

1. BACKGROUND

AE C656948 is a fungicide currently being developed by Bayer CropScience with potential uses in several crops.

The purpose of this study was to demonstrate that the "Analytical method 01051 for the determination of fluopyram (AE C656948) in drinking and surface water by HPLC-MS/MS"³, which is presented in Appendix 6, can be performed with acceptable recoveries for determination of the compound AE C656948 at an independent laboratory having no prior experience with the method. The method was developed by Bayer CropScience AG, Development-Residues, Operator and Consumer Safety, at their laboratory in Monheim, Germany and reported by Ralph Krebber.

On initially reviewing the analytical method it was noted that parts of the method did not meet the criteria in OPPTS 860.1340 (The Residue Analytical Method), specifically: the method as written required the use of a sample of the untreated matrix as a blank for use in preparation of the matrix-matched standards.

As OPPTS 850.7100 (d)(2)(i) states that the laboratory conducting the ILV must use the method exactly as it is written, the analysis was performed as described in the method and then the samples were reanalyzed using calibration solutions prepared in deionized water. If similar results are obtained in this study, the ILV will be considered successful.

A method detection limit (MDL) and calculated LOQ was determined using the data generated from the analyses using calibration solutions prepared in deionized water.

The study was performed in accordance with United States Environmental Protection Agency (EPA) Pesticide Assessment Guidelines and Good Laboratory Practices (and Ecological Effects Test Guidelines OPPTS 850.7100¹ and Residue Chemistry Test Guidelines, OPPTS 860.1340²). This validation fulfills the requirement that properly validated methods of analysis be utilized for the generation of pesticide residue data and for tolerance enforcement.

2. EXPERIMENTAL DETAILS

This study was conducted following an approved protocol. All amendments to the protocol were signed and dated by the Study Director and the Sponsor's Representative.

This study was initiated on January 2, 2008. The experimental phase of the study began on January 10, 2008 and concluded on January 16, 2008.

The following personnel were involved in the conduct of this study:

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2.1 Test Substance

The test substance for this study was AE C656948. See Appendix 3 for complete nomenclature and chemical structure.

2.2 Analytical Reference Substances

The test substance also served as the analytical reference substance. See Appendix 3 for complete nomenclature, chemical structure and reference information for the reference substance. The test substance was stored in a freezer until used to prepare fortification and calibration solutions. The stock solutions were stored in a refrigerator at an average temperature of $\leq 10^{\circ}\text{C}$.

2.3 Test System

The test system will be sub-samples of drinking (finished water) and surface water (raw water) The drinking water was obtained from a tap in Stilwell, Kansas (Bayer research Park) The surface water was obtained from Bayer CropScience Study Number 04RAOAY001⁴, Surface Water Monitoring for Residues of Oxadiazon in High Use Areas in the United States. Characterization data for the water is presented in Appendix 5.

Spiked waters were maintained in a refrigerator except when removed to prepare for analysis.

2.4 Method Summary

Each analytical set included one reagent blank, two unfortified control samples, five samples fortified at the LOQ (0.05 ng/mL(ppb)) and five samples fortified at 10x LOQ (0.5 ng/mL)

Twenty milliliters of the water sample was pipetted into a 25-mL disposable jar. Fortified samples were prepared by adding 0.01 mL or 0.1 mL of standard solution for each fortification level: 0.05 and 0.50 ng/mL. A 0.1 mL aliquot of 10 ng/ml solution was used to fortify the 0.05 ng/mL samples and a 0.01 mL aliquot of 1000 ng/mL solution was used for the

0.5 ng/mL samples. After mixing well, an aliquot of the sample was transferred into an HPLC vial for analysis by electrospray LC/MS/MS.

2.5 Instrumentation

- Sciex API 4000 LC/MS/MS System (Applied Biosystems)
- Shimadzu LC-10AD VP HPLC Pumps (2) with a High Pressure Mixer and Shimadzu SCL-10A VP Pump Controller
- Perkin Elmer Series 200 LC Autosampler

2.6 HPLC Conditions

Column: Phenomenex Aqua™ 5 μ C18 125Å,
Length 150 mm x 2.0 mm i.d.,
Particle size 5 μ m,
Part. No.: 00F-4299-B0

Column oven temperature: Ambient

Injection Volume: 20 μ L (100 μ L loop)
Mobile phase: A: water / acetonitrile / acetic acid (900/100/0.1; v/v/v)
B: acetonitrile / acetic acid (1000/0.1; v/v)

Flow rate (column): 0.4 mL/min

Retention times:
AE C656948: approx. 6.0 min

HPLC Gradient Parameters

Time [min]	% A	% B
0.00	55	45
4.50	55	45
5.00	10	90
9.00	10	90
9.10	55	45
12.50	55	45
12.60	System controller	Stop

2.7 MS/MS Conditions

Collision Gas Setting (CAD) [L/min]	10
Curtain Gas Setting (CUR) [L/min]	35
Ion Source Gas 1 (GS1) [L/min]	25
Ion Source Gas 2 (GS2) [L/min]	45
Turbo Gas Temperature (TEM)	750 °C
Interface Heater (ihe)	On
Resolution of Q1 and Q3	Unit, Low

Compound dependent:	AE C656948	
	Primary	Confirmatory
Q1 Mass [amu]	397	397
Q3 Mass [amu]	173	145
Dwell [msec]	250	250
Ionization Mode	Positive	Positive
Ion Spray Voltage (IS) [V]	5500	5500
Entrance Potential (EP) [V]	11	13
Declustering Potential (DP) [V]	62	54
Collision Energy (CE) [V]	39	43
Collision Cell Exit Potential (CXP) [V]	12	14

2.8 Calculations

An example calculation for AE 656948 from sample drinking water LOQ spike 1, which was analyzed during the study, is shown below. This sample was fortified with 0.05 ppb AE C656948. The chromatogram used in this example is presented in Appendix 2 (Chromatogram 5).

The standards were fit to the linear equation: $Y = MX + B$

where: X is the concentration of the reference standard in ng/mL

M is the calibration line slope

B is the calibration line intercept

Y is the peak area

The calibration points were weighted 1/x to provide a better fit near the limit of detection. The example shown below is for the calculation of AE C656948.

After regression coefficients were calculated, the residue in parts per billion was determined. The parts per billion (ppb) of AE C656948 in the water was calculated using the following equation,

$$\text{AE C656948 found (ppb)} = \frac{(Y-B)}{M}$$

From the above equations:

$$\text{AE C656948 found} = \frac{(21884.1 - 688)}{502,000} = 0.04222 \text{ ng/mL}$$

Y	M	B
21884.1	502,000	688

The slope and intercept were obtained from the calibration curve generated by Analyst and presented in Appendix 1 (Figure 7).

Therefore, when analyzed against calibration solutions prepared using deionized water, sample drinking water LOQ spike 1 contains 0.04222 ng/mL AE C656948.

As the sample was fortified with known amounts of analyte prior to extraction, the percent recovery was determined using the following equation:

$$\% \text{ Recovery} = \frac{\text{analyte found in spike (ppb)}}{\text{analyte added (ppb)}} \times 100$$

Therefore, for sample drinking water LOQ spike 1, the AE C656948 recovery may be calculated as follows:

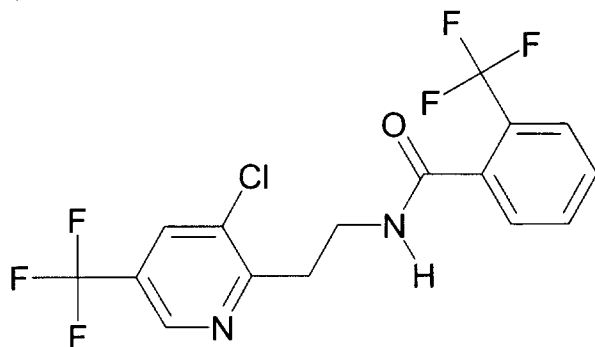
$$\% \text{ Recovery} = \frac{0.04222 \text{ ppb} \times 100}{0.05 \text{ ppb}} = 85\%$$

Remark: Calculations were performed using the LC/MS/MS software *Analyst (version 1.4.1)*. The example calculation was performed using the area values reported by the instrument. The instrument software carries additional figures not shown in the intermediate results. The instrument software calculated a recovery of 0.04219 ng/mL and a percent recovery of 84% for this sample. The *Analyst* results for this analytical set are presented in Appendix 4.

Appendix 3 Identity and Purity of the Test Material Used

AE C656948:

Structural formula:



Chemical code: AE C656948
Common Name: Fluopyram
CAS Number: [658066-35-4]
Chemical name: *N*-[2-[3-Chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)benzamide
Empirical formula: C₁₆ H₁₁ Cl F₆ N₂ O
Molecular weight: 396.7 g/mol
Vial: K-1377
Purity: 99.8%
Expire Date: 5/15/2012