

Analytical Method for the Determination of MKH 6562 and metabolites NODT, sulfonic acid, and sulfonamide in Soil by High-Performance Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

1.0 SUMMARY

An analytical method was developed to quantify MKH 6562, sulfonic acid, sulfonamide and *N,O*-dimethyltriazolinone (NODT) in soil using high-performance liquid chromatography electrospray tandem mass spectrometry (LC-MS/MS). The method was validated using control soil from the dissipation study sites at Washington¹ and Oklahoma². Soil was extracted with acetonitrile / 0.2 M acidified ammonium acetate (4 : 1, v/v) at room temperature in a mechanical shaker for an hour. The extracts were centrifuged for about 10 min. An aliquot of the centrifuged extracts (8 mL) were transferred to a culture tube and stable isotope internal standards were added. The solution was evaporated to dryness and reconstituted to 1 mL with HPLC mobile phase. The resultant solution was analyzed by LC-MS/MS, and quantitation was done against known amount of deuterated internal standards.

2.0 INTRODUCTION

The previous analytical method³ was developed to measure MKH 6562, sulfonic acid, sulfonamide and NODT 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 MKH 6562 and its three metabolites at the LOQ of 0.5 ppb.

3.0 EXPERIMENTAL

3.1 Equipment

Equivalent equipment may be substituted:

- Various general laboratory glassware and utensils
- Nylon Acrodisc[®] (0.45- μ m, 13 mm, Part No. 4551T, Gelman)
- Borosilicate glass disposable culture tube, 20x150mm (Fisher Scientific 14-961-33)
- Disposable 3-mL plastic syringe (Part No. 309586, Becton Dickinson)
- HPLC vials and caps (2-mL, Wheaton #223682)
- I-Chem vials, 60-mL (I-Chem S236-0060)
- Analytical Balance (Mettler A163)
- Balance, Top loader, capable of weighing to the nearest 0.01 g
- Mechanical reciprocating shaker (Eberbach No. 6010)
- SP Vortex mixer (Baxter S8223-1)
- Centrifuge (Damon/IEC Model DPR-6000)
- Turbo Vap LV (Zymark)
- Phenomenex Synergi Max-RP column, 75 x 4.6 mm, 4 μ m, 80 Å, Part Number: 00C-4337-E0
- TSQ 7000 LC/Tandem Mass Spectrometer with ESI or APCI interface and gradient HPLC

3.2 Reagents and Solvents

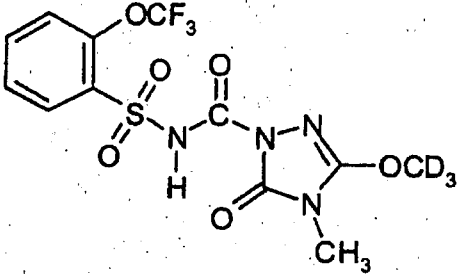
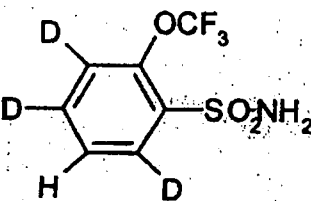
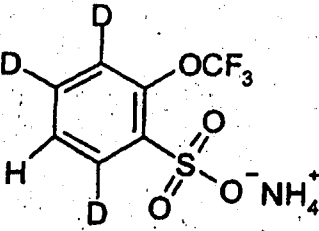
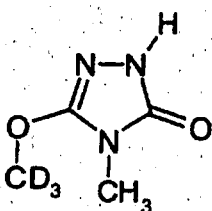
Use as a guide, equivalent reagents or solvents may be substituted:

- Methanol (MeOH; HPLC Grade, Burdick & Jackson #230-4)
- Acetonitrile (ACN; HPLC Grade, Burdick & Jackson #015-4)
- Water (HPLC Grade, Burdick & Jackson #365-4)
- Hydrochloric acid (12 N or 37%, Mallinckrodt #2062)
- 1% hydrochloric acid. E.g. Add 495 mL of water into a 500 mL graduated cylinder and follow by adding 5 mL of 12 N hydrochloric acid.
- Ammonium acetate (Fisher Scientific #A639-500)
- 100 mM NH₄OAc in methanol. E.g. Add 0.77 g of ammonium acetate to 100-mL volumetric flask and dilute to the mark with methanol. Sonicate and shake.
- Reconstitution solvent; Water/100 mM NH₄OAc in methanol (95:5, v/v). E.g. Add 950 mL HPLC grade water and mix with 50 mL of 100 mM NH₄OAc in methanol.
- Mobile phase A; Water/100 mM NH₄OAc in methanol (95:5, v/v)
Same as reconstitution solvent, see above.
- Mobile phase B; 5 mM NH₄OAc in methanol
Add 50 mL of 100 mM NH₄OAc in methanol and mix with 950 mL methanol.
- 0.2 M ammonium acetate with 1% HCl. E.g. Add 7.7 g of ammonium acetate into a 500 mL graduated cylinder and dilute it to 500 mL with 1% HCl. Shake.
- Extraction solvent: ACN/0.2 M NH₄OAc with 1% HCl (4:1, v/v). E.g. Combine 800 mL of ACN and 200 mL of 0.2 M NH₄OAc with 1% HCl.

3.3 Structures

<p>Common Name</p> <p>Standard Ref.</p> <p>Empirical Formula</p> <p>Molecular Weight</p> <p>Purity</p> <p>Expiration Date</p>	<p>MKH 5730 (acid form of MKH 6562)</p> <p>K-640 or equivalent</p> <p>$C_{12}H_{11}F_3N_4O_6S$</p> <p>396.04 g/mol</p> <p>98.8%</p> <p>05-02-06</p>	
<p>Common Name</p> <p>Standard Ref.</p> <p>Empirical Formula</p> <p>Molecular Weight</p> <p>Purity</p> <p>Expiration Date</p>	<p>MKH 6562 sulfonamide</p> <p>K-826 or equivalent</p> <p>$C_7H_6F_3NO_3S$</p> <p>241.00 g/mol</p> <p>100%</p> <p>8-13-03</p>	
<p>Common Name</p> <p>Standard Ref.</p> <p>Empirical Formula</p> <p>Molecular Weight</p> <p>Purity</p> <p>Expiration Date</p>	<p>MKH 6562 sulfonic acid (ammonium salt)</p> <p>K-643 or equivalent</p> <p>$C_7H_8F_3NO_4S$</p> <p>259 g/mol</p> <p>100%</p> <p>05-02-04</p>	
<p>Common Name</p> <p>Standard Ref.</p> <p>Empirical Formula</p> <p>Molecular Weight</p> <p>Purity</p> <p>Expiration Date</p>	<p>NODT (N,O-dimethyltriazolinone)</p> <p>K-751 or equivalent</p> <p>$C_4H_7N_3O_2$</p> <p>129.05 g/mol</p> <p>85.5%</p> <p>04-20-06</p>	

Internal standards

Common Name Standard Ref. Empirical Formula Molecular Weight Purity Expiration Date	MKH 5730-methoxy- <i>d</i> ₃ K-705 or equivalent C ₁₂ H ₈ D ₃ F ₃ N ₄ O ₆ S 399.05 99.1% 8-24-04	
Common Name Standard Ref. Empirical Formula Molecular Weight Purity Expiration Date	MKH 6562 sulfonamide- <i>d</i> ₃ K-701 or equivalent C ₇ H ₃ D ₃ F ₃ NO ₃ S 244.02 97% 11-07-05	
Common Name Standard Ref. Empirical Formula Molecular Weight Purity Expiration Date	MKH 6562 sulfonic acid- <i>d</i> ₃ (ammonium salt) K-706 or equivalent C ₇ H ₅ D ₃ F ₃ NO ₄ S 262.03 85.1% 10-9-03	
Common Name Standard Ref. Empirical Formula Molecular Weight Purity Expiration Date	N,O-Dimethyltriazolinone- <i>d</i> ₃ K-704 or equivalent C ₄ H ₄ D ₃ N ₃ O ₂ 132.07 99.3% 3-25-08	

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 Native Analyte Solutions

Prepare 100 µg/mL stock solutions of MKH 6562, MKH 6562 sulfonamide, MKH 6562 sulfonic acid and *N,O*-dimethyltriazolinone (NODT) (see Section 3.3 for standard reference numbers) by weighing 10 mg of each (corrected for purity) using an analytical balance (with 0.1 mg readout) and adding the corresponding volumetric amount of acetonitrile. For MKH 6562 sulfonic acid, prepare with 1:1 acetonitrile/water (v/v). If the purity of a standard is <99%, weigh the appropriate amount to correct for the lower purity.

100 µg/mL stock solution of MKH 6562 in acetonitrile

E.g. Add 10 mg of MKH 6562 to 100-mL volumetric flask and dilute to the mark with acetonitrile.

100 µg/mL stock solution of MKH 6562 sulfonamide in acetonitrile

E.g. Add 10 mg of MKH 6562 sulfonamide to 100-mL volumetric flask and dilute to the mark with acetonitrile.

100 µg/mL stock solution of MKH 6562 sulfonic acid in 1:1 acetonitrile/water (v/v)

E.g. Add 10 mg of MKH 6562 sulfonic acid to 100-mL volumetric flask and dilute to the mark with 1:1 acetonitrile/water (v/v).

100 µg/mL stock solution of NODT in acetonitrile

E.g. Add 10 mg of NODT to 100-mL volumetric flask and dilute to the mark with acetonitrile.

Label the solutions to reflect the actual concentration of the analyte. Store all solutions in a freezer (< -7 °C) and protect from light when not in use.

3.5.1.2 Mixed Native Analyte Solutions

Prepare the mixed solutions from the stock solutions of the individual native analytes (Section 3.5.1.1) as follows (allow the stock solutions to equilibrate to room temperature prior to use):

10 $\mu\text{g/mL}$ mixed native solution

E.g. Add 10 mL of each solution of MKH 6562, MKH 6562 sulfonamide, MKH 6562 sulfonic acid and *N,O*-dimethyltriazolinone (NODT) from Section 3.5.1.1 to a 100-mL volumetric flask and dilute to the mark with acetonitrile.

1 $\mu\text{g/mL}$ mixed native solution

E.g. Add 10 mL of 10- $\mu\text{g/mL}$ mixed solutions of MKH 6562, MKH 6562 sulfonamide, MKH 6562 sulfonic acid and *N,O*-dimethyltriazolinone (NODT) to a 100-mL volumetric flask and dilute to the mark with acetonitrile.

0.1 $\mu\text{g/mL}$ mixed native solution

E.g. Add 10 mL of 1- $\mu\text{g/mL}$ mixed solutions of MKH 6562, MKH 6562 sulfonamide, MKH 6562 sulfonic acid and *N,O*-dimethyltriazolinone (NODT) to a 100-mL volumetric flask and dilute to the mark with acetonitrile.

0.01 $\mu\text{g/mL}$ mixed native solution

E.g. Add 10 mL of 0.1- $\mu\text{g/mL}$ mixed solutions of MKH 6562, MKH 6562 sulfonamide, MKH 6562 sulfonic acid and *N,O*-dimethyltriazolinone (NODT) to a 100-mL volumetric flask and dilute to the mark with acetonitrile.

Label the solutions to reflect the actual concentration of the analyte. Store all solutions in a refrigerator ($<8\text{ }^{\circ}\text{C}$) and protect from light when not in use.

3.5.1.3 Internal Standard Stock Solutions

Prepare 100 $\mu\text{g/mL}$ stock solutions (nominally 0.1 mg/mL) of MKH 6562- d_3 , MKH 6562 sulfonamide- d_3 , MKH 6562 sulfonic acid- d_3 and *N,O*-dimethyltriazolinone- d_3 (NODT) (see Section 3.3 for standard reference numbers) by weighing 10 mg of each using an analytical balance and adding the corresponding volumetric amount of acetonitrile. For MKH 6562 sulfonic acid- d_3 , prepare in 1:1 acetonitrile/water (v/v). If the purity of a standard is $<99\%$, weigh the appropriate amount to correct for the lower purity.

Label the solutions to reflect the actual concentration of the analyte. Store all solutions in a freezer ($<-7\text{ }^{\circ}\text{C}$) and protect from light when not in use. If the purity of a standard is $<99\%$, weigh the appropriate amount to correct for the lower purity.

100 $\mu\text{g/mL}$ stock solution of MKH 6562- d_3

E.g. Add 10 mg of MKH 6562- d_3 to 100-mL volumetric flask and dilute to the mark with acetonitrile.

100 $\mu\text{g/mL}$ stock solution of MKH 6562 sulfonamide- d_3

E.g. Add 10 mg of MKH 6562 sulfonamide- d_3 to 100-mL volumetric flask and dilute to the mark with acetonitrile.

100 µg/mL stock solution of MKH 6562 sulfonic acid- d_3

E.g. Add 10 mg of MKH 6562 sulfonic acid- d_3 to 100-mL volumetric flask and dilute to the mark with 1:1 acetonitrile/water (v/v).

100 µg/mL stock solution of NODT- d_3

E.g. Add 10 mg of *N,O*-dimethyltriazolinone- d_3 (NODT) to 100-mL volumetric flask and dilute to the mark with acetonitrile.

Label the solutions to reflect the actual concentration of the analyte. Store all solutions in a freezer (<-7 °C) and protect from light when not in use.

3.5.1.4 Mixed Internal Standard Solution

Prepare mixed internal standard solution from the stock solutions of the individual internal standard (Section 3.5.1.3) as follows (allow the stock solutions to equilibrate to room temperature prior to use):

1 µg/mL mixed internal standard solution

E.g. Add 1 mL of each solution of MKH 6562- d_3 , MKH 6562 sulfonamide- d_3 , MKH 6562 sulfonic acid- d_3 and *N,O*-dimethyltriazolinone- d_3 (NODT) from Section 3.5.1.3 to a 100 mL volumetric flask and dilute to the mark with acetonitrile.

0.1 µg/mL mixed internal standard solution

E.g. Add 10 mL of the 1 µg/mL mixed solutions of MKH 6562- d_3 , MKH 6562 sulfonamide- d_3 , MKH 6562 sulfonic acid- d_3 and *N,O*-dimethyltriazolinone- d_3 (NODT) to a 100-mL volumetric flask and dilute to the mark with acetonitrile.

This is the internal standard spiking solution.

Label the solutions to reflect the actual concentration of the analyte. Store all solutions in a refrigerator (<8 °C) and protected from light when not in use.

3.6 Sample Extraction

Figure 1 shows the analytical scheme for the extraction of MKH 6562 and its metabolites from soil. The detailed stepwise procedure is summarized as follows:

Step 1. Weigh about 10 ± 0.2 g soil into a 60-mL I-Chem glass vial.
(If fortification is needed, please refer to Section 3.5.6)

Step 2. Add 20-mL of extraction solvent [ACN/0.2 M NH_4OAc with 1% HCl (4:1, v/v)] into the glass vial.

- Step 3. Shake the sample for about an hour in a mechanical shaker at ~130 cycles/min at ambient temperature.
- Step 4. Remove the glass vial from the shaker and centrifuge it at about 2300 rpm (~1127 g) for about 10-15 min.
- Step 5. Pipet 8 mL of the extract into disposable culture tube or equivalent. (*Aliquot factor = 8/20*)
- Step 6. Add 100 µL of 0.1 µg/mL of mixed internal standard into the same culture tube.
- Step 7. Vortex the culture tube for ~15 s.
- Step 8. With TurboVap, evaporate the extract aliquot to ~1 mL at water bath temperature of ~40 °C. Add ~2 mL of methanol and then continue to dryness.
- Step 9. Reconstitute the residue with 1 mL of 95:5 water/100 mM ammonium acetate in methanol. Vortex for ~15 s and then sonicate for ~2 minutes.
- Step 10. Filter the extract with 0.45-µm filter (Nylon Acrodisc, 13 mm and 0.45 µm Gelman or equivalent) into a HPLC vial.
- Step 11. Store these in a freezer until ready for LC-MS/MS. (Also, keep the remaining extract from step 4 in a freezer)

3.7 LC-MS/MS Analysis

These conditions are suggested based on the instrument and model used. LC and/or MS conditions may be changed if deemed necessary to obtain acceptable chromatographic performance or MS sensitivity.

3.7.1 HPLC Conditions

ThermoFinnigan P-4000 quaternary pump with a ThermoFinnigan degasser and A 3000 autosampler.

Column:	Phenomenex Synergi Max-RP column, 75 x 4.6 mm, 4 µm, 80 Å Part No. 00C-4337-E0
Injection volume:	35 µL (loop size 20 µL)
Column temp:	40 °C
Flow rate:	400 µL/min
Mobile Phase A:	95:5 water/100 mM ammonium acetate in methanol
Mobile Phase B:	5 mM ammonium acetate in methanol
Split ratio:	4:1 (i.e., 80% to waste and 20% to MS)

Gradient:

Time (min)	%B
0	10
1.0	10
8.0	90
9.0	90
9.1	10
11.0	10

Approximate retention time:	NODT	-	5.1 min
	MKH 6562 sulfonic acid	-	6.2 min
	MKH 6562	-	6.9 min
	MKH 6562 sulfonamide	-	7.7 min

3.7.2 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:

Instrument	ThermoFinnigan TSQ 7000 triple quadrupole
Interface:	Atmospheric pressure API II in electrospray ionization (ESI) mode
Scanning Mode:	Selected Reaction Monitoring (SRM)
Capillary temp:	300 °C
Spray Voltage:	4.5 kV
Sheath Gas:	Nitrogen; 80-100 psi
Auxiliary Gas:	Nitrogen; 25-45 mL/min
Collision Gas:	Argon at ~2.2-2.4 mtorr

3.8 Calibration Curve of MKH 6562

3.8.1 General Definitions

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

3.8.2 Calibration Curve Solutions

Prepare five data-point solvent calibration curve of MKH 6562 as follows:

- Cal 5: *25 ppb sample equivalent (i.e. 100 ng/mL)*
E.g. Add 0.5 mL of 10 $\mu\text{g/mL}$ of mixed native standards and 0.5 mL of 1 $\mu\text{g/mL}$ of mixed internal standards to a 50-mL volumetric flask. Dilute to 50 mL with water/100 mM NH_4OAc in MeOH (95:5, v/v).
- Cal 4: *5 ppb sample equivalent (i.e. 20 ng/mL)*
E.g. Add 1.0 mL 1 $\mu\text{g/mL}$ of mixed native standards and 0.5 mL of 1 $\mu\text{g/mL}$ of mixed internal standards to a 50-mL volumetric flask. Dilute to 50 mL with water/100 mM NH_4OAc in MeOH (95:5, v/v).
- Cal 3: *1 ppb sample equivalent (i.e. 4 ng/mL)*
E.g. Add 0.2 mL of 1 $\mu\text{g/mL}$ of mixed native standards and 0.5 mL of 1 $\mu\text{g/mL}$ of mixed internal standards to a 50-mL volumetric flask. Dilute to 50 mL with water/100 mM NH_4OAc in MeOH (95:5, v/v).
- Cal 2: *0.5 ppb sample equivalent (i.e. 2 ng/mL)*
E.g. Add 1 mL of 0.1 $\mu\text{g/mL}$ of mixed native standards and 0.5 mL of 1 $\mu\text{g/mL}$ of mixed internal standards to a 50-mL volumetric flask. Dilute to 50 mL with water/100 mM NH_4OAc in MeOH (95:5, v/v).
- Cal 1: *0.25 ppb for sample equivalent (i.e. 1 ng/mL)*
E.g. Add 0.5 mL of 0.1 $\mu\text{g/mL}$ of mixed native standards and 0.5 mL of 1 $\mu\text{g/mL}$ of mixed internal standards to a 50-mL volumetric flask. Dilute to 50 mL with water/100 mM NH_4OAc in MeOH (95:5, v/v).

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

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) as follows:

✓ 0.5 ppb Adding 50 μ L of a 100 ng/mL fortification solution into 10 g soil.

✓ 5 ppb Adding 50 μ L of a 1 μ g/mL fortification solution into 10 g soil.

3.10 Quantitation of Analyte

Quantitation of the native analyte was based on duplicate, five data point solvent calibration curves with a concentration range from 0.25 to 25 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 weighted (1/X) linear regression curve was used for quantitation of MKH 6562, MKH 6562 sulfonamide and MKH 6562 sulfonic acid.

$$\frac{\text{Native Area}}{\text{Internal Standard Area}} = \text{Slope} \times \text{Standard Concentration (ppb)} + y \text{ intercept}$$

$$\text{Residue Level (ppb)} = \left(\frac{\text{Native Area}}{\text{Internal Standard Area}} - y \text{ intercept} \right) \times \frac{1}{\text{Slope}}$$

For NODT, 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

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

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

Table 1 MS/MS Selected Ions.

Compound	Precursor Ion (amu / Mode)	Product Ion (amu)	Scan Time (s)	Collision Energy (eV)
NODT	130 / +	114.5	0.3	-20
NODT-d ₃	133 / +	114.5	0.3	-20
MKH 6562 acid	241 / -	85	0.5	35
MKH 6562 acid-d ₃	244 / -	85	0.5	35
MKH 6562	395 / -	127.6	0.3	20
MKH 6562-d ₃	398 / -	130.6	0.3	20
MKH 6562 amide	240 / -	85	0.3	28
MKH 6562 amide-d ₃	243 / -	85	0.3	28

Table 2 Mass spectrometer Instrument Control Language (ICL).

```
HPLC=m62soil2, ICL=m62soil2, Column=syneri Max RP
#Monitored NODT(130), MKH 6562(395), acid(241) and amide(240)

apion; on; pos; cent; minfwidth=30; merge=80; apause; valveon
capht=300;spray=4.5;emult=1600;vsend: #quads: #rest
vsend: #quads: #m62soil
while rt<3
    go; stop; end
aresume; valveoff; cidon
while rt<5.7
    dau 130,114.6,115.1,0.3,-20;go;stop
    dau 133,114.6,115.1,0.3,-20;go;stop;end
neg
while rt<6.5
    dau 241,84.5,85.2,0.5,35;go;stop
    dau 244,84.5,85.2,0.5,35;go;stop;end
while rt<9
    dau 395,127.6,128.3,0.3,20;go;stop
    dau 398,130.6,131.3,0.3,20;go;stop
    dau 240,84.5,85.0,0.3,28;go;stop
    dau 243,84.5,85.0,0.3,28;go;stop;end
apause;valveon;cidoff
```

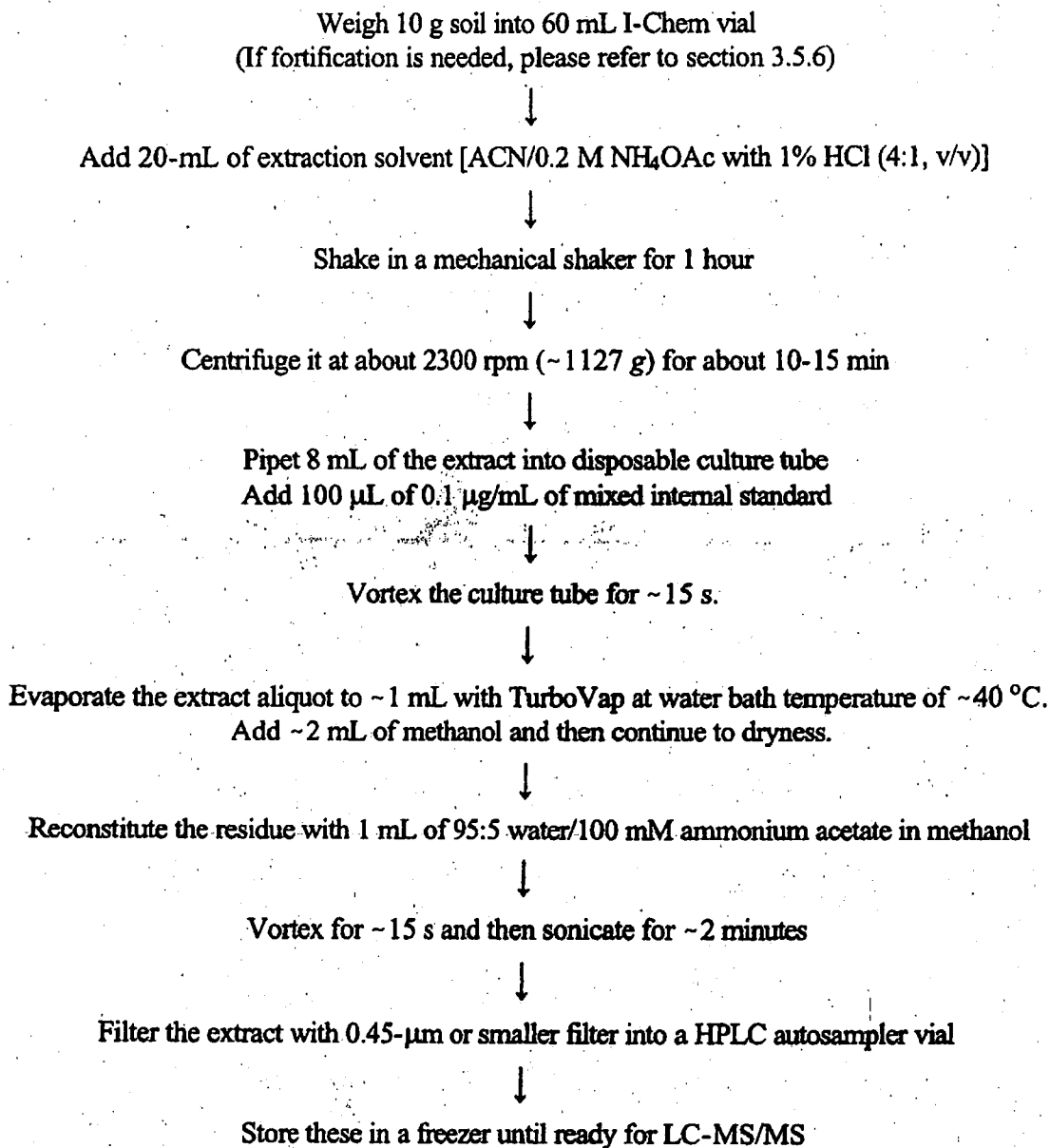


Figure 1 Analytical scheme for the extraction of MKH 6562 and its metabolites from soil