

## VALIDATION OF BASF METHOD No. D0502:

### I. INTRODUCTION AND SUMMARY

#### A. PURPOSE OF STUDY

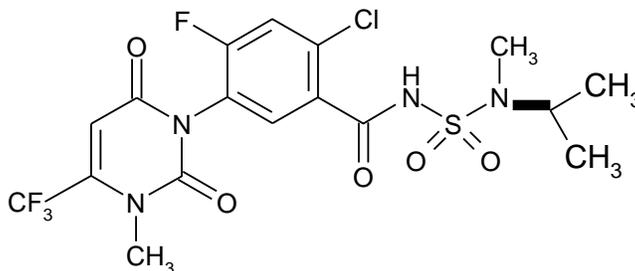
BAS 800 H is a new herbicide that will be used in tree orchards, vineyards, and numerous field and row crops in the US. A residue analytical method with a limit of quantitation of 0.001 mg/kg for the active ingredient and its metabolites in water was developed to determine the residue in water for ground water monitoring and eco-toxicological studies. This study was conducted to validate BASF Analytical Method D0502. Recovery ranges and standard deviations were determined from fortified control water samples. Recoveries of all analytes were determined in three different types of water.

### II. MATERIALS/METHODS

#### A. TEST AND REFERENCE SUBSTANCES

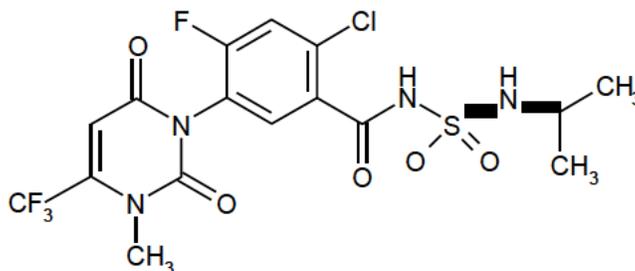
##### Fortification Compound

BASF Code Name:	BAS 800 H
BASF Registry Number:	4054449
CAS Number:	372137-35-4
Molecular Formula:	C <sub>17</sub> H <sub>17</sub> ClF <sub>4</sub> N <sub>4</sub> O <sub>5</sub> S
Molecular Weight:	500.9
Lot No.:	L67-140
Purity	99.9%
Expiration date:	July 01, 2008
Structural Formula:	

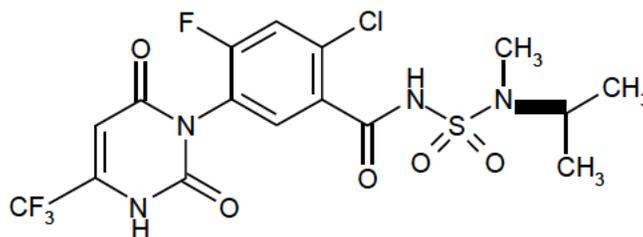


## II. MATERIALS/METHODS (Continued)

BASF Code Name: M800H01  
BASF Registry Number: 4118561  
Molecular Formula:  $C_{16}H_{15}ClF_4N_4O_5S$   
Molecular Weight: 486.8  
Lot No.: L74-62  
Purity: 98.8%  
Expiration date: February 1, 2008  
Structural Formula:

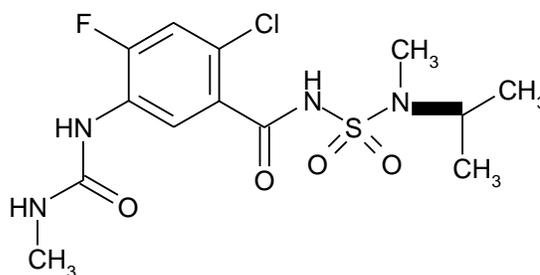


BASF Code Name: M800H02  
BASF Registry Number: 4118416  
Molecular Formula:  $C_{16}H_{15}ClF_4N_4O_5S$   
Molecular Weight: 486.8  
Lot No.: L67-186  
Purity: 99.2%  
Expiration date: March 1, 2009  
Structural Formula:

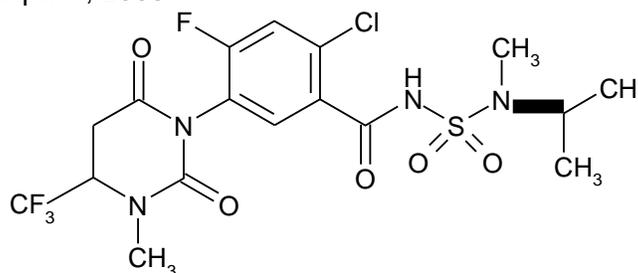


## II. MATERIALS/METHODS (Continued)

BASF Code Name: M800H07  
BASF Registry Number: 4775453  
Molecular Formula:  $C_{13}H_{18}ClFN_4O_4S$   
Molecular Weight: 380.8  
Lot No.: L67-196  
Purity: 95.4%  
Expiration date: March 1, 2009  
Structural Formula:



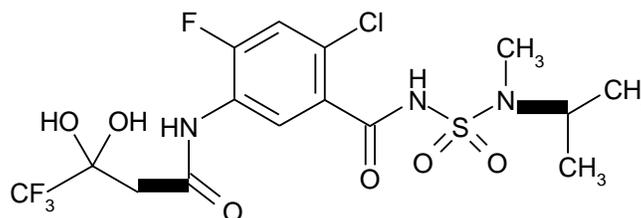
BASF Code Name: M800H08  
BASF Registry Number: 4773881  
Molecular Formula:  $C_{17}H_{19}ClF_4N_4O_5S$   
Molecular Weight: 502.9  
Lot No.: L74-66  
Purity: 97.2%  
Expiration date: April 1, 2008  
Structural Formula:



## II. MATERIALS/METHODS (Continued)

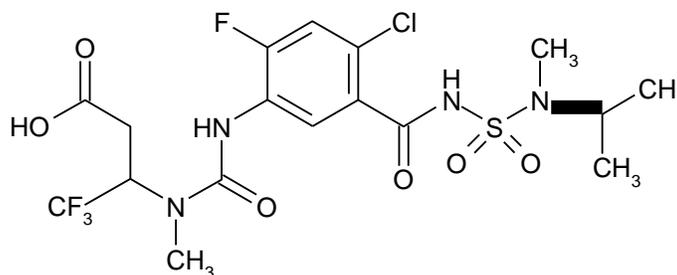
BASF Code Name: M800H015  
BASF Registry Number: 5264357  
Molecular Formula:  $C_{15}H_{18}ClF_4N_3O_6S$   
Molecular Weight: 479.9  
Lot No.: L74-80  
Purity: 94.5%  
Expiration date: June 1, 2008

Structural Formula:



BASF Code Name: M800H022  
BASF Registry Number: 5216337  
Molecular Formula:  $C_{17}H_{21}ClF_4N_4O_6S$   
Molecular Weight: 520.9  
Lot No.: L74-56  
Purity: 94.1%  
Expiration date: March 1, 2008

Structural Formula:



### Reference Standards (used for calibration)

Same as fortification compounds (Section II A)

Standard substances are stored in a freezer (<-5<sup>0</sup>C) until use. Characterization, purity and stability were determined prior to use for this study. Details of these determinations are available to BASF and are located at Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany.

## II. MATERIALS/METHODS (Continued)

### ***Standard Solution Stability/ Extract Stability***

Test and reference substance solutions were stored in a refrigerator at 4°C and were refrigerated during their use in this study. During the course of this study the stability of fortification and LC-MS/MS calibration standard solutions was examined and the summary of the results are provided in this report. Stock solutions (1 mg/mL) were made fresh every three months and further diluted to proper concentration. The stability of the stock solution in methanol was examined in the validation study on soil (**Reference 1**) and the result is provided in the table shown below. Dilutions of stock standards for fortifications were made fresh every month. The following table shows the stability of the analytes in various solvent systems used within the method.

<b>SOLUTION</b>	<b>STABILITY (DAYS)</b>
Stock solutions of BAS 800 H and its metabolites, M800H01, M800H02, M800H07, M800H08, M800H15, M800H22 in methanol	90
Fortification solutions of BAS 800 H and its metabolites, M800H01, M800H02, M800H07, M800H08, M800H15, M800H22 in methanol	50
Sample Extracts and LC-MS/MS calibration standards of BAS 800 H and its metabolites, M800H01, M800H02, M800H07, M800H08, M800H15, M800H22 in methanol-water (10:90, v/v)	50

The stability of the test and reference substances in the fortified water matrices were not examined separately. The fortified water samples are the same as the calibration standards solution

### **B. TEST SYSTEM**

The test systems consisted of untreated water samples: De-ionized, well, pond and salt-water. These water samples were used to validate method D0502.

### **C. SAMPLE STORAGE AND HANDLING**

Bulk water samples were stored frozen (<-5°C) before analysis.

### **D. EXPERIMENTAL DESIGN**

Control water samples were fortified by applying standard solutions directly to the water prior to extraction with a volumetric pipet. The fortified control samples were analyzed to determine the recoveries of each analyte.

## II. MATERIALS/METHODS (Continued)

Initially three validation sets were conducted with the method that analyzed only parent molecule. At a later point six additional sets were added by an amendment to include the analysis of parent molecule and the metabolites. Each validation set consisted of a reagent blank, two unfortified matrix controls, 5 matrix control samples fortified at the LOQ (0.001 ppm) and 5 matrix control samples fortified at 10 times LOQ (a total of 13 samples per matrix type). All of these sets were subsequently analyzed with the method described in Section E.

### E. METHOD OF ANALYSIS

BASF Analytical Method D0502 was developed and validated initially to determine only the parent compound (BAS 800 H) in salt, deionized and well water for the use of eco-tox studies. The technical procedure (Version: May 13, 2005) of this method is attached to this report as **Appendix A**.

This original method was further modified to incorporate the all other relevant metabolites. This modified method was designed to determine the residues as individual analytes and will be used for the residue analysis of water samples collected from aquatic dissipation or ground water monitoring studies. The method was developed for analyzing deionized, pond and well water. Salt water was not a necessary matrix for ground water monitoring studies. This method was developed to determine the residues of BAS 800 H, M800H01, M800H02, M800H07, M800H08, M800H15, and M800H22 in water matrices using LC-MS/MS. The method was designed to determine the residues as individual analytes and will be used for the residue analysis of water samples collected from aquatic dissipation or ground water monitoring studies. The technical procedure of this method is attached to this report as **Appendix A** (Version: April 15, 2008)

A brief description of the method is provided below:

The water samples (1.0 g aliquot) are diluted with methanol (0.1 mL). The residues are determined by LC-MS/MS quantitation.

The transitions monitored for quantitation ion are: m/z 501.0 → 348.9, 487.0 → 365.9, 487.0 → 335.0, 381.0 → 229.0, 503.1 → 351.1, 480.0 → 420.1, and 521.00 → 369.0 for analytes BAS 800 H, M800H01, M800H02, M800H07, M800H08, M800H15 and M800H22, respectively.

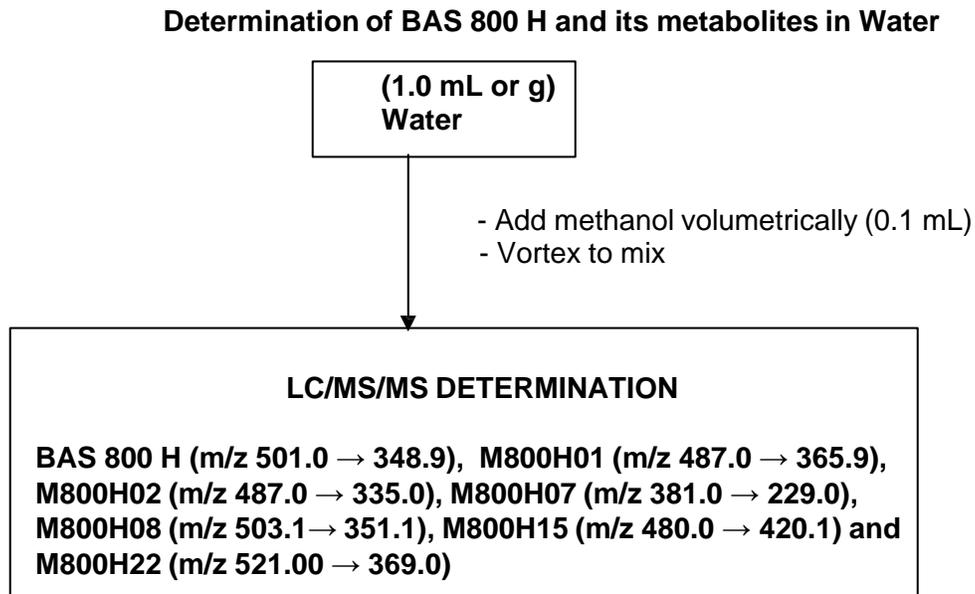
The transitions monitored for secondary ion (confirmation purposes) are: m/z 501.00 → 459.0, 487.0 → 445.0, 487.0 → 445.0, 381.0 → 338.96, 505.1 → 353.1, 480.0 → 310.0, and 521.00 → 172.0 for analytes BAS 800 H, M800H01, M800H02, M800H07, M800H08, M800H15 and M800H22, respectively.

A flow diagram of the analytical procedures is provided in **Figure 1**.

Typical recovery calculations for the LC/MS/MS quantitation are shown in **Figure 2**.

Specific chromatographic conditions are listed with each analysis set. Typical chromatographic parameters are provided in the technical procedure (**Appendix A**).

**Figure 1. Flow Diagram of Analytical Method No. D0502 in Water**



**Figure 2. Typical Recovery Calculation (BASF Method D0502)**

Sample Number 132692-7-E Control water sample fortified with 0.001 ppm of BAS 800 H, and its metabolites, M800H01, M800H02, M800H07, M800H08, M800H15, M800H22

Sample Number 132659-1B, C: Controls unfortified.

Calculations are shown only for BAS 800 H

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

**Residue calculation for BAS 800 H for the Sample Number 132692-7-E:**

Slope (m): 12,209.5868

Intercept (b): -626.2349

correl.coeff. = 0.9992

Peak Area = 11,122.0

BAS 800 H Found (ng/mL) =  $\frac{\text{peak area} - \text{intercept}}{\text{Slope}}$

BAS 800 H Found (ng/mL) =  $11,122.0 - (-626.2349) / 12,209.5868$   
= 0.9622

Residue (ppm of BAS 800 H) =

$\frac{\text{BAS 800 H Found (ng/mL)} \times \text{Final Volume (mL)}}{\text{Sample Weight (g)} \times A_F \times 1000}$  (to convert to ppm)

Sample Weight = 0.1 g (0.1 mL) [Density of water = 1]

Final Volume = 1.1 mL

Aliquotation Factor ( $A_F$ ) = 100%

Residue (ppm of BAS 800 H) =  $\frac{0.9622 \text{ ng/mL} \times 1.1 \text{ mL}}{0.1000 \text{ g} \times 100\% \times 1000}$  = 0.001058 ppm

Recovery of BAS 800 H (%) =  $\frac{\text{Residue (ppm of BAS 800 H)} - \text{Residue in Control (ppm)}}{\text{Amount Fortified (ppm)}} \times 100$

Amount Fortified = 0.001 ppm

Residue in Control = none detected

Recovery of BAS 800 H (%) =  $\frac{0.001058}{0.001} \times 100$  = 106%

Full computer/calculator precision in any intermediate calculations is used and the final values are only rounded for reporting purpose. Percent recoveries of all other analytes were calculated in similar fashion.

**Tables I through X: Procedural Recovery Data for BAS 800 H, and its metabolites, M800H01, M800H02, M800H07, M800H08, M800H15, M800H22**

**Table XI: Summary of Standard Data**

**Tables XII through XV: Summary of Statistical Data**

Unless otherwise noted, the following parameters were used for all analyses:

**Master sheet 1-3:**

Sample size: 10 mL (g)

- Final volume (mL) = 11 mL
- Aliquot factor = Aliquot (mL) taken /Final volume (mL): 1/2 = 0.5
- Dilution factor = 1, 0.1 for 0.001 and 0.01 ppm samples, respectively

**Master sheet 7-12:**

Sample size: 1.0 mL (g)

- Injection volumes: 50 µL
  - Final volume (LOQ) = 1.1 mL
  - Aliquot factor = 100%
  - Dilution factor = 1/10 = 0.1 for 10 times LOQ samples
- 
- Amount of analyte (pg) found = (peak area - intercept)/slope [obtained from calibration curve]
  - Values in these tables may have been rounded off for reporting purpose, but not for further calculation. Rounding occurs only in the final result, the "percent recovery" column
  - If no signal was detected, ND was reported
  - Calculations used to obtain "mg injected", "ppm calculated" and percent recoveries are shown in page 20 (**Figure 2**).
  - Recoveries were corrected for control background, if present, the average of two control values were used. If no signal or signal with amount less than LOD (20% of the LOQ) was detected for control samples, not applicable (NA) was reported in the recovery column.
  - Sample Number: Master Sheet Number (Study Number-Sequential Number for master sheet – sequential number) for the samples
  - Reagent blank: Has shown no interference peaks at retention time of all analytes
  - Information of the aliquot factor, final volumes and dilution factor used to calculate recoveries are shown above

## **ABSTRACT**

BASF Method D0502 is developed to determine the residues of BAS 800 H and its metabolites, M800H01, M800H02, M800H07 M800H08 M800H15, and M800H22 in water using LC-MS/MS at BASF Corporation, Research Triangle Park, NC.

The water samples (1.0 g aliquot) are diluted with methanol (0.1 mL). The residues are determined by LC-MS/MS quantitation.

The method has a limit of quantitation (LOQ) of 0.001 mg/kg in water.

## 1. Introduction

### 1.1 Scope of the method

BAS 800 H a new herbicide that will be used for corn, cereals and other crops in the US and Canada. A residue analytical method with a limit of quantitation of 0.001 mg/kg for the active ingredient in water is developed to determine the residue in water from ground water monitoring and eco-toxicological studies.

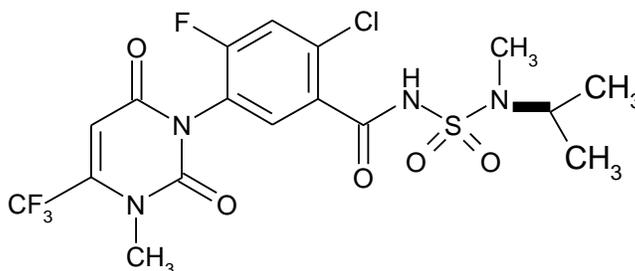
## 2. Materials

Standard substances are stored in a freezer (<-5°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.

### 2.1. Test and Reference Substance

#### 2.1.1 Fortification Compound

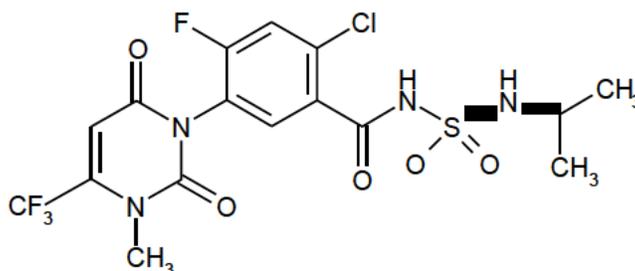
BASF Code Name: BAS 800 H  
BASF Registry Number: 4054449  
CAS Number: 372137-35-4  
Molecular Formula: C<sub>17</sub>H<sub>17</sub>ClF<sub>4</sub>N<sub>4</sub>O<sub>5</sub>S  
Molecular Weight: 500.9  
Lot No.: L67-140, and L67-190  
Purity: Expiration 99.9% and 99.6%  
date: Structural July 01, 2008 and April 01, 2009  
Formula:



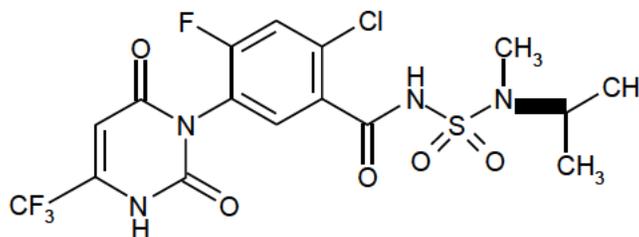
BASF Code Name: M800H01  
BASF Registry Number: 4118561  
Molecular Formula: C<sub>16</sub>H<sub>15</sub>ClF<sub>4</sub>N<sub>4</sub>O<sub>5</sub>S

## 2. Materials (Continued)

Molecular Weight: 486.8  
Lot No.: L74-62  
Purity: 98.8%  
Expiration date: February 1, 2008  
Structural Formula:



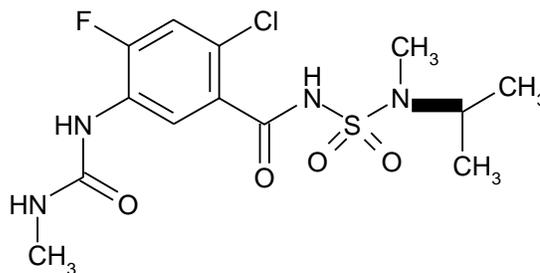
BASF Code Name: M800H02  
BASF Registry Number: 4118416  
Molecular Formula: C<sub>16</sub>H<sub>15</sub>ClF<sub>4</sub>N<sub>4</sub>O<sub>5</sub>S  
Molecular Weight: 486.8  
Lot No.: L67-186  
Purity: 99.2%  
Expiration date: March 1, 2009  
Structural Formula:



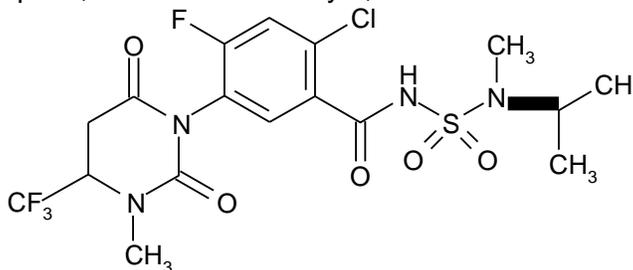
BASF Code Name: M800H07  
BASF Registry Number: 4775453  
Molecular Formula: C<sub>13</sub>H<sub>18</sub>ClFN<sub>4</sub>O<sub>4</sub>S  
Molecular Weight: 380.8  
Lot No.: L67-196  
Purity: 95.4%  
Expiration date: March 1, 2009

## 2. Materials (Continued)

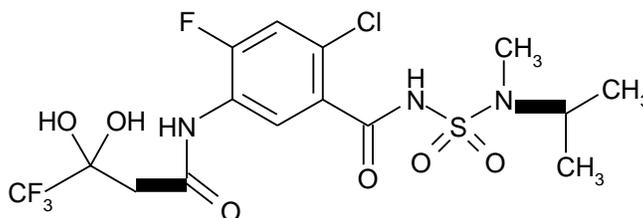
Structural Formula:



BASF Code Name: M800H08  
BASF Registry Number: 4773881  
Molecular Formula: C<sub>17</sub>H<sub>19</sub>ClF<sub>4</sub>N<sub>4</sub>O<sub>5</sub>S  
Molecular Weight: 502.9  
Lot No.: L74-66  
Purity: 97.2%  
Expiration date: April 1, 2008 and February 1, 2010  
Structural Formula:

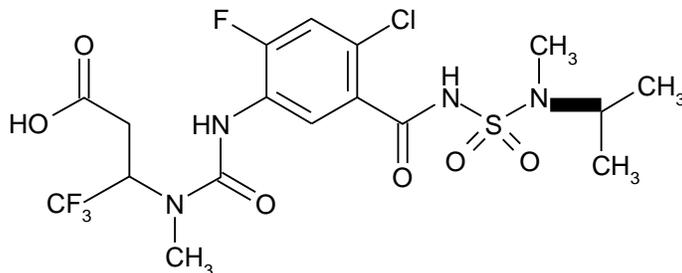


BASF Code Name: M800H15  
BASF Registry Number: 5264357  
Molecular Formula: C<sub>15</sub>H<sub>18</sub>ClF<sub>4</sub>N<sub>3</sub>O<sub>6</sub>S  
Molecular Weight: 479.9  
Lot No.: L74-80  
Purity: 94.5%  
Expiration date: June 1, 2008  
Structural Formula:



## 2. Materials (Continued)

BASF Code Name: M800H22  
BASF Registry Number: 5216337  
Molecular Formula:  $C_{17}H_{21}ClF_4N_4O_6S$   
Molecular Weight: 520.9  
Lot No.: L74-56  
Purity: Expiration  
date: Structural 94.1%  
Formula: March 1, 2008



### 2.2.2 Reference Standard (used for calibration)

Same as fortification compound (section 2.1.1)

BASF has retained a reserve sample of these chemicals, and has documentation at the BASF Agricultural Products Center, Research Triangle Park, North Carolina, specifying the location of the synthesis and characterization information for these compounds.

## 2. Materials (Continued)

### 2.2 Equipment -- Suggested Sizes/Suppliers, Manufacturers

Method Step	Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
3.1	Alphanumeric well plate tube	1.4 mL	Matrix	4253
2.3, 2.4	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.	Bottle, Amber glass	Qorpak , 2 oz, 4 oz and 8 oz with Teflon®-lined screw cap	Qorpak	
3.1	Centrifuge	Refrigerated Centrifuge Model CS-6KR	Beckmann	
Various	Cylinder, Graduated	Various sizes	Various	
Various	Flask, Erlenmeyer, 24/40	1000 mL	Various	
Various	Flask, Volumetric	100, 50, 25 ,10 and 5 mL	Various	
3.3	Liquid Handling System	Quadra96®, Model 320 or Quadra 3 <sub>NS</sub> ®	Tomtec Inc.	
3.3	MicroMan pipettes	10-1000 µL	Gilson	M-25,M-50, M-250,M-1000
3.3	Multitube Vortexer	VX-2500	VWR	58816-116
Various	Pipet, Volumetric	0.5, 1-10, 25 mL	Various	
Various	Pasteur Pipet, disposable	various size	VWR	
Various	Pipet tips	polypropylene	Matrix Inc.	196-205
Various	Reagent reservoir	Dimpled polypropylene	Tomtec Inc.	
Various	Spatula		Various	
Various	Stopper, Teflon®	24/40	Various	
Various	Ultrasonic Bath	Model FS 7652H	Fisher Scientific	

**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
Formic Acid	98%	E.M. Science	FX0440-7
Methanol	High Purity	B & J	230-4
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
<p><b>Solution I:</b> Methanol-water, 10:90, v/v                      Add 100 mL of methanol into a 1L volumetric flask. Dilute to the mark with water and mix well to ensure complete homogeneous solution.</p> <p><b>Note:</b> Heat may occur while mixing.</p>	2.4.2.3 and 3.3

Solvent Mixtures	Method Step
<p><b>LC-MS/MS Mobile Phase A:</b>                      Water with 0.1 % formic acid and 4 mM ammonium formate:                      Add 1.0 mL of formic acid (98 %), about 50 –100 mL of water and 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.</p>	3.5
<p><b>LC-MS/MS Mobile Phase B:</b>                      Methanol with 0.1 % formic acid and 4 mM ammonium formate:                      Add 1.0 mL of formic acid (98 %), about 50 –100 mL of methanol and 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 methanol and mix well to ensure complete homogeneous solution.</p>	3.5

## 2. Materials (Continued)

### 2.4 Standard Solutions and their Storage Stability

#### 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 method validation 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 solution of BAS 800 H and its metabolites, M800H01, M800H02, M800H07, M800H08, M800H15 and M800H22 (1 mg/mL):

Prepare a 1.0 mg/mL stock solution by weighing an appropriate amount of analyte into a volumetric flask. Dissolve with methanol and dilute to mark. For example, to prepare a 10 mL stock solution of BAS 800 H, place 10.0 mg of BAS 800 H into a 10 mL volumetric flask. Dissolve and dilute to mark with methanol. Sonicate and vortex to ensure a complete homogeneous solution.

##### 2.4.2.2 Standards for Fortifications

Prepare a 10 µg/mL standard solution for fortification by transferring 1.0 mL of stock solution (2.4.2.1) into a 100 mL volumetric flask. Dilute to mark with methanol and vortex to ensure a complete homogeneous solution. Prepare serial dilutions of this combined solution as needed. Suggested concentrations of standards for fortifications are 10, 1.0, 0.1, and 0.01 µg/mL in methanol.

##### 2.4.2.3 Calibration Standard Solutions for LC-MS/MS Analysis: 10.0, 2.0, 1.0, and 0.4 ng/mL in methanol-water (10:90, v/v)

Prepare a 10.0 ng/mL calibration standard solution by transferring 0.5 mL of 1.0 µg/mL of fortification solution (2.4.2.2) with a volumetric pipet into a 50 mL volumetric flask and dilute to the mark with methanol-water (10:90, v/v). Vortex to ensure a homogeneous solution. Prepare serial dilutions of this solution as needed. Suggested concentrations of standards are 10.0, 2.0, 1.0, and 0.4 pg/µL in methanol-water (10:90, v/v).

**NOTE:**

- Use amber bottles 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 samples received from the field are kept frozen ( $<-5^{\circ}\text{C}$ ) before analysis. Add 1.0 mL ( $1.0 \pm 0.1\text{g}$ ) of water sample volumetrically into a 1.4 mL Alphanumeric well plate tube (Matrix).

#### 3.2 Fortification and extraction

3.2.1 For the fortification samples, add an appropriate volume of standard solution of BAS 800 H to the respective control sample by a volumetric pipet. For example, for a 0.001 ppm fortification sample, pipet 0.1 mL of the 0.01  $\mu\text{g}/\text{mL}$  standard fortification solution (2.4.2.2) onto 1.0 mL (1.0 g) of control water sample. For unfortified control (or treated field sample) and reagent blank sample, add 0.1 mL of methanol into the samples.

Attach a stopper and vortex to mix. Mix well to obtain a homogeneous extract.

**NOTE:** Extreme caution should be taken while mixing the samples. Vigorous mixing in stages will avoid sample loss due to pressure build-up. Also, not enough mixing will cause low recoveries.

Hold the extract for sample preparation for LC-MS/MS analysis (Section 3.3).

#### 3.3 Sample Preparation for LC-MS/MS Analysis

3.3.1 **For control and 0.001 ppm fortifications**, sample extracts in Section 3.2.1 are ready for the analysis.

3.3.2 **For 0.01 ppm fortifications and higher ppm**, take 0.1 mL of the sample solution (Section 3.2.1) and dilute to 1 mL with methanol-water (10:90, v/v) or any desired concentration to adjust the concentration within the calibration curve

A flow chart of the analytical procedure is presented in **Figure 1**.

### 3.5. Instrumentation

Suggested LC-MS/MS Operating condition:

**LC-MS/MS Method A:** Used only for BAS 800 H

Instrument:	PE Sciex API 3000 Mass Analyzer												
Inlet [HPLC System]:	PE Series 200 Micro Pump system with Series 200 Auto sampler												
Software Version:	Analyst 1.1												
Column:	Thermo RP Prism 5 $\mu$ , 50 X 2.1 mm, [P/N 32105-052130]												
Injection:	Typically 10 $\mu$ L												
Mobile Phase: [Gradient]	<p>A = water with 4 mM ammonium formate and 0.1 % formic acid                      B = methanol with 4 mM ammonium formate and 0.1 % formic acid</p> <table border="0"> <thead> <tr> <th><u>Time (min.)</u></th> <th><u>Composition</u></th> </tr> </thead> <tbody> <tr> <td>0.0</td> <td>70% A + 30% B</td> </tr> <tr> <td>2.0</td> <td>10% A + 90% B (Switching valve)</td> </tr> <tr> <td>4.0</td> <td>10% A + 90% B</td> </tr> <tr> <td>4.1</td> <td>70% A + 30% B</td> </tr> <tr> <td>5.0</td> <td>70% A + 30% B</td> </tr> </tbody> </table> <p>Run every 5.0 minutes</p>	<u>Time (min.)</u>	<u>Composition</u>	0.0	70% A + 30% B	2.0	10% A + 90% B (Switching valve)	4.0	10% A + 90% B	4.1	70% A + 30% B	5.0	70% A + 30% B
<u>Time (min.)</u>	<u>Composition</u>												
0.0	70% A + 30% B												
2.0	10% A + 90% B (Switching valve)												
4.0	10% A + 90% B												
4.1	70% A + 30% B												
5.0	70% A + 30% B												
Flow Rate:	300 $\mu$ L/minute												
Expected Retention Times	~ 2.8 minutes												
Transitions:	m/z 501 $\rightarrow$ 459 (Quantitation ion) and m/z 501 $\rightarrow$ 349												
Ionization Mode:	Positive ion; Turbospray (500°C)												

**LC-MS/MS Method B:** Used for all analytes

Instrument:	PE Sciex API 3000 Mass Analyzer																		
Inlet [HPLC System]:	PE Series 200 Micro Pump system with Series 200 Auto sampler																		
Software Version:	Analyst 1.1																		
Column:	Varian Inertsil Phenyl 5 $\mu$ , 100 X 2.0 mm, [P/N A0301100X020]																		
Injection:	Typically 50 $\mu$ L																		
Mobile Phase: [Gradient]	<p>A = water with 4 mM ammonium formate and 0.1 % formic acid                      B = methanol with 4 mM ammonium formate and 0.1 % formic acid</p> <table border="1"> <thead> <tr> <th><u>Time (min.)</u></th> <th><u>Composition</u></th> </tr> </thead> <tbody> <tr> <td>0.0</td> <td>95% A + 5% B</td> </tr> <tr> <td>0.5</td> <td>95% A + 5% B</td> </tr> <tr> <td>2.0</td> <td>40% A + 60% B</td> </tr> <tr> <td>3.5</td> <td>30% A + 70% B</td> </tr> <tr> <td>4.5</td> <td>5% A + 95% B</td> </tr> <tr> <td>7.5</td> <td>5% A + 95% B</td> </tr> <tr> <td>7.6</td> <td>95% A + 5% B</td> </tr> <tr> <td>8.5</td> <td>95% A + 5% B</td> </tr> </tbody> </table> <p>Run every 8.5 minutes</p>	<u>Time (min.)</u>	<u>Composition</u>	0.0	95% A + 5% B	0.5	95% A + 5% B	2.0	40% A + 60% B	3.5	30% A + 70% B	4.5	5% A + 95% B	7.5	5% A + 95% B	7.6	95% A + 5% B	8.5	95% A + 5% B
<u>Time (min.)</u>	<u>Composition</u>																		
0.0	95% A + 5% B																		
0.5	95% A + 5% B																		
2.0	40% A + 60% B																		
3.5	30% A + 70% B																		
4.5	5% A + 95% B																		
7.5	5% A + 95% B																		
7.6	95% A + 5% B																		
8.5	95% A + 5% B																		
Flow Rate:	250 $\mu$ L/minute																		

**3. ANALYTICAL PROCEDURES (Continued)**

Analytes	Expected Retention Times (minutes)	Transitions (m/z):	
		Quantitation ion	Secondary ion
BAS 800 H	5.98	501.0 → 348.9	501.00 → 459.0
M800H01	5.8	487.0 → 365.9	Not used
M800H02	5.8	487.0 → 335.0	Not used
M800H07	5.36	381.0 → 229.0	381.0 → 338.9
M800H08	5.79	503.1 → 351.1	505.1 → 353.1
M800H15	5.53	480.0 → 420.1	480.0 → 282.0
M800H22	5.69	521.00 → 369.0	521.00 → 172.0
Ionization Mode:	Positive ion; Turbospray (400°C)		

### 3. ANALYTICAL PROCEDURES (Continued)

#### LC-MS/MS Method C: Secondary Chromatographic method Used for M800H01 and M800H02

Instrument:	PE Sciex API 3000 Mass Analyzer																		
Inlet [HPLC System]:	PE Series 200 Micro Pump system with Series 200 Auto sampler																		
Software Version:	Analyst 1.1																		
Column:	Inertsil ODS-3 5 $\mu$ , 150 X 2.0 mm,																		
Injection:	Typically 50 $\mu$ L																		
Mobile Phase: [Gradient]	A = water with 4 mM ammonium formate and 0.1 % formic acid B = methanol with 4 mM ammonium formate and 0.1 % formic acid  <table border="1"> <thead> <tr> <th><u>Time (min.)</u></th> <th><u>Composition</u></th> </tr> </thead> <tbody> <tr> <td>0.0</td> <td>95% A + 5% B</td> </tr> <tr> <td>1.0</td> <td>95% A + 5% B</td> </tr> <tr> <td>3.0</td> <td>75% A + 25% B</td> </tr> <tr> <td>5.0</td> <td>75% A + 25% B</td> </tr> <tr> <td>7.0</td> <td>10% A + 90% B</td> </tr> <tr> <td>9.0</td> <td>10% A + 90% B</td> </tr> <tr> <td>9.1</td> <td>95% A + 5% B</td> </tr> <tr> <td>11</td> <td>95% A + 5% B</td> </tr> </tbody> </table> Run every 11 minutes	<u>Time (min.)</u>	<u>Composition</u>	0.0	95% A + 5% B	1.0	95% A + 5% B	3.0	75% A + 25% B	5.0	75% A + 25% B	7.0	10% A + 90% B	9.0	10% A + 90% B	9.1	95% A + 5% B	11	95% A + 5% B
<u>Time (min.)</u>	<u>Composition</u>																		
0.0	95% A + 5% B																		
1.0	95% A + 5% B																		
3.0	75% A + 25% B																		
5.0	75% A + 25% B																		
7.0	10% A + 90% B																		
9.0	10% A + 90% B																		
9.1	95% A + 5% B																		
11	95% A + 5% B																		
Flow Rate:	500 $\mu$ L/minute																		

Analytes	Expected Retention Times (minutes)	Transitions (m/z):	
		Quantitation ion	Secondary ion
M800H01	5.8	487.0 $\rightarrow$ 365.9	487.0 $\rightarrow$ 445.0
M800H02	5.8	487.0 $\rightarrow$ 335.0	487.0 $\rightarrow$ 445.0
Ionization Mode:	Positive ion; Turbospray (400°C)		

### 3. ANALYTICAL PROCEDURES (Continued)

**NOTE:**

1. The LC-MS/MS instrument and 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 a different type of instrument.

#### 3.6 Calibration Procedures

Calculation of results is based on peak area or peak height measurements using a calibration curve. The calibration curve is obtained by direct injection of 50 µL of BAS 800 H standards for LC-MS/MS in the range of 0.4 pg/µL to 10.0 pg/µL. In a given injection run, the same injection volume is used for all samples and standards. Typical standard amounts injected on-column range as follows: 20, 50, 100, and 500 pg.

Calibration curves are prepared by plotting the peak area versus the weight using a linear least squares working curve in the form of  $y = bx + c$ .

The transitions monitored (Quantitation ion) are  $m/z$  501.0 → 348.9, 487.0 → 365.9, 487.0 → 335.0, 381.0 → 229.0, 503.1 → 351.1, 480.0 → 420.1, and 521.00 → 369.0 for analytes BAS 800 H, M800H01, M800H02, M800H07, M800H08, M800H15 and M800H22, respectively.

The confirmatory (secondary) ions are :  $m/z$  501.00 → 459.0, 381.0 → 338.91, 505.1 → 353.1 480.0 → 282.0, and 521.00 → 172.0 for BAS 800 H, M800H07, M800H08, M800H15 and M800H22, respectively. Analytes M800H01 and M800H02 has same retention time and same mass transition. Therefore,  $m/z$  487.0 → 445.0 has not been used for the confirmatory method. A separate chromatographic method is used for the confirmation purpose.

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. ANALYTICAL PROCEDURES (Continued)

#### 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 800 H. The limit of detection has not been determined, but set at 20 % of the limit of quantitation [e.g. at the LOQ (0.001 ppm), if the amount of analyte is 45 pg/50 µL injection, LOD is 9 pg/50 µL injection]. The lowest standard for each analyte in the calibration curve has good detectability (signal to noise ratio greater than 3:1).

#### 4. Calculation of Results

##### 4.1 Principle

Calculation of results is based on peak height or area measurements. The recoveries and residues of BAS 800 H and its metabolites in µg/g (ppm) are calculated with the following formulas:

$$\text{Residue in ppm} = \frac{\text{ng found per injection}}{\text{mg injected}}$$

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

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

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

$$\text{mg injected} = \frac{\text{Sample weight (g) extracted}}{\text{Final extraction volume (mL)}} \times \mu\text{L injected} \times \text{Dilution factor (F1)}$$

**Dilution Factor (F1)** = 1 and 0.1 for 0.001 and 0.01 ppm fortification samples, respectively.

#### 5. Time Requirement for Analysis

The time required for a set of 13 samples (10 fortified, 2 controls and one reagent blank) is approximately 4 person-hours (without the sample clean up) and is 8 person-hours (with the sample clean up) provided that no special problems arise, such as matrix interference and LC-MS/MS analysis and data processing times that could be automated and unattended

#### 6. Confirmatory Techniques

The method of determination is LC-MS/MS, which is a highly selective and self-confirmatory detection technique. However two transitional ions were monitored during analysis for peak confirmation

## **7. Potential problems**

The glassware used for the method should be thoroughly rinsed with methanol and to prevent contamination.

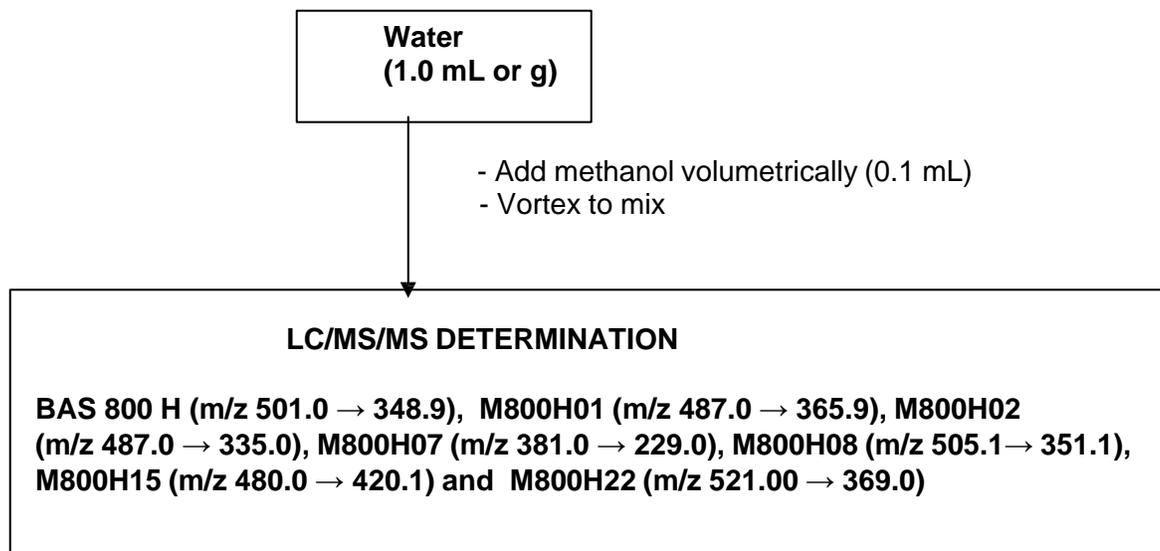
Peak enhancement could be a potential problem without sufficient sample clean-up. It is highly recommended to perform instrument check routinely during LC-MS/MS analysis for standard peak enhancement or suppression. The instrument check sample is basically prepared by adding known amount of standard to the control matrix at the limit of quantitation (0.001 ppm level). It is recommended to clean the LC-MS thoroughly, if peak enhancement or suppression has been observed. 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.

## **9. Safety and Health Considerations**

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

### Figure 1: Flow Diagram of Analytical Method No. D0502

#### Determination of BAS 800 H and its metabolites in Water:



## **ABSTRACT**

BASF Method D0502 is developed to determine the residues of BAS 800 H in water using LC-MS/MS at BASF Corporation, Research Triangle Park, N.C.

The water samples (10 g aliquot) are diluted with methanol (1 mL). The resulting solutions are further diluted with water-methanol (90:10, v/v) and are filtered through a PTFE syringe filter. In case of salt-water samples, reverse phase (RP C<sub>18</sub>) solid phase extraction (SPE) is used for the sample clean-up. The residues are determined by HPLC-MS/MS quantitation.

The method has a limit of quantitation (LOQ) of 0.001 mg/kg in water.

## 1. Introduction

### 1.1 Scope of the method

BAS 800 H a new herbicide that will be used for corn, cereals and other crops in the US and Canada. A residue analytical method with a limit of quantitation of 0.001 mg/kg for the active ingredient in water is developed to determine the residue in water from eco-toxicological studies.

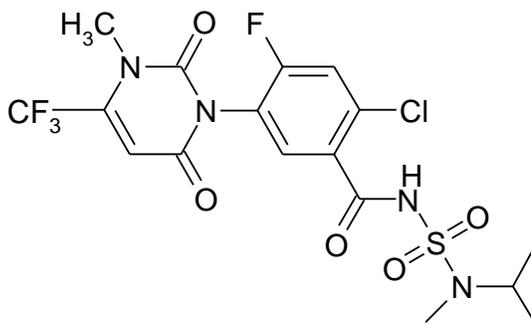
## 2. Materials

Standard substances are stored in a freezer (<-5<sup>0</sup>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.

### 2.1. Test and Reference Substance

#### 2.1.1 Fortification Compound

BASF Code Name: BAS 800 H  
BASF Registry Number: 4054449  
Chemical Name: N'-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methyl sulfamide  
Molecular Formula: C<sub>17</sub>H<sub>17</sub>ClF<sub>4</sub>N<sub>4</sub>O<sub>5</sub>S  
Molecular Weight: 500.86  
Lot No.: L67-140  
Purity: 99.9%  
Structural Formula:



BASF has retained a reserve sample of these chemicals, and has documentation at the BASF Agricultural Products Center, Research Triangle Park, North Carolina, specifying the location of the synthesis and characterization information for these compounds.

#### Reference Standard (used for calibration)

Same as fortification compound (section 2.1.1)

## 2. Materials (Continued)

### 2.2 EQUIPMENT -- SUGGESTED SIZES/SUPPLIERS, MANUFACTURERS

Method Step	Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
2.3; 2.4	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.	Bottle, Amber glass	Qorpak , 2 oz, 4 oz and 8 oz with Teflon®-lined screw cap	Qorpak	
Various	Cylinder, Graduated	Various sizes	Various	
Various	Flask, Volumetric	100, 50, 25,10 and 5 mL	Various	
3.3	Gelman PTFE acrodisc	0.45 um, 13 mm and 22 mm	Gelman Science	4543 & 4219
Various	Hot Plate, Magnetic Stirring		Various	
Various	Pipet, Volumetric	0.5, 1-10, 25 mL	Various	
Various	Pasteur Pipet, disposable	Various size	VWR	
3.3	Syringes, plastic, disposable	1 mL	Various	
Various	Ultrasonic Bath	Model FS 7652H	Fisher Scientific	
3.3	Vials, HPLC Screw caps	9 mm; 2.0 mL	Sun Brokers International	502-130, 502-235
Various	Vortex mixer	Genie 2	Fisher Scientific Co	12-812
3.3	Vacubrand vacuum pump/controller	Model HS501-D	Elnik Systems, Inc.	
3.5	LC-MS/MS	API 3000 Mass Analyzer	PE Sciex	
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	

## 2. Materials (Continued)

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

### 2.3 Reagents and Chemicals -- Suggested Sources

#### 2.3.1 Chemicals

Chemical	Grade	Manufacturer/ Supplier	Catalog Number
Ammonium Formate	MicroSelect >99%	Fluka	09735
Formic Acid	98%	E.M. Science	FX0440-7
Methanol	High Purity	B & J	230-4
Pre-pack SPE Column, C <sub>18</sub>	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
<p><b>Solution I:</b> Methanol-water, 10:90, v/v</p> <p>Add 100 mL of methanol into a 1L volumetric flask. Dilute to the mark with water and mix well to ensure complete homogeneous solution.</p> <p><b>Note:</b> Heat may occur while mixing.</p>	2.4.2.3 and 3.3
<p><b>Solution II:</b> 0.1% formic acid (98 %) in water</p> <p>Add 1.0 mL of formic acid (98%) into a 1L volumetric flask. Dilute to the mark with water and mix well to ensure complete homogeneous solution. The pH of the solution is about 3.</p>	3.2.2

## 2. Materials (Continued)

Solvent Mixtures	Method Step
<p><b>LC-MS/MS Mobile Phase A:</b>                      Water with 0.1 % formic acid and 4 mM ammonium formate</p> <p>Add 1.0 mL of formic acid (98 %), about 50 –100 mL of water and 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.</p>	<p>3.5</p>
<p><b>LC-MS/MS Mobile Phase B:</b>                      Methanol with 0.1 % formic acid and 4 mM ammonium formate</p> <p>Add 1.0 mL of formic acid (98 %), about 50 –100 mL of methanol and 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 methanol and mix well to ensure complete homogeneous solution.</p>	<p>3.5</p>

### 2.4 Standard Solutions and their Storage Stability

#### 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 method validation 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 solution of BAS 800 H (1 mg/mL):

Prepare a 1.0 mg/mL stock solution by weighing an appropriate amount of BAS 800 H into a volumetric flask. Dissolve with methanol and dilute to mark. For example, to prepare a 10 mL stock solution of BAS 800 H, place 10.0 mg of BAS 800 H into a 10 mL volumetric flask. Dissolve and dilute to mark with methanol. Sonicate and vortex to ensure a complete homogeneous solution.

## 2. Materials (Continued)

### 2.4.2.2 Standards for Fortifications

Prepare a 10 µg/mL standard solution for fortification by transferring 1.0 mL of stock solution (2.4.2.1) into a 100 mL volumetric flask. Dilute to mark with methanol and vortex to ensure a complete homogeneous solution. Prepare serial dilution's of this combined solution as needed. Suggested concentrations of standards for fortifications are 10, 1.0, 0.1 and 0.01 µg/mL, in methanol.

### 2.4.2.3 Calibration Standard Solutions for LC-MS/MS Analysis: 10.0, 4.0, 2.0, 1.0, 0.4 and 0.2 ng/mL in methanol-water (10:90, v/v)

Prepare a 10.0 ng/mL calibration standard solution by transferring 0.5 mL of 1.0 µg/mL of fortification solution (2.4.2.2) with a volumetric pipet into a 50 mL volumetric flask and dilute to the mark with methanol-water (10:90, v/v). Vortex to ensure a complete homogeneous solution. Prepare serial dilutions of this solution as needed. Suggested concentrations of standards are 10.0, 4.0, 2.0, 1.0, 0.4 and 0.2 pg/µL, in methanol-water (10:90, v/v).

**NOTE:**

- Use amber bottles with Teflon®-lined screw caps as storage containers for standard solutions. It is also recommended to turn off the hood light, if possible, while working with these 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 samples received from the field are kept frozen (<-5°C) before analysis. Add 10 mL (10 g) of water sample volumetrically into a Erlenmeyer flask with a glass stopper using a 10 mL volumetric pipet.

### 3.2 Fortification

3.2.2 For the fortification samples, add an appropriate volume of standard solution of BAS 800 H to the respective control sample by a volumetric pipet. For example, for a 0.001 ppm fortification sample, pipet 1 mL of the 0.01 µg/mL standard fortification solution (2.4.2.2) onto 10 mL (10 g) of control water sample. For unfortified control (or treated field sample) and reagent blank sample, add 1 mL of methanol into the samples.

Attach a stopper and vortex to mix. Mix well to obtain a homogeneous extract.

**NOTE:** Extreme caution should be taken while mixing the samples. It is recommended initially to hand mix the extract in the volumetric flask with occasional venting by opening the stopper followed by the mixing with Vortex

mixer. Vigorous mixing in stages will avoid sample loss due to pressure build-up. Also, not enough mixing will cause low recoveries.

### 3.2.2 De-ionized and Well water analysis

Vortex the extract in the volumetric flask. Transfer a 1 mL aliquot of the extract volumetrically into a 25 mL vial (VWR Catalog number GLC-01008) and proceed to sample preparation (Section 3.4.1) with the extract.

### 3.2.3 Salt Water Analysis

Vortex the extract in the volumetric flask and transfer a 1 mL aliquot of the extract into a 25 mL vial (VWR Catalog number GLC-01008). Hold the extract for sample clean-up (Section 3.3).

## 3.3 Sample Clean-up:

### 3.3.1 Solid Phase Extraction (C<sub>18</sub>)

#### 3.3.1.1 Column Preparation and Conditioning

Connect a pre-pack C<sub>18</sub> SPE (500 mg) to a solid phase extraction manifold.

Condition the coupled column by passing through 5 mL of methanol followed by 6 mL of 0.1% formic acid (98 %) in water. Allow the solvents to pass through just below the bed (top frit) of C<sub>18</sub> column. Do not allow the column to go dry as well as make sure there is no methanol left above the bed line of the column.

**NOTE:**

1. Vacuum (~ 600-700 mbar) is applied for the extraction using solid phase extraction manifold. Obtain a steady flow (8-12 mL/minute) before the extraction. It is advisable to "stabilize" the vacuum to obtain good recoveries of the analytes.
2. Use 25 mL vial (VWR Catalog number GLC-01008) for collection.

#### 3.3.1.2 Loading, Washing and Elution

Add 4.0 mL of 0.1% formic acid (98 %) in water into the extract from section 3.2.3 and mix gently with a Pasteur pipet. Vortex gently to obtain a homogeneous extract.

**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 column. Apply the vacuum and allow the solvents to pass through. Collect the eluent into the same vial that contains the conditioning solvent. Add 2 mL of 0.1% formic acid (98 %) in water to the vial that contained

### 3. Analytical Procedure (Continued)

the extract from section 3.2.3 and vortex to wash. Add the wash to the top of the column. Apply the vacuum and allow the solvents to pass through (just enough to pass through the top frit of the column). Do not allow the column to go dry, as well as make sure there is no 0.1% formic acid (98 %) in water left above the bed line of the column.

Add 5 mL of deionized water to the top of the C<sub>18</sub> column. Apply the vacuum and allow all the solvents to pass through.

Disconnect the vacuum. Remove the collection vial and discard all the solutions. Remove the water droplets from the inside of the column using the paper tissues. Dry the column for about 5 minutes.

Replace the needles from the SPE manifold with clean and dry ones. Also replace the collection vials to collect the eluent that is obtained after the following step.

Add 5 mL of methanol to the top of the C<sub>18</sub> column. Apply the vacuum and allow the solvents to pass through and collect the eluent.

Evaporate off the combined eluent at about 40°C under a stream of nitrogen using a N-evaporator. Remove the samples immediately after evaporation and proceed to step 3.4.2.

#### **NOTE:**

1. It is recommended to apply constant vacuum about 600-700 mbar throughout the entire extraction procedure
2. The water bath temperature of the N-evaporator should be at about 40°C. The evaporation time of the extract is about 40-60 minutes.
3. Use of a wide mouth vial (~ 25 mL capacity, VWR catalog number GLC 01008) accelerates the evaporation
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

### 3.4 Sample Preparation for LC-MS/MS Analysis

Typically the following procedures are used to prepare the samples for analysis:

#### 3.4.1 De-ionized and Well Water Analysis

Add 1.0 mL of methanol-water (10:90, v/v) to the sample extract from Section 3.2.2 and vortex to obtain a homogeneous extract.

#### 3.4.2 Salt Water Analysis

Add 2.0 mL of methanol-water (10:90, v/v) to the sample extract from Section 3.3.1.2 and vortex to mix. Sonicate for 3 minutes and vortex to obtain a homogeneous extract.

- 3.4.3 **For control and 0.001 ppm fortifications**, filter the sample solution from Section (3.4.1 or 3.4.2) through a syringe filter (a 0.45 micron PTFE membrane filter fitted to 1.0 mL disposable plastic syringe) and collect the filtrate (about 1-2 mL) into an injection vial.
- 3.4.4 **For 0.01 ppm fortifications**, take 1 mL of the sample solution (3.4.1 or 3.4.2) and dilute to 10 mL with methanol-water (10:90, v/v). Filter the solution through a syringe filter (a 0.45 micron PTFE membrane filter fitted to 1.0 mL disposable plastic syringe) and collect the filtrate (about 1-2 mL) into an injection vial.

A flow chart of the analytical procedure is presented in **Figure 1**.

### 3.5. Instrumentation

Suggested LC-MS/MS Operating condition:

Instrument:	PE Sciex API 3000 Mass Analyzer												
Inlet [HPLC System]:	PE Series 200 Micro Pump system with Series 200 Auto sampler												
Software Version:	Analyst 1.1												
Column:	Thermo RP Prism 5 $\mu$ , 50 X 2.1 mm, [P/N 32105-052130]												
Injection:	Typically 10 $\mu$ L												
Mobile Phase: [Gradient]	<p>A = water with 4 mM ammonium formate and 0.1 % formic acid                      B = methanol with 4 mM ammonium formate and 0.1 % formic acid</p> <table border="0"> <thead> <tr> <th><u>Time (min.)</u></th> <th><u>Composition</u></th> </tr> </thead> <tbody> <tr> <td>0.0</td> <td>70% A + 30% B</td> </tr> <tr> <td>2.0</td> <td>10% A + 90% B (Switching valve)</td> </tr> <tr> <td>4.0</td> <td>10% A + 90% B</td> </tr> <tr> <td>4.1</td> <td>70% A + 30% B</td> </tr> <tr> <td>5.0</td> <td>70% A + 30% B</td> </tr> </tbody> </table> <p>Run every 5.0 minutes</p>	<u>Time (min.)</u>	<u>Composition</u>	0.0	70% A + 30% B	2.0	10% A + 90% B (Switching valve)	4.0	10% A + 90% B	4.1	70% A + 30% B	5.0	70% A + 30% B
<u>Time (min.)</u>	<u>Composition</u>												
0.0	70% A + 30% B												
2.0	10% A + 90% B (Switching valve)												
4.0	10% A + 90% B												
4.1	70% A + 30% B												
5.0	70% A + 30% B												
Flow Rate:	300 $\mu$ L/minute												
Expected Retention Times	~ 2.8 minutes												
Transitions:	m/z 501 $\rightarrow$ 459 (Quantitation ion) and m/z 501 $\rightarrow$ 349												
Ionization Mode:	Positive ion; Turbospray (500 $^{\circ}$ C)												

### 3. ANALYTICAL PROCEDURES (Continued)

**NOTE:**

4. The LC-MS/MS instrument and equipment listed was used for method development and validation. Other equivalent hardware may be used. The use of a guard column is optional.
5. 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.
6. 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 a different type of instrument.

#### 3.6 Calibration Procedures

Calculation of results is based on peak area or peak height measurements using a calibration curve. The calibration curve is obtained by direct injection of 10  $\mu\text{L}$  of BAS 800 H standards for LC-MS/MS in the range of 0.2  $\text{pg}/\mu\text{L}$  to 4.0  $\text{pg}/\mu\text{L}$ . In a given injection run, the same injection volume is used for all samples and standards. Typical standard amounts injected on-column range as follows: 2, 4, 10, and 40  $\text{pg}$ .

Calibration curves are prepared by plotting the peak area versus the weight using a linear least squares working curve in the form of  $y = bx + c$ . The transitions monitored are  $m/z$  501  $\rightarrow$  459 (Quantitation ion) and  $m/z$  501  $\rightarrow$  349. The quantitation of the analyte is performed on 501  $\rightarrow$  459.

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 800 H. The limit of detection has not been determined, but set at 20 % of the limit of quantitation [e.g. at the LOQ (0.001 ppm), if the amount of analyte is 4.55  $\text{pg}/10 \mu\text{L}$  injection, LOD is 0.9  $\text{pg}/10 \mu\text{L}$  injection]. The lowest standard for each analyte in the calibration curve has good detectability (signal to noise ratio greater than 3:1).

#### 4. Calculation of Results

##### 4.1 Principle

Calculation of results is based on peak height or area measurements.

The recoveries and residues of BAS 800 H in  $\mu\text{g/g}$  (ppm) are calculated with the following formulas:

$$\text{Residue in ppm} = \frac{\text{ng found per injection}}{\text{mg injected}}$$

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

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

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

$$\text{mg injected} = \frac{\text{Sample weight (g) extracted}}{\text{Final extraction volume (mL)}} \times \text{Aliquot Factor} \times \mu\text{L injected} \times \text{Dilution factor (F1)}$$

$$\text{Aliquot factor} = \frac{\text{Aliquot taken}}{\text{Final volume}} = \frac{1 \text{ mL}}{2 \text{ mL}} = 0.5$$

**Dilution Factor (F1)** = 1 and 0.1 for 0.001 and 0.01 ppm fortification samples, respectively.

## **6. Time Requirement for Analysis**

The time required for a set of 13 samples (10 fortified, 2 controls and one reagent blank) is approximately 4 person-hours (without the sample clean up) and is 8 person-hours (with the sample clean up) provided that no special problems arise, such as matrix interference and LC-MS/MS analysis and data processing times that could be automated and unattended.

## **7. Confirmatory Techniques**

The method of determination is LC-MS/MS, which is a highly selective and self-confirmatory detection technique. However two transitional ions were monitored during analysis for peak confirmation

## **8. Potential problems**

The glassware used for the method should be thoroughly rinsed with methanol to prevent contamination.

Peak enhancement could be a potential problem without sufficient sample clean-up. It is highly recommended to perform instrument check routinely during LC-MS/MS analysis for standard peak enhancement or suppression. The instrument check sample is basically prepared by adding known amount of standard to the control matrix at the limit of quantitation (0.001 ppm level). It is recommended to clean the LC-MS thoroughly, if peak enhancement or suppression has been observed. 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.

## **9. Safety and Health Considerations**

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

**Figure 1: Flow Diagram of Analytical Method No. D0502 (Method D0502, parent only procedure)**

*Determination of BAS 800 H in Water*

