

2.0 INTRODUCTION

2.1 Purpose of the Study

The purpose of this study is to perform an Independent Laboratory Validation of BASF Analytical Method D0903: "Determination of BAS 700 F (Reg. No. 5094351) and its Metabolites, M700F001 (Reg. No. 5069089) and M700F002 (Reg. No. 5435595) in Soil at LOQ 0.001 mg/kg"

2.2 Summary of the Results

The independent laboratory validation of the BASF Analytical Method D0903 was successfully completed in the first trial for soil. Method clarification was not necessary, prior to the first trial, for successful completion of the method validation.

3.0 REFERENCE SUBSTANCE AND SAMPLE HISTORY

The reference substances, BAS 700 F and its metabolites, M700F001 and M700F002 were received at ADPEN Laboratories, Inc. on March 26, 2009, May 15, 2009 and July 17, 2009, respectively. See Figure 1 for the structure and detailed information including lot number, purity, storage conditions, and expiration dates. The reference standard of BAS 700 F was stored in refrigerator E109, which had a temperature range of 1 to 6°C for the duration of the study. The reference standards of the metabolites, M700F001 and M700F002, 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 5°C. Sample extracts for this study were stored under refrigerated conditions in refrigerator E20. The temperature range during the course of this study for refrigerator E20 was 4 to 5°C.

Control soil sample was sent from BASF Corporation and received by ADPEN Laboratories on August 13, 2009. The sample received from BASF was stored in freezer E24. The temperature range of freezer E24 during the course of this study was -20 to -18°C. Prior to analysis a unique sample number was assigned to each weighing of the sample. The unique sample number consisted of a year code, client code, project code and a unique sample number (i.e. 2K9-903-374091-ILV-1).

4.0 PROCEDURE - METHOD SYNOPSIS

BASF Analytical Method D0903 was used to determine residues of BAS 700 F and its metabolites, M700F001 and M700F002, in soil in order to validate the method. The following is a brief summary of the validation procedure:

Residues of BAS 700 F and its metabolites are extracted in a 50 mL centrifuge tube by adding 25 mL of methanol:HPLC water (1:1 v/v) to 5 g of soil. The sample is vortexed, shaken for 30 minutes in a mechanical shaker and centrifuged at 3400 RPM for 10 minutes at 25°C. A 0.5-mL aliquot is transferred into a 15-mL centrifuge tube and brought up to 1 mL with methanol-water (5:95 v/v) in 0.1% formic acid. The sample is vortexed and passed through a syringe filter (13 mm; 0.45 µm) and collected into an autosampler vial. High fortifications (0.01 ppm) require a 1:10 dilution in methanol-water (5:95, v/v) in 0.1% formic acid. The sample is ready for injection on the LC-MS/MS system.

5.0 LIMIT OF QUANTITATION AND DETECTION

The limit of quantitation (LOQ) for residues of BAS 700 F and its metabolites is 0.001 mg/kg (ppm). The limit of detection (LOD) is 20% of the LOQ, equivalent to 0.0002 ppm.

Sub-samples were fortified with BAS 700 F and its metabolites at the LOQ (0.001 mg/kg (ppm)) and at 10 times the LOQ (0.01 ppm) and analyzed using BASF Analytical Method D0903.

6.0 CALIBRATION, CALCULATIONS AND STATISTICS

Residues of BAS 700 F and its metabolites were quantitated using external calibration standards. A calibration curve for the analyte was generated by plotting the detector's response (peak area) versus the amount (ng) of standard injected for the primary ion. 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 ion using Applied Biosystem's Analyst® data system. The confirmatory ion can be used as an alternate means of calculating residues should interferences be found with the primary quantitation ion as well as confirmation of the residues. No interferences were observed in this validation study. Recovery results were computed for each set of samples and reported in spreadsheet data reports with Microsoft Office Excel®. The residue data reports 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 also performed using Excel. Results were rounded to three significant figures only for reporting purposes. No calculations were made with rounded numbers.

One amendment was issued for this study. This amendment corrects the location information for the control soil sample that is listed in the study protocol. No deviations were issued for this analytical study.

8.0 COMMUNICATION / CONTACT

There was no communication prior to the commencement of this independent laboratory validation. This independent laboratory method validation of BASF Analytical Method D0903 was successfully completed and the Study Monitor was informed of the successful completion of the study at the end of the first trial on August 21, 2009.

9.0 CONCLUSION

In summary, the ILV was completed successfully on the first trial. Therefore, BASF Analytical Method D0903 is suitable for determining residues of BAS 700 F and its two metabolites, M700F001 and M700F002, in soil down to a level of 0.001 mg/kg (ppm). The method is well-written and contains a fair amount of comments to guide the analyst through the procedure for the first time. Recommendations presented in Table 5 should be incorporated in the final analytical method.

Original facility related data are archived at ADPEN Laboratories, Inc., Jacksonville, Florida. Certified copies of the raw data and a copy of this report and the protocol will be temporarily archived at ADPEN Laboratories, Inc., Jacksonville, Florida. The original final report, the protocol and protocol amendment and certified copies of facility related data and original raw data will be archived at BASF Crop Protection, Research Triangle Park, North Carolina.

10.0 REFERENCES

1. BASF Draft Analytical Method D0903: "Determination of BAS 700 F (Reg. No. 5094351) and its Metabolites, M700F001 (Reg. No. 5069089) and M700F002 (Reg. No. 5435595) in Soil at LOQ 0.001 mg/kg", Manasi Saha, Version dated June 16, 2009, BASF Reg. Doc No.2009/7003272

TABLE 4. Typical LC/MS Instrument Parameters for the Analysis of BAS 700 F, M700F001 and M700F002

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 Software
Column:	Unison UK-C18 3 μ m, 75 x 3 mm
Injection:	50 μ L

Mobile Phase:	A = 0.1% formic acid in water B = 0.1% formic acid in acetonitrile		
Gradient:	Time (minute)	Composition (%)	
		A	B
	0.00	70.0	30.0
	1.00	60.0	40.0
	2.00	60.0	40.0
	3.00	30.0	70.0
	3.10	0.0	100.0
	5.90	0.0	100.0
	6.00	70.0	30.0
8.50	70.0	30.0	
Flow Rate:	500 μ L/minute		

Analytes	Expected Retention Times (minutes)	Transitions (m/z):	
		Quantitation ions	
BAS 700 F	5.42	382.0 \rightarrow 362.0	382.0 \rightarrow 342.0
M700F001	1.32	175.0 \rightarrow 91.0	175.0 \rightarrow 111.0
M700F002	1.09	161.1 \rightarrow 141.0	161.0 \rightarrow 116.9
Scan Type:	MRM		
Ion Source:	TurboSpray		
Source Temperature:	600 $^{\circ}$ C		
Ionization Mode:	BAS 700 F	Positive	
	M700F001 M700F002	Negative	

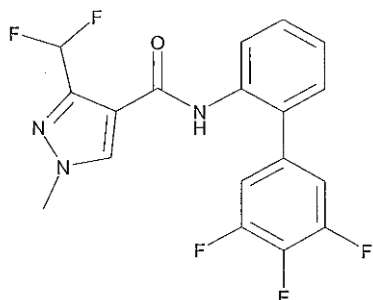
TABLE 5. Recommendations for BASF Draft Analytical Method D0903

Recommendations:

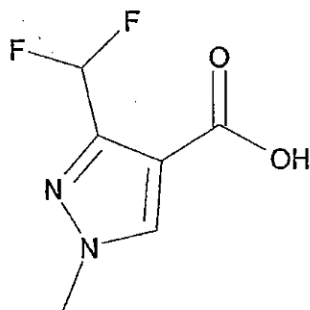
1. It is recommended to establish suitable equilibrium time at the end of the HPLC method to produce consistent analyte retention times. Comparable HPLC systems may vary in the time necessary to equilibrate between sample injections.
2. Keep aware of instrument sensitivity that may lessen due to numerous sample injections. Regular cleaning of the mass spec will help insure enough sensitivity to comply with method specifications for the lowest standard to have a signal to noise ration of 3:1.

12.0 FIGURES SECTION

FIGURE 1. Structure of the Test and Reference Substance

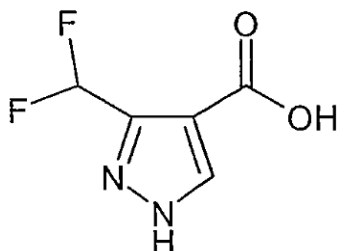


BASF Code Name: BAS 700 F
BASF Registry Number:: 5094351
CAS Number: 907204-31-3
Molecular Formula: C₁₈H₁₂F₅N₃O
Molecular Weight: 381.31 g/mol
Lot No.: L80-28
Purity 99.7%
Expiration date: April 01, 2010
Storage conditions: Refrigerator



BASF Code Name: M700F001
BASF Registry Number: 5069089
CAS Number: 176969-34-9
Molecular Formula: C₈H₆F₂N₂O₂
Molecular Weight: 176.1
Lot No.: L80-68
Purity 99.2%
Expiration date: August 01, 2010
Storage conditions: Freezer

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



BASF Code Name:	M700F002
BASF Registry Number:	5435595
CAS Number:	151734-02-0
Molecular Formula:	C ₅ H ₄ F ₂ N ₂ O ₂
Molecular Weight:	162.1 g/mol
Lot No.:	L80-56
Purity	98.2%
Expiration date:	May 01, 2010
Storage conditions:	Freezer

Note: The purity of this material was determined under GLP's by BASF Corporation. BASF has retained a reserve sample of these chemicals, and has documentation at the BASF Agricultural Research 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 700 F

Residue 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, found in the analytical method, were used for quantitation:

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

c) $\text{ppm} = \frac{\text{ng found}}{\text{mg injected}}$

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

As an example, calculations to obtain the percent recovery in a control soil sample fortified with BAS 700 F in sample μ number R0805430019 (Lab Code Number 2K9-903-374091-ILV-9). The calculations are shown below:

a) $\text{ng found} = \frac{24612 - (670)}{4.83e + 006} = 0.00496 \text{ ng}$

b) $\text{mg of sample injected} = \frac{50 \mu\text{L}}{500 \text{ mL}} \times 5 \text{ g} \times \frac{1 \text{ mL}}{1000 \mu\text{L}} \times \frac{1000 \text{ mg}}{1 \text{ g}} = 0.500 \text{ mg}$

c) $\text{ppm} = \frac{0.00496 \text{ ng}}{0.500 \text{ mg}} = 0.00992 \text{ ppm}$

d) Percent recovery = $\frac{(0.00992 - (0.00000))}{0.01} \times 100 = 99.2\%$