2.0 INTRODUCTION

2.1 Purpose of the Study

The purpose of this study is to demonstrate BASF Analytical Method L0143/01: "Determination of BAS 700 F and its Metabolites, M700F001, M700F002 and M700F007 in Water by HPLC/MS-MS" (Reference 1) can be performed with acceptable recoveries by an outside facility.

This independent laboratory validation was conducted in compliance with the US Environmental Protection Agency (EPA) Good Laboratory Practices (GLP) standards, 40 CFR 160.

2.2 Study Design

The study was conducted according to OPPTS 850.7100 Data Reporting for Environmental Chemistry Methods (Reference 2); and under OPPTS 835.6200 Aquatic Field Dissipation (Reference 3); OPPTS 835.7100 Prospective Ground Water Monitoring (Reference 4); EPA Pesticide Assessment Guideline, Subdivision N 164-2 (Reference 5); and SANCO/3029/99 rev 4 (Reference 6).

The laboratory had up to three trials to complete the method successfully. If necessary, clarification of the method could be requested prior to each trial, but never during the trial. Any communications would be documented.

The analytical set consisted of a reagent blank, two un-spiked control pond water samples, 5 control pond water samples fortified at 0.03 ppb (0.03 µg/L), which is the LOQ, and 5 control pond water samples fortified at 10×LOQ (0.3 ppb). A total of 13 samples were used for the method validation first trial set. Samples were assigned unique numbers according to ADPEN Standard Operating Procedures.

Control pond water was provided by BASF to be used as the test system matrix. Fortifications were made with BAS 700 F and its metabolites M700F001, M700F002 and M700F007. Acceptable recovery (mean) ranges were to be 70-110 % as defined in EC guidance document and reported in Method L0143/01.

As a means to control bias, calibration standards were injected with the analytical set to ensure detector linearity and stable response.

3.0 MATERIALS

3.1 Reference Materials

The reference substances BAS 700 F, M700F001, M700F002 and M700F007 were received at ADPEN Laboratories, Inc. on August 13, 2010. Figure 1 presents the structure and detailed information for each reference substance including lot number, purity, storage conditions, and expiration dates. The reference standards were stored in freezer E109, which had a temperature range

of -22 to -18°C for the duration of the study. Standard solutions prepared for this study were stored under refrigerated conditions in refrigerator E51. The temperature range during the course of this study for refrigerator E51 was 2 to 4°C. Sample extracts for this study were stored under refrigerated conditions in refrigerator E20. The temperature during the course of this study for refrigerator E20 was 7°C.

3.2 Test System

A control pond water sample (surface water) was sent from BASF Corporation to be used as the control matrix for validation. This water type was chosen because it would be harder to work with in comparison to other water types. Control pond water was received by ADPEN Laboratories on August 13, 2010. While in the custody of ADPEN Laboratories, Inc., the sample was stored in freezer E16. The temperature range of freezer E16 during the course of this study was -15.5 to -10°C. Prior to analysis, a unique sample number was assigned to each validation sample. The unique sample number consisted of a year code, client code, project code and a unique sample number (i.e. 2K10-903-395759-ILV-1).

4.0 METHODS

4.1 Summary of Analytical Procedure

BASF Analytical Method L0143/01 was used to determine residues of BAS 700 F and its metabolites in pond water. The method used is presented in Appendix C. The following is a brief summary of the analytical procedure:

Residues were extracted in a 150 mL centrifuge tube by adding 0.3 mL of formic acid to 50 mL (or 50 g) of water. The sample was shaken to make a homogenous mixture. A Phenomenex Strata X-AW SPE column was conditioned with 5mL of methanol (MeOH) and followed with 5mL of 1% formic acid in water. The sample was added to the column at a rate of approximately 1 drop per second and not allowed to go dry. The column as washed with 5mL of 1% formic acid in water and vacuum dried for about 1 minute to remove all water. The column was eluted with 10mL of 90:10 MeOH:formic acid into a 50 mL glass centrifuge tube. The extract was evaporated to dryness under nitrogen at 50°C and reconstituted in 1.5mL of 1:1 MeOH:DI water. The sample was vialed and injected on the HPLC-MS/MS system. Instrument parameters are presented in Table 5.

4.2 Limit of Quantitation and Limit of Detection

The method's limit of quantitation (LOQ) for all analytes is 0.03 µg/L (ppb). The method's limit of detection (LOD) is 20% of the LOQ, which is equivalent to 0.006 ppb.

Validation samples were fortified with BAS 700 F and its metabolites at the LOQ (0.03 ppb) and at ten times the LOQ (0.3 ppb).

4.3 Calibration

A seven-point standard curve was prepared by injecting calibration standard solutions at appropriate concentrations ranging from 0.0002 ng to 0.050 ng. Calibration standards were injected to bracket every 2-5 sample injections.

4.4 Calculations

Residue concentrations of BAS 700 F and its metabolites were calculated using Analyst 1.5.1 data system from a generated standard calibration curve. The data system derived a linear equation for the fit of the standard curve by plotting the standard concentration (ng) on the x-axis versus the respective detector's response (peak area) on the y-axis. The correlation coefficient (r^2) for the calibration curves were greater than 0.990 for all analytes. Peak integration was performed using Analyst 1.5.1. Excel is used to calculate the ppb and percent recovery and to present the data in a report format. Typical calibration curves are presented in Figure 2.

The following equations are used for residue calculations within Analyst:

a) Calibration curve: $y = mx + b$ Solving for x: $x = \frac{y - b}{m}$

Where, m = slope
b = y intercept
x = Analyte found (ng)
y = Peak Area

The following equations are used for residue and recovery calculations within Excel:

b) Amount of sample injected (g) = $\frac{\text{injection size}}{\text{final volume}} \times \text{sample weight} \times \frac{1\ \text{mL}}{1000\ \mu\text{L}}$

c) $\text{ppb} = \frac{\text{ng found}}{\text{g injected}}$

d) Percent recovery = $\frac{(\text{ppb in the sample} - \text{ppb in the control})}{\text{ppb added}} \times 100$

As an example, below are the calculations to obtain the percent recovery in control water sample number 10081301 fortified with BAS 700 F (Workorder number: WO-10090203; Lab code number: 100902003-001C):

a) Analyte found (ng) = $\frac{31827 - (5.55e+3)}{3.03e+007} = 0.00087$ ng

b) Amount of sample injected (g) = $\frac{10\mu\text{L}}{15\ \text{mL}} \times 50\ \text{g} \times \frac{1\ \text{mL}}{1000\ \mu\text{L}} = 0.0333$ g

c) $\text{ppb} = \frac{0.00087\ \text{ng}}{0.0333\ \text{g}} = 0.0261$ ppb

Average ppb found in control samples 100902003-001A and 100902003-001B = 0.0000 ppb

d) Percent recovery = $\frac{(0.0261 - 0.0000)}{0.03} \times 100 = 87.0\%$

The response of the primary and secondary ion transitions was sufficient for calculating residues. No interferences were found with the primary or secondary quantitation ions. Use of the secondary ion for confirmation is acceptable.

TABLE 5. Typical LC/MS Instrument Parameters for the Analysis of BAS 700 F, M700F001, M700F002, and M700F007

HPLC System:	Agilent 1200 HPLC System with Binary Pump, High Performance Autosampler and Heated Column Compartment
MS/MS Instrument:	Applied Biosystems™ API 4000 QT (Q-Trap) Analyst 1.5.1 Software
Column:	Waters Atlantis® T3, 3 µm
Injection:	10 µL

Mobile Phase:	A = 0.1% formic acid in methanol B = 0.1% formic acid in water		
Gradient:	Time (minute)	Composition (%)	
		A	B
	0.00	10.0	90.0
	1.50	10.0	90.0
	7.00	40.0	60.0
	8.10	100.0	0.0
	11.50	100.0	0.0
	11.60	10.0	90.0
	15.00	10.0	90.0
Flow Rate:	500µL/minute		

Analytes	Transitions (m/z):	
	Quantitation ions (Expected Retention Times in minutes)	
BAS 700 F	382.0 → 362.0 (12.1)	382.0 → 342.0 (12.1)
M700F001	175.0 → 91.0 (6.64)	175.0 → 111.0 (6.66)
M700F002	161.0 → 141.0 (9.35)	161.0 → 97.0 (9.34)
M700F007	176.0 → 156.0 (6.79)	176.0 → 136.0 (6.79)
Scan Type:	MRM	
Ion Source:	Turbo Spray	
Source Temperature:	600°C	
Ionization Mode:	Positive and Negative	

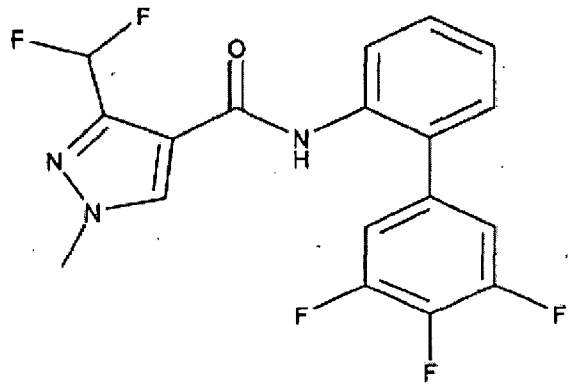
TABLE 6. Recommendations for BASF Analytical Method L0143/01

Recommendations:

1. The samples can be diluted if matrix suppression is observed.

FIGURE 1. Structure of the Test and Reference Substances

BASF Code Name:	BAS 700 F
BASF Registry Number:	5094351
Molecular Formula:	$C_{18}H_{12}F_5N_3O$
Molecular Weight:	381.3 g/mol
Lot No.:	L80-28
Purity:	99.7%
Expiration date:	August 01, 2012
Structural Formula:	



BASF Code Name:	M700F001
BASF Registry Number:	5069089
Molecular Formula:	$C_6H_6F_2N_2O_2$
Molecular Weight:	176.1 g/mol
Lot No.:	L80-68
Purity:	99.2%
Expiration date:	August 01, 2011
Structural Formula:	

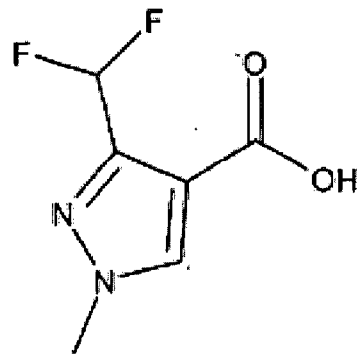
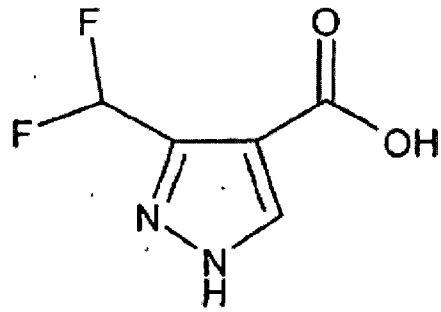
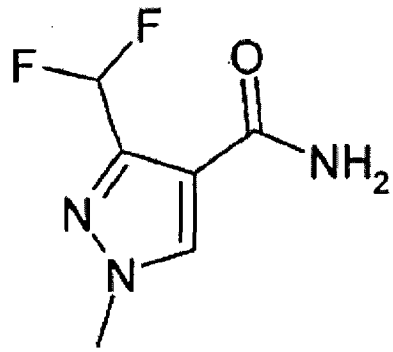


FIGURE 1. Structure of the Test and Reference Substances (continued)

BASF Code Name:	M700F002
BASF Registry Number:	5435595
Molecular Formula:	$C_5H_4F_2N_2O_2$
Molecular Weight:	162.1 g/ml
Lot No.:	L80-20
Purity:	99.2%
Expiration date:	July 01, 2011
Structural Formula:	



BASF Code Name:	M700F007
BASF Registry Number:	5621781
Molecular Formula:	$C_6H_7F_2N_3O$
Molecular Weight:	175.1 g/mol
Lot No.:	L81-108
Purity:	99.4%
Expiration date:	April 01, 2011
Structural Formula:	



Note: 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.