

1.0 INTRODUCTION

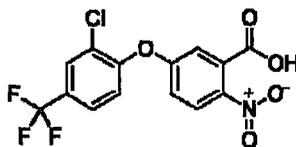
This report details the independent laboratory validation (ILV) of BASF Analytical Method A 9208 entitled "Determination of Acifluorfen, Acifluorfen Amine, Acifluorfen Acetamide and Des-Carboxy Acifluorfen in Soil," which is summarized in BASF Report No. ER94017 "Analytical Phase Of Protocol B92126 1992 BLAZER Herbicide Dissipation In A Rice Paddy Analysis Of Soil Samples At ADPEN Laboratories, Inc." [1]. 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 Substance

The test/reference substance was purchased from Chem Service company, and were certified by JRF America.

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:	



2.2 Test System

Sandy Soil and Clay Soil were received at the test facility on July 13, 2011. The GLP characterization of the Soils were performed at AGVISE Laboratories, Northwood, ND. Summary of the results for soil characteristic analysis are listed in **Table 1**.

The test soils were sieved through a 2mm-sieve and stored frozen during the ILV trial. The test soils were identified with a unique identification number at the testing facility.

2.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined in BASF report ER94017 (Appendix D, 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 successfully for the two soil matrices, Sandy Soil and Clay Soil specified in this study. For each matrix, the validation set consisted of 13 samples: one reagent blank, two control samples and five control samples fortified at the LOQ (5 ng/g) and five control samples fortified at 10× LOQ (50 ng/g). For each matrix, each validation set was divided into two sub-sets for LOQ and 10× LOQ. 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 10 grams of representative soil sample. The reagent blank sample consisted of the different reagents applied through the entire extraction procedure according to the method without any soil.

Fortification:

The LOQ and 10×LOQ recovery samples were fortified with 50 µL and 500 µL, respectively, from a 1.0 µg/mL fortification solution containing acifluorfen standard.

Modifications:

A centrifuge step (10 minutes at 3000 rpm) was added in the partition between DCM (dichloromethane) and petroleum ether to separate the two phases.

Extraction :

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

1. Weigh 10 grams of representative soil sample into a 250-mL plastic bottle.
2. Fortify control sample(s) with an appropriate known amount of acifluorfen standard solution.
3. Add 75 mL extraction solution 1 [10% Acetone in (0.5M KCl:0.1M NaOH)] to sample bottle. Check sample pH (should be >11). Polytron for 1 minute at 20000 rpm.
4. Centrifuge for 5 minutes at 3000 rpm. Decant extract into a 500 mL separatory funnel.
5. Add 75 mL extraction solution 2 [Acetone:1N HCl (9:1)] to sample bottle. Polytron for 1 minute at 20000 rpm.
6. Centrifuge for 5 minutes at 3000 rpm. Combine extracts.
7. Add 5 mL 1N HCl to the combined extracts and check pH (pH should be <3).
8. Add 50 mL methanol to sample bottle. Polytron for 1 minute at 20000 rpm.

9. Centrifuge for 5 minutes at 3000 rpm. Combine extracts.
10. Add 90 mL DCM (dichloromethane) to sample funnel, shaking thoroughly for ~30 seconds and then allow the mixture to settle to obtain two clear layers of solution.
11. Slowly drain the organic layer (lower layer), and pass it through a Phase Separator filter paper to a Rapidvap beaker.
12. Repeat step 10 and step 11 once more, combine the organic layer to the beaker.
13. Discard aqueous phase. Transfer organic phase back to the separatory funnel.
14. Add 40 mL petroleum ether, shake gently for approximately 5 minutes. Transfer into 250 mL plastic bottles and centrifuge at 3000 rpm for 10 minutes.
15. Transfer sample back into 500 mL separatory funnel and let sample sit for ~5 minutes.
16. Slowly drain the organic layer (lower layer) and pass it through a Phase Separator filter paper to the Rapidvap beaker.
17. Discard petroleum ether.
18. Add 50 mL ACN to sample.
19. Evaporate the filtrate using a Rapidvap with a temperature of 40°C until all organic solvent is removed and less than 3 mL of extract remains.
20. Transfer sample to a 10 mL graduated test tube. Rinse beaker several times with MeOH and add to the test tube. Dilute the final volume to 10 mL with MeOH.
21. Dilute all samples 25x with MeOH:H₂O 10:90 solution and transfer to an HPLC vial for LC/MS/MS determination.

2.4.2 Data Acquisition and Reporting

Residues of acifluorfen were quantitated by external standards. A calibration curve was generated by plotting the 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.

Because the derivatization procedure was eliminated, acifluorfen was directly quantified by LC/MS/MS. The calibration curve was obtained by direct injection of 10 µL of the acifluorfen standards by LC/MS/MS in the range of 25 ng/mL to 0.1 ng/mL. In a given injection run, the same injection volume was used for all samples and standards. Peak integration and quantitation were performed using Applied Biosystem Analyst software version 1.5.1. Concentration of ppb calculations and recovery results were computed for each set of samples by Applied Biosystem Analyst software version 1.5.1.

The statistics include the calculation of averages, standard deviations, relative standard deviations, and confidence intervals which were calculated using Microsoft Office Excel 2007®.

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

Soils 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
3.10	70	30

Typical Analyte LC Retention Times:

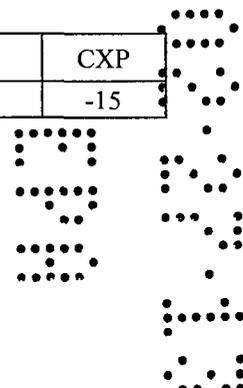
Analyte	Approx. Retention Time (Minutes)
Acifluorfen	3.5

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



9.0 APPENDIX

Calculations

A calibration curve was prepared by injecting constant volumes of six standard calibration solutions of Acifluorfen. The standards ranged from 25 to 0.1 ng/mL for LC/MS/MS analysis.

Analyte peak area and concentrations of analyte in the samples were generated using Analyst 1.5.1 data handling software using linear regression of $1/x^2$ weighting. The regression functions were used to calculate a best fit line (from a set of standard concentrations in ng/mL versus peak area response).

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 fortified Sandy and Clay Soil samples that were fortified with known amounts of analyte prior to extraction. Percent recovery and ppb found are calculated as shown:

$$\text{ng/mL injected} = [(\text{sample peak area} - \text{intercept})/\text{slope}]$$

$$\text{ppb Found} = \frac{\text{ng/mL injected} \times \text{final volume (mL)} \times \text{Dilution Factor}}{\text{sample weight (g)}}$$

Percent recovery calculation:

$$\% \text{ Recovery} = \frac{(\text{ppb in fortified sample} - \text{ppb in control}) \times \text{sample weight (g)}}{\text{ng/mL fortified} \times \text{weight (g)}} \times 100\%$$

Example from set 1B (Sandy Soil 17229)

Acifluorfen fortified at the LOQ (5ppb) level in Sandy Soil sample;

Sample ID: Soil 17229_LOQ_3

Peak area = 4734.73 Slope = 30800 Intercept = -273

$$[(4734.73 + 273) / 30800] = 0.1626 \text{ ng/mL}$$

Dilution factor = 25

$$\text{ppb Found} = \frac{0.1626 \text{ ng/mL} \times 10 \text{ mL} \times 25}{9.95 \text{ g}}$$

$$= 4.085 \text{ ng/g}$$

$$\% \text{ Recovery} = \frac{4.085 \text{ ng/g} \times 9.95 \text{ g}}{5 \text{ ng/mL} \times 10 \text{ g}} \times 100 \%$$

$$= 81.3 \%$$

Example from set 2B (Clay Soil 17231)

Acifluorfen fortified at the LOQ (5ppb) level in Clay Soil sample;

Sample ID: Soil 17231_LOQ_3

Peak area = 3877.77 Slope = 20100 Intercept = -554

$$[(3877.77 + 554) / 20100] = 0.220 \text{ ng/mL}$$

Dilution factor = 25

$$\text{ppb Found} = \frac{0.220 \text{ ng/mL} \times 10 \text{ mL} \times 25}{9.95 \text{ g}}$$

$$= 5.540 \text{ ng/g}$$

$$\% \text{ Recovery} = \frac{5.540 \text{ ng/g} \times 9.95 \text{ g}}{5 \text{ ng/mL} \times 10 \text{ g}} \times 100 \%$$

$$= 110 \%$$