Analytical Method for the Determination of MKH 6562 and Three Metabolites in Ground Water by High-Performance Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)

1.0 SUMMARY

An analytical method was developed to quantify MKH 6562 and three of its metabolites in water using high-performance liquid chromatography electrospray tandem mass spectrometry (LC-ESI/MS/MS). The metabolites measured included MKH 6562 sulfonic acid, MKH 6562 sulfonamide and *N,O*-dimethyltriazolinone (NODT). Deuterated internal standards of MKH 6562 and its metabolites are added to the water sample (50 mL). The water samples are acidified with hydrochloric acid and extracted with C_{18} solid phase extraction (SPE) cartridge. The extracts are concentrated to dryness, reconstituted with mobile phase, filtered with 0.45-µm nylon Acrodisc® and analyzed by LC-ESI/MS/MS.

The method was validated using control well water obtained from North Dakota (a farm yard, ~6 miles from Agvise Research Farm). Water (50 mL) was fortified at 0.03 ppb (7 replicates) and 0.05 ppb (7 replicates).

2.0 INTRODUCTION

MKH 6562 (sodium salt of 4,5-dihydro-3-methoxy-4-methyl-5-oxo-N-[[2-(trifluoromethoxy) phenyl]sulfonyl]-1H-1,2,4-triazole-1-carboxamide) is an experimental herbicide being developed by the Agricultural Division of Bayer Corporation for use on wheat grown in western Canada and the north central United States.

3.0 EXPERIMENTAL

3.1 Equipment

- Volumetric flasks (10- , 100-, 500- and 1000-mL)
- Volumetric pipettes (Baxter or equivalent)
- Graduated cylinders (50- and 100-mL)
- Syringes, gas-tight type (25-, 50-, 100-, 250- and 500-µL)
- Pipetman (Gilson or equivalent)
- Pasteur pipettes (Kimble or equivalent)
- 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)

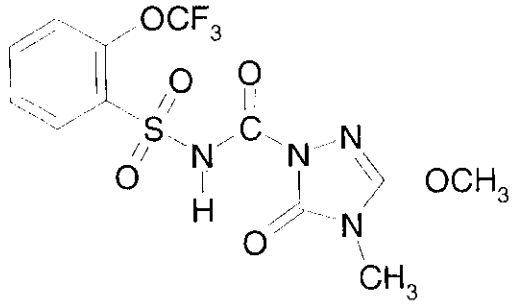
- Analytical Balance (Mettler A163 or equivalent)
- Balance, Top loader, capable of weighing to the nearest 0.01 g
- C_{18} Solid-phase extraction cartridges (2 g sorbent, MegaBond Elut, Varian #1225-6015)
- Solid phase 12- or 24-port vacuum manifold (J. T. Baker #7208-00 or equivalent)
- Turbo Vap LV (Zymark) or equivalent
- Keystone Scientific Betasil C_{18} column, 100 x 2.0 mm, 5 µm, 100Å, Part Number: 105-701-2-CPF
- TSQ 7000 LC/Tandem Mass Spectrometer with ESI or APCI interface and gradient HPLC, or equivalent (Finnigan Corp)

3.2 Reagents and Solvents

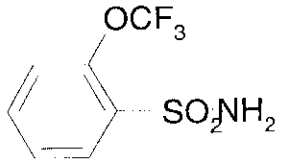
- Methanol (MeOH; HPLC Grade, Burdick & Jackson #230-4 or equivalent)
- Acetonitrile (ACN; HPLC Grade, Burdick & Jackson #015-4 or equivalent)
- Water (HPLC Grade, Burdick & Jackson #365-4 or equivalent)
- Hydrochloric acid (12 N or 37%, Mallinckrodt #2062 or equivalent)
- 1 N hydrochloric acid. Add ~50 mL HPLC grade water follow by ~ 8.3 mL of 12 N hydrochloric acid to water and dilute to 100 mL with HPLC grade water.
- Ammonium hydroxide (~29.5 %, J. T. Baker #9721-3 or equivalent)
- 5% ammonium hydroxide. Add 5-mL of concentrated ammonium hydroxide (~29.5%) and diluted to 100-mL with HPLC grade water.
- Elution solvent, methanol: 5% ammonium hydroxide, (9:1, v:v) in water. Add 900 mL methanol and mix with100-mL of 5% ammonium hydroxide in water.
- Ammonium acetate (Fisher Scientific #A639-500 or equivalent)
- 100 mM NH_4OAc in methanol : Add 0.77 g of ammonium acetate to 100-mL volumetric flask and dilute to the mark with methanol.
- Reconstitution solvent; Water : 100 mM NH_4OAc in methanol (19:1, v:v). Add 950 mL HPLC grade water and mix with 50 mL of 100 mM NH_4OAc in methanol.
- Mobile phase A; Water : 100 mM NH_4OAc in methanol (19:1, v:v)
  Same as reconstitution solvent, see above.
- Mobile phase B; 5 mM NH_4OAc in methanol
  Add 50 mL of 100 mM NH_4OAc in methanol and mix with 950 mL methanol.

3.3 Analytical Standards

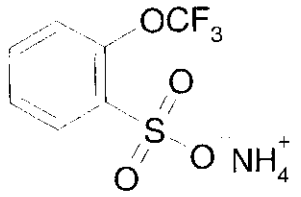
Common Name:	**MKH 5730 (acid form of MKH 6562)**
Bayer Ref.#:	K-640
Empirical Formula:	$C_{12}H_{11}F_3N_4O_6S$
Molecular Weight:	396.3
Purity:	99.0%
Expiration Date:	5/14/01
Chemical Name:	4,5-Dihydro-3-methoxy-4-methyl-5-oxo-N-[[2-(trifluoromethoxy) phenyl]sulfonyl]-1H-1,2,4-triazole -1-carboxamide
CAS Number:	145026-88-6



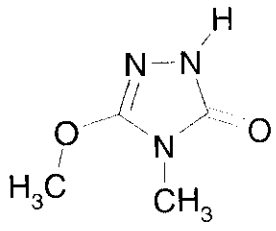
Common Name:	**MKH 6562 sulfonamide**
Bayer Ref.#:	K-826
Empirical Formula:	$C_7H_6F_3NO_3S$
Molecular Weight:	241.2
Purity:	100%
Expiration Date:	08/13/03
Chemical Name:	2-(Trifluoromethoxy)benzenesulfonamide



Common Name:	**MKH 6562 sulfonic acid (ammonium salt)**
Bayer Ref.#:	K-643
Empirical Formula:	$C_7H_8F_3O_4SN$
Molecular Weight:	259
Purity:	100%
Expiration Date:	05/02/04
Chemical Name:	2-(Trifluoromethoxy)benzenesulfonic acid

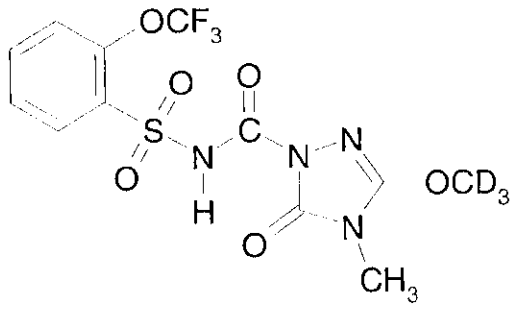


Common Name:	**Methyoxy triazolinone or N,O-Dimethyltriazolinone (NODT)**
Bayer Ref.#:	K-751
Empirical Formula:	$C_4H_7N_3O_2$
Molecular Weight:	129.1
Purity:	98%
Expiration Date:	04/01/01
Chemical Name:	2,4-Dihydro-5-methoxy-4-methyl-3H-1,2,4-triazol-3-one

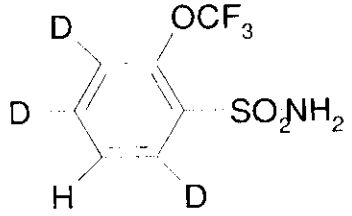


The following are the internal standards that were used in this study:

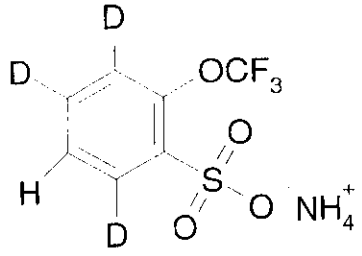
Common Name:	**MKH 5730-methoxy-d_3**
Bayer Ref.#:	K-705
Empirical Formula:	$C_{12}H_8D_3F_3N_4O_6S$
Molecular Weight:	399.32
Purity:	98.3%
Expiration Date:	07/01/99



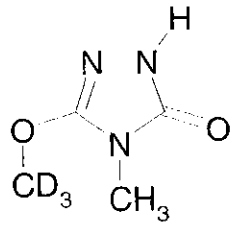
Common Name:	**MKH 6562 sulfonamide- d_3**
Bayer Ref.#:	K-701
Empirical Formula:	$C_7H_3D_3F_3NO_3S$
Molecular Weight:	244
Purity:	97.9%
Expiration Date:	06/21/00



Common Name:	**MKH 6562 sulfonic acid-d_3 (ammonium salt)**
Bayer Ref.#:	K-706
Empirical Formula:	$C_7H_5D_3F_3O_4SN$
Molecular Weight:	261
Purity:	88%
Expiration Date:	07/08/00



Common Name:	**N,O-Dimethyltriazolinone-methoxy-d_3**
Bayer Ref.#:	K-704
Empirical Formula:	$C_4H_4D_3N_3O_2$
Molecular Weight:	132
Purity:	99.3%
Expiration Date:	06/27/00



3.4 Location and Personnel

The development and validation (non-GLP) of the method was conducted at Bayer Research Park (BRP), Stilwell, Kansas from February to April 1999. All raw data for this study are archived at BRP (Notebook Number: 99B89). The names of the individuals involved in this study are listed in Appendix 1.

3.5 Procedures

3.5.1 Preparation of Standards and Reagents

3.5.1.1 Native Analyte Solutions

Prepare 100-ppm stock solutions (nominally 0.1 mg/mL) 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 10-20 mg of each 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.

E.g.: Add 10 mg of MKH 6562 to 100-mL volumetric flask and dilute to the mark with acetonitrile.
Add 10 mg of MKH 6562 sulfonamide to 100-mL volumetric flask and dilute to the mark with acetonitrile.
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).
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 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 µg/mL mixed native solution
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 µg/mL mixed native solution
Add 10 mL of mixed solutions of 10-µg/mL 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 µg/mL mixed native solution
Add 10 mL of mixed solutions of 1-µg/mL 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 µg/mL mixed native solution
Add 10 mL of mixed solutions of 0.1-µg/mL 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 °C) and protect from light when not in use.

3.5.1.3 Internal Standard Stock Solutions

Prepare 100-ppm 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 10-20 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.

E.g.: Add 10 mg of MKH 6562-d_3 to100-mL volumetric flask and dilute to the mark with acetonitrile.
Add 10 mg of MKH 6562 sulfonamide-d_3 to 100-mL volumetric flask and dilute to the mark with acetonitrile.
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).
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
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
Add 10 mL of mixed solutions of 1 µg/mL 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.

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.

3.5.2 Calibration Curve

Prepare a five-point solvent calibration curve using 0.01-, 0.03-, 0.05-, 0.1- and 0.5-ppb standards (concentration in sample equivalent for a 50-mL sample), each containing 0.1-ppb internal standards (concentration in sample equivalent for a 50-mL sample) as shown in Table 1. The concentration of each solution is based on a 50-mL sample equivalent for a final volume of 1 mL. Analyze each standard solution by LC/MS/MS in **triplicate** (three injections for each solution).

0.5 ng/mL standard (equivalent to 0.01 ppb sample): Prepare by adding 250 µL of the 0.1-µg/mL mixed native solution from Section 3.5.1.2 to a 50-mL volumetric flask, 250 µL of the 1-µg/mL mixed internal standards from Section 3.5.1.4, 1.0 mL of acetonitrile and bringing to volume with water:100 mM ammonium acetate in methanol 19:1 (v:v).

1.5 ng/mL standard (equivalent to 0.03 ppb sample): Prepare by adding 750 µL of the 0.1-µg/mL mixed native solution from Section 3.5.1.2 to a 50-mL volumetric flask, 250 µL of the 1-µg/mL mixed internal standards from Section 3.5.1.4, 500 µL of acetonitrile and bringing to volume with water:100 mM ammonium acetate in methanol 19:1 (v:v).

2.5 ng/mL standard (equivalent to 0.05 ppb sample): Prepare by adding 125 µL of the 1-µg/mL mixed native solution from Section 3.5.1.2 to a 50-mL volumetric flask, 250 µL of the 1-µg/mL mixed internal standards from Section 3.5.1.4, 1.125 mL of acetonitrile and bringing to volume with water:100 mM ammonium acetate in methanol 19:1 (v:v).

5.0 ng/mL standard (equivalent to 0.10 ppb sample): Prepare by adding 250 µL of the 1-µg/mL mixed native solution from Section 3.5.1.2 to a 50-mL volumetric flask, 250 µL of the 1-µg/mL mixed internal standards from Section 3.5.1.4, 1.0 mL of acetonitrile and bringing to volume with water:100 mM ammonium acetate in methanol 19:1 (v:v).

25 ng/mL standard (equivalent to 0.50 ppb sample): Prepare by adding 125 µL of the 10-µg/mL mixed native solution from Section 3.5.1.2 to a 50-mL volumetric flask, 250 µL of the 1-µg/mL mixed internal standards from Section 3.5.1.4, 1.125 mL of acetonitrile and bringing to volume with water:100 mM ammonium acetate in methanol 19:1 (v:v).

3.5.3 Sample Extraction

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

1. Measure 50-mL aliquots of water samples into the 100-mL graduated cylinders.

2. Add 50-µL of the 0.1 µg/mL mixed internal standard solution (Section 3.5.1.4) and shake to mix the solutions. This is equivalent to a nominal 0.1-ppb solution based on a 50-mL sample size.

3. Add 1-mL of 1 N hydrochloric acid and shake.

4. Condition a 2g (12 cc) MegaBond C_{18} SPE cartridge (Varian, Part 1225-6015) by rinsing it with 10 mL MeOH followed by 10-15 mL of HPLC-grade water. Do not allow the cartridge to go to dryness.

5. Pass the water sample through the cartridge at a rate of 20-30 mL/min.

6. Add 10 mL HPLC-grade water onto the cartridge. Purge the cartridge with air and dry the cartridge under vacuum for 1-2 minutes.

7. Elute the sample with 10 mL of MeOH: 5% ammonium hydroxide (9:1, v:v) and collect it in a 13-mL centrifuge tube.

8. Set nitrogen evaporator or turbo-evaporator at 40-45 °C, evaporate the sample to dryness.

9. Reconstitute to 1.0 mL with water:100 mM ammonium acetate in methanol 19:1 (v:v).

10. Filter the extract with nylon acrodisc (Gelman, Part No. 4438) into HPLC vial.

11. Store the extract in a freezer (< -7 °C) until ready for LC/ESI/MS/MS analysis.

3.6 Instrumentation

3.6.1 HPLC Conditions

The general operation conditions of HPLC are as follows:

Instrument:	Thermo Separation's Consta Metric 3200 MS, 3500 MS
Column:	Betasil; C_{18}, 100 x 2 mm, 5 µm, 100 Å Part No. 105-701-2-CPF, Keystone Scientific
Column Oven:	35 °C
Flowrate:	0.3 mL/min
Split ratio:	4:1 (4 parts to waste and 1 part to MS)
Injection volume:	50 µL
Mobile Phase A:	19:1 water:100 mM ammonium acetate in methanol
Mobile Phase B:	5 mM ammonium acetate in methanol

Gradient:

Time (min)	%B
init	0
2.0	0
9.0	95
10.0	95
10.5	0
16.0	0

Approximate retention times: *N,O*-dimethyltriazolinone ~4.9 min
MKH 6562 sulfonic acid ~6.5 min
MKH 6562 ~7.1 min
MKH 6562 sulfonamide ~7.4 min

3.6.2 MS Conditions

The general operating conditions of MS are as follows (These conditions may be modified to optimize the systems):

Instrument:	Finnigan TSQ 7000
Capillary Temp:	330 °C
Sheath Gas:	N_2 at 90 psi
Auxiliary Gas:	N_2 at 10-20 mL/min
Electronic Multiplier:	1700 V
Polarity:	Positive ion for *N,O*-dimethyltriazolinone Negative ion for MKH 6562, MKH 6562 sulfonic acid and MKH 6562 sulfonamide

The analytes, daughter ions and collision voltages are shown in Table 2. The Instrument Control Language (ICL) codes are shown in Table 3.

3.7 Method Validation

3.7.1 Recovery Validations

Table 4 shows the amount of sample fortification in each level. The detailed stepwise procedure is summarized as follows:

1. Measure sixteen 50-mL-aliquots of control groundwater. Designate two samples as control matrices, seven samples as 0.03-ppb spikes, seven samples as 0.05-ppb spikes and a reagent blank (HPLC-grade water).

2. Fortify the 50-mL groundwater at 0.03-ppb (seven replicates), and 0.05-ppb (seven replicates) as follows:

   0.03-ppb — Spike 150-µL of 0.01 µg/mL mixed standard (3.5.1.2) into the 50-mL groundwater.

   0.05-ppb — Spike 25-µL of 0.1 µg/mL mixed standard (3.5.1.2) into the 50-mL groundwater.

3. No fortification is needed for the reagent blank or control groundwater (duplicate).

4. Follow steps 2-11 in Section 3.5.3.

4.0 QUANTITATION

Quantitation is based on the use of average response factor (average of 15 data). Each analyte is quantitated by using its deuterated analog.

4.1 Response Factor

The response factor for the analyte is calculated according to the following formula:

$$RF = \frac{(Area_{Nat})}{(Area_{Is})(Conc_{Nat})}$$

where: RF = Response factor
$Area_{Nat}$ = Area of response for the daughter ion from the native standard
$Area_{Is}$ = Area of response for the daughter ion from the internal standard
$Conc_{Nat}$ = Concentration of the native standard (ng/mL)

The average response factor is calculated as follows:

$$RF_{Avg} = \frac{\sum_{i=1}^{15} RF_i}{15}$$

4.2 Analyte Concentration

The analyte concentration is calculated as follows:

$$\text{Analyte Conc (ppb)} = \text{Conc}_{Nat} = \frac{(\text{Area}_{Nat})}{(RF_{Avg})(\text{Area}_{Is})}$$

where: RF_{Avg} = Average response factor
Area_{Nat} = Area of response for the native daughter ion for the sample
Area_{Is} = Area of response for the internal standard daughter ion for the sample
Conc_{Nat} = Calculated amount (ppb in the sample), uploaded from the MS

4.3 Recovery in Spiked Validation Samples

$$\% \text{ Recovery} = \frac{\text{Conc}_{Nat}}{\text{Spike Level}} \times 100\%$$

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. Preparation of Detector Linear Response and Calibration Standards

Combine and dilute to 50 mL with water:100 mM ammonium acetate in MeOH 19:1 (v:v)								
Native Conc. in 50-mL water[1]	Sample equivalent in 50-mL water[2]	Amount of Native Added per 50-mL Sample Equivalent		Internal Std Conc. in 50-mL water	Internal Std Sample Equivalent in 50-mL water	Internal Standard Added per 50-mL Sample Equivalent[3]		Amount of ACN
ng/mL	ppb	ng	µL Solution	ng/mL	ppb	ng	µL Solution[4]	mL
0	0	0	0	5	0.1	250	250	1.25
0.5	0.01	25	250[5]	5	0.1	250	250	1.0
1.5	0.03	75	750[5]	5	0.1	250	250	0.5
2.5	0.05	125	125[6]	5	0.1	250	250	1.125
5.0	0.10	250	250[6]	5	0.1	250	250	1.0
25	0.50	1250	125[7]	5	0.1	250	250	1.125

[1] Sample concentration levels in 50-mL sample equivalents.
[2] The final volume is 1 mL.
[3] Internal standard concentration is 0.1 ppb (sample equivalents) in all samples.
[4] 1.0-µg/mL mixed internal standard spiking solution from Section 3.5.1.4.
[5] 0.1-µg/mL mixed native solution from Section 3.5.1.2.
[6] 1.0-µg/mL mixed native solution from Section 3.5.1.2.
[7] 10.0-µg/mL mixed native solution from Section 3.5.1.2.

Table 2. MS/MS Selected Ions

Substance	Mode	m/z Parent	m/z Daughter	Collision energy (eV)
MKH 5730	ESI-	395	127.6	16
MKH 5730-methoxy-d_3	ESI-	398	130.6	16
MKH 6562 sulfonamide	ESI-	240	84.5	24
MKH 6562 sulfonamide-d_3	ESI-	243	84.5	24
MKH 6562 sulfonic acid	ESI-	241	84.5	25
MKH 6562 sulfonic acid-d_3	ESI-	244	84.5	25
N,O-dimethyltriazolinone	ESI+	130	114.5	-20
N,O-dimethyltriazolinone-methoxy-d_3	ESI+	133	114.5	-20

ESI = Electrospray Ionization

Table 3. The Instrument Control Language (ICL) commands used in the MS

```
apion; on; pos; cidon; cent; minfwidth=30; merge=80
apause, valveon
while rt<3
        go; stop; end
aresume; valveoff
while rt<5.75
        dau 130, 114.5, 115, 1, -20; go; stop
        dau 133, 114.5, 115, 1, -20; go; stop; end
neg;
while rt<6.75
        dau 241, 84.5, 85, 0.5, 25; go; stop
        dau 244, 84.5, 85, 0.5, 25; go; stop; end
while rt<9.0
        dau 395, 127.6, 128, 0.3, 16; go; stop
        dau 398, 130.6, 131, 0.3, 16; go; stop
        dau 240, 84.5, 85, 0.3, 24; go; stop
        dau 243, 84.5, 85, 0.3, 24; go; stop; end
apause; valveon
```

Table 4. Sample Fortification

Sample Fortification Level, (ppb)	Amount of Native Added per 50 mL sample			Native Conc. in final Soln (1-mL)	Amount of Internal Std Added per 50 mL Sample			Internal Std Conc. in final Soln (1-mL)
	ng	µL Spike Solution	Original Conc. (ng/mL)	ng/mL	ng	µL Spike Solution	Original Conc. (ng/mL)	ng/mL
Control	0	0	0	0	5	50 [3]	0.1	5
0.03	1.5	150 [1]	0.03	1.5	5	50 [3]	0.1	5
0.05	2.5	25 [2]	0.05	2.5	5	50 [3]	0.1	5

[1] 0.01-µg/mL mixed native solution from Section 3.5.1.2.
[2] 0.1-µg/mL mixed native solution from Section 3.5.1.2.
[3] 0.1-µg/mL mixed internal standard from Section 3.5.1.4.

Measure 50-mL ± 0.5 mL of groundwater samples into 100-mL graduated cylinders.

↓

Add 50-µL of the 0.1 µg/mL mixed internal standard solution (Section 3.5.1.4) and shake.

↓

Acidify the sample with 1-mL of 1 N hydrochloric acid and shake.

↓

Condition a 2 g (12 cc) MegaBond C_{18} SPE cartridge (Varian) by rinsing it with 10-mL MeOH followed by 10-15 mL water. Do not allow the cartridge to go to dryness.

↓

Pass the sample through the cartridge at a rate of 20-30 mL/min.

↓

Add 10 mL HPLC grade water onto the cartridge. Purge the cartridge with air and dry the cartridge under vacuum for 1-2 minutes.

↓

Elute the sample with 10 mL of MeOH : 5% ammonium hydroxide (9:1, v:v), and collect it in a centrifuge tube.

↓

Evaporate the sample to dryness with nitrogen or turbo-evaporator (40-45 °C).

↓

Reconstitute it to 1.0 mL with water:100 mM ammonium acetate in methanol (19:1, v:v). Filter the extract with nylon acrodisc (Gelman) into HPLC vial.

↓

Analyze by LC/ESI/MS/MS.

Figure 1. Analytical scheme for sample analyses (See Section 3.5.3 for method details)