1.0 INTRODUCTION

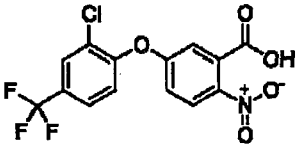
Described in this report is the independent laboratory validation (ILV) of BASF Analytical Method A9206 entitled "Determination of Acifluorfen, Acifluorfen Amine, Acifluorfen Acetamide and Des-Carboxy Acifluorfen in Rice Paddy Water," which is summarized in BASF Report No. ER94017 "1992 Blazer Herbicide Aquatic use Dissipation Study" as performed by JRF America, 2650 Eisenhower Avenue, Audubon, PA 19403 USA.

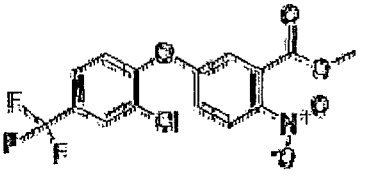
The goal of this study was to satisfy the harmonized guidelines for the OPPTS Residue Chemistry Test Guidelines OCSPP 850.6100[2] .

2.0 MATERIALS AND METHODS

2.1 Test Reference and Control Substances

The test/reference substances were purchased from Chem Service company, and were certified by JRF America. The following test/reference substances were used:

Common Name:	Acifluorfen
CAS Name:	5-(2-chloro-4-trifluoromethylphenoxy)-2-Nitro-benzoic acid
CAS Registry No.:	50594-66-6
Batch No.:	468-131B
Purity:	99.5%
Reassay Date:	March 13, 2013
Storage Conditions:	Ambient
Source:	ChemService
Structure:	

Common Name:	Acifluorfen Methyl Ester
CAS name:	Methyl-5-(2-chloro-4-trifluoromethylphenoxy)-2-nitrobenzoate
CAS Registry No.:	50594-67-7
Batch No.:	476-100B
Purity:	96.2%
Reassay Date:	March 13, 2013
Storage Conditions:	Ambient
Source:	ChemService
Structure:	

2.2 Test System

Surface water was collected from brandywine creek in Deleware, and ground water was obtained from well water located at 591 West Boot Road,West Chester, PA 19380. The characterized data for water samples is presented in Appendix B.

2.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined in BASF report ER94017 (Appendix C, analytical report). Identical or equivalent apparatus and materials were used.

2.4 Method and Method Modifications

2.4.1 Sample Validation Sets, Fortification and Extraction Procedure

Sample validation sets:

The method was validated in surface water and ground water as specified in the study protocol. For each matrix, the validation set consisted of 13 samples: one reagent blank, two control samples and five control samples fortified at the LOQ level and five control samples fortified at 10× LOQ level. Reagents blanks were run within the study to adequately demonstrate that the reagents did not interfere with the quantification of the analytes. Each control and control fortified sample contained 100 grams of water sample. The reagent blank sample contained 100 g MilliQ water.

Fortification:

The LOQ and 10×LOQ recovery samples were fortified with 100 µL and 1000 µL, respectively, of a 1.0 µg/mL fortification solution containing acifluorfen standard.

Extraction :

The following extraction steps were followed for each sample based on the flowchart described in BASF report ER 94107 :

1. Transfer a 100-gram representative sample to a 250-mL separatory funnel.
2. Fortify recovery sample(s) with an appropriate known amount of acifluorfen standard solution. Swirl to mix the fortification standards into the sample(s).
3. Add 800 µL of 1N HCl to the sample. Swirl to mix the acid into the sample(s).
4. Add 40 mL DCM/ACN (dichloromethane/acetonitrile 1:1 v/v) to sample, shaking thoroughly for approximately 30 seconds and then allowing the mixture to settle (~10 minutes) to obtain two clear layers of solution.
5. Remove the organic layer (lower layer), and pass it through a filter paper to a RotaVap container.
6. Repeat step 4 and 5 twice more, and combine the organic layers in the container.
7. Evaporate the filtrate using a RotaVap at a temperature of 40°C until all organic solvent is removed and less than 1 mL of extract remains.

8. Methylate by adding 10 mL of 0.02 M TMS-diazomethane in hexane, 2 ml of acetone to the container and mix.
9. After allowing approximately 75 minutes at room temperature for the reaction, add 800 µL of toluene.
10. Evaporate the mixture using a RapidVap at temperature of 40°C until dryness.
11. Redissolve the residue in 1 mL of toluene, sonicate the container and transfer to a GC vial for gas chromatographic determination.

2.4.2 GC/ECD Detector Instrumentation

A GC/ECD system from Hewlett Packard was employed for this ILV study.

GC/ECD: Hewlett-Packard 6890 Plus gas chromatograph equipped with an Agilent 7683 series injector and Hewlett-Packard 6890 Plus ECD detector. The system is controlled and the data processed by Agilent GC ChemStation™ Revision B.04.02.

GC System Operating Parameters:

GC Column	J&W DB-5 capillary column, 1.0 micron film thickness, 30 meter x 0.32 mm internal diameter.
Column temperature program:	Initial value 130°C (~0.5 minutes); 1st program rate 30°C/ min to 250°C hold until ~6 min; 2nd program rate 30°C/ min to 290°C hold until ~1 min; 10°C/ min to 290°C hold until ~17.2 min; final run time ~30 minutes
Injection volume:	1 µL
Carrier Gas:	Helium – 2.8 mL/ min
Inlet Temperature:	275°C splitless
Column Head Pressure	16.5 psi
Detector Temperature:	350 °C

Analyte Typical Retention Time

Analyte	Approximate Retention Time (min.)
Acifluorfen Methyl Ester	8.4-8.9

2.4.3 Data Acquisition and Reporting

Residues of acifluorfen were quantitated by external standards. A calibration curve was generated by plotting the detector's response in peak area versus the concentration (ppb) of standard injected. The data system derived an equation for the standard curve and this equation was used to calculate intercept and slope of the linear regression curve.

The calibration curve was obtained by direct injection of 1.0 µL of the acifluorfen methyl ester standards into the GC/ECD in the range of 10 ng/mL to 500 ng/mL. In a given injection run, the same injection volume was used for all samples and standards.

A statistics includes the calculation of averages, standard deviations, and relative standard deviations which were calculated using Microsoft Office Excel 2003®.

2.4.4 Modifications

BASF Analytical Method A9206 was followed as written in report BASF Report ER94017. No modifications occurred during the study.

2.4.5 Extended ILV at LOQ 0.1 ppb

Per request of the Sposor, the ILV was conducted again and the method extended to an LOQ of 0.1 ppb. The extended ILV used the same original method except the derivatization step was eliminated and LC/MS/MS was used for the analysis. The test system, number of samples and analytical sets were the same as the previous ILV.

Fortification:

The LOQ and 10×LOQ recovery samples were fortified with 100 µL and 1000 µL, respectively, from a 0.1 µg/mL fortification solution containing acifluorfen standard.

Modifications:

1. The deviatization step in the original method was not performed.
2. The analysis was perfomed on an LC/MS/MS system. The system conditions were presented after "Extraction".
3. As acifluorfen was directly analyzed, the calibration standards were prepared as acifluorfen instead of acifluorfen methyl ester.

Extraction :

The following extraction steps were followed for each sample based on the flowchart described in BASF report ER 94107 :

1. Transfer a 100-gram representative sample to a 250-mL separatory funnel.
2. Fortify recovery sample(s) with an appropriate known amount of acifluorfen standard solution. Swirl to mix the fortification standards into the sample(s).
3. Add 800 µL of 1N HCl to the sample. Swirl to mix the acid into the sample(s).
4. Add 40 mL DCM/ACN (dichloromethane/acetonitrile 1:1 v/v) to sample, shaking thoroughly for approximately 30 seconds and then allowing the mixture to settle (~10 minutes) to obtain two clear layers of solution.
5. Remove the organic layer (lower layer), and pass it through a phase separation paper to a RotaVap container.
6. Repeat step 4 and 5 twice more, combine the organic layers in the container.

7. Evaporate the filtrate using a RotaVap at temperature of 40°C until all organic solvent is removed and less than 1 mL of extract remains.
8. Transfer sample to a 10 mL graduated test tube. Rinse beaker several times with ACN and add to the test tube. Dilute the final volume to 10 mL with ACN.
9. Transfer or further dilute sample to an HPLC vial for LC/MS/MS determination.

LC/MS/MS Analysis

Instrument: **Waters Acquity UPLC with Applied BioSystem/MDS Sciex 4000 Q-trap® LC/MS/MS**
Applied Biosystems Analyst Software version 1.5.1

Waters Acquity UPLC Conditions

Column	Acquity BEH C18, 5.0 cm x 2.1 mm, 1.7 um
Column Oven Temperature	Ambient
Flow rate	0.3 mL min^{-1}
Injection volume	10 µL
Stop Time	5.00 minutes
Mobile phase	Solvent A = 0.1% formic acid in water Solvent B = 0.1% formic acid in methanol

Mobile Phase Program (linear gradient changes)

Time (min.)	% A	% B
0.00	70	30
0.20	70	30
2.00	10	90
3.00	10	90
3.1	70	30

Typical Analyte LC Retention Times:

Analyte	Approx. Retention Time (Minutes)
Aciflurofen	2.9

Acquisition Ions and Compound Dependent Parameters

Analyte	Mass Transition	Dwell	DP	CE	CXP
Acifluorfen	359.9→315.8	150	-55	-12	-15

Typical MS/MS Voltage Conditions Used

Ionization Mode:	Turbospray
Scan Type	MRM
Polarity	negative
Resolution Q1	unit
Resolution Q3	unit

Curtain gas (N_2)	30
GS1	20
GS2	50
CAD gas (N_2)	medium
Ion Spray (V)	-4200
Temperature (°C)	450
EP	-10

Residues of acifluorfen were quantitated by external standards. Because the derivatization procedure was eliminated, acifluorfen was directly quantified on LC/MS/MS. The calibration curve was obtained by direct injection of 10 µL of the acifluorfen standards into LC-MS/MS in the range of 0.25 ng/mL to 25 ng/mL. Peak integration and quantitation were performed using Applied Biosystem Analyst software version 1.5.1. Concentration of ppm calculations and recovery results were computed for each set of samples by Applied Biosystem Analyst software version 1.5.1.

9.0 APPENDIX

A. Calculations

A calibration curve was prepared by injecting constant volumes of six standard calibration solutions of Acifluorfen Methyl Ester. The standards ranged from 10 to 500 ng/mL for GC analysis.

Analyte peak areas were generated using Agilent GC ChemStation™ Revision B.04.02 software. The regression functions were used to calculate a best fit line (from a set of standard concentrations in ng/mL versus peak area response) and to determine concentrations of the analyte in samples using Excel spread sheets. For LC/MS/MS, the same calculations were performed using the instrument's Analyst software, version 1.5.1.

The equation used for the least squares fit was: $y = mx + b$, where y=peak area, x = ng/mL, m= slope and b= y-intercept.

The example below is based on the Acifluorfen measurement in a fortified ground water sample that was fortified with known amounts of analyte prior to extraction. Percent recovery and ng/mL found are calculated as shown:

ng/mL injected = [(sample peak area – intercept)/slope]

$$\text{ng/mL} = \frac{\text{ng/mL injected} \times \text{final volume (mL)} \times \text{Dilution Factor x acid equivalent [0.963]}}{\text{sample mass (mL)}}$$

Percent recovery calculation:

% Recovery = (ng/mL found – ng/ml in control)/ ng/mL fortified x 100%

As acifluorfen was directly measured, there was no acid equivalent factor for LC/MS/MS data.

Example from the GC data set:

Acifluorfen fortified at the LOQ (1ppb) level in ground water sample;

UTC_LOQ_1

Peak area = 153747 Slope = 1341.3 Intercept = 4101.5

[(153747 - 4101.5)/ 1341.3] = 111.6 ng/mL

Aliquot factor = 1 , Dilution factor = 1

$$\frac{111.6\ \text{ng/mL} \times 1\ \text{mL} \times 1 \times 0.963}{100\text{mL}}$$

$$= 1.074\ \text{ng/mL}$$

$$\frac{1.074\ \text{ng/ml}}{1\ \text{ppb}} = 107.4\%\ \text{recovery}$$