Analytical Method for the Determination of O-Desmethyl MKH 6562 (Metabolite of MKH 6562) in Soil by High-Performance Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

1.0 SUMMARY

An analytical method was developed to determine O-desmethyl MKH 6562 in soil using high-performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS). Soils (25-g) were spiked with the deuterated internal standard of O-desmethyl MKH 6562. The analyte was simultaneously extracted and derivatized with methanol/water (9:1, v/v) to MKH 6562 N-methyl carbamate (NMC) using a Accelerated Solvent Extraction (ASE) at 80 °C for 5 min. The extracts were evaporated to dryness and reconstituted to 1 mL with HPLC solvent . The concentrated extracts were filtered with 0.45-µm nylon Acrodiscs® and analyzed by LC-MS/MS operated in negative electrospray-ionization mode (ESI).

The limit of quantitation (LOQ) was established at 0.5 ppb in the tested soils. Based on 0.5 ppb fortification level, the method detection limit (MDL) for the soil in Oklahoma and Washington was calculated to be 0.139 and 0.072 ppb, respectively.

2.0 INTRODUCTION

The previous analytical method[3] was developed to measure O-desmethyl MKH 6562 at the limit of quantitation (LOQ) of 1.0 ppb. Due to the low application rate of MKH 6562, the method was redeveloped and the method presented in this study was designed to measure the O-desmethyl MKH 6562 at the LOQ of 0.5 ppb.

3.0 EXPERIMENTAL

3.1 Equipment

Equivalent equipment may be substituted:

- Volumetric flasks (5-,10-, 100- and 500-mL)
- Volumetric pipettes (Baxter or equivalent)
- Graduated cylinders (10-, 50- and 100-mL)
- Syringes, gas-tight type (25-, 50-, 100-, 250- and 500-µL)
- Pipetman (Gilson or equivalent)
- Pasteur pipettes (Kimble or equivalent)
- Glass funnels
- Nylon Acrodisc® (0.45-µm, 25 mm, Part No. 4438,Gelman)

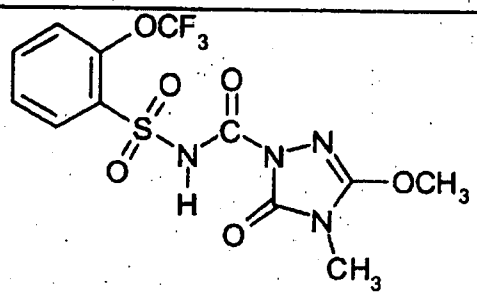
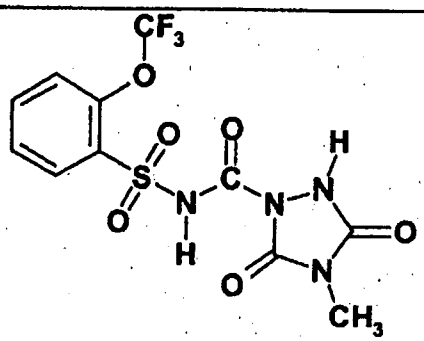
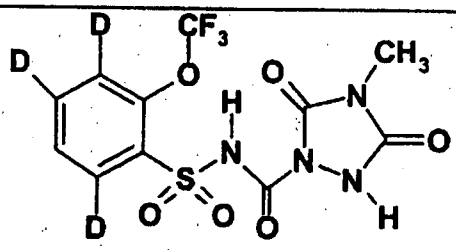
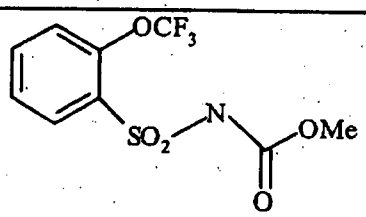
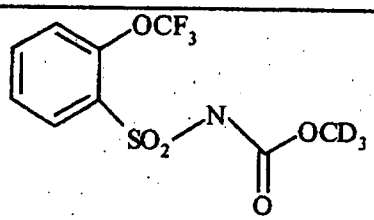
- Disposable 3-mL plastic syringe (Part No. 309586, Becton Dickinson)
- Autosampler vials (2-mL, Wheaton #223682 or equivalent)
- Vials, 60-mL VOA (volatile organic analysis, I-Chem S236-0060 or equivalent)
- Analytical Balance (Mettler A163 or equivalent)
- Balance, Top loader, capable of weighing to the nearest 0.01 g
- pH meter (Orion Model SA 520 or equivalent)
- Turbo Vap LV (Zymark)
- Turbo Vap LV ASE compatible rack (Part No. 60911)
- Accelerated Solvent Extractor ASE 200 (Dionex Corp, Sunnyvale, CA)
- 15 x 33 mL Extraction Cells (Part No. 048763, Dionex Corp, Sunnyvale, CA)
- Cellulose filters for extraction caps (Part No. 049458, Dionex Corp, Sunnyvale, CA)
- Luna C18(2), 5 µm, 100Å, 100 x 4.6 mm (Phenomenex, Torrance, CA)
  Product Code: 00ED-4525-E0
- TSQ 7000 LC/Tandem Mass Spectrometer with ESI interface and gradient HPLC, or equivalent (Finnigan Corp)

3.2 Reagents and Solvents

Use as a guide, equivalent reagents or solvents may be substituted:

- Methanol (MeOH; HPLC Grade, Burdick & Jackson)
- Acetonitrile (ACN, HPLC Grade, Burdick & Jackson)
- Water (HPLC Grade, Burdick & Jackson)
- Acetonitrile/Water (1:1, v/v). Add 250 mL of acetonitrile into 500-mL volumetric flask and dilute to the mark with HPLC water.
- Ammonium hydroxide - 30% (J. T. baker)
- Ammonium acetate (EM Science or equivalent)
- 5 mM Ammonium acetate. Weigh 0.385 g of ammonium acetate and dilute to 1 L with HPLC water.
- 10 mM Ammonium acetate at pH 9. Weigh 0.77 g of ammonium acetate and dilute to 1L with HPLC water. Adjust the pH to 9 by adding 10-15 drops of 30% ammonium hydroxide. Check the pH with pH meter.
- Hydromatrix (Varian, Part No. 198003)
- Extraction solvent, methanol/water (9:1, v/v). Add 900 mL methanol to a flask containing 100 mL water.

3.3 Structures

Common Name Standard Ref. Empirical Formula Molecular Weight Purity Expiration Date	MKH 5730 (acid form of MKH 6562) K-640 or equivalent $C_{12}H_{11}F_3N_4O_6S$ 396.04 g/mol 98.8% 05-02-06	
Common Name Standard No. Empirical Formula Molecular Weight Purity Expiration Date	O-Desmethyl MKH 6562 K-661 $C_{11}H_9N_4O_6F_3S$ 382.3 99.7% 3-30-2004	
Common Name Standard No. Empirical Formula Molecular Weight Purity Expiration Date	O-Desmethyl MKH 6562-phenyl-3,4,6-d_3 K-792 $C_{11}H_6D_3N_4O_6F_3S$ 385.0 92.8% 3-21-2005	
Common Name Standard No. Empirical Formula Molecular Weight Purity Expiration Date	N-methyl carbamate K-789 $C_9H_8NO_5F_3S$ 299.0 97.2% 3-24-2006	
Common Name Standard No. Empirical Formula Molecular Weight Purity Expiration Date	N-methyl carbamate-d_3 K-790 $C_9H_5D_3NO_5F_3S$ 302.2 97.4% 3-20-2006	

3.4 Safety and Health

The toxicity of each chemical used in this method has not been precisely determined, and thus each compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest reasonable or possible level by whatever means available.

3.5 Procedures

3.5.1 Preparation of Standards and Reagents

3.5.1.1 O-desmethyl MKH 6562 Native Solution

Prepare a 100-, 1- and 0.1-µg/mL stock solution of O-desmethyl MKH 6562 in 10 mM ammomnium acetate buffered at pH 9 as follows:
Caution: O-Desmethyl MKH 6562 is NOT stable at pH < 9. Make sure that the 10 mM ammonium acetate is buffered to pH 9 with ammonium hydroxide.[4] *The standard solution is given a 1 month expiration, and must be stored in a refrigerator.* If the purity of a standard is <99%, weigh the appropriate amount to correct for the purity.

100 µg/mL solution of O-desmethyl MKH 6562
E.g. Add 10 mg of O-desmethyl MKH 6562 to a 100-mL volumetric flask and dilute to the mark with 10 mM ammonium acetate buffered at pH 9.
1 µg/mL solution of O-desmethyl MKH 6562 (Used in OK soil)
E.g. Pipet 1 mL of the 100-µg/mL O-desmethyl MKH 6562 into a 100-mL volumetric flask and dilute to the mark with 10 mM ammonium acetate buffered at pH 9.
0.1 µg/mL solution of O-desmethyl MKH 6562 (Used in OK soil)
E.g. Pipet 0.1 mL of the 100-ppm O-desmethyl MKH 6562 into a 100-mL volumetric flask and dilute to the mark with 10 mM ammonium acetate buffered at pH 9.

Label these solutions appropriately to reflect the actual concentration of the analyte.

3.5.1.2 O-Desmethyl MKH 6562-phenyl-3,4,6-d_3 internal standard

Prepare a 100- and 1-µg/mL solution of O-desmethyl MKH 6562-phenyl-3,4,6-d_3 internal standard 6562 in 10 mM ammomnium acetate buffered at pH 9 as follows:
Caution: O-Desmethyl MKH 6562-phenyl-3,4,6-d_3 is NOT stable at pH < 9. Make sure that the 10 mM ammonium acetate is buffered to pH 9 with ammonium hydroxide.[4] *The standard solution is given a 1 month expiration, and must be stored in a refrigerator.* If the purity of the standard is <99%, weigh the appropriate amount to correct for the purity.

100 µg/mL solution of O-desmethyl MKH 6562-phenyl-3,4,6-d_3
E.g. Add 10 mg of O-desmethyl MKH 6562-phenyl-3,4,6-d_3 to 100-mL volumetric flask and dilute to the mark with 10 mM ammonium acetate buffered at pH 9.

1 µg/mL solution of O-desmethyl MKH 6562-phenyl-3,4,6-d_3

E.g. Pipet 1 mL of the 100-ppm O-desmethyl MKH 6562-phenyl-3,4,6-d_3 into a 100-mL volumetric flask and dilute to the mark with 10 mM ammonium acetate buffered at pH 9.

Label these solutions appropriately to reflect the actual concentration of the analyte.

3.5.1.3 Mixed solution of O-desmethyl MKH 6562 and its internal standard

Prepare a mixed solution of native and internal of O-desmethyl MKH 6562 in 10 mM ammomnium acetate buffered at pH 9 as follows:

0.0625 µg/mL of O-desmethyl and 0.25 µg/mL of O-desmethyl MKH 6562-phenyl-3,4,6-d_3

E.g. Add 0.0625 mL of 100 µg/mL of O-desmethyl MKH 6562 and about 0.25 mL of 100 µg/mL of O-desmethyl MKH 6562-phenyl-3,4,6-d_3 into 100 mL volumetric flask flask and dilute to the mark with 10 mM ammonium acetate buffered at pH 9. (This is used for method validation in Washington soil)

0.625 µg/mL of O-desmethyl and 0.25 µg/mL of O-desmethyl MKH 6562-phenyl-3,4,6-d_3

E.g. Add 0.625 mL of 100 µg/mL of O-desmethyl MKH 6562 and about 0.25 mL of 100 µg/mL of O-desmethyl MKH 6562-phenyl-3,4,6-d_3 into 100 mL volumetric flask flask and dilute to the mark with 10 mM ammonium acetate buffered at pH 9. (This is used for method validation in Washington soil)

0.625 µg/mL of O-desmethyl and 0.50 µg/mL of O-desmethyl MKH 6562-phenyl-3,4,6-d_3

E.g. Add 0.625 mL of 100 µg/mL of O-desmethyl MKH 6562 and about 0.50 mL of 100 µg/mL of O-desmethyl MKH 6562-phenyl-3,4,6-d_3 into 100 mL volumetric flask flask and dilute to the mark with 10 mM ammonium acetate buffered at pH 9. (This is used for concurrent recoveries in sample analysis with soil from Washington)

3.5.1.4 N-methyl carbamate solution

Prepare a 100-µg/mL solution of N-methyl carbamate in methanol as follows:
If the purity of the standard is <99%, weigh the appropriate amount to correct for the purity. Since the molecular weight of N-methyl carbamate and O-desmethyl MKH 6562 are 299.0 and 382 g/mole, respectively, the equivalent weight of O-desmethyl MKH 6562 to each gram of N-methyl carbamate is 1.278 (assume the mole ratio is one).

100 µg/mL of N-methyl carbamate (127.8 µg/mL O-desmethyl MKH 6562)

E.g. Add 5 mg of N-methyl carbamate to 50-mL volumetric flask and dilute to the mark with methanol. This 100 µg/mL of N-methyl carbamate is equivalent to 127.8 µg/mL of O-desmethyl MKH 6562.

0.782 µg/mL of N-methyl carbamate (1.0 µg/mL O-desmethyl MKH 6562)

E.g. Add 782 µL of 100 µg/mL of N-methyl carbamate to 100-mL volumetric flask and dilute to the mark with methanol.

3.5.1.5 N-methyl carbamate-d_3 internal standard

Prepare a 100-µg/mL of N-methyl carbamate-d_3 internal standard in methanol as follows:
If the purity of the standard is <99%, weigh the appropriate amount to correct for the purity. Since the molecular weight of N-methyl carbamate-d_3 and O-desmethyl MKH 6562 are 302 and 382.0 g/mole, respectively, the equivalent weight of O-desmethyl MKH 6562 to each gram of N-methyl carbamate-d_3 is 1.265 (assume the mole ratio is one).

100 µg/mL of N-methyl carbamate-d_3 (126.5 µg/mL O-desmethyl MKH 6562)
E.g. Add 5 mg of N-methyl carbamate-d_3 to 50-mL volumetric flask and dilute to the mark with methanol. This 100 µg/mL of N-methyl carbamate-d_3 is equivalent to 126.5 µg/mL of O-desmethyl MKH 6562.

7.905 µg/mL of N-methyl carbamate-d_3 (10 µg/mL O-desmethyl MKH 6562)
E.g. Add 7.905 mL of 100 µg/mL of N-methyl carbamate-d_3 to 100-mL volumetric flask and dilute to the mark with methanol.

3.5.2 Extraction of Samples by Accelerated Solvent Extraction (ASE)

Figure 1 shows the analytical scheme for the extraction of O-desmethyl MKH 6562 from soil. The detailed stepwise procedure is as follows:

1. Screw an end cap onto the end of the 33-mL extractor body which is closest to the Dionex logo. (This is designated as the bottom of the extractor cell)

2. Insert a disposable cellulose filter in the bottom of the 33-mL extractor cell with an insertion tool.

3. Weigh 2-3 g of Hydromatrix and load it into the 33-mL extractor cell with a funnel.

4. Weigh 25 ± 0.5 g (wet weight) of sample and load it into the 33-mL extractor cell with a glass funnel.

5. Add 50 µL of the 1-µg/mL O-desmethyl MKH 6562-phenyl-3,4,6-d_3 internal standard into the 25-g soil. (Note: Perform for sample analysis only, not for method validation)

6. Label the corresponding 60-mL collection vial and place into the vial tray.

7. Operate the ASE under the following conditions:

(Due to the instability of O-desmethyl MKH 6562, extractions must be done within three hours after fortification. Therefore, samples may need to put on the ASE in two or more groups).

Solvent: methanol:water (9:1, v/v)
(The above solvent is used for both extraction and derivatization of O-desmethyl MKH 6562 to MKH 6562 N-methyl carbamate)

Pressure:	1500 psi
Temperature:	80 °C
Static Time:	5 min
Cycle:	1
Flush Volume:	50%
Purge Time:	60 s

8. After extraction, remove the I-Chem vials from the vial tray and place them in a Turbo Vap LV.

9. Evaporate the extract to dryness under nitrogen in a Turbo Vap LV (equipped with ASE compatible rack number 60911) at 55 - 60 °C.

10. Reconstitute the extract with 1.0 mL of HPLC mobile phase (i.e., 20% MeOH and 80% 5 mM ammonium acetate in water)

11. With a disposable syringe, filter the extract (about 2-3 mL) from the I-Chem vial into an HPLC autosampler vial through a 0.45-µm nylon Acrodisc®.

12. Store the extracts in a freezer until ready for LC-MS/MS analysis.

3.6 HPLC-MS/MS Analysis

3.6.1 HPLC Conditions

Column:	Luna C18(2), 5 µm, 100Å, 100 x 4.6 mm (Product Code: 00ED-4252-E0, Phenomenex, Torrance, CA)
Flow (column):	0.8 mL/min
Split Ratio:	4:1
Flow (interface):	200 µL/min
Injection Volume:	20-50 µL
Column Temp:	35 °C
Mobile Phase:	A: 5 mM NH_4OAc in water
	B: 5 mM NH_4OAc in MeOH

Time (min)	% Solvent A	% Solvent B
0	80	20
6	10	90
7	10	90
7.5	80	20
10.0	80	20

Approx. retention times: MKH 6562 N-methyl carbamate ≈5 min

3.6.2 ESI/MS/MS Conditions

The MS/MS selected ions for the analytes and the mass spectrometer instrument control language (ICL) procedures are shown in Tables 1 and 2 respectively. The general operating conditions of MS is as follows:

Capillary Temperature:	300 °C
Sheath Gas/pressure (psi):	N_2/90-100 psi
Auxiliary Gas Flow (mL/min):	N_2/15-20 mL/min
Collision Induced Dissociation (CID) Gas:	2.5 mtorr/argon
Scan Time (s):	0.3 s (for each ion)
Polarity:	Negative

3.7 Calibration Curve of N-Methyl Carbamate

3.7.1 General Definitions

There are two definitions of standard concentration used in this method. The first is defined in terms of "µg/mL" or "ng/mL", which describes the concentration of stock solutions and fortification solutions. The second definition is in terms of "µg/g" (ppm) or "ng/g" (ppb) of original matrix sample, which applies to all quantification and linearity standard solutions. This definition takes into account any aliquoting, concentration, or dilution of samples during sample preparation. Any concentration specified as "ppm" or "ppb" is a sample-equivalent concentration.

3.7.2 Calibration Curve Solutions

Prepare five data-point solvent calibration curve of MKH 6562 N-methyl carbamate (NMC) as follows:

Solution for 0.2-ppb 0-desmethyl sample equivalent standard (5 ng/mL):

E.g. Prepare by adding 250 µL of the 0.782-µg/mL NMC (i.e., 1.0-µg/mL of 0-desmethyl) and 250 µL of the 7.905-µg/mL NMC-d_3 (i.e., 10-µg/mL of 0-desmethyl IS) into a 50 mL

volumetric flask and dilute to the mark with 80:20 (5 mM ammonium acetate(aq)/MeOH). The internal standard concentration is equivalent to 2.0 ppb (i.e, 50 ng/mL).

Solution for 0.5-ppb 0-desmethyl sample equivalent standard (12.5 ng/mL):

E.g. Prepare by adding 625 µL of the 0.782-µg/mL NMC (i.e.,1.0-µg/mL of 0-desmethyl) and 250 µL of the 7.905-µg/mL NMC-d_3 (i.e., 10-µg/mL of 0-desmethyl IS) into a 50 mL volumetric flask and dilute to the mark with 80:20 (5 mM ammonium acetate(aq)/MeOH).

Solution for 1.0-ppb 0-desmethyl sample equivalent standard (25 ng/mL):

E.g. Prepare by adding 1.25 mL of the 0.782-µg/mL NMC (i.e., 1.0-µg/mL of 0-desmethyl) and 250 µL of the 7.905-µg/mL NMC-d_3 (i.e., 10-µg/mL of 0-desmethyl IS) into a 50 mL volumetric flask and dilute to the mark with 80:20 (5 mM ammonium acetate(aq)/MeOH).

Solution for 2.0-ppb 0-desmethyl sample equivalent standard (50 ng/mL):

E.g. Prepare by adding 2.5 mL of the 0.782-µg/mL NMC (i.e., 1.0-µg/mL of 0-desmethyl) and 250 µL of the 7.905-µg/mL NMC-d_3 (i.e., 10-µg/mL of 0-desmethyl IS) into a 50 mL volumetric flask and dilute to the mark with 80:20 (5 mM ammonium acetate(aq)/MeOH).

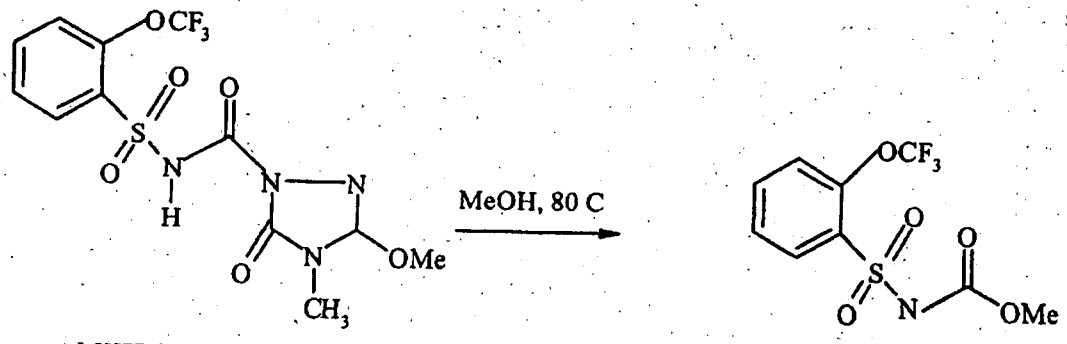
Solution for 10-ppb 0-desmethyl sample equivalent standard (250 ng/mL):

E.g. Prepare by adding 12.5 mL of the 0.782-µg/mL NMC (i.e., 1.0-µg/mL of 0-desmethyl) and 250 µL of the 7.905-µg/mL NMC-d_3 (i.e., 10-µg/mL of 0-desmethyl IS) into a 50 mL volumetric flask and dilute to the mark with 80:20 (5 mM ammonium acetate(aq)/MeOH).

Analyze each standard solution by LC-MS/MS in duplicate (two injections for each solution).

3.8 Side Reaction (Derivatization of MKH 6562 parent to NMC)

It should be noted that MKH 6562 (parent) also react with methanol at 80 °C to form N-methyhl carbamate to a certain extent. Accordingly, both Oklahoma and Washington soils were fortified with MKH 6562 and processed through the method. The rate of conversion to N-methyl carbamate was determined to be approximately 10% (See Tables 5 and 6). The analyst should be aware of this "side-reaction" and if needed to test the degree of conversion of MKH 6562 parent to NMC.



MKH 6562 Parent

N-methyl carbamate

3.9 Method Validation

The method was validated with soil from Oklahoma and Washington spiked at 0.5 ppb (seven replicates) and 5 ppb level (five replicates) with O-desmethyl MKH 6562.

1. For Oklahoma soil, samples (25 g) were fortified at the 0.5-ppb level by adding 125 µL of the 0.1-µg/mL native O-desmethyl MKH 6562 standard and followed by 50 µL of the 1.0-µg/mL internal standard solution (O-desmethyl MKH 6562-phenyl-3,4,6-d_3). Similarly, soil samples (25 g) were fortified at the 5-ppb level by adding 125 µL of the 1-µg/mL native O-desmethyl MKH 6562 standard and followed by 50 µL of the 1.0-µg/mL internal standard solution (O-desmethyl MKH 6562-phenyl-3,4,6-d_3).

2. For Washington soil, samples (25 g) were fortified at 0.5 ppb level by adding 200 µL of mixed standards containing the 0.0625-µg/mL native O-desmethyl MKH 6562 standard and 0.25-µg/mL internal standard solution (O-desmethyl MKH 6562-phenyl-3,4,6-d_3). Similarly, soil samples (25 g) were fortified at the 5 ppb level by adding 200 µL of mixed standards containing the 0.625-µg/mL native O-desmethyl MKH 6562 standard and 0.25-µg/mL internal standard solution (O-desmethyl MKH 6562-phenyl-3,4,6-d_3).

3. As noted previously, MKH 6562 parent will also react with methanol at 80 °C and form N-methyl carbamate. To test the degree of conversion of MKH 6562 to N-methyl carbamate, both Oklahoma and Washington soils were fortified with MKH 6562 parent at 2 ppb (five replicates) and 10 ppb (five replicates) level as follows:

   a) Soil samples (25 g) were fortified at 2 ppb by adding 50 µL of the 1.0-µg/mL MKH 6562 native and followed by 50 µL of the 1.0-µg/mL O-desmethyl MKH 6562-phenyl-3,4,6-d_3 internal standard.
   b) Soil samples (25 g) were fortified at 10 ppb by adding 250 µL of the 1.0-µg/mL MKH 6562 native and followed by 50 µL of the 1.0-µg/mL O-desmethyl MKH 6562-phenyl-3,4,6-d_3 internal standard.

3.10 Quantitation

Quantitation of the native analyte was based on duplicate, five data point solvent calibration curves with a concentration range from 0.2 to 10 ppb. The peak area ratio of native to internal standard of each compound was plotted with its standard concentration. The slope and intercept from a quadratic weighted $1/X^2$ was used for quantitation.

$$\frac{\text{Native Area}}{\text{Internal Standard Area}} = AX^2 + BX + C$$

where A, B and C are the coefficient of the quadratic equation
X is the concentration of the residue.

$$X = \frac{-B + \sqrt{B^2 - 4AC}}{2A}$$

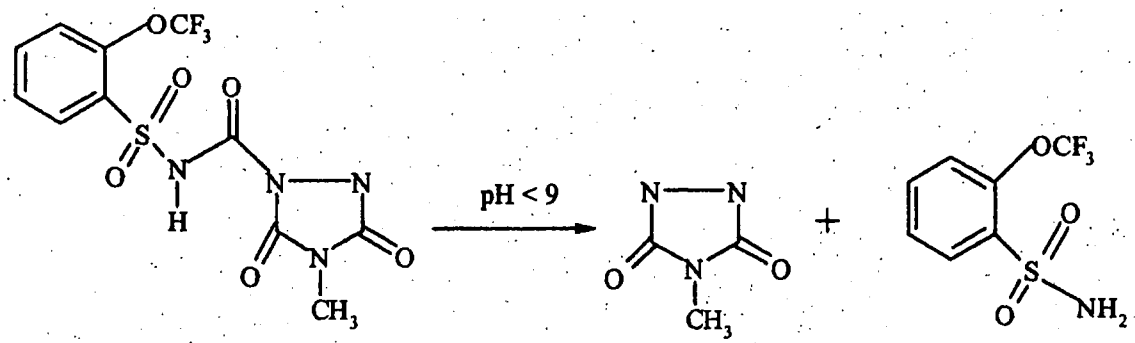
3.10.1 Recovery in Spiked Validation Samples

$$\%\ Recovery = \frac{(Conc_{NAT})}{(\text{Spiked Level})} \times 100\%$$

where $Conc_{NAT}$ = Calculated amount (ppb in the sample), uploaded from the MS
Spike Level = Concentration (ppb) at which the matrix spike was prepared.

4.1 General Considerations

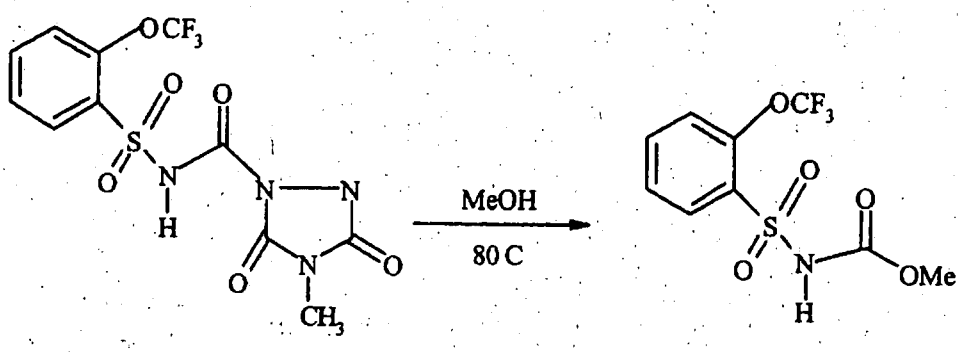
1. From the hydrolysis study[4], O-desmethyl MKH 6562 was determined to be unstable at pH<9 and readily undergone hydrolysis to form N-methyl triazolinone (NMT) and MKH 6562 sulfonamide according to the following reaction:



O-desmethyl MKH 6562 NMT Sulfonamide

To minimize the above reaction, O-desmethyl MKH 6562 was prepared in 10 mM ammonium acetate buffered to pH 9 with ammonium hydroxide.

2. Since the O-desmethyl MKH 6562 was not very sensitive to either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) mass spectrometry, it was converted to MKH 6562 N-methyl carbamate (NMC) and detected by negative electrospray ionization mass spectrometry.



O-desmethyl MKH 6562 N-methyl carbamate

3. The method validation for Washington soil used fortification solution contains both native O-desmethyl MKH 6562 standard and internal standard of O-desmethyl MKH 6562-phenyl-3,4,6-d_3. For Oklahoma soil, samples were fortified with native O-desmethyl MKH 6562 standard followed by internal standard of O-desmethyl MKH 6562-phenyl-3,4,6-d_3. There should be no significant difference in both techniques.

4.3 Reaction of MKH 6562 to N-methyl carbamate

As noted previously, parent MKH 6562, whose structure is very similar to O-desmethyl MKH 6562, also derivatized with methanol to form NMC. As shown in Tables 5 and 6, there is about 10% of MKH 6562 converted to NMC. This may be especially critical in the early sampling intervals (e.g. Day 0, 3, 7 or 14) when a significant proportion of the residues in the soil may be MKH 6562.

Table 1 MS/MS Selected Ions

Substance	Principle	m/z Parent	m/z Daughter	Collision energy (eV)
MKH 6562 N-methyl carbamate derivatized from O-Desmethyl MKH 6562	ESI-	298	85	23
MKH 6562 N-methyl carbamate - phenyl-3,4,6-d_3 derivatized from O-desmethyl MKH 6562-phenyl-3,4,6-d_3 (Internal Standard)	ESI-	301	85	23

ESI- = Electrospray Ionization (negative)

Table 2. Mass spectrometer Instrument Control Language (ICL).

TSQ 7000 Method Sequence Analysis of O-desmethyl MKH 6562 as MKH 6562 N-methyl carbamate
#HPLC=odes, ICL=odesn3, column= Luna C18, 100x4.6 mm #monitored odesmethyl as methyl carbamate (298) apion;on;neg;cent; minfwidth=30;merge=80;apause;valveon capatr=300;spray=4.5;emult=1600;vsend:.#quads:.#rest vsend:.#quad:.#odes while rt<2 go;stop;end aresume;valveoff;cidon while rt<8 dau 298,84.7,85.3,0.3,23;go;stop dau 298,84.7,85.3,0.3,23;go;stop end apause;valveon;cidoff

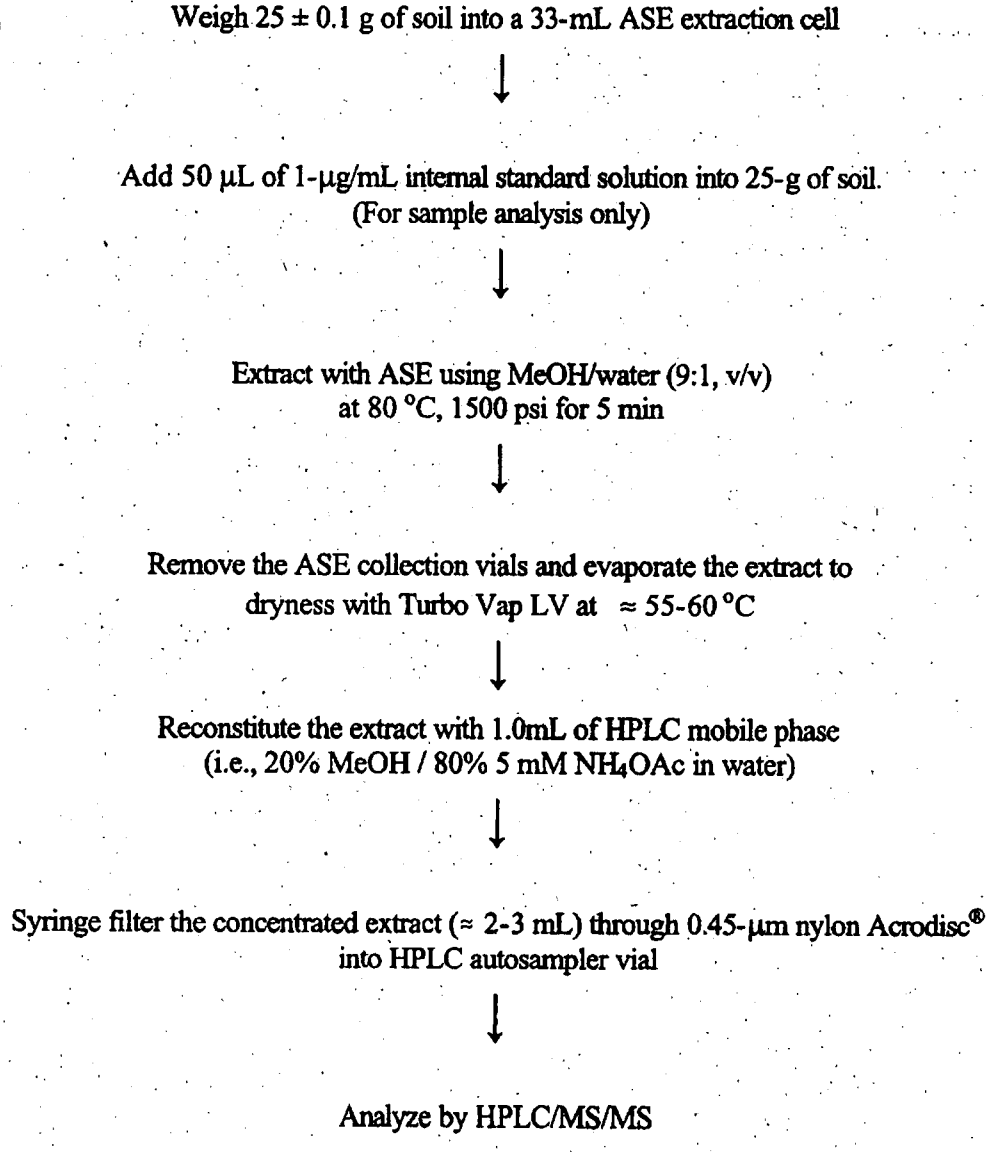


Figure 1 Analytical scheme for sample analyses.