

VALENT U.S.A. CORPORATION
VALENT TECHNICAL CENTER
Dublin, California

**DETERMINATION OF IMAZOSULFURON
IN THATCH**

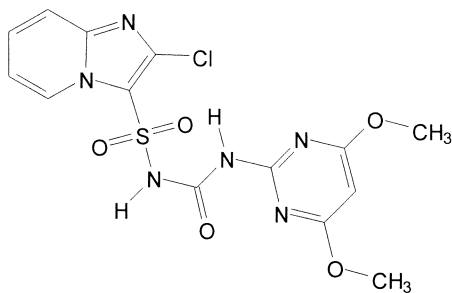
Method: **RM-42C-1**

Date: July 18, 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 in thatch samples. Briefly, the method involves extraction with an aqueous acetonitrile mixture (4:1 acetonitrile/0.2 M sodium bicarbonate) and filtration to remove the solids. The extract is evaporated to remove the acetonitrile and obtain an aqueous residue, partitioned into methylene chloride, then cleaned up using an OASIS HLB cartridge. The imazosulfuron residues are eluted from the cartridge with aqueous methanol (50% methanol), diluted and analyzed by LC/MS-MS.

II. ANALYTICAL STANDARDS



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

Imazosulfuron Standard, 1.0 mg/mL Stock solution.

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

Imazosulfuron Standard, 10.0 µg/mL solution (in acetone).

Pipet 1.0 mL of the 1.0 mg/mL Imazosulfuron Standard into a 100 mL volumetric flask. Dilute to volume with acetone. Store in a freezer.

Fortification Solution, 1.0 µg/mL (in acetonitrile)

Pipet 10.0 mL of the 10.0 µg/mL Standard into a 100 mL volumetric flask. Dilute to volume with acetonitrile. Store in a freezer.

Prepare the following analytical standards with each set of samples for analysis:

Imazosulfuron 0.100 $\mu\text{g}/\text{mL}$ Calibration Standard

Pipet 0.2 mL of the 1.0 $\mu\text{g}/\text{mL}$ Fortification Solution into a 10 mL vial and add 1.8 mL of 40% methanol/water.

Imazosulfuron 0.050 $\mu\text{g}/\text{mL}$ Calibration Standard

Pipet 1.0 mL of the 0.100 $\mu\text{g}/\text{mL}$ Imazosulfuron Standard into a 10 mL vial and add 1.0 mL of 40% methanol/water.

Imazosulfuron 0.020 $\mu\text{g}/\text{mL}$ Calibration Standard

Pipet 1.0 mL of the 0.050 $\mu\text{g}/\text{mL}$ Imazosulfuron Standard into a 10 mL vial and add 1.5 mL of 40% methanol/water.

Imazosulfuron 0.010 $\mu\text{g}/\text{mL}$ Calibration Standard

Pipet 1.0 mL of the 0.020 $\mu\text{g}/\text{mL}$ Imazosulfuron Standard into a 10 mL vial and add 1.0 mL of 40% methanol/water.

Imazosulfuron 0.005 $\mu\text{g}/\text{mL}$ Calibration Standard

Pipet 1.0 mL of the 0.010 $\mu\text{g}/\text{mL}$ Imazosulfuron Standard into a 10 mL vial and add 1.0 mL of 40% methanol/water.

Note: Similar dilutions may be made to generate appropriate standards.

III. REAGENTS

Acetonitrile - Pesticide quality

Formic Acid, 96% - Reagent grade

Methanol - Pesticide quality

Methylene chloride – Pesticide quality

Sodium Bicarbonate – Reagent grade

Sodium Chloride – Reagent Grade

Sodium Sulfate, Anhydrous – Reagent Grade

Water – Deionized

Water – HPLC grade

IV. REAGENT SOLUTIONS

Acetonitrile, with 0.05% Formic acid (HPLC solvent)

40% Methanol in water

Mix 40 mL of methanol with 60 mL of deionized water. [This preparation may be scaled as necessary.] Store at room temperature.

Saturated Sodium Chloride solution

Add approximately 30 g of sodium chloride to 100 mL of deionized water. Stir the solution until no additional sodium chloride goes into solution. Store at room temperature over excess sodium chloride.

Sodium Bicarbonate Solution (0.2 M).

Dissolve 16.8 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 (HPLC solvent)

Add 0.5 mL of formic acid per liter of HPLC grade water. Store at room temperature.

V. EQUIPMENT and SUPPLIES

Autosampler vials, screw-top with Teflon-coated septums

Balances, Analytical and Top Loading

Centrifuge Tubes, graduated, screw-capped, 15 mL capacity

Filter Paper, Folded, Grade 313, 15 cm diameter

Funnels, Glass Filtering, 75 mm top diameter

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

Heated Water Bath (temperature <40°C)

Jars, Teflon lined screw-caps, 8 oz. (250 mL) capacity

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

Pipettes, Graduated or Volumetric suitable of accurately delivering 0.5 – 10 mL

Rotary Vacuum Evaporators

Round-bottom Flasks – 50, 100 and 250 mL

Vacuum Manifold for SPE Cartridges, Baker (or equivalent)

Vials, Teflon lined screw-caps, 20 and 40 mL capacity

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).

HPLC Conditions:

Column: Synergi 2.5u POLAR RP 100A, 50 mm x 2.0 mm
(Phenomenex part # 00B-4336-B0)

Mobile Phases: Acetonitrile, 0.05% formic acid
Water, 0.05% formic acid

Flow Rate: 250 μ L/minute

Injection Volume: 20 μ L

Gradient Program:

Time, Min	% ACN, 0.05% HOOCH	%Water, 0.05% HOOCH
0	10	90
3.0	10	90
4.0	40	60
7.5	40	60
9.0	95	5
16.0	95	5
18.0	10	90
20.0	10	90

LC/MS Interface Conditions:

Interface: Electrospray Ionization

Polarity: Positive

Imazosulfuron

Source CID Collision Energy: 16

Q2 Collision Gas Pressure: 1.5 mTorr

Scan type: SRM

Parent Ion Mass: 413.0

Quantitation Ion Mass: 156.1

Collision Energy: 34

Retention Times: 10.1 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 and Extraction

Thoroughly mix the crop sample. Weigh 10.0 g (± 0.1 g) of the sample into a 250 mL glass screw-capped jar. At this point, if required by the testing facility, a control sample to be used for method recoveries may be fortified with imazosulfuron (*see Note 1*).

Add 10 mL of 0.2 M sodium bicarbonate solution to the sample, mix, and allow to sit for 10 minutes. Add 40 mL of acetonitrile to the sample jar. Place the jar on a reciprocating mechanical shaker and shake at high speed for 30 minutes. Filter the sample through Whatman GF/A filter paper on a vacuum filter setup (Büchner funnel on a side-arm vacuum flask, prewet the filter paper with a small amount of acetonitrile before applying the vacuum and adding the sample). Celite® may be added to the sample (approximately 5 g, one teaspoon) to aid in the filtration, if necessary. Transfer the filter cake and paper back to the sample jar. Repeat the extraction twice more, using 10 mL of 0.2 M sodium bicarbonate and 40 mL of acetonitrile for each extraction, shaking the sample for 30 minutes, filtering and combining the filtrates in the vacuum flask. Rinse the sample jar and filter cake twice with ca. 10 mL of acetonitrile. Transfer the sample extract to a 500 mL round bottom flask using two or three 5 to 10 mL acetonitrile rinses of the vacuum flask, and evaporate the acetonitrile from the sample using a rotary evaporator with a warm ($<40^{\circ}\text{C}$) water bath (approximately 10 to 15 mL of water remains).

2. Methylene Chloride Partition

Transfer the sample to a 250 mL separatory funnel using two 10 mL saturated sodium chloride rinses of the round bottom flask, and adding the rinses to the separatory funnel. Rinse the round bottom flask with 100 mL of methylene chloride, and add to the sample in the separatory funnel. Shake the sample for one minute, and allow the phases to separate. Drain the lower methylene chloride phase through approximately 25 g of sodium sulfate suspended on glass wool in a filter funnel, collecting the methylene chloride in a clean 500 mL round bottom flask. Repeat the methylene chloride partition twice more, using 50 mL portions of methylene chloride, and drying and combining the methylene chloride portions in the round bottom flask. Evaporate the sample using a rotary evaporator with a warm ($<40^{\circ}\text{C}$) water bath, until 5 to 10 mL of methylene chloride remains. Transfer the sample to a 50 or 100 mL round bottom flask using small portions of methylene chloride to rinse the larger round bottom flask. Evaporate the sample to dryness, add 5 mL of methanol and sonicate the sample to dissolve the residues. Add 5 mL of 0.2 M sodium bicarbonate, and carefully evaporate the sample to remove the methanol from the sample.

3. OASIS HLB Cleanup

Place an OASIS HLB cartridge onto a vacuum manifold or suitable cartridge holder, and condition the cartridge by passing 2 x 5 mL methanol through the cartridge, then equilibrate the cartridge by passing 4 x 5 mL of water through the cartridge. Allow each portion to pass completely through the cartridge before adding the next portion. The OASIS HLB cartridges can be eluted without the use of a vacuum (by allowing gravity to elute the cartridge), and once the HLB sorbent has been conditioned and equilibrated, there is no need to keep the cartridge wet prior to sample loading. If necessary, a light vacuum (5 – 10 mm Hg) may be applied to facilitate the cartridge elution.

Transfer the sample to the HLB cartridge, rinse the round bottom flask twice with 5 mL of deionized water, and pass the water portions through the cartridge. Add 1 mL of methanol to the round bottom flask, sonicate and 9 mL of deionized water. Transfer this rinse to the HLB cartridge, and discard the rinses. Place a 15 mL graduated centrifuge tube under the cartridge, add 4 mL of methanol to the round bottom flask, sonicate, add 6 mL of deionized water, and transfer the solution to the cartridge, collecting the eluant (containing the imazosulfuron residues) in the 15 mL graduated centrifuge tube.

Add methanol to the sample to set the total eluant volume to 10.0 mL (typically less than 0.5 mL). Transfer a 250 μ L aliquot of the sample to a screw-top autosampler vial, and dilute with 250 μ L of deionized water for imazosulfuron analysis by LC/MS-MS (Step 4). This solution should be analyzed within 5 days, and should be stored in a freezer if not analyzed immediately.

Prepare the analytical standards as described previously, and store the standards under the same conditions as the samples.

4. LC/MS-MS Analysis for Imazosulfuron

Instrument calibration is performed using a linear fit with a non-zero intercept. The linearity of the instrument response is verified on a daily basis.

Condition the instrument with at least six injections of a sample extract. Analyze a range of at least four standard concentrations *within the analytical sequence* to establish the linear response of the LC/MS-MS (five concentrations are recommended), including a 0.005 μ g/mL (or less) standard. A typical set of standards would include concentrations of 0.100, 0.050, 0.020, 0.010 and 0.005 μ g/mL (with an injection volume of 20 μ L). 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 for the standard injection to be considered acceptable.

Continuing reference standards (mid-level linearity standard) are analyzed as part of the

analytical sequence. Typically, the sequence is constructed with the following order: reference standard (i. e. the 0.020 µg/mL), 2 to 4 sample extracts, a linearity standard or reference standard, 2 to 4 sample extracts, ..., and a reference standard. *The sequence must begin and end with a reference standard injection, and the analytical set should have at least one additional reference standard injection in the midst of the sequence.* 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 methanol/water, 4:1) 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 so that the standard concentration is plotted as the *Y* value, and the response as the *X* value.

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

Standard	Number of Entries in Data Set
0.100 µg/mL	1 (0.10/0.10)
0.050 µg/mL	2 (0.050/0.10)
0.020 µg/mL	5 (0.020/0.10)
0.010 µg/mL	10 (0.010/0.10)
0.005 µg/mL	20 (0.005/0.10)

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

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

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

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

where:

- b* = slope [from regression analysis]
- X* = Sample response (Peak Area)
- a* = intercept [from regression analysis]
- C* = Final volume (20.0 mL)
- D* = Dilution factor, used if the sample extract is diluted prior to analysis
- E* = Sample weight (10.0 g)

VIII. LIMIT OF DETECTION

The limit of detection (LOD) of this method is 0.01 ppm ($\mu\text{g/g}$), based on a 10.0 g sample with a 20 mL final volume and a 0.005 $\mu\text{g/mL}$ linearity standard in the linearity verification.

$$\text{Limit of Detection} = \frac{20 \text{ mL Final Volume} \times 0.005 \mu\text{g/mL}}{10.0 \text{ g}} = 0.01 \mu\text{g/g} = 0.01 \text{ ppm}$$

IX. DISCUSSION

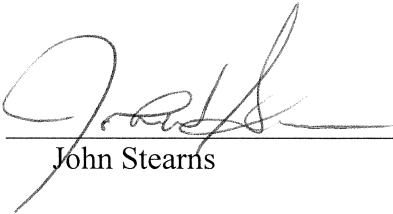
The method was verified by fortifying thatch samples with imazosulfuron at 0.02 and at 0.1 ppm, and then analyzing the sample extracts by LC/MS-MS. The mean recoveries were 79.6% \pm 6.5% CV for imazosulfuron. Example chromatograms are shown in *Figures 1* through *6*. The recovery data are presented in Attachment 1.

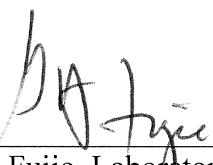
X. NOTES

1. Valent Standard Operating Procedure VR-002 specifies that fortified control samples are to be analyzed with each set of samples. If the testing facility does not require concurrent analysis of fortified control samples, or if an untreated control (UTC) sample is not available, this method requirement may be waived.

Typically, samples are fortified at 0.02 ppm and/or 0.1 ppm. For example, add 0.20 mL of the 1.0 $\mu\text{g/mL}$ Fortification solution (in acetonitrile) or 1.0 mL of the 1.0 $\mu\text{g/mL}$ Fortification Standard solution (in acetonitrile) to a 10.0 g sample. Method recoveries must be 70 to 120% to be acceptable, unless approved by the supervising chemist responsible for the analysis. Fortifications should include a range of fortification levels encompassing the range of residues found in the treated samples, and must include a level equal to or greater than the largest residue found.

XI. SIGNATURES

Written by  Date 18-Jul-07
John Stearns

Approved by  Date 7/19/07
Glenn Fujie, Laboratory Manager


Reviewed by  Date July 18, 07
Michelle Chen, QAU

Figure 1
0.005 µg/mL standard

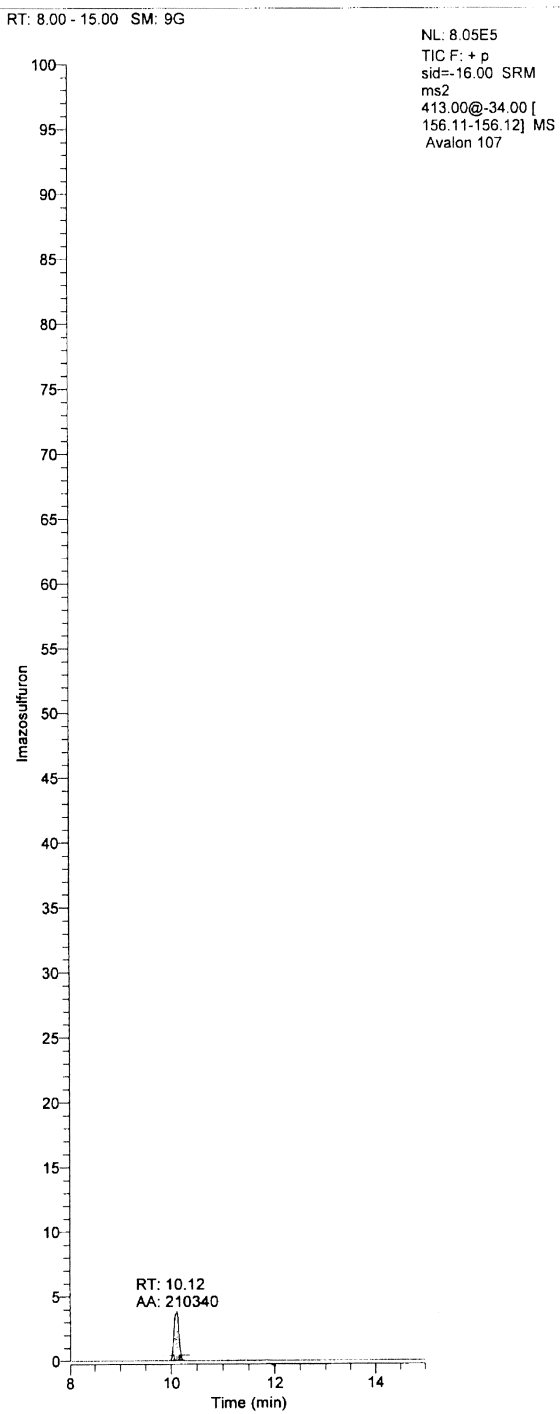


Figure 2
0.02 µg/mL standard

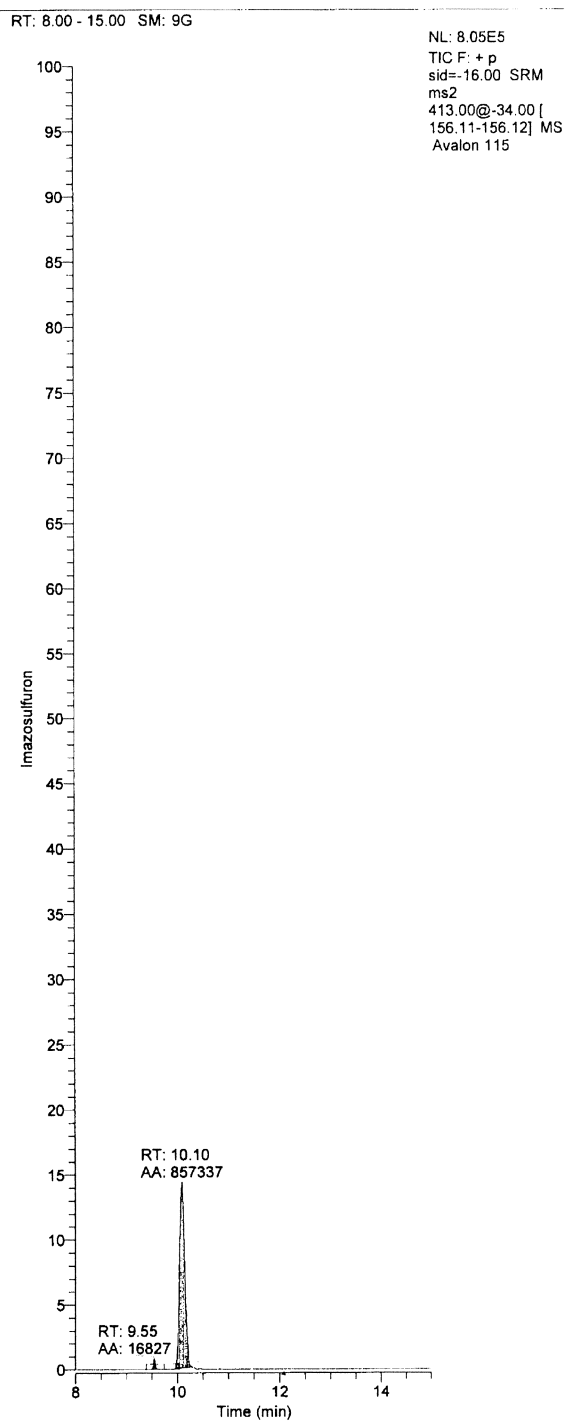


Figure 3
0.10 µg/mL std

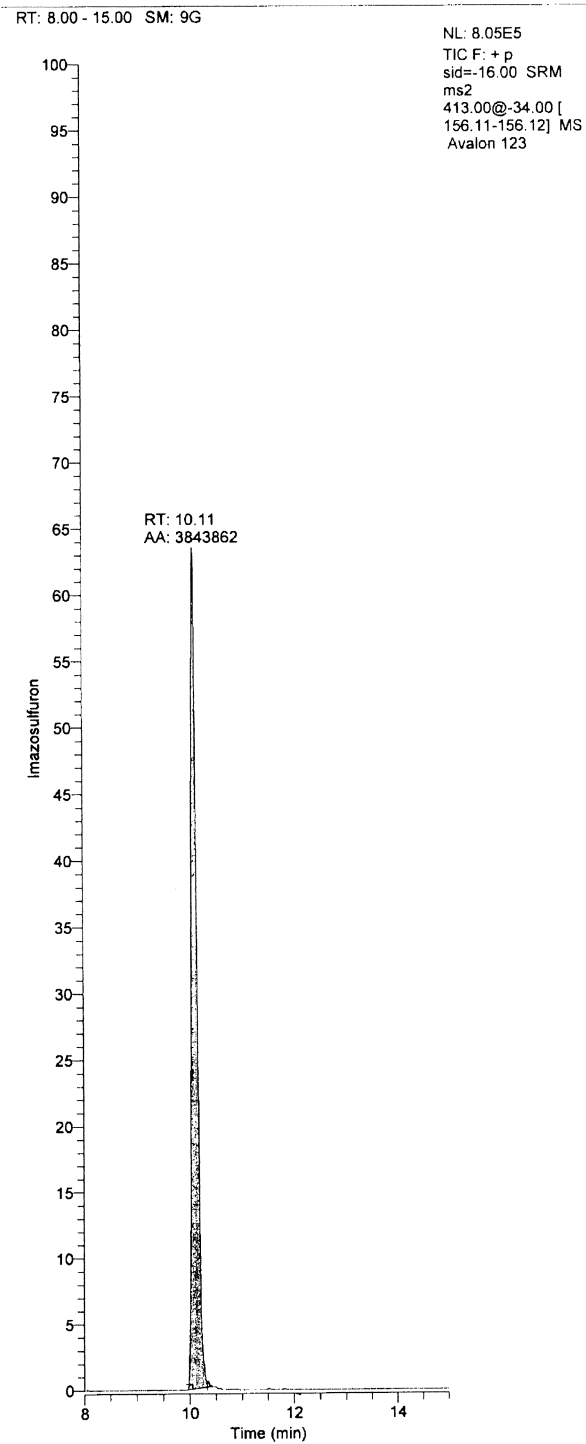
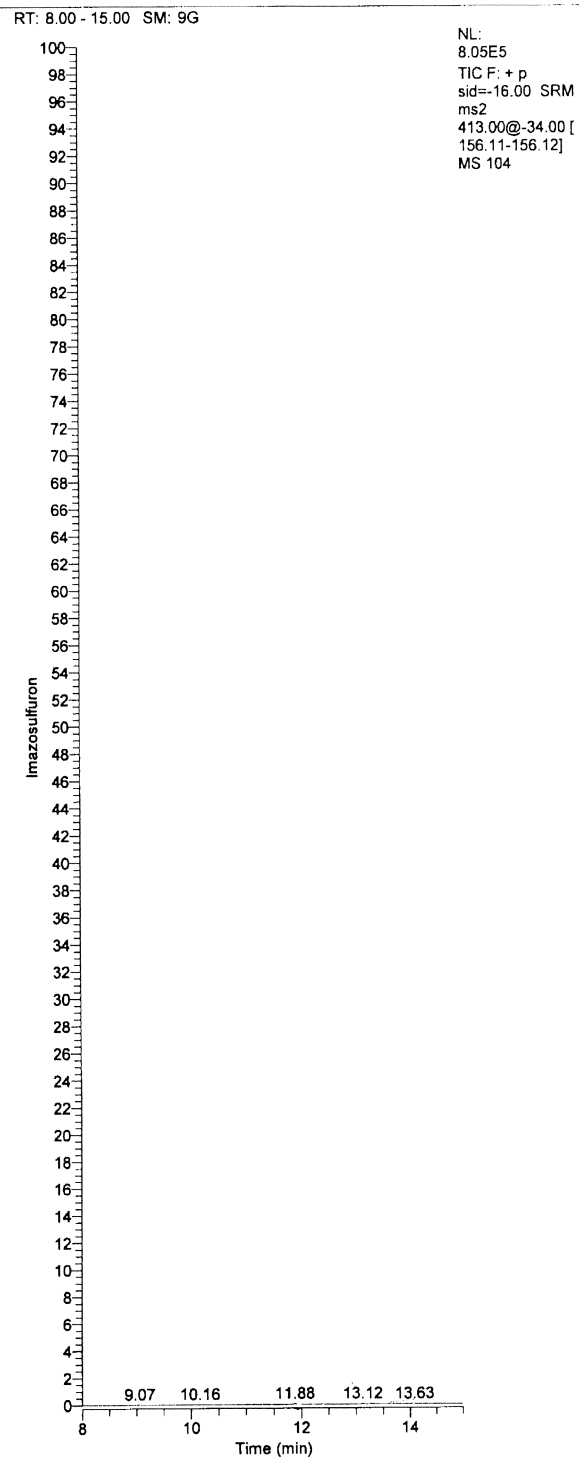
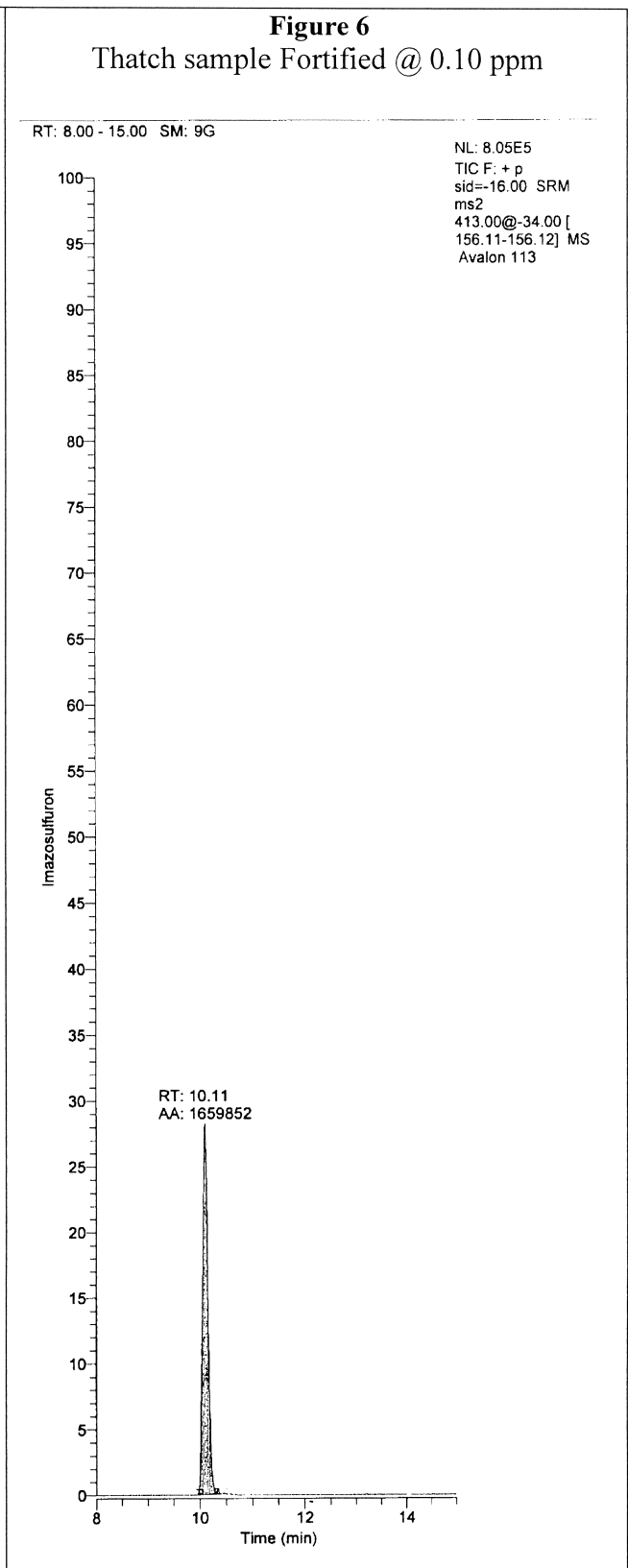
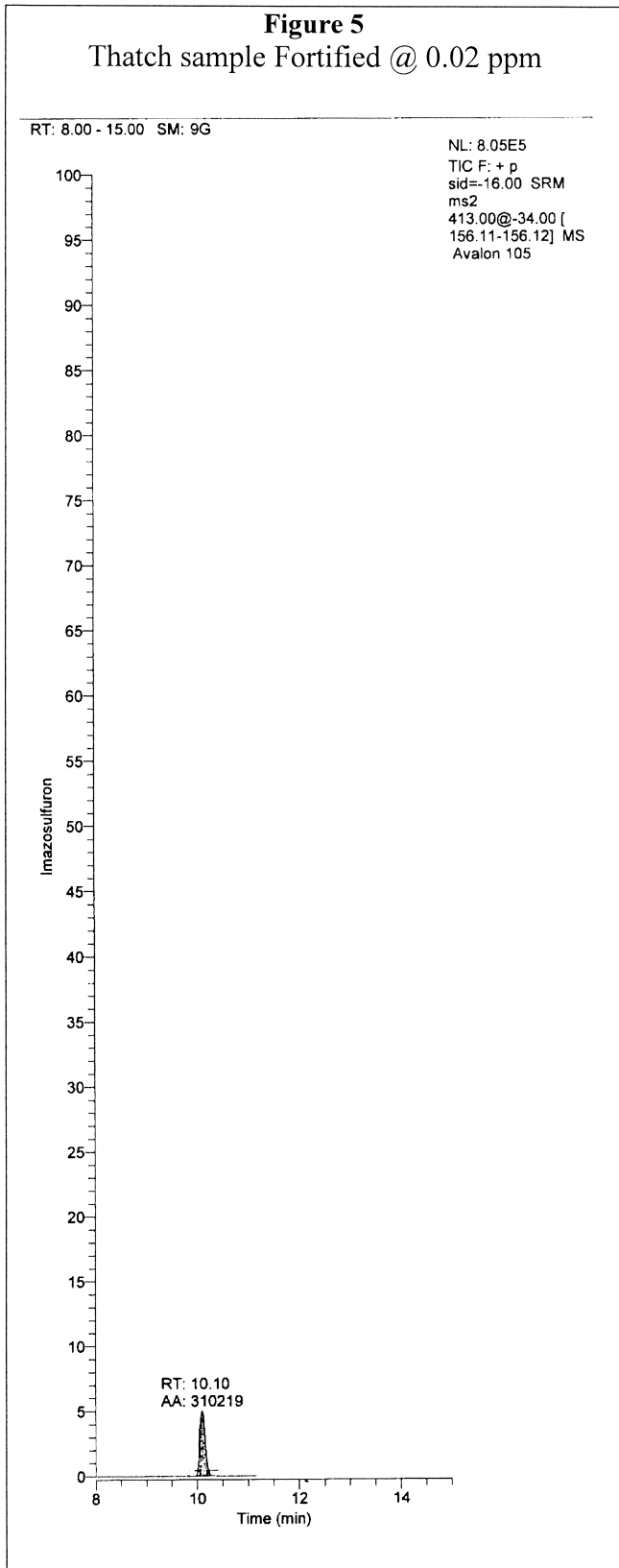


Figure 4
Untreated thatch sample





ATTACHMENT 1

VALENT U.S.A. CORPORATION
VALENT TECHNICAL CENTER
Dublin, CA

VALIDATION REPORT FOR RESIDUE METHOD **RM-42C-1**
REPRODUCIBILITY OF ANALYSIS (REF. SOP VR-002)

Sample Matrix	Sample ID	Fortification Level (ppm)	Extraction Date	Result (ppm)	Percent Recovery
Thatch	utc	0	2007-Jun-13	0.000	---
Thatch	Ft 1	0.02	2007-Jun-13	0.015	73.0
Thatch	Ft 2	0.02	2007-Jun-13	0.014	71.2
Thatch	Ft 3	0.02	2007-Jun-13	0.015	76.2
				average:	73.5
				%CV:	3.4%
Thatch	Ft 4	0.10	2007-Jun-13	0.080	79.8
Thatch	Ft 5	0.10	2007-Jun-13	0.087	86.8
Thatch	Ft 6	0.10	2007-Jun-13	0.083	83.1
Thatch	Ft 7	0.10	2007-Jun-13	0.084	83.5
Thatch	Ft 8	0.10	2007-Jun-13	0.083	83.0
Thatch	Ft 9	0.10	2007-Jun-13	0.080	80.2
				average:	82.7%
				%CV:	3.1%
Overall Average =					79.6%
Overall %CV =					6.5%
n =					9


Approved by

18-JUL-07
Date


Reviewed by

July 18, 07
Date