2.0 INTRODUCTION

2.1 Purpose of the Study

The purpose of the study was to demonstrate that BASF Analytical Methods 574/0 as written in "Validation of the BASF Analytical Method 574/0 for the Determination of Residues of BAS 650 F in Tap and Surface Water Samples" (Reference 1) and L0113 as written in "Validation of Analytical Method L0113 for the Determination of BAS 650 F Metabolites M650F01, M650F02, M650F03 and M650F04 in Water Samples" (Reference 2) could be performed successfully at an outside facility with no prior experience with the methods.

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 860.1340 Residue Analytical Method (Reference 3); OPPTS 835.6200 Aquatic Field Dissipation (Reference 4); OPPTS 835.7100 Prospective Ground Water Monitoring (Reference 5); EPA Pesticide Assessment Guideline, Subdivision N 164-2 (Reference 6); and SANCO/3029/99 rev 4 (Reference 7).

The analytical set consisted of a reagent blank, two un-spiked control pond (surface water) water samples, 5 control pond water samples fortified at 0.05 µg/kg (0.05 ppb), which is the LOQ, and 5 control pond water samples fortified at 10×LOQ (0.5 ppb). A total of 26 samples (13 for BAS 650 F and 13 for metabolites) were used for the method validation first trial set.

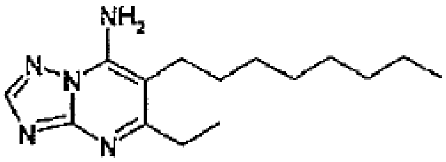
Control pond water was provided by BASF to be used as the test system matrix. Fortifications were made with BAS 650 F, M650F01, M650F02, M650F03 and M650F04. Acceptable recovery (mean) ranges were 70-110 % as defined in EC guidance documents and reported in methods 574/0 and L0113.

3.0 MATERIALS

3.1 Reference Materials

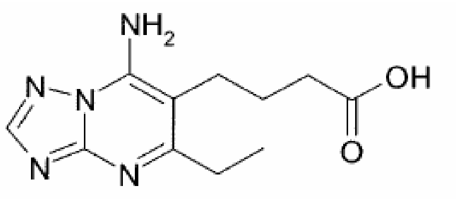
The reference substance BAS 650 F was received at ADPEN Laboratories, Inc. on July 17, 2009. The reference standards were stored in freezer E-109, which had a temperature range of -24 to -17°C for the duration of the study. Standard solutions prepared for this study were stored in refrigerator E-51, which had a temperature range during the course of this study of 2 to 4°C.

BASF Code Name:	BAS 650 F
BASF Registry Number:	4993353
Lot Number:	L71-166
Molecular Formula:	$C_{15}H_{25}N_5$
Molecular Weight:	275.4 g/mol
Purity:	99.7%
Expiration date:	December 01, 2011

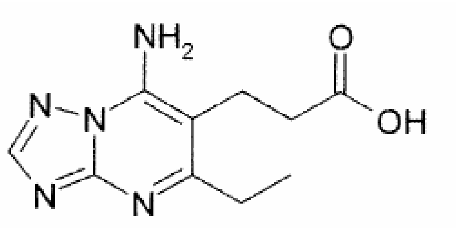


The reference substances, M650F01, M650F02, M650F03 and M650F04 were received at ADPEN Laboratories, Inc. on August 13, 2010. The reference standards were stored in freezer E-109, which had a temperature range of -21 to -18°C for the duration of the study. Standard solutions prepared for this study were stored in refrigerator E-51, which had a temperature range during the course of this study of 2 to 4°C. Sample extracts for this study were stored in refrigerator E-20, which had a temperature range during the course of this study of 5 to 7°C.

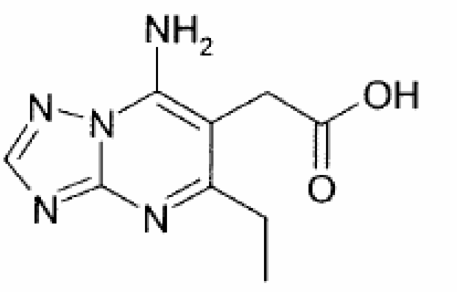
BASF Code Name: M650F01
BASF Registry Number: 5178872
Lot Number: L71-122
Molecular Formula: $C_{11}H_{15}N_5O_2$
Molecular Weight: 249.3 g/mol
Purity: 83.0%
Expiration date: March 01, 2011



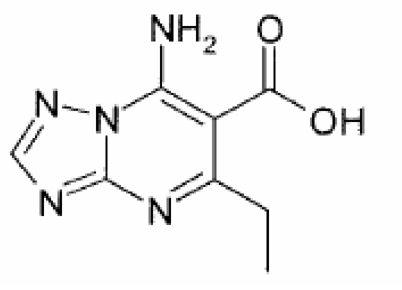
BASF Code Name: M650F02
BASF Registry Number: 5178871
Lot Number: L71-120
Molecular Formula: $C_{10}H_{13}N_5O_2$
Molecular Weight: 235.3 g/mol
Purity: 75.8%
Expiration date: March 01, 2011



BASF Code Name: M650F03
BASF Registry Number: 5178870
Lot Number: L71-154
Molecular Formula: $C_9H_{11}N_5O_2$
Molecular Weight: 221.2 g/ml
Purity: 83.5%
Expiration date: March 01, 2011



BASF Code Name: M650F04
BASF Registry Number: 5211623
Lot Number: L74-106
Molecular Formula: $C_8H_9N_5O_2$
Molecular Weight: 207.2
Purity: 99.2%
Expiration date: August 01, 2011



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

3.2 Sample Receipt and Storage Conditions

Two control pond water samples (surface water) were sent from BASF Corporation and received by ADPEN Laboratories, Inc and entered into Order ID 100902002. The first sample was received on August 13, 2010 and the second on December 09, 2010. The control pond water samples were given unique sample IDs (10081301 and 10120901) and stored in freezer E16, which had a temperature range during the course of this study of -18 to -10°C. The water samples were also given unique ADPEN lab codes (100902002-001 and 100902002-002). Twelve sub samples were aliquoted from each original sample and unique lab codes were assigned by adding a suffix of “A” through “L”. Aliquots “A” and “B” were used as controls and aliquots “C” through “L” were used for fortifications. Water characterizations for each control pond water sample are presented in Figure 17.

4.0 METHODS

4.1 Summary of the Analytical Procedure

BAS 650 F

BASF Analytical Method 574/0 was used to determine residues of BAS 650 F in pond water. The analytical method is presented in Appendix C. The following is a brief summary of the analytical procedure:

Sodium chloride (10 g) was added to the water sample (100 g) via funnel and mixed well to completely dissolve the salt. Dichloromethane (25 mL) was added to the sample and agitated vigorously by mechanical means for 30 minutes at approximately 250 rpm. The sample was allowed to settle and separate. The sample was sonicated and/or centrifuged to eliminate emulsion if necessary. Exactly 10 mL of the organic layer was aliquoted through the aqueous and evaporated in a roto-evaporator at 40°C. The residues were reconstituted with 50:50 acetonitrile:deionized water (20 mL). Dilutions were made if necessary and the sample was ready for analysis using LC-MS/MS monitoring the primary and secondary ion transitions as described in the instrument operating conditions in Section 4.3.1.

M650F01, M650F02, M650F03 and M650F04

BASF Analytical Method L0113 was used to determine residues of M650F01, M650F02, M650F03 and M650F04 in pond water. The method is presented in Appendix D. The following is a brief summary of the analytical procedure:

A water sample (10 g) was adjusted to a pH of 7 using either hydrochloric acid or ammonia. The sample is shaken well to ensure homogeneity. A solid phase extraction (SPE) column was conditioned with acetonitrile (6 mL) followed by deionized water (6 mL). The water sample was passed through the column at a rate of approximately 1 to 2 mL per minute and the column was vacuum dried. The sample was eluted into a 25-mL glass centrifuge tube with 9:1 aceonitrile:25% ammonia (10 mL) at a rate of approximately 1 to 2 mL per minute. The sample was evaporated to almost dryness at 50°C with a rotary evaporator and evaporated to dryness further under nitrogen. The sample was reconstituted with acetonitrile (200µL) and deionized water (1.8 mL) and sonicated. The sample was ready for analysis using LC-MS/MS monitoring the primary and secondary ion transitions as described in the instrument operating conditions in Section 4.3.1.

4.2 Limit of Quantitation and Limit of Detection

The limit of quantitation (LOQ) for BASF Analytical Method 574/0 for residues of BAS 650 F is 0.05 µg/kg (ppb). The limit of detection (LOD) is set at 20% of the LOQ, equivalent to 0.01 µg/kg (ppb).

The LOQ for BASF Analytical Method L0113 for residues of M650F01, M650F02, M650F03 and M650F04 is 0.05 µg/kg (ppb). The LOD is set at 20% of the LOQ, equivalent to 0.01 µg/kg (ppb).

4.3 Instrumentation

4.3.1 Typical Instrument Operating Conditions

<u>BAS 650 F</u>

HPLC System:	Agilent 1100 HPLC System with Binary Pump, High Performance Autosampler and Heated Column Compartment		
Column:	Xterra® MS, C18, 3.5 µm, 4.6 x 50mm		
Injection:	50 µL		
Mobile Phase:	A = 0.1% acetic acid in water B = 0.1% acetic acid in acetonitrile		
Gradient:	Time (minute)	Composition (%)	
		A	B
	0.00	90.0	10.0
	7.00	0.0	100.0
	9.00	0.0	100.0
	9.10	90.0	10.0
	12.00	90.0	10.0
Flow Rate:	400 µL/minute		

MS/MS Instrument:	Applied Biosystems™ API 3000 Analyst Software (version 1.5.1)			
Analytes	Transitions (m/z): Quantitation ions (Expected Retention Times in minutes)			
BAS 650 F	276.2 → 149.1 (7.43)		276.2 → 176.2 (7.42)	
	Scan Type	Ion Source	Source Temperature	Ionization Mode
	MRM	Turbo Spray	450°C	Positive

M650F01, M650F02, M650F03 and M650F04

HPLC System:	Agilent 1200 HPLC System with Binary Pump, High Performance Autosampler and Heated Column Compartment		
Column:	Xterra® MS, C18, 3.5 µm, 4.6 x 50mm		
Injection:	50 µL		
Mobile Phase:	A = 0.1% acetic acid in water B = 0.1% acetic acid in acetonitrile		
Gradient:	Time (minute)	Composition (%)	
		A	B
	0.00	90.0	10.0
	7.00	0.0	100.0
	9.00	0.0	100.0
	9.10	90.0	10.0
	13.00	90.0	10.0
Flow Rate:	500 µL/minute		

MS/MS Instrument:	Applied Biosystems™ API 4000 QT (Q-Trap) Analyst 1.5.1 Software			
Analytes	Transitions (m/z): Quantitation ions (Expected Retention Times in minutes)			
M650F01	250.2 → 232.0 (4.02)		250.2 → 149.1 (4.02)	
M650F02	236.0 → 176.1 (2.91)		236.0 → 218.2 (2.89)	
M650F03	222.2 → 176.1 (2.11)		222.2 → 204.1 (2.13)	
M650F04	208.2 → 190.2 (2.51)		208.2 → 123.0 (2.51)	
	Scan Type:	Ion Source:	Source Temperature:	Ionization Mode:
	MRM	Turbo Spray	550°C	Positive

4.3.2 Calibration

A minimum of a six-point standard curve was prepared by injecting standard solutions at concentrations ranging from 0.00125 ng to 0.25000 ng. Calibration standards were injected to bracket every 2-5 sample injections. Representative chromatograms of calibrations standards for each analyte are presented in Figures 2 through 6.

4.4 Calculations

Calculations for residue concentrations of the BAS 650 F and its metabolites were made using Analyst® 1.5.1 which generated a standard curve based on linear regression. The data system derived an equation for the fit of the standard curve by plotting the standard concentration (ng) on the x-axis versus the detector's response (peak area) on the y-axis. Concentrations of the analyte, found in sample analysis, were calculated from the standard curve equation. The correlation coefficient (r) for the calibration curves were greater than 0.990 for all analytes. Microsoft® 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 1.

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

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

e) Corrected ppb found = ppb in the sample – ppb in the control

d) Percent recovery = $\frac{\text{Corrected ppb found}}{\text{ppb added}} \times 100$

As an example, below are the calculations to obtain the percent recovery of a control water sample (Sample ID: 10120901) fortified with BAS 650 F.

Lab code: 100902002-002C:

a) ng found = $\frac{2752 - (261)}{5.43\text{e} + 005}$ = 0.00458 ng

b) Amount of sample injected = $\frac{50 \mu\text{L}}{50 \text{ mL}} \times 100.90 \text{ g} \times \frac{1 \text{ mL}}{1000\ \mu\text{L}}$ = 0.1009 g

c) ppb = $\frac{0.00458 \text{ ng}}{0.1009 \text{ g}}$ = 0.04539 ppb

e) Corrected ppb found = 0.04539 – 0.00000 [1]

d) Percent recovery = $\frac{0.04539}{0.05} \times 100$ = 90.8%

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[1] Control water samples, 100902002-002A and 100902002-002B, were averaged and used as ppb found in the control.

As an example, below are the calculations to obtain the percent recovery of a control water sample (Sample ID: 10081301) fortified with M650F01.

Lab code: 100902002-001C:

a) ng found = $\frac{17517-(3.49e+003)}{5.53e+006}$ = 0.00254 ng [1]

b) Amount of sample injected = $\frac{50\mu L}{10\ mL}\times 10\ g\times\frac{1\ mL}{1000\ \mu L}$ = 0.0500 g

c) ppb = $\frac{0.00254\ ng}{0.0500\ g}$ = 0.05080 ppb

e) Corrected ppb found = 0.05080 – 0.00000 [2]

d) Percent recovery = $\frac{0.05080}{0.05}\times 100$ = 101.6%

4.5 Statistics

Statistical methods used included regression analysis within Analyst 1.5.1 and calculations of means, range, standard deviations and percent relative standard deviations within Microsoft Excel® 2007. All calculations were made using the entire number (no rounding).

TABLE 6. Recommendations

BASF Analytical Method 574/0:

1. No recommendations were made.

BASF Analytical Method L0113:

1. Sample extracts may require dilution if matrix suppression is observed.