BASF Crop Protection
BASF Agricultural Research Center ▪ Research Triangle Park, North Carolina, USA

BASF
The Chemical Company

FINAL TECHNICAL PROCEDURE

Analytical Method Number D1001

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

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This technical procedure consists of 22 pages

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1. Introduction

1.1 Scope of the method

BAS 670 H is a herbicide used for corn in the US, Canada and Europe. For registration of the herbicide and for establishing the DT50/90 values from aquatic field dissipation study in this use pattern, residue analytical method (D1001) in water and sediment is developed, with a limit of quantitation of 0.001 mg/kg for the active ingredient and its metabolites M670H01, M670H05 and M670H10 at BASF Corporation, Research Triangle Park, N.C.

The method (D1001) has a limit of quantitation of 0.001 mg/kg in water and sediment for each analyte. A brief description of the analytical procedure is described below:

Water:
A 2 mL water sample aliquot extraction is performed by using a solid phase extraction cartridge (Reverse Phase, OASIS™ HLB). Then 0.05% ammonium hydroxide in water is added to the eluent from the HLB column. The organic solvent is removed and the volume is adjusted with 0.05% ammonium hydroxide in water and the residues are determined by HPLC-MS/MS in negative ion mode.

Sediment:
A 10 g sediment sample aliquot was extracted three times by shaking with methanol-phosphate buffer, (1:1,v/v). The combined extract was concentrated to 10 mL. A post-extraction sample clean-up was performed with an aliquot (2 mL, 20 %) of the extract by using a solid phase extraction cartridge (Reverse Phase, OASIS™ HLB). Then 0.05% ammonium hydroxide in water is added to the eluent from the HLB column. The organic solvent is removed and the volume is adjusted with 0.05% ammonium hydroxide in water and the residues are determined by HPLC-MS/MS in negative ion mode.

2. Materials

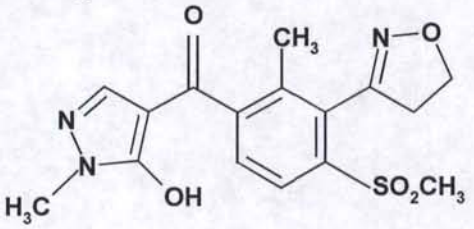
Standard substances are stored in a freezer (<-5^{0}C) until use. Information on the characterization of these substances is available from BASF and is located at the Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany. Detail description of the Test and Reference Substances is provided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

2. Materials (Continued)

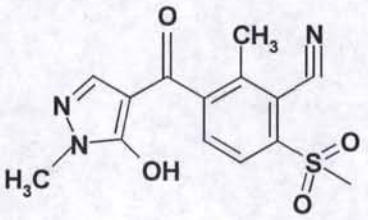
2.1. TEST AND REFERENCE SUBSTANCES

2.1.1 Fortification Compounds

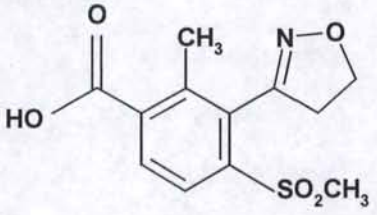
BASF Code Name: BAS 670 H
BASF Registry Number: 375080
Molecular Formula: $C_{16}H_{17}N_3O_5S$
Molecular Weight: 363.4 g/mol
Structural Formula:



BASF Code Name: M670H01
BASF Registry Number: 417882
Molecular Formula: $C_{14}H_{13}N_3O_4S$
Molecular Weight: 319.3g/mol
Structural Formula:

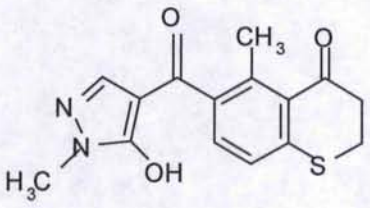


BASF Code Name: M670H05
BASF Registry Number: 388010
Molecular Formula: $C_{12}H_{13}NO_5S$
Molecular Weight: 283.3g/mol
Structural Formula:



2. Materials (Continued)

BASF Code Name:	M670H10
BASF Registry Number:	4969168
Molecular Formula:	$C_{15}H_{14}N_2O_3S$
Molecular Weight:	302.4 g/mol
Lot No.:	L67-124
Purity	96.8%
Expiration date:	October 1, 2010
Structural Formula:	



2.1.2 Reference Standards (used for calibration)

Same as fortification compounds (section 2.1.1)

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	5, 10, 25 and 50 mL	Various	
Various	Flask, Flat Bottom	250 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	Nitrogen-Evaporator	Model 112	Organomation	11250
Various	Pipet, Volumetric	0.5, 1-10, 15, 25 mL	Various	
Various	Pipet, disposable	1, 5 and 10 mL, Pyrex	VWR	
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, 40 mL	VWR	
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/MS	API 4000	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
Ammonium Formate	MicroSelect >99%	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, OASIS™ HLB	3 c.c, 60 mg	Waters	WAT 094226
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 a 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 a 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 a homogeneous solution. The pH of the solution is 9.0.	3.3.6
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 a homogeneous solution. **Note:** Significant amount of salt precipitation may occur while standing. This solution should be prepared fresh prior to the extraction.	3.3

2. Materials (Continued)

Solution V: Add 10 mL of formic acid (98%) and 250 mL of **Solution III** into a 1L Erlenmeyer flask. Mix well to ensure a homogeneous solution. The pH of the solution is about 3.	3.3.8, 3.3.9
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	3.2.4, 3.2.5, 3.3.9, 3.3.10
Solution VII: 9% formic acid in water: Add 9 mL of formic acid (98%) into a 100 mL volumetric flask and mix well to ensure complete homogeneous solution	3.2.2
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 a homogeneous solution.	3.4
LC-MS Mobile Phase B: Methanol with 4 mM ammonium formate	3.4

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 the mark. For example, to prepare a 10 mL stock solution, place 10.0 mg of BAS 670 H into a 10 mL volumetric flask. Dissolve and dilute to the mark with methanol. Sonicate and vortex to ensure a homogeneous solution.

M670H01

Prepare a 1.0 mg/mL M670H01 stock solution by weighing an appropriate amount of M670H01 into a volumetric flask. Dissolve with methanol and dilute to the mark. For example, to prepare a 10 mL stock solution, place 10.0 mg of M670H01 into a 10 mL volumetric flask. Dissolve and dilute to the mark with methanol. Sonicate and vortex to ensure a homogeneous solution.

2. Materials (Continued)

M670H05

Prepare a 1.0 mg/mL M670H05 stock solution by weighing an appropriate amount of M670H05 into a volumetric flask. Dissolve with water and dilute to the mark. For example, to prepare a 10 mL stock solution, place 10.0 mg of M670H05 into a 10 mL volumetric flask. Dissolve and dilute to the mark with water. Sonicate and vortex to ensure a homogeneous solution.

M670H10

Prepare a 1.0 mg/mL M670H10 stock solution by weighing an appropriate amount of M670H10 into a volumetric flask. Dissolve with methanol and dilute to the mark. For example, to prepare a 25 mL stock solution, place 25.0 mg of M670H10 into a 25 mL volumetric flask. Dissolve and dilute to the mark with methanol. Sonicate and vortex to ensure a homogeneous solution.

2.4.2.2 Mix Standards for Fortifications

BAS 670 H, M670H01, M670H05, and M670H10 in Water

Prepare a 10 µg/mL mixed standard solution for fortification by combining 1.0 mL of each of the BAS 670H, M670H01, M670H05, and of M670H10 stock solutions (2.4.2.1) into a 100 mL volumetric flask. Dilute to the mark with DI water. Sonicate and vortex to ensure a homogeneous solution. The serial dilutions of this mixed standard were also made using DI water to yield a concentration range from 0.01µg/mL to 10 µg/mL.

2.4.2.3 Injection Standard Solutions of BAS 670 H, M670H01, M670H05, and M670H010 for LC-MS/MS Analysis (Calibration Standards): 5.0, 1.0, 0.5, 0.25 and 0.125 ng/mL in 0.05% Ammonium Hydroxide (Solution VI)

Prepare a 5.0 ng/mL mixed injection standard solution by transferring an appropriate amount of the 1 µg/mL of fortification solution (2.4.2.2) with a volumetric pipet into a volumetric flask and dilute to the mark with **Solution VI**.

2. Materials (Continued)

Do not use the 0.1 µg/mL for the preparation of 5.0 ng/mL mixed injection standard. The proposed procedure for the preparation of the 5.0 ng/mL mixed injection standard maintains the required concentration of methanol for LC-MS/MS analysis. Prepare serial dilutions of this solution as needed from 5.0 ng/mL in **Solution VI**. Suggested concentrations of mixed calibration standards are 5.0, 1.0, 0.5, 0.25 and 0.125 ng/mL 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 water and sediment samples received from the field stored frozen (<-5°C) before analysis.

3.2 Analytical Procedure for Water

3.2.1 Weighing and Fortification

Weigh 2 g to the nearest tenth of a gram or volumetrically measure a 2 mL aliquot of the water sample into a culture tube.

For the fortification samples, add an appropriate volume of standard solution of BAS 670 H, M670H01, M670H05, and M670H10 to the respective control sample by a volumetric pipet. For example, for a 0.001 ppm fortification sample, pipet 0.02 mL of the 0.1 µg/mL standard fortification solution (2.4.2.2) onto 2.0 mL (2.0 g) of control water sample using a micro-pipet. Attach a cap and vortex to mix. Mix well to obtain a homogeneous extract and proceed to Spep 3.2.3.

3.2.2 Column Preparation and Conditioning

Connect a pre-pack HLB SPE (3 cc, 60 mg) column on to a solid phase extraction manifold without any needle. Condition the column by passing through 1 mL methanol followed by 1 mL of 9% Formic Acid (**Solution VII**) using gravity. Allow the solvents to pass through just below the bed (top frit) of column. Do not allow the column to go dry.

Load:
Add 0.2 mL of 9% formic acid in DI water (**Solution VII**) to a 2 mL aliquot of the water sample in Step 3.2.1 and mix gently with an aid of a Pasteur pipet or vortex. Transfer (load) the acidic extract in portion (not more than half full) to the top of the conditioned column.

3. Materials (Continued)

3.2.3 Column Loading, Washing and Elution

NOTE:
Do not vortex at high speed which may cause splashing of the sample out of the vessel.

Wash:
Add 1 mL of **Solution VII** to the vial that contained the sample extract originally and vortex to wash. Add the wash to the top of the column. Using gravity, allow all of the solvent to pass through to remove last drop of **Solution VII** out of the needle tip. [In case, there are significant drop of liquid adhere to the barrel of the column, wipe it out with an aid of a Kimwipe]. Collect the eluent in to the same vial that contains the load and conditioning solvent.

Add 3.0 mL of deionized water to the top of the column and elute under gravity. Remove the collection vial and discard all the solutions. Add the needles from the SPE manifold. Replace also the collection vials with a 15 mL graduated centrifuge tube to collect the eluent that is obtained after the following step.

Elute:
Add 7 mL of methanol to the top of the column and allow all of the solvents to pass through. Collect the eluent in the 15 mL graduated centrifuge tube and proceed to Step 3.2.4.

NOTE:
• If there is a change of lot number for HLB Column, it is recommended to conduct column profile with known amount of analytes fortified into the extract before HLB clean-up. • Do not allow the sample to go to dryness. This will cause low recoveries of BAS 670 H and its metabolites

3.2.4 Sample Preparation for LC-MS/MS Determination
Add 2 mL of **Solution VI** in to the eluent obtained in Step 3.2.3 and concentrate the extract down to 2 mL volume level under nitrogen at about 60°C. Remove and allow the samples to cool to room temperature. Adjust the volume to 5 mL with **Solution VI**. Swirl, sonicate and vortex to ensure a complete homogeneous solution. Proceed to Section 3.2.4a.

3. Materials (Continued)

3.2.4a **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) into an injection vial.

3.2.4b **For procedurals and samples with residue higher than 0.001ppm-**
Dilute the samples from 3.2.4a with **Solution VI** to the appropriate volume to fit into the calibration curve. Vortex to ensure a homogeneous solution.

The samples are ready for injection.

NOTE:
A 0.45µm PTFE membrane filter from VWR (VWR P/N: 28145-497) will be used if the solution is cloudy or has insoluble particulate matter in solution prior to transferring the solution into an auto-sampler vial for analysis.

3.3 Analytical Procedure for Sediment

3.3.1 Weighing and Fortification

Weigh a 10 g or to the nearest tenth of a gram aliquot of the sediment sample into a 50 mL Teflon® centrifuge bottle or 50mL polypropylene free standing centrifuge tube. (VWR p/n: 82018-052) and proceed to Step 3.3.2

3.3.2 For the fortification samples, add an appropriate volume of mixed standard solution of BAS 670 H, M670H01, M670H05, and M670H10 to the respective control sample by volumetric pipet. For example, for a 0.001 ppm fortification sample, pipet 1 mL of the 0.01 µg/mL mixed standard solution of all analytes onto a control sediment sample.

3.3.2 Add 12.5 mL **Solution IV** into the centrifuge tube containing the sediment and vortex to mix the solvent with the sediment. Place the centrifuge tube horizontally in the shaker and shake at 300 RPM for 30 minutes. Centrifuge at about 2500 rpm for 10 minutes at room temperature. Attach a funnel fitted with whatman filter paper into a 250 mL flat bottom flask, transfer the supernatant by decantation through the funnel and collect. Rinse the filter paper with approx. 5 mL of **Solution IV** (use a disposable pipet) and collect.

3.3.3 Add 12.5 mL of **Solution IV** into the sediment marc, and vortex to loosen the sediment and allow to mix to consistency. Mix well to obtain a homogeneous suspension. Repeat the extraction step above (3.3.2) for 30 minutes. After centrifugation, transfer the supernatant into the above 250 mL flat bottom flask by decantation through the funnel and collect. Rinse the filter paper with approx. 5 mL of **Solution IV** (use a disposable pipet) and collect.

3. Materials (Continued)

3.3.4 Add 12.5 mL of **Solution IV** into the sediment marc, vortex to loosen the sediment and allow to mix to consistency. Mix well to obtain a homogeneous suspension. Repeat the extraction step above (3.3.2) for 30 minutes. After centrifugation, transfer the supernatant into the above 250 mL flat bottom flask by decantation through the funnel and collect. Rinse the filter paper with approx. 5 mL of **Solution IV** (use a disposable pipet) and collect.

NOTE: In case Zymark Turbo Vap is used for the concentration step use following procedure
1. The section 3.3.2, 3.3.3, and 3.3.4 –. The sample solution is then filtered through a #4 Whatman filter paper in a plastic funnel into a graduated 200 mL Zymark Turbo Vap evaporation tube with a 1 mL endpoint.

2. Section 3.3.5 – A Turbo Vap II from Zymark is used to evaporate the filtered solution to under 10 mL using nitrogen gas with a bath temperature 60°C.

3. Section 3.3.6 – Vortex mixing is also used to help ensure the dry residue on the side of the tubes was re-dissolved adequately. The solution is then quantitatively transferred out of the Zymark tubes into a wide mouth 10 mL volumetric flask.

3.3.5 Attach a rotary evaporator trap (see note) to the 250 mL flat bottom flask and evaporate the extract carefully to less than 10 mL, 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).

3.3.6 Swirl and sonicate the extract to dissolve the dry residue from the side of the 250 mL flat-bottom flask and transfer the extract to a volumetric flask (10 mL). Rinse the 250 mL flask with **Solution III.**

Bring the volume of the extract and the rinse to 10 mL with the buffer solution **Solution III.** **Mix well and to obtain a homogeneous extract**.

NOTE:
- It is absolutely necessary to use a 250 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 50 mL of extract to about 10 mL.
- Do not 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 sediment sample aliquot.
- To determine how much 10 mL of solution represents in a 250 mL flask during rotary evaporation, it is suggested that the analyst add 10 mL of water into an empty flask prior to conducting step 3.3.5 and compare. This will give the analyst a "picture" of how much 10 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. Materials (Continued)

3.3.7 Vortex and sonicate the extract in the volumetric flask and transfer a 2 mL aliquot of the extract (3.3.6) into a 12 mL vial or a disposable glass culture tube . Hold the extract for sample clean-up (Section 3.3.8). This step could be a stopping point of the method, if required.

3.3.8 Column Preparation and Conditioning (Reverse Phase, OASIS HLB)

Connect a pre-pack HLB SPE (3 cc, 60 mg) column on to a solid phase extraction manifold without the needles.

Condition the column by passing through 1 mL methanol followed by 1 mL of 9% formic acid in water. Allow the solvents to pass through just below the bed (top frit) of column using gravity. A small pipet bulb can be used to provide positive pressure to the top of the SPE column containing solution to help start the flow through the SPE column. Do not allow the column to go dry.

NOTE:
If the sediment 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:

Connect an empty glass column fitted with a frit (VWR Catalog No. 7121-06) on the top of the HLB SPE column and attach the coupled column to a solid phase extraction manifold. Apply vacuum and add some (enough to make 1 inch bed) silanized glass wool in the column. Add about 1 g of celite to the top of the packed glass wool. It is recommended to apply constant vacuum about 800 mbar throughout the entire extraction procedure. Lower pressure maybe needed for some matrices, but a constant flow rate of 1-2 mL a minute should be followed.

3.3.9 Column Loading, Washing and Elution

Load:
Add 0.2 mL of 9% formic acid in water to a 2 mL aliquot of the sediment sample extract and mix gently with an aid of a Pasteur pipet or vortex. Transfer (load) the acidic extract in portion (not more than half full) to the top of the conditioned column.

NOTE:
Do not vortex at high speed which may cause splashing of the sample out of the vessel.

3. Materials (Continued)

Wash:
Add 1 mL DI water to the flak containing the extracts, vortex to rinse and transfer the solution to the top of the column. Add 2.0 mL of deionized water to the top of the column and allow all of the solvent to pass through. Remove the collection vial and discard all the solutions.

Add the needles to the SPE manifold. Replace also the collection vials with a 15 mL graduated centrifuge tube to collect the eluent that is obtained after the following step.

Elute:
Add 7 mL of methanol to the top of the column and allow all of the solvents to pass through. Collect the eluent into the 15 mL graduated centrifuge tube and proceed to Step 3.3.10

NOTE:
- If there is a change of lot number for HLB Column, it is recommended to conduct column profile with known amount of analytes fortified into the extract before HLB clean-up.
- Do not allow the sample to go to dryness. This will cause low recoveries of BAS 670 H and its metabolites

3.3.10 Sample Preparation for LC-MS/MS Determination

Add 2 mL of **Solution VI** in to the eluent obtained in Step 3.2.3 and concentrate the extract down to 2 mL volume level under nitrogen at about 60°C. Remove and allow the samples to cool to room temperature. Adjust the volume to 5 mL with **Solution VI**. Swirl and vortex to ensure a complete homogeneous solution. Proceed to Section 3.3.10a.

3.3.10a 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) into an injection vial.

3.3.10b For procedurals and samples with residue higher than 0.001ppm-
Dilute the samples from 3.2.4a with **Solution VI** to the appropriate volume to fit into the calibration curve. Vortex to ensure a homogeneous solution.

The samples are ready for injection.

NOTE:
A 0.45µm PTFE membrane filter from VWR (VWR P/N: 28145-497) will be used if the solution is cloudy or has insoluble particulate matter in solution prior to transferring the solution into an auto-sampler vial for analysis.

3. Materials (Continued)

3.4 Instrumentation: Suggested LC-MS/MS Operating condition:

Instrument:	Waters Quattro Micro
Inlet [HPLC System]	Waters Alliance 2695
Data System:	Masslynxx 4.1
Column:	Phenomenex Columbus C_{18}, 5µ, 100 X 2.0 mm
Injection:	Typically 40 µL
Mobile Phase: [Gradient]	A = 4 mM ammonium formate in DI Water B = 4 mM ammonium formate in Methanol **Time (min.) Composition** 0.0 85% A + 15% B 0.7 85% A + 15% B 2.5 50% A + 50% B 3.25 2% A + 98% B 6.25 2% A + 98% B 6.5 85% A + 15% B 12.0 85% A + 15% B Run every 12.0 minutes
Flow Rate:	450 µL/minute

	BAS 670 H	M670H01	M670H05	M670H10
Expected Retention Times	3.25 minutes	2.25 minutes	1.25 minutes	4.50 minutes
Transitions Monitored - m/z:	362.0 →333.9 (Primary)	318.1 →193.8 (Primary)	282.0 →238.1 (Primary)	301.1 →177.1 (Primary)
Ionization Mode:	Negative ion for all analytes; Desolvation (400°C)			

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.
   The Phenomenex Gemini-NX C_{18} 5µm (100 X 2.0mm) HPLC column was used in the *Independent lab validation*

3. Materials (Continued)

3.5 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, M670H01, M670H05, and M670M10 standards for LC-MS/MS in the range of 0.125 ng/mL to 5 ng/mL. In a given injection run, the same volume is used for all samples and standards. Typical standard amounts injected on-column range as follows: 2.5, 5, 10, 20, and 100 pg.

Prepare calibration curves by plotting the peak area (monitoring transitions 362.2 →334.0, 282.0 →237.9, and 301.0 →177.0, for mixed BAS 670 H, M670H01, M670H05, and M670H10, respectively, versus the concentration 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.

3.6 Limit of Quantitation and Limit of Detection

The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.001 ppm for all analytes will be tested in different sediment and water types. The LOQ of 0.001 ppm is used to capture lower concentration of residues and to obtain reasonable dissipation pattern. The limit of detection (LOD) has not been determined, but it is set at about 20 % of the limit of quantitation [e.g. at the LOQ (0.001 ppm), if the amount of analyte is 0.4 ng/mL per injection, then the LOD is 0.08 ng/mL per injection]. In addition, a minimum signal to noise ratio (S/N) of 3:1 is used for the lowest standard in the calibration curves.

4. Calculation of Results

4.1 Principle

Calculation of results is based on peak height or area measurements. Typical recovery calculations for the LC-MS/MS quantitation are shown below.

Following equations were used to calculate procedural recoveries (%):

$$\textbf{Percent recovery (\%)} = \frac{\text{(Residue (ppm) for fortified sample - Residue (ppm) for control sample)}}{\text{Amount (ppm) fortified}} \times 100$$

$$\textbf{Sample Residue (ppm)} = \frac{\text{ng value calculated from calibration curve}}{\text{mg sample injected}}$$

ng found per injection = Amount of analyte calculated from calibration curve

Calibration curve: $ng = \frac{\text{Peak Area - intercept}}{\text{slope}}$

$$\textbf{mg injected} = \frac{\text{Sample weight (g) extracted} \times \mu\text{L injected} \times F1 \times F2}{Fv\ (mL)}$$

Fv = Final volume (mL) of the extract in the extraction solvent mixture

$$F1\ \text{(First dilution factor)} = \frac{\text{Aliquot (mL) taken from final extract}}{\text{Dilution volume (mL)}}$$

F2 (Second dilution factor): Equals 1, 0.1 and 0.01 for 0.001, 0.01 and 0.1 ppm fortification samples, respectively

Full computer/calculator precision in any intermediate calculations is used and the final values are only rounded for reporting purpose..

5. Time Requirement for Analysis

The time required for a set of 13 samples (10 fortified, 2 controls and one reagent blank) is approximately 12 person-hours, or 1.5 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, M670H01, M670H05, M670H10 and M670H15 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 sediment, the sediment marc has to be broken completely after the first centrifugation in the extraction step in order to obtain acceptable recovery. ***The glassware 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 an 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 and M670H10 could be enhanced or suppressed due to a heavy load of matrix (sediment residue) in the LC-MS/MS analysis. As a result, increased/decreased sensitivity (high/low signal) of the target analytes (especially M670H10)) 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/MS thoroughly. Some of the cleaning procedures 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.

Figure 1: Flow Diagram for Analytical Method No. D01001 in Sediment (BAS 670 H and itsMetabolite (M670H01, M670H05 and M670H10)

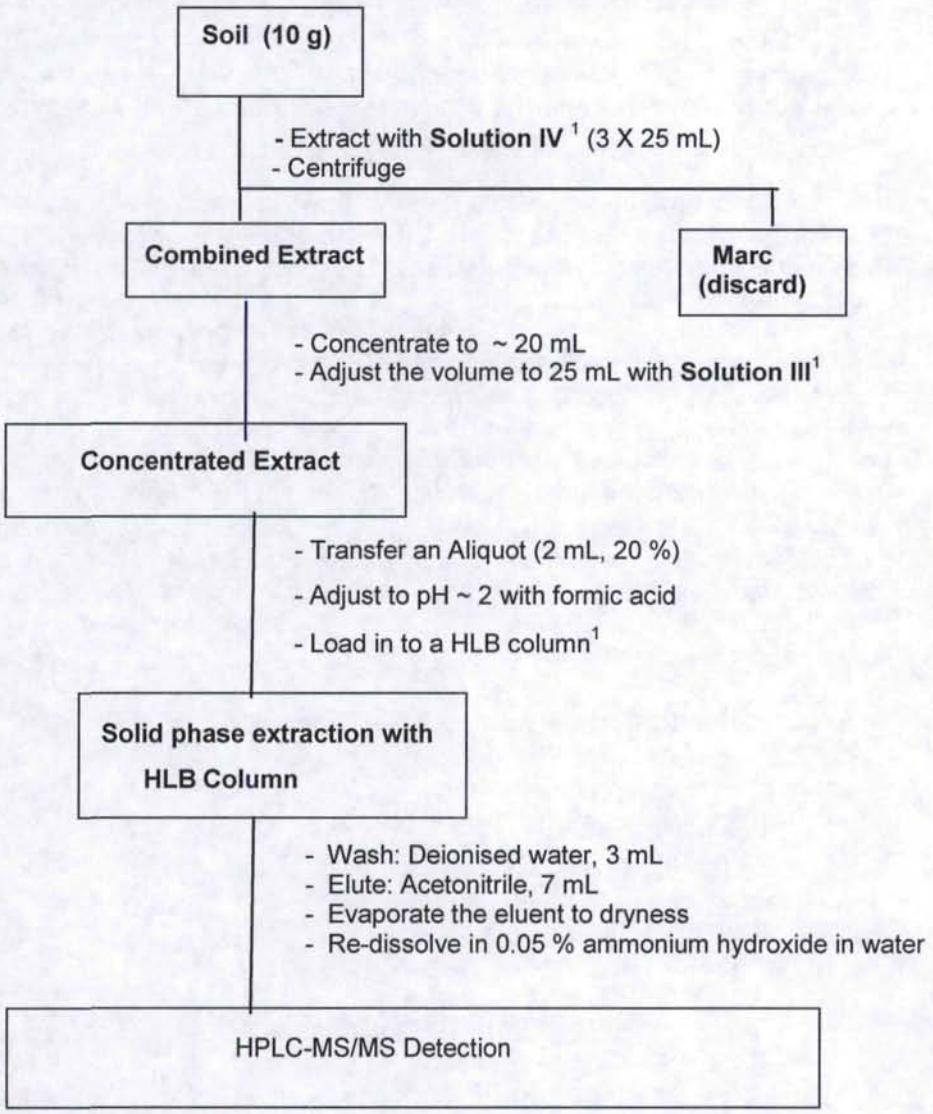


Figure 2: Flow Diagram for Analytical Method No. D01001 in Water (BAS 670 H and its Metabolites (M670H01, M670H05 and M670H10)