I. INTRODUCTION

A. Purpose of the Study

The purpose of this study is to demonstrate that BASF Method D0502, entitled "THE DETERMINATION OF RESIDUES OF BAS 800 H AND ITS METABOLITES IN WATER USING LC-MS/MS" [1] can be performed successfully at an outside facility with no experience with the method.

B. Summary of the Results

The independent laboratory validation of the BASF method was successfully completed in the first trial. Clarification of the method was necessary before the start of the first trial, no additional communication was necessary to complete the method validation. After completion of the first trial, communication between the performing laboratory and the study monitor was conducted and the first injected set was chosen to be reported for the successful completion of the method validation and is described in Section VI.

II. REFERENCE SUBSTANCE AND SAMPLE HISTORY

The reference substance, BAS 800 H and its metabolites were received at ADPEN Laboratories, Inc. on 9/18/2007. See Figure 1 for the structure and detailed information including lot number, purity, storage conditions, and expiration date of BAS 800 H and its metabolites. Reference substance was stored in refrigerator E109 which had a temperature range of 0 to 14°C for the duration of time the reference substance was stored there, through the duration of the study. Standard solutions prepared for this study were also stored under refrigerated conditions in refrigerator E51. The temperature range during the course of this study for refrigerator E51 was 0 to 3°C.

A control water sample was sent from BASF Corporation and received by ADPEN Laboratories on 05/23/08. Upon receipt, the sample was stored in refrigerator E57 according to instructions received with shipping information. The temperature range of refrigerator during the course of this study was 4 to 8°C. Method D0502 states water samples should be kept frozen therefore on June 10, 2008 the sample was moved to freezer E16 after receiving a letter of clarification from BASF. The temperature range of freezer during the course of this study was -13 to -17°C. Prior to analysis, the sample was assigned a unique sample code and each sub-sample was assigned a lab code. The sample code consisted of the project code and a sample number (i.e. 2K8-903-351551-01). Sample extracts were stored in refrigerator E20 while waiting for sample processing, LC-MS/MS analysis or completion of project. The temperature range during the course of this study for refrigerator E20 was 4 to 7°C.

III. PROCEDURE - METHOD SYNOPSIS

BASF Analytical Method D0502 is used to determine residues of BAS 800 H and its metabolites in water matrices as investigated for this validation study. The following is a brief summary of the method procedure:

A 1.0 mL sample aliquot is diluted with 0.1 mL methanol, vortex mixed and the residues are determined using LC-MS/MS. The detailed analytical method can be found in Appendix B.

IV. LIMIT OF QUANTITATION AND DETECTION

The limit of quantitation (LOQ) of the method is the lowest fortification level tested. The LOQ of the analytical method for all analytes (BAS 800 H, and its metabolites, M800H01, M800H02, M800H07, M800H08, M800H15 and M800H22) is 0.001 ppm (0.001 mg/kg). The standards equivalent to the LOQ level is at least 10 times the background noise; therefore, the 0.001 ppm fortification level is an acceptable limit of quantitation for all analytes. Sub-samples of water were fortified with BAS 800 H and its metabolites at 0.001 ppm (LOQ) and 0.01 ppm and analyzed using BASF Analytical Method D0502. The limit of detection was determined to be 0.4 ng/µL; the smallest standard injected which was better than three times the background noise.

V. CALIBRATION, CALCULATIONS AND STATISTICS

Residues of BAS 800 H and its metabolites were quantitated by external standards. Calibration curves for the analytes were generated by plotting the detector's response (peak area) versus the concentration (ng) of standard injected for the primary ions (quantitation transition ions). The data system derived an equation for the fit of the standard curve and this equation was used to calculate intercept and slope of the linear regression curve. Peak integration and quantitation were performed using the quantitation ions by computer using Applied Biosystem's Analyst® data system. The confirmatory ions can be used as a secondary means of calculating residues should interferences be found with the primary quantitation ion. No interferences were observed in this validation study. Recovery results were computed for each set of samples by Microsoft's Excel® and reported in spreadsheet data reports which are presented in Appendix A. Equations used for quantitation are presented in Figure 2. Statistical treatment of the data included calculation of averages and standard deviations. These calculations were performed using Excel. Results were rounded off for reporting purposes but not for any calculations.

Analyst is a registered trademark of Applied Biosystems.
Excel is a registered trademark of the Microsoft Corporation.

TABLE 13. Typical LC/MS Instrument Parameters for BAS 800 H Analysis

Instrument:	Applied Biosystems API 3000 LC-MS/MS with Analyst 1.4.2 Software
Inlet [HPLC System]:	Agilent 1100 HPLC System with Binary Pump, High Performance Autosampler and Heated Column Compartment set at 50ºC
Column:	Inertsil Ph 5um 2.1X100 mm S/N: 8FS50098
Injection:	Typically 50 μL

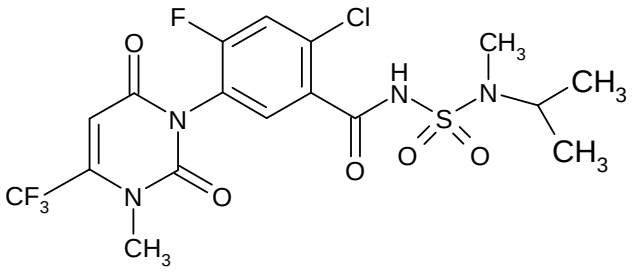
Mobile Phase:	A = water with 0.1 % formic acid and 4 mM ammonium formate B = methanol with 0.1 % formic acid and 4 mM ammonium formate		
[Gradient]	**Time (minute)**	**Composition (%)**	
		A	**B**
	0.0	95	5
	0.5	95	5
	2.0	40	60
	3.5	30	70
	4.5	5	95
	7.5	5	95
	7.6	95	5
	9.5	95	5
Flow Rate:	250μL/minute		

Analytes	Expected Retention Times (minutes)	Transitions (m/z):
		Quantitation ion
BAS 800 H	7.55	501.0 → 348.9
M800H01	7.33	487.0 → 365.9
M800H02	7.34	487.0 → 335.0
M800H07	6.76	381.0 → 229.0
M800H08	7.31	503.1→ 351.1
M800H15	7.05	480.0 → 420.1
M800H22	7.18	521.00 → 369.0
Ionization Mode:	Positive ion; Turbospray (400°C)	

FIGURE 1. Structure of the Test and Reference Substances

Test Substances:

BASF Code Name:	BAS 800 H
BASF Registry Number:	4054449
CAS Number:	372137-35-4
Molecular Formula:	$C_{17}H_{17}ClF_4N_4O_5S$
Molecular Weight:	500.9
Lot No.:	L67-190
Purity	99.6%
Expiration date:	April 1, 2009
Storage:	Refrigerator
Structural Formula:	



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, 2010
Storage:	Refrigerator
Structural Formula:	

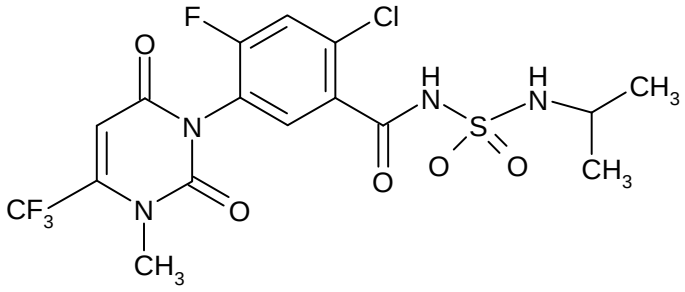
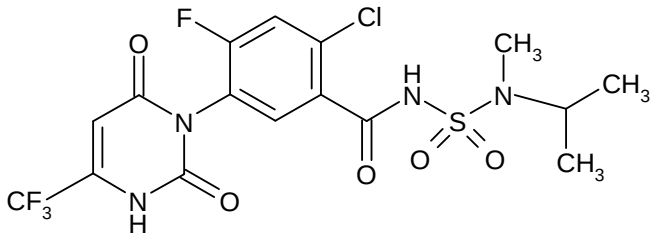


FIGURE 1. Structure of the Test and Reference Substances (continued)

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
Storage:	Refrigerator
Structural Formula:	



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
Storage:	Refrigerator
Structural Formula:	

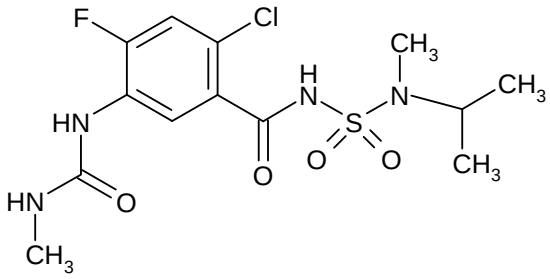
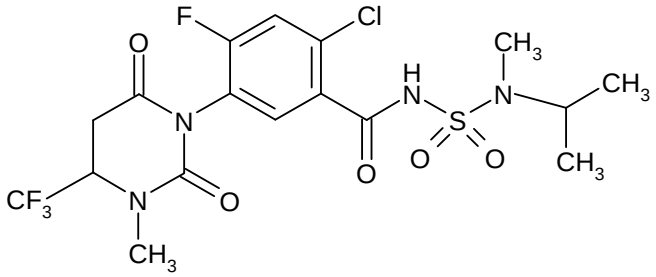


FIGURE 1. Structure of the Test and Reference Substances (continued)

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:	February 1, 2010
Storage:	Refrigerator
Structural Formula:	



BASF Code Name:	M800H15
BASF Registry Number:	5264357
Molecular Formula:	$C_{15}H_{18}ClF_4N_3O_6S$
Molecular Weight:	479.8
Lot No.:	L74-80
Purity	94.5%
Expiration date:	July 1, 2010
Storage:	Refrigerator
Structural Formula:	

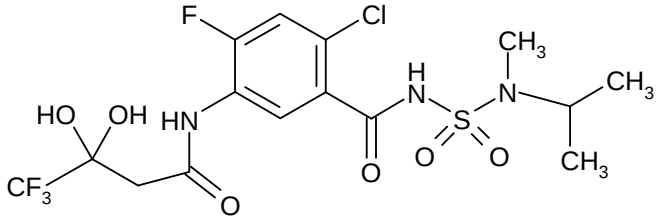
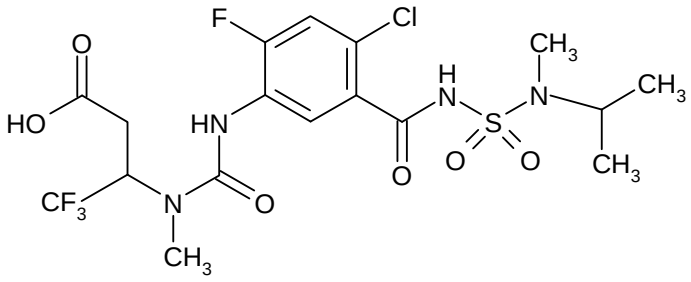


FIGURE 1. Structure of the Test and Reference Substances (continued)

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	94.1%
Expiration date:	February 1, 2010
Storage:	Refrigerator
Structural Formula:	



Note: A purity statement will accompany the shipment of these materials. BASF has retained a reserve sample of these chemicals, and has documents at the BASF Agricultural Products Center, Research Triangle Park, North Carolina specifying the location of the synthesis and characterization information for these compounds.

FIGURE 2. Calculations for the Quantitation of BAS 800 H

Recovery results are calculated by comparison to the standard curves obtained from a linear regression analysis of the data found by the data system. The equation for the fit of the standard curve was used to calculate intercept and slope of the linear regression curve. The intercept and the slope were used in the equation used for quantitation. Excel is used to calculate the ppm and percent recovery and to present the data in a report format. The following equations were taken from the method and were used for quantitation:

a) $$\text{mg injected} = \frac{\text{Sample weight}}{\text{Final extraction volume}} \times \mu\text{L injected} \times \text{Dilution Factor}$$

b) $$\text{Residue in ppm} = \frac{\text{ng found}}{\text{mg injected}}$$

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

As an example, calculations to obtain BAS 800 H percent recovery values using sample 2K8-903-351551-12 (recovery sample) are shown below:

a) $$\text{mg injected} = \frac{1.0\text{ g}}{1.1\text{mL}} \times 50\ \mu\text{L} \times 0.1 = 4.54\text{ mg}$$

b) $$\text{Residue in ppm} = \frac{0.0523\text{ ng}}{4.54\text{ mg}} = 0.01151\text{ ppm}$$

c) $$\text{Percent recovery (\%)} = \frac{0.01151\text{ ppm} - 0.00013}{0.01\text{ ppm}} \times 100 = 113.8$$