipettor, Automatic - capable of accurately dispensing volumes of 0.2 to 2.5 mL

Pipettes, Serological, Disposable – 10 mL

Pipettes, Volumetric – 10.0, 5.0, 4.0, and 1.0 mL

Polypropylene Tubes, 50 mL with caps – BD Falcon Blue Max #2098 or equivalent

Reciprocating Mechanical Shaker, (Erbach or equivalent)

Refrigerator

Rotary Vacuum Evaporators

Round-bottom Flasks – 250 and 100 mL

Vacuum Manifold for SPE Cartridges, Baker (or equivalent)

Waters Oasis HLB 12 cc (500 mg) LP Extraction Cartridge (Part No. 186000116; Box of 20)

VI. INSTRUMENTATION

High Performance Liquid Chromatograph with Mass Selective Detector (LC/MS-MS) -

Finnigan TSQ Quantum with electrospray ionization interface. Conditions shown below are suggested for this analysis (other conditions may be used as appropriate).

Column:	Synergi 4μ Polar-RP, 50mm x 2.0mm (Phenomenex part # 00B-4336-B0)
Mobile Phases:	Acetonitrile, 0.05% formic acid Water, 0.05% formic acid
Flow Rate:	250 μL/minute
InjectionVolume:	20 μL

Gradient Program:

Time, Min	% ACN, 0.05% HOOCH	%Water, 0.05% HOOCH
0	10	90
1.0	10	90
4.0	40	60
5.0	40	60
6.0	80	20
9.0	80	20
10.5	10	90
13	10	90

LC/MS-MS Conditions:

Interface:	Electrospray Ionization
Polarity:	Positive
Segment 1 (ADPM) -	
Source CID Collision Energy:	15
Q2 Collision Gas Pressure:	1.0 mTorr
Scan type:	SRM
Parent Ion Mass:	156.0
Quantitation Ion Mass	100.0
Collision Energy:	33
Segment 2 (HMS and IPSN) -	
Source CID Collision Energy:	14
Q2 Collision Gas Pressure:	0.9 mTorr
Scan type:	SRM
* Scan Event 1 (IPSN) -	
Parent Ion Mass:	231.7
Quantitation Ion Mass	152.1
Collision Energy:	38
* Scan Event 2 (HMS) -	
Parent Ion Mass:	399.0
Quantitation Ion Mass	142.1
Collision Energy:	22
Segment 3 (Imazosulfuron) -	
Source CID Collision Energy:	16
Q2 Collision Gas Pressure:	0.9 mTorr
Scan type:	SRM
Parent Ion Mass:	413.0
Quantitation Ion Mass	156.1
Collision Energy:	34

Retention Times: 1.9 minutes for ADPM
4.3 minutes for IPSN
4.8 minutes for HMS
8.0 minutes for Imazosulfuron (*Figure 1*)

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-MS instruments. Each set of chromatograms must be clearly labeled with the LC/MS-MS parameters used.

VII. ANALYTICAL PROCEDURES

1. Sample Setup

Thoroughly mix the soil sample. Weigh 5.0 g (±0.1 g) of the sample into a 50 mL polypropylene centrifuge tube [e.g.- Falcon Blue Max]. At this point, if required by the testing facility, a control sample to be used for method recoveries may be fortified with a mixture of the analytes (*see Note 1*).

2. Extraction with Acetonitrile/Water

Add 7.5 mL of sodium bicarbonate solution (0.02 M) into the tube. [*For sediment (water saturated), add 4.0 mL of sodium bicarconate solution (0.04 M) into the tube.*] Agitate briefly and let the sample stand for 20-30 minutes. Add 30 mL of acetonitrile to the tube, cap tightly, and place horizontally on a reciprocating mechanical shaker. Shake at high speed for 1 hour.

Place the tube into the centrifuge, and centrifuge for 15-20 minutes at 4000 rpm (at ambient temperature) to separate the solids. Decant the supernatant solution into a second polypropylene tube (labeled), cap the tube, and store the extract (typically on the bench or in a refrigerator).

Add 7.5 mL of sodium bicarbonate solution (0.02 M) into the tube. [*For sediment (water saturated), add 4.0 mL of sodium bicarconate solution (0.04 M) into the tube.*] Agitate briefly and let the sample stand for 20-30 minutes. Add 30 mL of acetonitrile to the tube, cap tightly, and place horizontally on a reciprocating mechanical shaker. Shake at low speed overnight.

Place the tube into the centrifuge, and centrifuge for 15-20 minutes at 4000 rpm (at ambient temperature) to separate the solids.

Place labeled 250 mL round-bottom flasks onto cork rings, and place gravity filter funnels into the flasks. Place folded filter paper into each funnel.

For each sample, pour the initial extract into the filter paper and collect the filtered extract in the 250 mL round-bottom flask. Once the sample extract has drained, decant the extract from the second extraction into the filter paper and combine the extracts in the 250 mL round-bottom flask. The combined extracts may be stored for up to 3 days in a refrigerator or freezer.

3. Oasis HLB Column Cleanup

Place Oasis HLB cartridges onto a vacuum manifold or a rack, and carefully precondition them by passing through 2 x 5 mL methanol (draining the first rinse to the top of the frit, adding the second rinse, and draining the second rinse to the top of the frit).

The flow through the Oasis HLB cartridge only requires gravity, and care should be taken to ensure that each sequential rinse passes completely through the cartridge before adding the next rinse.

Remove each cartridge and rinse the barrel of the cartridge (walls and frit) with 1 or 2 water rinses (each immediately discarded by inverting the cartridge) to ensure that no methanol is present. Place the cartridges back on the manifold or rack and pass 4 x 5 mL of water through each cartridge.

As before, care should be taken to ensure that each sequential rinse passes completely through the cartridge before adding the next rinse.

Remove the acetonitrile in the 250 mL round-bottom flasks by rotary evaporation using a heated water bath (temperature 35 to 40°C) to obtain an aqueous residue. Typically, the samples are rotary evaporated approximately 1 to 2 minutes after the liquid trap (adjacent to the round-bottom flask) clouds with water vapor. Add 40 mL of ethyl acetate, and continue rotary evaporation to obtain approximately 5 mL of aqueous residue. [This volume should be checked against a 250 mL round-bottom flask containing 5 mL of water.] Some aqueous soil sample extracts may still foam after the volatile organics have been removed. *Removal of all of the organic solvent is necessary to ensure that the analytes are collected on the cartridge.*

Transfer the aqueous residue onto the Oasis HLB cartridge. Add 5 mL deionized water to the 250 mL round-bottom flask, and sonicate briefly (rotating the flask) to dislodge residues. Allow the extract in the cartridge to drain to the frit, and then add the water rinse to the cartridge. Add 5 mL of deionized water again to the round-bottom flask and swirl to rinse the sides of the flask. When the liquid in the cartridge (the first rinse) reaches the frit, add the second rinse to the cartridge.

Add 5.0 mL of methanol to the 250 mL round-bottom flask (preferably while the second water rinse is passing through the cartridge), and briefly sonicate the flask to dissolve the residues. Add 1.0 mL of water to the round-bottom flask (making a 5:1 methanol/water mixture).

When the second water rinse has drained to the frit, place the cartridge into a 100 mL round-bottom flask on a cork ring (or other support). [The top of the cartridge will be supported by the top edge of the 24/40 joint.] Discard any accumulated eluant from loading and rinsing the cartridge.

Transfer the methanol/water rinse to the Oasis HLB cartridge, and elute the residues into the 100 mL round-bottom flask. Add a second 5.0-mL portion of methanol to the 250 mL round-bottom flask, swirl to rinse the sides of the flask, add 1.0 mL of water to the round-bottom flask, and then add this rinse to the cartridge when the first rinse has drained to the frit. After this eluant has passed through the cartridge, the combined eluant may be stored in a stoppered flask (or in a capped vial) in a freezer. (*Note: eluant samples should not be stored for more than a 3 days due to possible degradation of imazosulfuron*).

Rotary evaporate the sample (temperature 35 to 40°C) to reduce the volume to approximately 1 mL. Transfer the aqueous residue into a graduated vial with a Pasteur pipet, add 3.0 mL methanol into the round-bottom flask, and sonicate (rotating the flask) to dissolve the residues. Transfer the methanol rinse into the graduated vial with a Pasteur pipet, and add approximately 5

mL water to the round-bottom flask. Swirl the water in the flask to rinse the sides, and then transfer the water rinse to the graduated vial. The flask may be rinsed with another 1-2 mL of water, and the rinse (or water) added to the graduated vial to set the volume to 10 mL. Mix the final volume using the Pasteur pipet (by filling and emptying the pipet with the solution). Transfer a portion of the sample into an autosampler vial for LC/MS-MS analysis (Step 4). The samples may be stored in a freezer. (*Note: analysis of the samples within 5 days is recommended due to the possible degradation of imazosulfuron*).

4. LC/MS-MS Analysis

Instrument calibration is performed using a linear fit with a non-zero intercept. The calibration is performed with linearity standards that are distributed within each analytical sequence.

Condition the instrument with at least six injections of a sample extract. Analyze five linearity standard concentrations *within the analytical sequence* to generate the linear calibration for the LC/MS-MS, including a 5 µg/L (or less) standard. A typical set of standards would include concentrations of 250, 100, 50, 25, and 5 µg/L (with an injection volume of 20 µL). The coefficient of determination (r^2) is calculated from the linearity standards (see Step 5), and this value must be greater than 0.99 for the instrument response to be considered linear over the range of concentrations. In addition, the concentration calculated from the peak area of each of the standards, using the slope and intercept from the linear fit, must be within 15% of the corresponding standard concentrations

Additional 50 µg/L reference standards (or continuing calibration standards) are also analyzed as part of the analytical sequence. Typically, the sequence is constructed with the following order: a reference standard (50 µg/L), 2 to 4 sample extracts, a reference standard or linearity standard, 2 to 4 sample extracts, ..., and a reference standard. *The sequence must begin and end with reference standards.* The coefficient of variation of the reference standard responses must be 10% or less for the analysis set to be acceptable.

If the peak area observed for a sample is greater than the peak area of the highest linearity standard, the sample extract must be diluted and the diluted extract analyzed. The sample extract must be diluted (with 3:7 methanol/water, v/v) such that the peaks obtained are within the documented linear response range of the LC/MS-MS.

5. Calculations

To calculate the linear fit, the peak area and the concentration of each of the standards is input into an Excel spreadsheet.

Excel calculates the slope for the regression line as $b = \dfrac{n\sum xy - (\sum x)(\sum y)}{n\sum x^2 - (\sum x)^2}$

and calculates the intercept for the regression line as $a = \overline{Y} - b\overline{X}$

The slope and the intercept are calculated using a linear regression weighed by 1/concentration. Typically, the linear regression is based on standard concentration and Peak Units (Area/10^6), and replicate entries are included in the data set prior to performing the linear regression in Excel (to provide weighting by 1/concentration). For example:

Linearity Standard	Number of Entries in Data Set
250 µg/L	2
100 µg/L	5
50 µg/L	10
25 µg/L	20
5 µg/L	100

For each analyte, the concentration in the sample is calculated as follows:

$$\text{Sample Concentration, } (\mu g/g) = \frac{[(b \times X) + a] \times C \times D}{E \times 1000}$$

where:
b = slope [from regression analysis]
X = Sample response (Peak Area)
a = intercept [from regression analysis]
C = Final volume (10.0 mL)
D = Dilution factor, used if the sample extract is diluted prior to analysis
E = Sample weight (5.0 g)

VIII. LIMIT OF DETECTION

The limit of detection (LOD) of this method is 0.01 ppm (µg/g), based on a 5.0 g sample with a 10 mL final volume and a 5 µg/L (0.005 µg/mL) linearity standard in the linearity verification.

$$\text{Limit of Detection} = \frac{10\text{ mL Final Volume} \times 0.005\ \mu g/mL}{5.0\ g} = 0.01\ \mu g/g = 0.01\ \text{ppm}$$

ADPM-IPSN HPLC Conditions:

Column: Luna (C18), 3µm, 50mm x 3.0mm
(Phenomenex part # 00B-4251-Y0)

Mobile Phases: Acetonitrile, 0.05% formic acid
Water, 0.05% formic acid
Flow Rate: 250 µL/minute
Injection Volume: 20 µL

Gradient Program:

Time, Min	% ACN, 0.05% HOOCH	%Water, 0.05% HOOCH
0	10	90
2.0	10	90
9.0	90	10
11	90	10
12	10	90
17	10	90

LC/MS-MS Conditions:

Interface: Electrospray Ionization
Polarity: Positive

Segment 1 (ADPM) -
Source CID Collision Energy: 15
Q2 Collision Gas Pressure: 1.0 mTorr
Scan type: SRM
Parent Ion Mass: 156.0
Quantitation Ion Mass 100.0
Collision Energy: 33

Segment 2 (IPSN) -
Source CID Collision Energy: 14
Q2 Collision Gas Pressure: 1.0 mTorr
Scan type: SRM
Parent Ion Mass: 231.7
Quantitation Ion Mass 152.1
Collision Energy: 38

Retention Times: 3.2 minutes for ADPM
6.3 minutes for IPSN (*Figure 5*)

Imazosulfuron-HMS HPLC Conditions:

Column:	Luna (C18), 3µm, 50mm x 3.0mm (Phenomenex part # 00B-4251-Y0)
Mobile Phases:	Acetonitrile, 0.05% formic acid Water, 0.05% formic acid
Flow Rate:	250 µL/minute
Injection Volume:	20 µL

Gradient Program:

Time, Min	% ACN, 0.05% HOOCH	%Water, 0.05% HOOCH
0	50	50
1.0	50	50
7.0	90	10
9.0	90	10
10	50	50
115	50	50

LC/MS-MS Conditions:

Interface:	Electrospray Ionization
Polarity:	Positive

Segment 1 (Imazosulfuron) -

Source CID Collision Energy:	16
Q2 Collision Gas Pressure:	0.9 mTorr
Scan type:	SRM
Parent Ion Mass:	413.0
Quantitation Ion Mass	156.1
Collision Energy:	34

Segment 2 (HMS) -

Source CID Collision Energy:	10
Q2 Collision Gas Pressure:	0.9 mTorr
Scan type:	SRM
Parent Ion Mass:	399.0
Quantitation Ion Mass	142.1
Collision Energy:	22

Retention Times: 3.0 minutes for Imazosulfuron
4.2 minutes for HMS (*Figure 6*)