1.0 ABSTRACT

The purpose of this study was to independently validate Valent U.S.A. Corporation analytical method RM-42S-1-1, *Determination of Imazosulfuron, ADPM, HMS and IPSN in Soil.*

Residues of imazosulfuron, ADPM, HMS and IPSN were extracted twice from the soil with an aqueous acetonitrile mixture containing sodium bicarbonate (80:20 acetonitrile: 0.2*M* sodium bicarbonate, v/v). The extract was centrifuged and filtered to remove solids, and then rotary evaporated to obtain an aqueous mixture. The residues were loaded onto an Oasis HLB cartridge, washed with deionized water, and then eluted with methanol:water (5:1, v/v). The eluant was rotary evaporated to near dryness, and then redissolved in methanol:water (3:7, v/v). Extracts and standards were analyzed by liquid chromatography using a tandem mass spectrometer (LC/MS-MS). For each analyte, a six-point linear calibration curve (with 1/x weighting - plotting the peak area versus concentration) was used to quantify residues. The limit of quantitation (LOQ) for the each analyte was 0.02 ppm, µg/g (20 ng/g, ppb).

Two validation sample sets were extracted. Each validation set included a reagent blank, two unfortified control samples, five control samples fortified with each analyte at 0.02 ppm (LOQ), five control samples fortified with each analyte at 0.2 ppm (10X LOQ) and instrument calibration working standards.

One of the LOQ fortification extracts in the first validation set went nearly to dryness at the first rotary evaporation step, and recoveries for two of the analytes (imazosulfuron and ADPM) were low. The results from this fortification sample (for all of the analytes) were not included in the statistical analyses for the first trial. A subset that included three LOQ fortifications was added to this validation set due to the loss of this one fortified sample. However, as there was no recovery of imazosulfuron from the three fortification samples in this subset, a second validation sample set was extracted.

The performance data from the second trial of the method, to determine imazosulfuron, ADPM, HMS and IPSN in soil, met the EPA guideline criteria and supported an LOQ of 0.02 ppm for each analyte.

2.0 MATERIALS

2.1. EQUIPMENT

A list of equipment used in the second method validation trial is shown below. Similar equipment from other suppliers may also be used.

Autosampler vials, 1.8 mL, with Teflon®-lined septum caps (Chromatography Research Supplies)
Balance, analytical, capable of weighing 0.0001 g (Mettler)
Balance, top loading, capable of weighing to 0.01 g (Mettler)
Centrifuge (IEC Centra-8)
Filter paper, 12.5 cm diameter, Whatman No. 1
Glass centrifuge tubes, graduated, 15-mL
Graduated cylinders, assorted volumes
Gravity filter funnels, approximately 100 mm diameter
Heated water bath, temperature <40°C (Organomation)
Rotary evaporator (Buchi RE-121 Rotavapor)
Pasteur pipettes
Pippettor, automatic, capable of accurately dispensing volumes of up to 5.0 mL
Pippettor, automatic, µL (Eppendorf Repeater® Pro)
Polypropylene centrifuge tubes, graduated, 50-mL, with caps (VWR)
Polypropylene centrifuge tubes, graduated, 15-mL (Fisher)
Reciprocating mechanical shaker
Refrigerator
Flat-bottom flasks, 250 and 100 mL
Sonicator (Branson 5210)
Syringes, µL
Vacuum manifold for SPE cartridges plus disposable valve liners (Supelco)
Volumetric flasks
Waters Oasis® HLB 12 cc (500 mg) LP extraction cartridge

2.2 REAGENTS

A list of reagents used in the second method validation trial is shown below. All reagents used were Fisher brand but other suppliers can also be used, provided the reagents are sufficiently pure.

Acetone, pesticide quality
Acetonitrile (ACN), pesticide quality
Ethyl acetate, pesticide quality
Formic acid, 96%, reagent grade
Methanol, pesticide quality (Fisher)
Sodium bicarbonate, reagent grade
Water, deionized
Water, HPLC grade

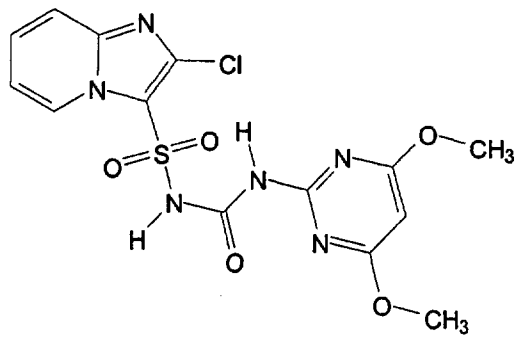
2.3 REAGENT SOLUTIONS

ACN with 0.05% formic acid (mobile phase "B")
Methanol:water 3:7 (v/v)
Sodium bicarbonate solution, 0.02*M*
Water with 0.05% formic acid (mobile phase "A")

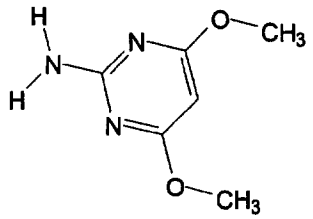
2.4 TEST SUBSTANCES / ANALYTICAL REFERENCE STANDARDS

The test substances/analytical reference standards were supplied by Valent Technical Center, Dublin, CA. Copies of the certificates of quality are included in Appendix 3.

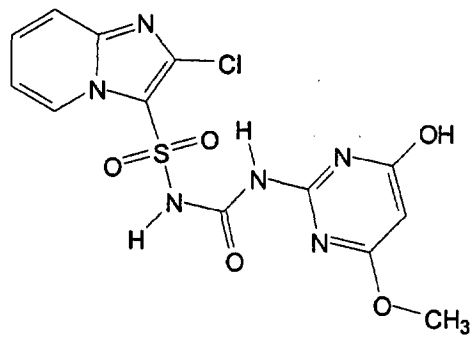
Common name:	Imazosulfuron
Chemical name:	1-(2-chloroimidazo[1,2-α]pyridin-3-ylsulfonyl)-3-(4,6-dimethoxyprimidin-2-yl)urea
CAS number:	122548-33-8
Lot number:	AS 2146a
Stated purity:	99.9%
Expiration date:	January 3, 2009
Storage conditions:	Under nitrogen in freezer
Structure:	



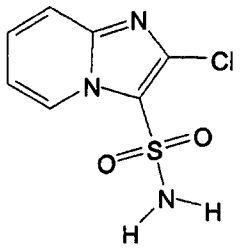
Common name: ADPM
Chemical name: (4,6-dimethoxypyrimidine-2-yl)amine
CAS number: NA
Lot number: AS 2137a
Stated purity: 100%
Expiration date: July 13, 2008
Storage conditions: Under nitrogen in freezer
Structure:



Common name: HMS
Chemical name: 1-(2-chloroimidazo[1,2-α]pyridin-3-ylsulfonyl)-3-(4-hydroxy-6-methoxyprimidin-2-yl)urea
CAS number: NA
Lot number: AS 2183a
Stated purity: 96.3%
Expiration date: February 15, 2008
Storage conditions: Under nitrogen in freezer
Structure:



Common name: IPSN
Chemical name: 1-(2-chloroimidazo[1,2-α]pyridin-3-ylsulfonyl)amine
CAS number: NA
Lot number: AS 2147a
Stated purity: 100%
Expiration date: December 14, 2008
Storage conditions: Under nitrogen in freezer
Structure:



All imazosulfuron single analyte stock standard solutions were stored in a freezer. ADPM, HMS and IPSN single analyte stock standard solutions were all stored refrigerated.

The mixed analyte fortification standard solution was stored in a freezer and the mixed analyte instrument calibration working standards were stored refrigerated when needed.

2.5 LC/MS-MS INSTRUMENTATION

Analysis was performed using a high pressure liquid chromatograph with a tandem mass spectrometer (LC/MS-MS). The following equipment was used:

Perkin Elmer (PE) ISS 200 autosampler
Shimadzu SCL-AvpLC pump system controller
(2) Shimadzu LC-10ADvp pumps
Harvard Apparatus #PHD 2000 infusion syringe pump
Sciex API 3000 LC/MS-MS triple quadrapole mass spectrometer
Sciex Turbo Ion Spray (TIS) sample introduction unit for the 3000 mass spectrometer, operated in positive ion mode
Peak Scientific Instruments High Purity Nitrogen Generator
PE Sciex "Analyst" software version 1.2
HP LaserJet 4100 printer

2.6 TEST SYSTEM AND SAMPLE STORAGE

An untreated control soil sample was supplied by Valent U.S.A Corporation (Valent). The sample was a sub-sample of a site qualification sample from a terrestrial field soil dissipation study of imazosulfuron on bare soil (Valent Project No. 28759). The soil sample had a unique Valent number and was also identified with a unique North Coast Laboratories, Ltd. (NCL) sample number on receipt at the laboratory. The sample was stored frozen (nominally ≤-10°C) in a limited access freezer, except when in use. The dates of events for the sample validation sets are presented in Table 7.3.

2.7 SAFETY AND HEALTH

This method should be performed by trained analytical chemists. A copy of the material safety data sheet is presented in Appendix 4 and documents the hazards associated with the use of these chemicals.

3.0 ANALYTICAL METHOD

3.1 PRINCIPLE OF THE METHOD

Imazosulfuron residues were extracted twice from the soil with an aqueous acetonitrile mixture containing sodium bicarbonate (80:20 acetonitrile:0.2*M* sodium bicarbonate, v/v). The extract was centrifuged and filtered to remove solids, and then rotary evaporated to obtain an aqueous mixture. The residues were loaded onto an Oasis HLB cartridge, washed with deionized water, and then eluted with methanol:water 5:1 (v/v). The eluant was evaporated to near dryness, and then redissolved in methanol:water 3:7 (v/v).

Extracts and standards were analyzed using a high pressure liquid chromatograph with a tandem mass spectrometer (LC/MS-MS). Six-point linear calibration curves (with 1/x weighting - plotting the peak area versus concentration) were used to quantify imazosulfuron, ADPM, HMS and IPSN. The primary transitions (most abundant product ions) for the analytes were used for quantitation.

3.2 LIMITS OF QUANTITATION (LOQ)

The LOQ in soil for each analyte (imazosulfuron, ADPM, HMS and IPSN) was 0.02 ppm, µg/g (20 ng/g, ppb).

3.3 VALIDATION SAMPLE SETS

Two trials were conducted and each validation set consisted of the following samples:

Instrument calibration working standards
1 reagent blank
2 unfortified control samples
5 samples fortified with analytes at 0.02 µg/g, ppm (20 ng/g, ppb), LOQ
5 samples fortified with analytes at 0.2 µg/g, ppm (200 ng/g, ppb), 10X LOQ

An additional subset, consisting of one reagent blank, one control sample and three LOQ fortifications, was also extracted as part of the first trial (see section **3.10**).

3.4 PREPARATION OF SINGLE ANALYTE IMAZOSULFURON, ADPM, HMS AND IPSN STANDARD STOCK SOLUTIONS

3.4.1 1,000 µg/mL (ng/µL, ppm), single analyte, imazosulfuron, ADPM, HMS and IPSN standard stock solutions

For the calculations involved in the preparation of these standard solutions, see section **3.15.2**.

An aliquot of the respective neat analyte, 0.010 g – 0.011 g, was weighed out and, taking into account the percent purity, was brought to volume with the appropriate amount of solvent to yield a 1,000 µg/mL standard solution. The imazosulfuron, ADPM and IPSN standard stock solutions were made up in acetone and the HMS standard stock solution was made up in a mixture of 5 mL water:2.5 mL methanol:2.5 mL acetone. The imazosulfuron standard solution was stored in a freezer and the ADPM, HMS and IPSN standard solutions were stored refrigerated.

3.4.2 100 µg/mL and 25 µg/mL (ng/µL, ppm), single analyte, imazosulfuron, ADPM, HMS and IPSN standard stock solutions

These standard stock solutions were prepared by serial dilution from the respective 1,000 µg/mL standard stock solution. A 100 µg/mL standard stock solution was prepared by transferring a 1.0 mL aliquot of the respective 1,000 µg/mL standard stock solution into a 10 mL volumetric flask and filling the flask to the mark with solvent. A 25 µg/mL standard stock solution was prepared by transferring a 2.5 mL aliquot of the respective 100 µg/mL standard stock solution into a 10 mL volumetric flask and filling the flask to the mark with

solvent. The imazosulfuron, ADPM and IPSN standard stock solutions were made up in acetone and the HMS standard stock solutions were made up in a methanol. The imazosulfuron standard solution was stored in a freezer and the ADPM, HMS and IPSN standard solutions were stored refrigerated.

3.5 PREPARATION OF MIXED ANALTE IMAZOSULFURON, ADPM, HMS AND IPSN FORTIFICATION AND INSTRUMENT CALIBRATION WORKING STANDARD SOLUTIONS

3.5.1 1.0 µg/mL (ng/µL, ppm) mixed analyte fortification standard solution

A 1.0 ng/µL mixed analyte fortification standard stock solution was prepared by transferring 0.40 mL aliquots of each of the 25 ng/µL stock standard solutions into one 10 mL volumetric flask and filling the flask to the mark with acetone. This standard was stored in a freezer and was prepared every two weeks.

3.5.2 Mixed analyte instrument calibration working standard solutions

A 0.25 ng/µL (µg/mL, ppm) mixed analyte standard solution was prepared by transferring 0.10 mL aliquots of each of the 25 ng/µL stock standard solutions into one 10 mL volumetric flask and filling the flask to the mark with methanol:water 3:7 (v/v). This standard was stored refrigerated and was prepared weekly.

Six levels of mixed analyte instrument calibration working standards, at 0.5X, 1X, 2.5X, 5X, 10X and 25X LOQ, were used for calibration and were prepared from the 0.25 ng/µL (µg/mL, ppm) standard solution. The calibration standards were named with respect to the concentration of analyte in the fortified samples, and also accounted for the final extract volume (see calculations section **3.15.4**).

The mixed analyte instrument calibration working standards were prepared in autosampler vials, by dilution with 3:7 methanol:water (v/v), as tabulated below:

Calibration standard analyte concentrations relative to the sample	Concentration of mixed analyte stock solution used (ng/µL)	Volume of mixed analyte stock solution used (µL)	Final volume of standard* (mL)	In-solution analyte concentrations (ng/mL)
0.5X LOQ = 10 ng/g	0.25	20	1.0	5.0
1X LOQ = 20 ng/g	0.25	40	1.0	10
2.5X LOQ = 50 ng/g	0.25	100	1.0	25
5X LOQ = 100 ng/g	0.25	200	1.0	50
10X LOQ = 200 ng/g	0.25	400	1.0	100
25X LOQ = 500 ng/g	0.25	1000	1.0	250

* diluted with 3:7 methanol:water (v/v)

The instrument calibration working standards were prepared weekly and stored refrigerated when not in use.

3.6 PREPARATION OF SAMPLES AND WEIGHING

The soil sample was homogenized at Valent and shipped frozen, on dry ice, *via* FedEx overnight service, to NCL. Immediately prior to the first extraction, the sample was transferred to a larger plastic bag and then thoroughly mixed in this bag. Sub-samples, 5.0 g (± 0.02 g), of this prepared sample were weighed into 50-mL polypropylene centrifuge tubes.

3.7 PREPARATION OF FORTIFICATION SAMPLES

An LOQ fortification at 0.02 µg/g, ppm (20 ng/g, ppb) on a 5.0 g soil sample was prepared by adding, using a microliter automatic pippettor, 100 µL of the 1.0 ng/µL mixed analyte fortification standard solution.

A 10X LOQ fortification at 0.2 µg/g, ppm (200 ng/g, ppb) on a 5.0 g soil sample was prepared by adding, using a microliter automatic pippettor, 1,000 µL of the 1.0 ng/µL mixed analyte fortification standard solution.

3.8 EXTRACTION PROCEDURE

NOTES. Prior to the extraction:

- Methanol lot to be used was checked for low pH
- pH for water lot to be used was checked
- All glassware was rinsed with:
  - 1*M* hydrochloric acid, one rinse
  - Tap water, eight rinses
  - Acetone, three rinses
  - Methylene chloride, one rinse
  - Methanol, two rinses
- The elution pattern for the Waters Oasis® HLB 12 cc (500 mg) LP extraction cartridges was checked.

3.8.1 Extraction with ACN:water and rotary evaporation

A 7.5-mL aliquot of 0.02*M* sodium bicarbonate solution was added to the polypropylene tube containing the soil sample, the mixture was briefly agitated

and then allowed to stand for 20-30 minutes. ACN, 30 mL, was added to the tube, the tube capped and then shaken on a reciprocating mechanical shaker at high speed for about 1 hour.

The tube was centrifuged for 15-20 minutes at 4000 rpm (at room temperature) to separate the solids. The supernatant was decanted into a second polypropylene tube, the tube capped and the extract stored at room temperature.

A second 7.5-mL aliquot of 0.02*M* sodium bicarbonate solution was added to the soil pellet in the original polypropylene tube, the mixture was briefly agitated and then allowed to stand for 20-30 minutes. ACN, 30 mL, was added to the tube, the tube capped and then shaken on a reciprocating mechanical shaker at low speed overnight.

The tube was centrifuged for 15-20 minutes at 4000 rpm (at room temperature) to separate the solids.

A gravity filter funnel containing a folded filter paper was placed into a 250-mL flat-bottom flask. The initial extract was poured into the filter paper and the filtered extract collected in the flat-bottom flask. Then the extract from the second extraction was decanted into the filter paper, combining the filtered extracts in the flask.

The flat-bottom flask containing the combined extract was place on a rotary evaporator and the acetonitrile was removed using a heated waterbath at a temperature of 35-40°C. The samples were rotary evaporated approximately 1 to 2 minutes after the liquid trap (adjacent to the flat-bottom flask) clouded with water vapor. A 40-mL aliquot of ethyl acetate was added to the aqueous extract and rotary evaporation was continued until approximately 5 mL of the aqueous extract remained.

3.8.2 Oasis® HLB column cleanup

Preparation of the Waters Oasis® HLB cartridge:

- A Waters Oasis® HLB cartridge was placed onto a manifold and carefully preconditioned with two 5-mL rinses of methanol; the methanol was allowed to drain under gravity. The first rinse was drained to the top of the frit and then the second rinse was added, draining it to the top of the frit.
- The cartridge was removed and the barrel of the cartridge (walls and frit) was rinsed with two water rinses (and the rinse immediately discarded) to

ensure that no methanol was present. The cartridge was placed back on the manifold and rinsed with four sequential 5-mL water rinses.

The aqueous extract was transferred onto the Oasis® HLB cartridge. Five (5) mL of deionized water were added to the flat-bottom flask and the contents were sonicated for about 30 seconds, rotating the flask to dislodge residues. After the extract in the cartridge had drained to the frit, the water rinse was added to the cartridge. The flat-bottom flask was rinsed with a second 5-mL aliquot of water, the contents swirled to rinse the sides of the flask, and after the first water rinse reached the frit, the second water rinse was added to the cartridge.

After the second water rinse had drained to the frit, the cartridge was placed into a 100-mL flat-bottom flask, the top of the cartridge being supported by the top edge of the 24/40 joint. Any accumulated eluant from loading and rinsing the cartridge was discarded.

Five (5.0) mL of methanol were added to the 250-mL flat-bottom flask and the contents sonicated for about 30 seconds to dissolve the residues. One (1) mL of water was added to the 250-mL flat-bottom flask, resulting in a 5:1 methanol: water mixture.

The methanol:water extract was transferred to the Oasis® HLB cartridge and the residues eluted into the 100-mL flat-bottom flask. A second 5.0-mL aliquot of methanol was added to the 250-mL flat-bottom flask, the contents were swirled to rinse the sides of the flask, and 1.0 mL of water was added. This second rinse was added to the cartridge after the first methanol:water rinse had drained to the frit.

The extract was then rotary evaporated, using a heated waterbath at a temperature of 35-40°C, and the volume reduced to approximately 1 mL. This aqueous extract was then transferred into a graduated glass centrifuge tube, using a Pasteur pipette. The flat-bottom flask was rinsed with 3.0 mL of methanol, sonicated for approximately 30 seconds to dissolve the residues, and then the rinse was transferred to the graduated tube using a Pasteur pipette. Approximately 5 mL of water were added to the flat-bottom flask, and the water was swirled to rinse the sides of the flask and then transferred to the graduated tube. The flask was then rinsed with an additional 1-2 mL of water and this rinse also added to the tube. The volume of the extract in the graduated tube was then brought to 10.0 mL with water and mixed using a Pasteur pipette. A portion of this final extract was transferred to an autosampler vial for analysis by LC/MS-MS.

3.9 LC/MS-MS OPERATION PARAMETERS

3.9.1 Conditions

Column: Phenomenex Synergi 4 micron Polar-RP 80A, 75 x 2 mm

Mobile phases: ACN, 0.05% formic acid ("B")
Water, 0.05% formic acid ("A")

Flow rate: 200 µL/min.

Injection volume: 20 µL

Gradient program:

Time (minutes from start of run)	% A	%B
0.00	90	10
1.00	90	10
4.00	60	40
5.00	60	40
6.00	20	80
9.00	20	80
10.50	90	10
13.00	90	10

The gradient and column are given as guidelines. Substitutions of column and adjustment of gradients may be required to achieve optimal separation and sensitivity.

Scan type: MRM

Polarity: Positive

Retention times: Imazosulfuron, ~7.1 min.
ADPM, ~3.5 min.
HMS, ~4.3 min.
IPSN, ~4.6 min.

3.9.2 Mass spectrometer mass calibration, optimization and operation

The sample was introduced into the mass spectrometer by atmospheric pressure ionization (API) pneumatically assisted electrospray operated in the positive ion mode (Turbo Ion Spray, for Sciex instruments). Mass calibration and peak widths

were periodically checked with Sciex PPG solution and adjusted as required. The mass spectrometer and flow injection analysis were optimized for the relevant analyte(s).

3.9.3 Monitored transitions

The primary transition was used for quantitation of each analyte. The transitions used were as follows:

Imazosulfuron, 413.0/156.0 amu
ADPM, 155.9/99.9 amu
HMS, 399.0/142.2 amu
IPSN, 231.9/152.0 amu

Copies of example chromatograms are included in Appendix 1.

3.9.6 Calibration procedures

Instrument calibration working standard solutions were prepared as described in section **3.5.4**. Calibration consisted of injecting six instrument calibration working standards throughout each run/sequence and calibrating (area counts versus concentration) using a linear regression curve with the 1/x weighting option. The standard concentrations were 0.5X, 1X, 2.5X, 5X, 10X and 25X LOQ for each analyte (10, 20, 50, 100, 200 and 500 ng/g, ppb, respectively). The correlation coefficients (r) of the standard regression curves were required to be at least 0.995 and the coefficients of variation for the standard responses were required to be <10% for each analyte/analytical set. The regressions were calculated by the PE Sciex "Analyst" software, version 1.2. The software also calculated the sample residue results printed on the chromatograms.

The method required that each sequence should begin and end with reference standards. None of the three analyses included these standards and a deviation was issued, see Appendix 3, Deviation #1.

3.10 TRIALS AND CONTACT WITH SPONSOR

For the first trial, NCL used the following supplies:

- Whatman No. 41 filter papers (that were prerinsed to waste with 5 mL of sodium bicarbonate solution), instead of Whatman No.1 filter papers
- Water Oasis HLB 6 cc (500 mg) extraction cartridges, instead of 12 cc (500 mg) cartridges

In the first trial sample set, one LOQ fortification showed low recoveries for imazosulfuron and ADPM. The Sponsor was contacted and NCL was instructed not to include the results of this fortification in the statistical analyses for any of the analytes, and to run an additional subset which included three LOQ fortifications.

The three LOQ fortifications included in the additional subset showed no recovery for imazosulfuron. On consultation with the Sponsor regarding these results and prior to preparations for a second trial, NCL was instructed to clean the glassware thoroughly (as documented in section **3.8** of this report), to check the pH of the methanol and water lots to be used, and to check the profile of the HLB cartridges. Additionally, NCL decided to order and use Whatman No.1 filter papers and 12 cc (500g) Water Oasis HLB cartridges as listed in the method.

The independent laboratory validation was successful on the second attempt when the method was performed exactly as written in RM-42S-1-1.

3.11 POTENTIAL INTERFERENCES

No interferences were detected for any of the analytes. The analyses were highly specific and provided signals that effectively identified imazosulfuron, ADPM, HMS and IPSN in soil.

Copies of example control sample chromatograms can be found in Appendix 1, Figures 9, 24, 39 and 54 for imazosulfuron, ADPM, HMS and IPSN analyses, respectively.

3.12 CONFIRMATION OF ANALYTE IDENTIFICATION

No additional confirmatory techniques were required because monitoring a single transition from the precursor ion to a product ion on the LC/MS-MS is considered highly specific for analyte identification.

Copies of the example chromatograms are included in Appendix 1.

3.13 TIME REQUIRED FOR EXTRACTIONS AND ANALYSES

An extractionist/analyst would be able to complete the extraction and analysis of a set of 12 samples in approximately eight hours, assuming that there were sufficient rotovaps available. However, it is noted that the extraction involves shaking of the samples overnight, so the extraction takes place over a two-day period. Data reprocessing/printing took approximately one hour. Results could be available within a 24-hour period.

3.14 POTENTIAL PROBLEMS, HAZARDS OR PRECAUTIONS

Potential problems encountered during the ILV:

- Section II, Analytical Standards, page 3, HMS Standard, 1.0 mg/mL Stock Solution:
  NCL had problems getting the HMS into solution as described in the method (in acetone:water). Instead, the standard was prepared in 5.0 mL water:2.5 mL methanol:2.5 mL acetone and sonicated in a warm (~40°C) water bath for about 15 minutes.

- Section VII, Analytical Procedures, 3., Oasis HLB Cleanup:
  When the extractionist noted that an extract was taken to near dryness at the rotary evaporation step before the addition of ethyl acetate, recoveries of imazosulfuron and ADPM were found to be low (trial #1).

- Section VII, Analytical Procedures, 3., Oasis HLB Cleanup:
  The incomplete removal of all organic solvent before extracts were applied to the Oasis HLB cartridge and/or glassware cleanliness were suspected to result in no imazosulfuron recovery (trial #1, additional subset).

Notes regarding RM-42S-1-1 write-up:

- Section II, Analytical Standards, page 3, Fortification Mix 1.0 µg/mL (in acetone):
  It is noted that "bi-weekly" means every two weeks (not twice a week); this standard solution should be prepared every two weeks.

- Section VII.3, Analytical Procedures, Oasis HLB Cleanup, page 9:
  Sonication times were not included in the method. Sonication times of about 30 seconds were used in the ILV.

3.15 CALCULATIONS

3.15.1 Threshold area counts

The area reject threshold was determined by the analyst to be at a level lower than 10% of the peak of the lowest calibration standard (0.5X LOQ) and which excluded baseline noise. No imazosulfuron, ADPM, HMS or IPSN residues were detected in the reagent blank or control sample extracts and are recorded as "ND" (not detectable) in the validation recovery data tables.

3.15.2 Calculation of the amount of solvent needed to bring a weighed amount of standard to the required concentration

$$\text{Volume of solvent} = \frac{\text{Weight of standard (adjusted for percent purity)}}{\text{Desired concentration}}$$

Example calculation
Preparation of the 1,000 ng/µL imazosulfuron standard stock solution:

Weight imazosulfuron = 0.0103 g
Percent purity = 99.9%

Weight imazosulfuron corrected for percent purity = (0.0103 g) (0.999)
= 0.01029 g
= 10,290,000 ng

$$\text{Volume of solvent needed} = \frac{10{,}290{,}000 \text{ ng}}{1{,}000 \text{ ng/µL}}$$

= 10,290 µL

= 10.29 mL

3.15.3 Calculations used in the preparation of fortification samples

An LOQ fortification for all analytes on a 5.0 g soil sample was prepared by fortifying the sample with 100 µL of the 1.0 ng/µL (ppm) mixed analyte fortification standard solution:

(100 µL) (1.0 ng/µL) / (5.0 g) = 20 ng/g (ppb)

A 10X LOQ fortification for all analytes on a 5.0 g soil sample was prepared by fortifying the sample with 1,000 µL of the 1.0 ng/µL (ppm) mixed analyte fortification standard solution:

(1,000 µL) (1.0 ng/µL) / (5.0 g) = 200 ng/g (ppb)

3.15.4 Calculations used in the preparation of a 1X LOQ, 20 ng/g (ppb), instrument calibration working standard

Example calculation:
The total amount of each analyte in the initial extract from a 1X LOQ (20.0 ng/g) fortification on a 5.0 g sample at 100% recovery was:

(5.0 g) (20.0 ng/g) = 100.0 ng

All the sample was taken through the extraction.

The final extract volume was 10.0 mL.

Thus the "in-solution" concentration of each analyte in the final extract from a 1X LOQ fortification was:

(100.0 ng) (1/10.0 mL) = 10 ng/mL

The final extract volume was incorporated into the preparation of the instrument calibration working standards. A 1X LOQ instrument calibration working standard was prepared by adding 40 µL of the 0.25 ng/µL standard stock to 960 µL of 3:7 methanol:water (v/v) in an autosampler vial (see section **3.5.2**). Therefore the "in solution" concentration of a 1X LOQ standard was:

[(40 µL) (0.25 ng/µL)] / (1.0 mL) = 10 ng/mL

This agrees with the calculated final extract "in-solution" concentration from a 1X LOQ fortification at 100% recovery.

Instrument calibration working standards at other concentrations were calculated in a similar manner.

3.15.5 Calculation of analyte concentrations

Calculations of the analyte concentrations were accomplished by PE Sciex "Analyst" software by interpolation along the respective curves of the linear regression equations (with 1/x weighting) generated by the calibration standards analyzed with the sample sets. The peak area was the dependent variable and the concentration of each calibration working standard solution, expressed as ng/g (ppb), was the independent variable. The quantitation values reported on the chromatograms and spreadsheets generated from the LC/MS-MS (as ng/g) were

the in-sample concentrations, and the sample residues were calculated/printed to four significant figures. The area counts and concentrations were recorded on the LC/MS-MS chromatograms and spreadsheets and were transferred to the residue data sheets. Copies of the residue data sheets from both trials are presented in Appendix 2. The results were transcribed to the tables of results where they are reported as µg/g (ppm).

Analyte concentrations on the chromatograms, in ng/g (ppb), were calculated by the software using the following equation:

$$Y = bX + a$$

Where: Y = area counts for analyte
X = concentration of analyte
a = intercept calibration coefficient
b = slope calibration coefficient

Example calculation
Concentration of imazosulfuron in the Trial 2, 1X LOQ fortification #5, 20.0 ng/g (ppb), extracted 07/26/07 and analyzed 07/28/07 (see Figure 12 in Appendix 1 and Table 7.2):

where Y = 128050
a = 8.55e+003
b = 7.00e+003

$$X = \frac{Y - a}{b}$$

$$X = \frac{128050 - (8.55e+003)}{7.00e+003}$$

$$= 17.07 \text{ ng/g (ppb)}$$

NOTE: The coefficients and area counts used by the by the PE Sciex "Analyst" software to calculate residues include more significant figures than the rounded numbers printed out in the data packages. Therefore, the software calculation cannot always be reproduced, using Excel/hand calculator and the printed area counts and coefficients, beyond the third/fourth significant figure. In the above example, however, the software calculation and hand calculation agreed.

3.15.6 Calculation of method fortification percent recovery

Method fortification recovery (%) =

$$\frac{\text{Residue found (ng/g)}}{\text{Fortification concentration (µg/kg)}} \text{ X } 100\%$$

Example calculation
Continuing with the example in section **3.15.5,** Trial 2, 1X LOQ imazosulfuron fortification #5, 20.0 ng/g (ppb), extracted 07/26/07 and analyzed 07/28/07 (see Figure 12 in Appendix 1 and Table 7.2):

$$\text{Percent Recovery} = \frac{17.07 \text{ ng/g}}{20 \text{ ng/g}} \text{ X } 100\%$$

$$= 85.35\%$$

$$= 85.4\%$$

3.15.7 Calculation of standard deviation (s) and relative standard deviation (RSD) for a mean

Standard deviations for the mean percent recoveries were calculated and designated as "s" and expressed as an absolute percent value.

$$\text{Relative standard deviation} = \frac{\text{standard deviation}}{\text{mean}} \text{ X } 100\%$$

Example calculation
Using the five replicates of the Trial 2, LOQ, 20 ng/g, imazosulfuron fortifications, see Table 7.2, where the unrounded mean recovery was 84.75% and the unrounded standard deviation was 4.377%:

$$\text{RSD} = \frac{4.377\%}{84.75\%} \text{ X } 100\%$$

$$= 5.165\%$$

$$= 5.2\%$$

3.15.8 Calculation of 95% confidence interval for a mean

$$95\%\ \text{confidence interval} = \text{mean} \pm \frac{t \times \text{standard deviation}}{\sqrt{n}}$$

where n = number of measurements/samples

t = student t variate for n-1 degrees of freedom at the 95% confidence interval (2.776 for 4 degrees of freedom and 2.262 for 9 degrees of freedom). See table C.3, page 267, Quality Assurance of Chemical Measurements, John Keenan Taylor, Lewis Publishers, Inc., 1987

Example calculation

Using the five replicates of the Trial 2, LOQ, 20 ng/g, imazosulfuron fortifications, see Table 7.2, where the mean recovery was 84.8% and the unrounded standard deviation was 4.377%:

$$95\%\ \text{confidence interval} = 84.8\% \pm \frac{(2.776)\,(4.377\%)}{\sqrt{5}}$$

$$= 84.8\% \pm 5.434\%$$

$$= 84.8\% \pm 5.4\%$$