TECHNICAL PROCEDURE

Analytical Method No. D0103

THE DETERMINATION OF RESIDUES OF BAS 670 H AND ITS METABOLITES IN SOIL USING LC-MS/MS

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This technical procedure consists of 15 pages

1. Introduction

1.1 Scope of the method

BAS 670 H is a new herbicide used for corn in the US, Canada and Europe. For registration of the herbicide and for establishing the DT50/90 values from field dissipation study in these use patterns, a residue analytical method with a limit of quantitation of 0.001 mg/kg for the active ingredient and its degradate (M670H05) in soil is developed.

The method (D0103) has a limit of quantitation of 0.001 mg/kg in soil for each analytes.

BASF Method D0103 was originally developed **(Reference 1)** as a LC-MS/MS residue method for the analysis of BAS 670 H and its metabolite, 3-(4,5-dihydro-isoxazol-3-yl)-4-methanesulfonyl-2-methylbenzoic acid (M670H05). This method was further modified to determine the residues of BAS 670 H and its metabolite, 3-(4,5-dihydro-isoxazol-3-yl)-4-methanesulfonyl-2-methylbenzoic acid (M670H05) in US type soil at BASF Corporation, Research Triangle Park, N.C.

A 20 g soil sample aliquot was extracted three times by shaking with methanol-phosphate buffer, 50:50,v/v. The combined extract was concentrated to 25 mL. A post-extraction sample clean-up was performed with an aliquot (40 %) of the extract by using solid phase extraction (coupled SAX/C_{18}) cartridges. The eluent from the C_{18} column is evaporated to dryness and re-dissolved with water containing 0. 05% ammonium hydroxide for HPLC-MS/MS determination.

2. Materials

Standard substances are stored in a freezer (<-5^{0}C) until use. Information on the characterization of these substances is available from BASF and is located at the Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany. Detail description of the Test and Reference Substances is provided in **Section II.**

2. Materials (Continued)

2.2 Equipment -- Suggested Sizes/Suppliers, Manufacturers

Method Step	Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
2.4.2.1	Balance, Analytical	Model AT100	Mettler	
Various	Balance, Top Loading	Model PM 4800	Mettler	
Various	Bar, Magnetic Stirring	2 inch lengths	Various	
2.4, 3.2.5	Bottle, Amber glass	Qorpak , 2 oz, 4 oz and 8 oz with Teflon®-lined screw cap	Qorpak	
3.2.1-3.2.4	Centrifuge	Refrigerated Centrifuge Model CS-6KR	Beckmann	
3.1, 3.2	Centrifuge Tubes (Teflon®)	50 mL	VWR	21009-477
3.2.1-3.2.4	Centrifuge Adapter	for 50 mL tubes	VWR	
Various	Cylinder, Graduated	Various sizes	Various	
3.2.1-3.2.4	Filter paper	7.0 cm, Whatman 4	VWR	1004070
Various	Flask, Erlen Meyer, 24/40	1000 mL	Various	
Various	Flask, Volumetric	10, 25 and 50 mL	Various	
Various	Flask, Flat Bottom	500 mL	Various	
3.2.2	Funnel, long stem; glass	top i.d (65 mm), stem o.d.(8.0 mm) and stem length (65 mm)	Various	
3.4	Gelman PTFE acrodisc	0.45 um, 13 mm	Gelman Science	4422
Various	Hot Plate, Magnetic Stirring		Various	
Various	Pipet, Volumetric	0.5, 1-10, 15, 25 mL	Various	
Various	Pipet, disposable	2 and 10 mL, Pyrex	VWR	53283-776
3.2.6, 3.3.1.3	Nitrogen-Evaporator	Model 112	Organomation Assoc.	11250
3.2.1-3.2.4	Laboratory Shaker	Model HS501-D	Janke and Kunkel	
Various	Rotary Evaporator	Buchi RE 111 or R-124 or Labconco 78892-00	Brinkmann (VWR)	

2. Materials (Continued)

Method Step	Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
Various	Rotary Evaporator Trap (Anticlimb)	250 mL, 24/40	Aldrich	Z16,405-4
3.3	Solid Phase Extraction Manifold		J.T. Baker or Baxter Healthcare Corporation	
Various	Spatula		Various	
Various	Stopper, Teflon®	24/40	Various	
3.4	Syringes, plastic, disposable	1 mL	Various	
Various	Ultrasonic Bath	Model FS 7652H	Fisher Scientific	
3.4	Vials, HPLC Snap caps	11 mm; 1.5 mL 11 mm; PE w/ TFE/GR;SILICONE	VWR Sun Brokers International	66010-539 500-352
Various	Vials, Collection, PTFE screw cap	1 oz	VWR	GLC-01008
Various	Vials, Collection, PTFE screw cap	12 mL	VWR	66009-985
Various	Votex mixer	Genie 2	Fisher Scientific Co	12-812
3.3	Vacubrand vacuum pump/controller	Model HS501-D	Elnik Systems, Inc.	
3.4	LC-MS	API 3000 Biomolecular Mass Analyzer	PE Sciex	

NOTE Other general laboratory glassware and equipment may be needed. Equipment with equivalent performance may be used, as required.

2. Materials (Continued)

2.3 Reagents and Chemicals -- Suggested Sources

2.3.1 Chemicals

Chemical	Grade	Manufacturer/ Supplier	Catalog Number
Acetonitrile	High Purity	B & J	015-4
Ammonium Formate	MicroSelect >99%	Fluka	09735
Ammonium hydroxide	20-30%	Fluka	09735
Celite	545 grade	J. T. Baker	3371-01
Formic Acid	98%	E.M. Science	FX0440-7
Glass wool, Silanized		J. T. Baker	7084-05
Methanol	High Purity	B & J	230-4
Sodium Phosphate, monobasic, diydrate	ACS Reagent grade	J.T. Baker	3819-01
Sodium Phosphate, dibasic, anhydrous	Reagent grade	J.T. Baker	3828-01
Pre-pack SPE Column, SAX	Mega BE, 12 mL, 2.0 g	Varian	12256021
Pre-pack SPE Column, C_{18}	Bond Elut, 6 mL, 500 mg	Varian	12102052
Water	High Purity	B & J	365-4

NOTE: Equivalent reagents and chemicals from other suppliers may be substituted.

2.3.2 Solvent Mixtures and their Preparation

Solvent Mixtures	Method Step
Solution I: Add 31.2 g of $NaH_2PO_4.2H_2O$ (MW 155) and 1L of water into a 1L Erlenmeyer flask. Mix well to ensure complete homogeneous solution. The molarity and pH of the solution is 0.2M and 4.0, respectively.	--
Solution II: Add 35.6 g of Na_2HPO_4, anhydrous (MW 142) and 1L of water into a 1L Erlenmeyer flask. Mix well to ensure complete homogeneous solution. The molarity and pH of the solution is 0.25M and 10.0, respectively.	--
Solution III: Add 27 mL of **Solution I**, 473 mL of **Solution II** and 500 mL of water into a 1L Erlenmeyer flask. Mix well to ensure complete homogeneous solution. The pH of the solution is 9.0.	3.2.6

2. Materials (Continued)

Solution IV (Extraction Solvent): Methanol- **Solution III**, 50:50, v/v: Add 500 mL of methanol and 500 mL of **Solution III** into a 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution. **Note:** Significant amount of salt precipitation may occur while standing. This solution should be prepared freshly prior to the extraction	3.2
Solution V: Add 10 mL of formic acid (98%) and 250 mL of **Solution III** into a 1L Erlenmeyer flask. Mix well to ensure complete homogeneous solution. The pH of the solution is about 3.	3.3.1.2, 3.3.1.3
Solution VI: 0.05% ammonium hydroxide in water: Add 0.5 mL of ammonium hydroxide (28-30%) into a 300 mL volumetric flask and mix well to ensure complete homogeneous solution.	2.4.2 and 3.4
Solution VII: Acetonitrile- **Solution VI** (9:1, v/v): Add 90 mL of acetonitrile and 10 mL of **Solution VI** into a 100 mL volumetric flask and mix well to ensure complete homogeneous solution.	3.3.1
LC-MS Mobile Phase A: Water with 4 mM ammonium formate: Add 252 mg of ammonium formate into a 1L volumetric flask. Mix well to ensure complete dissolution of the ammonium formate. Dilute to the mark with water and mix well to ensure complete homogeneous solution.	3.5
LC-MS Mobile Phase B: Methanol with 4 mM ammonium formate	3.5

2.4 Standard Solutions

2.4.1 Standard Solution Storage Stability

Standard solutions are kept refrigerated. The storage stability of standard solutions made in methanol and any other solvent will be established during the course of the study. BASF recommends that stock solutions (1 mg/mL) in methanol be made fresh every three months. Dilution of stock solutions should be stored refrigerated no longer than one month or according to their established storage stability in a particular solvent.

2.4.2 Standard Solutions

2.4.2.1 Stock solutions (1 mg/mL)

BAS 670 H

Prepare a 1.0 mg/mL BAS 670 H stock solution by weighing an appropriate amount of BAS 670 H into a volumetric flask. Dissolve with methanol and dilute to mark. For example, to prepare a 25 mL stock solution, place 25.0 mg of BAS 670 H into a 25 mL volumetric flask. Dissolve and dilute to mark with methanol. Sonicate and vortex to ensure a complete homogeneous solution.

2. Materials (Continued)

M670H05
Prepare a 1.0 mg/mL stock solution by weighing an appropriate amount of M670H05 into a volumetric flask. Dissolve with methanol and dilute to mark. For example, to prepare a 25 mL stock solution, place 25.0 mg of M670H05 into a 25 mL volumetric flask. Dissolve and dilute to mark with methanol. Sonicate and vortex to ensure a complete homogeneous solution.

2.4.2.2 Mix Standards for Fortifications

BAS 670 H and M670H05 in water
Prepare a 20 µg/mL mixed standard solution for fortification by combining 2.0 mL of each of the BAS 670 H and of M670H05 stock solutions (2.4.2.1) into a 100 mL volumetric flask. Dilute to mark with water. Sonicate and vortex to ensure a complete homogeneous solution. Prepare serial dilution's of this combined solution as needed. Suggested concentrations of mixed standards for fortifications are 20, 2.0, 0.2 and 0.02 µg/mL, in water.

2.4.2.3 Injection Standard Solutions of BAS 670 H and M670H05 for LC-MS/MS Analysis (Calibration Standards): 10, 5.0, 2.0, and 1.0 pg/µL in Solution VI.

Prepare a 10.0 pg/µL mixed injection standard solution by transferring an appropriate amount of the 0.2 µg/mL of each fortification solutions (2.4.2.2) with a volumetric pipet into a volumetric flask containing **Solution VI.** Add 5 mL of the 0.2 µg/mL of each fortification solutions into a 100 mL volumetric flask and dilute to the mark with **Solution VI**. Do not use the 0.02 µg/mL for the preparation of 10.0 pg/µL mixed injection standard. The proposed procedure for the preparation of the 10.0 pg/µL mixed injection standard maintains the required concentration of ammonium hydroxide for LC-MS/MS analysis. Prepare serial dilutions of this solution as needed. Suggested concentrations of mixed standards are 10, 5.0, 2.0 and 1.0 pg/µL, in **Solution VI.**

NOTE: Use amber bottles or clear vials with Teflon®-lined screw caps as storage containers for standard solutions. Suggested standard concentrations are listed here. A different concentration scheme may be used and additional standards may be prepared as needed.

3. Analytical Procedure

3.1 Sample Preparation

Bulk soil samples received from the field are homogenized using a blender or mill. Homogenized soil samples are stored frozen (<-5°C) before analysis. Weigh a 20 g

3. **Analytical Procedure (Continued)**

or to the nearest tenth of a gram aliquot of the soil sample into a 50 mL Teflon® centrifuge bottle.

3.2 Fortification and Extraction

3.2.1 For the fortification samples, add an appropriate volume of mixed standard solution of BAS 670 H and 3-(4,5-dihydro-isoxazol-3-yl)-4-methanesulfonyl-2-methylbenzoic acid (M670H05) to the respective control sample by volumetric pipet. For example, for a 0.001 ppm fortification sample, pipet 1 mL of the 0.02 µg/mL mixed standard solution of BAS 670 H and 3-(4,5-dihydro-isoxazol-3-yl)-4-methanesulfonyl-2-methylbenzoic acid onto a control sample.

3.2.2 Add 25 mL **Solution IV** in portion into the centrifuge bottle containing the soil and vortex to mix the solvent to the soil. Place the centrifuge bottle horizontally in the shaker and shake at 300 RPM for 30 minutes. Centrifuge at 3000 rpm for 5 minutes at 20°C. Attach a funnel fitted with whatman filter paper into a 500 mL flat bottom flask, transfer the supernatant by decantation through the funnel and collect. Rinse the filter paper with 5-10 mL of **Solution IV** (use a disposable pipet) and collect.

3.2.3 Add 25 mL aliquot of **Solution IV** into the soil marc, sonicate for 10 minutes and vortex to loosen the soil and allow to mix to consistency. Mix well to obtain a homogeneous suspension. Repeat the extraction step above (3.2.2) for 30 minutes. After centrifugation, transfer the supernatant into the above 500 mL flat bottom flask by decantation through the funnel and collect. Rinse the filter paper with 5-10 mL of **Solution IV** (use a disposable pipet) and collect.

3.2.4 Add 25 mL aliquot of **Solution IV** into the soil marc, sonicate and vortex to loosen the soil and allow to mix to consistency. Mix well to obtain a homogeneous suspension. Repeat the extraction step above (3.2.2) for 30 minutes. After centrifugation, transfer the supernatant into the above 500 mL flat bottom flask by decantation through the funnel. and collect. Rinse the filter paper with 5-10 mL of **Solution IV** (use a disposable pipet) and collect.

NOTE:
1. Centrifugation must be continued until the solid residue forms a compact pellet. If the soil extract is heavy (dark non-homogeneous appearance), centrifuge at 4000 rpm with small no of samples (2-4 samples) to obtain an adequate centrifugation. This will prevent column clogging (section 3.3).
2. In case of heavy clay soil, use a flat head spatula to break the soil marc before sonication.

3.2.5 Attach a rotary evaporator trap ***(Anticlimb; VWR Cat. No. 570200-0124)*** with the 500 mL flat bottom flask and evaporate the extract carefully to about 15-20 mL

3. Analytical Procedure (Continued)

using a rotary evaporator with the water bath temperature set approximately at 60°C (set vacuum initially at about 300 mbar and then gradually reduce to about 35 mbar).

3.2.6 Swirl and sonicate the extract to dissolve the dry residue from the side of the 500 mL flat-bottom flask and transfer the extract to a volumetric flask (25 mL). Rinse the 500 mL flask with **Solution III.**

Bring the volumes of the combined extracts to 25 mL with the buffer solution **Solution III.** **Mix well and to obtain a homogeneous extract**.

NOTE:
- It is absolutely necessary to use a 500 mL flat bottom flask and an anticlimb rotary evaporator trap (*(VWR Cat. No. 570200-0124)* to avoid bumping due to excessive frothing and to reduce the time for evaporation. It takes about 10-15 minutes to concentrate 75 mL of extract to about 20 mL.
- Do not to allow the samples to go to dryness. This causes low recovery. If the sample goes to dryness, do not proceed to the next step. Start over with a new soil sample.
- To determine how much 20-25 mL of solution represents in a 500 mL flask during rotary evaporation, it is suggested that the analyst add 20 mL of water into an empty flask prior to conducting step 3.2.7 and compare. This will give the analyst a "picture" of how much 20 mL of solution is and prevent over evaporation.
- Extract should be stored at room temperature. This is a good stopping point of the method. The extract should be sonicated and vortexed, before transferring an aliquot.

3.2.7 Vortex and sonicate the extract in the volumetric flask and transfer a 10 mL aliquot of the extract (3.2.6) into a 25 vial (VWR Catalog number GLC-01008). Hold the extract for sample clean-up (Section 3.3.1.3).

3.3 Sample Clean-up:

3.3.1 Solid Phase Extraction (SAX coupled with C_{18})

3.3.1.1 Column Preparation

Connect a pre-pack SAX SPE column on the top of a pre-pack C_{18} SPE column with an aid of a sampling adaptor. Connect the coupled column to a solid phase extraction manifold by attaching the C_{18} SPE column at the bottom.

3. **Analytical Procedure (Continued)**

NOTE:
1. If the soil extract is heavy (dark non-homogeneous appearance), there is a possibility of column clogging.
   In this case, use the following procedure to prepare the column:
   Apply vacuum and add some (enough to make I inch bed) silanized glass wool on the top of the frit of SAX SPE column. Add 1 g of celite on the top of the pcked glass wool

2. Vacuum (~ 600-500 mbar) is applied for the extraction using solid phase extraction manifold. It is advisable to "stabilize" on vacuum to obtain good recoveries of the analytes.

3. Use 25 mL vial (VWR Catalog number GLC-01008) for collection

3.3.1.2 **Column Conditioning**

Condition the coupled column by passing through 5 mL acetonitrile followed by 10 mL of buffer solution **Solution V**. **Allow the solvents to pass through just below the bed (top frit) of C_{18} column**. Do not allow the column to go dry as well as make sure there is no acetonitrile left above the bed line of the bottom column.

3.3.1.3 **Loading, Washing and Elution**

Add 1.0 mL of formic acid to the extract from section 3.2.7 and vortex gently to mix.

NOTE: Caution should be taken while vortexing the samples. Vigorous mixing will cause sample loss. Not enough mixing will cause low recoveries.

Transfer (load) the acidic extract to the top of the conditioned coupled columns. Apply the vacuum (~ 500 mbar) and allow the solvents to pass through. Collect the eluent in to the same vial that contains the conditioning solvent. Add 5 mL of **Solution V** to the vial that contained the extract from section 3.2.6 and vortex to wash. Add the wash to the top of the coupled column. Apply the vacuum (~ 500 mbar) and allow the solvents to pass through. Do not allow the column to go dry.

Disconnect the vacuum and detach the SAX SPE column (top) and the sampling adaptor. Add 4 mL of deionized water to the top of the C_{18} column. Apply the vacuum (~ 700 mbar) and allow the solvents to pass through. Do not allow the column to go dry. Disconnect the vacuum. Remove the collection vial and discard all the solutions. Replace the needles from the SPE manifold with clean and dry ones. Replace also the collection vials to collect the eluent that is obtained after the following step.

Add 10 mL of acetonitrile followed by 10 mL of **Solution VII** to the top of the C_{18} column. Apply the vacuum (~ 700 mbar) and allow the solvents to pass through. Do not allow the column to go dry in between elution. Evaporate off the combined

3. Analytical Procedure (Continued)

eluent at 50-60°C under a stream of nitrogen using a N-evaporator. Remove the samples immediately after evaporation.

NOTE:
1. It is recommended to apply constant vacuum about 500-600 mbar throughout the entire extraction procedure
2. The water bath temperature of the N-evaporator should be at about 60°C. The evaporation time of the extract is about 0.5 hour.
3. Use of a wide mouth vial (~ 25 mL capacity, VWR catalog number GLC 01008) accelerates the eveporation
4. It is recommended to clean the needles of the N-evaporation periodically. Use the following procedure
   a. Sonicate the needles in 50 % methanol and water
   b. Rinse with methanol
   c. Dry under slow stream of nitrogen

NOTE: (Optional)
1. If the recoveries are higher than expected (i>130 %) due to matrix enhancement (generally determined by fortifying the control extract with a known amount of standard solution), use the following procedure to prepare the extract prior to SAX-C_{18} clean-up (Section 3.3.1.1 through 3.3.1.3):

a. Connect an empty glass column fitted with a frit (VWR Catalog No. 7121-06) to a solid phase extraction manifold. Apply vacuum and add some (enough to make I inch bed) silanized glass wool in the column. Add about 1.5 g of celite to the top of the packed glass wool.

b. Add 1 mL of formic acid to the 10 mL of extract from Section 3.2.7 and gently mix the solution. Filter the solution through the celite. Add 5 mL of **Solution V** to the vial and vortex to wash. Add the wash to the top of the column. Use 25 mL vial (VWR Catalog number GLC-01008) for collection.

c. Perform Section 3.3.1.1 through 3.3.1.3, but do not acidify the extract again with 1 mL of formic acid prior to on to the SAX-C_{18} column.

3.4 Sample Preparation for LC-MS/MS Analysis

3.4.1 For LC-MS/MS determination, add 4 mL of **Solution VI** to the dry residue (Section 3.3.1.3). Swirl, sonicate and vortex to ensure a complete homogeneous solution. Typically the following procedures are used to prepare the samples for analysis:

3.4.2 **For control and 0.001 ppm fortifications**, filter the solution through a syringe filter (a 0.45 micron Gelman membrane filter fitted to 1.0 mL disposable plastic syringe). Transfer the sample solution (3.4.1) with a glass disposable pipette to the syringe and collect the filtrate (about 1-2 mL) into an injection vial.

3. Analytical Procedure (Continued)

3.4.3 **For 0.01 ppm fortifications**, take 1 mL of the sample solution (3.4.1) and dilute to 10 mL with **Solution VI**. Sonicate and vortex to ensure a homogeneous solution. Filter the solution into the injection vial using the procedure above. The sample is ready for injection.

3.5. Instrumentation

Suggested LC-MS/MS Operating condition:

Instrument:	PE Sciex API 3000 Biomolecular Mass Analyzer
Inlet [HPLC System]:	PE Series 200 Micro Pump system with Series 200 Autosampler
Data System:	Analyst 1.1
Column:	Phenomenex Columbus C_{18}, 5µ, 100 X 2.0 mm, [P/N 00D-4108-B0]
Injection:	Typically 20 µL
Mobile Phase: [Gradient]	A = water with 4 mM ammonium formate B = methanol with 4 mM ammonium formate Time (min.) Composition 0.0 85% A + 15% B 0.5 85% A + 15% B 1.0 77% A + 23% B 3.0 62% A + 38% B 4.0 27% A + 73% B 4.5 10% A + 90% B 5.0 10% A + 90% B 5.1 85% A + 15% B 5.6 85% A + 15% B Run every 5.6 minutes
Flow Rate:	300 µL/minute

	BAS 670 H	M670H05
Expected Retention Times	4:63 minutes	2:49 minutes
Transitions:	362.1 →334.0	282.0 →238.0
Ionization Mode:	Negative ion for all analytes; Turbospray (375°C)	

3. Analytical Procedure (Continued)

NOTE:

1. The equipment listed was used for method development and validation. Other equivalent hardware may be used. The use of a guard column is optional.
2. The recommended instrument parameters were found to be optimal for the instrument used for the method validation. The exact values used must be optimized for each instrument.
3. The recommended chromatographic systems were found to be optimal for the types of instrument used for the method validation. Different chromatographic systems might be necessary to be developed for different type of instrument.

3.6 Calibration Procedures

Calculation of results is based on peak area measurements using a calibration curve. The standard curve is obtained by direct injection of 20 µL of the mixed BAS 670 H and M670H05 standards for LC-MS/MS in the range of 1.0 pg/µL to 10.0 pg/µL. In a given injection run, the same volume is used for all samples and standards. Typical standard amounts injected on-column range as follows: 20, 40, 100 and 200 pg.

Prepare calibration curves by plotting the peak area (monitoring transitions 362.1→334, and 282→238 for mixed BAS 670 H and M670H05, respectively, versus the weight of mixed BAS 670 H and M670H05, respectively, using a linear least squares working curve in the form $y = bx + c$.

Establish the stability of the detection response by injecting several concentrations of standards. For analysis, alternate samples and standards. For each injection set, the set should begin and end with standard injections, and each standard level should be injected at least in duplicate.

Note: It is advisable to "stabilize" on column retention time of the analytes before injecting the first sample of an analytical series.

3.7 Limit of Quantitation and Limit of Detection

The limit of quantitation is defined as the lowest fortification level successfully tested. The limit of quantitation is 0.001 ppm for BAS 670 H and its metabolite, M670H05. The limit of detection has not been determined, but the lowest standard for each analyte in calibration curve has good detectability (signal to noise ratio greater than 3:1).

4. Calculation of Results

Typical recovery calculations for Method D0103 are shown in **Figure 2.**

5. Time Requirement for Analysis

The time required for a set of 13 samples (10 fortified, 2 controls and one reagent blank) is approximately 8 person-hours, or 1 calendar days, provided that no special problems arise, such as matrix interference.

6. Confirmatory Techniques

The method allows for the determination of BAS 670 H and M670H05 using LC-MS/MS which is a highly selective and self confirmatory detection technique. Therefore, no confirmatory technique is required.

7. Potential Problems

In case of clay soil, the soil marc has to be broken completely after the first centrifugation in the extraction step in order to obtain acceptable recovery. ***The glass ware used for the method should be thoroughly rinsed with water and methanol to prevent contamination. Peak enhancement is a potential problem without sufficient sample clean-up.***

It is also highly recommended to perform instrument check routinely during LC-MS/MS analysis for analyte peak enhancement or suppression. During method development, it was observed that the response of the BAS 670 H could be enhanced or suppressed due to a heavy load of matrix (soil residue) in the LC-MS/MS analysis. As a result increased/decreased sensitivity (high/low signal) of the target analytes (especially BAS 670 H)) and chromatograms with choppy base lines, were produced. These problems could be observed or diagnosed by an instrument check sample prior or during the actual sample analysis. The instrument check sample is basically prepared by adding known amount of standard to the control matrix at the limit of quantitation. (this case 1 ppb level). Once the problem is observed, it was absolutely required to clean the LC-MS thoroughly. Some of the cleaning procedure included exhaustive cleaning of the hardware, such as skimmer, fused silica for sample introduction, and several gradient systems to wash the column.

8. Safety and Health Considerations

All procedures involving organic solvents should be performed in a well-ventilated hood. Personal protective equipment (gloves, lab coats) should be worn while performing this method. Read all label statements and precautions.