

Test Material: Acifluorfen

MRID: 49085304

Title: BASF Analytical Method A9208; Determination of Acifluorfen, Acifluorfen-Amine, Acifluorfen-Acetamide, and Des-Carboxy-Acifluorfen in Soil.

MRID: 49085301

Title: Acifluorfen; Independent Laboratory Validation of BASF Method A 9208 for the Analysis of Acifluorfen in Soil; Final Report.

EPA PC Code: 114402

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Rich Lester

Signature:



Date: 2/18/14

Secondary Reviewer: Dan Hunt

Signature:



Date: 2/18/14

QC/QA Manager: Joan Gaidos

Signature:



Date: 2/18/14

Analytical method for acifluorfen, acifluorfen-amine, acifluorfen-acetamide, and des-carboxy-acifluorfen in soil

Reports: ECM: MRID 49085304. United Phosphorus, Inc. (2013). BASF Analytical Method A9208; Determination of Acifluorfen, Acifluorfen-Amine, Acifluorfen-Acetamide, and Des-Carboxy-Acifluorfen in Soil. Project ID: UPI-2013-003. Completion Date: February 26, 2013. 53 pages.

ILV: MRID 49085301. Patel, D. and Li, F. (2013). Acifluorfen; Independent Laboratory Validation of BASF Method A 9208 for the Analysis of Acifluorfen in Soil; Final Report. Performing Laboratory: JRF America, Audubon, Pennsylvania. Laboratory Project ID: AU-2012-03. Sponsor: United Phosphorus, Inc., King of Prussia, Pennsylvania. Study Completion Date: February 6, 2013. 50 pages.

Document No.: MRIDs 49085304 and 49085301

Guideline: 850.6100


Statements: ECM: The study was not conducted in compliance with FIFRA GLP standards (p. 3). A signed and dated Data Confidentiality statement was provided (p. 2). Quality Assurance and Certification of Authenticity statements were not provided.

ILV: The study was conducted in accordance with FIFRA GLP standards. Signed and dated Data Confidentiality, GLP Compliance, Quality Assurance, and Certification of Authenticity statements are provided (pp. 2-5).

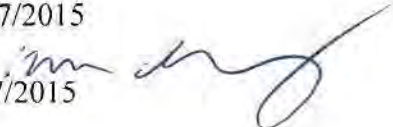
Classification: This analytical method is classified as supplemental – upgradeable. Modifications to the method by the ILV were not implemented in the ECM and the ILV did not analyze for the metabolites acifluorfen-amine, acifluorfen-acetamide, and des-carboxy-acifluorfen which are included in the ECM. The LOQ was not established using scientifically acceptable procedures. The soil used in the ECM was not characterized. Chromatograms were not provided for reagent blanks.

PC Code: 114402

Reviewer: Michelle Colletti, Chemist
US EPA

Signature: 
Date: 06/17/2015

Secondary Reviewer: Andrew Shelby, Physical Scientist
US EPA

Signature: 
Date: 06/17/2015

Executive Summary

This analytical method, BASF Analytical Method A9208, is designed for the quantitative determination of acifluorfen and its metabolites acifluorfen-amine, acifluorfen-acetamide, and

des-carboxy-acifluorfen in soil using HPLC with fluorescence detector for analysis of acifluorfen amine or GC/ECD following methylation for analysis of acifluorfen methyl ester (acifluorfen), acifluorfen-acetamide methyl ester (acifluorfen-acetamide), and des-carboxy-acifluorfen (see Table 1). The method is quantitative for acifluorfen, acifluorfen-amine, acifluorfen-acetamide, and des-carboxy-acifluorfen at the stated LOQ of 10 µg/kg. The ILV modified the method, adding a centrifugation step and eliminating the derivatization procedure, with subsequent analysis for acifluorfen by LC/MS/MS with a stated LOQ of 5 µg/kg (metabolites were not included in the ILV analysis). Lowest toxicological levels of concern in soil are not reported. No major issues were discovered by the independent laboratory for acifluorfen. An internal validation in the ECM was not performed on the updated method.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation					
Acifluorfen, Acifluorfen- amine, Acifluorfen- acetamide, Des- carboxy- acifluorfen ¹	49085304	49085301	Soil	Unknown ²	United Phosphorus, Inc.	ECM: HPLC with fluorescence detection (for acifluorfen-amine) or GC/ECD (for the remaining analytes) ILV: UPLC/MS/MS	ECM: 10 µg/kg ILV: 5 µg/kg

¹ The ILV did not analyze for acifluorfen-amine, acifluorfen-acetamide and des-carboxy-acifluorfen.

² The final report of the updated ECM was 2/26/2013; the original date of BASF Method A9208 was not reported.

I. Principle of the Method

BASF analytical method A9208 was developed for the analysis of acifluorfen and its metabolites (acifluorfen-amine, acifluorfen-acetamide, and des-carboxy-acifluorfen) in soil (pp. 13, 16). Soil samples are extracted using a Polytron homogenizer with each of the following solvents one at a time: 10% acetone in (0.5M KCl: 0.1M NaOH) solution (pH > 13.0), acetone in 1N HCl (9:1) and methanol. After each extraction, the soil samples are centrifuged for 5 min. at 3000 RPM and the extracts decanted. The first two combined extracts are acidified with 5 mL 1N HCl. The combined extracts are partitioned twice with 90 mL dichloromethane. The organic phase is removed through phase separation paper. The petroleum ether is added to the combined organic phases and again filtered through a phase separation paper to remove all traces of water. The acetonitrile is added prior to concentration and reconstituted to 10 mL with acetonitrile. An aliquot is removed for HPLC analysis for acifluorfen amine. The remaining sample is again concentrated and then methylated with trimethylsilyl diazomethane in hexane. The sample is reconstituted in toluene for GC analysis. Instrumental analysis is accomplished using an HPLC (Partisphere C18-ODS 3 column) with a fluorescence detector for analysis of acifluorfen-amine and a GC/ECD for analysis of residues of des-carboxy-acifluorfen, methylated acifluorfen as acifluorfen-methyl-ester and acifluorfen-acetamide as acifluorfen acid amide methyl ester (pp. 19-20). The limit of quantification is 10 ppb (µg/kg) for all analytes.

The BASF method A9208 was modified in the ILV by JRF America. The modified method added a centrifugation step to the partition step between dichloromethane and petroleum ether, modified the transfer step to use methanol followed by dilution with methanol:water (10:90, v:v) rather than acetonitrile, eliminated the derivatization procedure, and analyzed for acifluorfen directly by UPLC (Acquity BEH C18 column, 5.0 cm x 2.1 mm, 1.7 μ m) using a mobile phase gradient of 0.1% formic acid in water:0.1% formic acid in methanol (70:30 to 10:90, v:v) with MS/MS detection (pp. 11-13). The LOQ is 5 ppb (μ g/kg) for acifluorfen (metabolites were not included in the ILV analysis).

II. Recovery Findings

Mean recoveries for all analytes and soil types were within guideline requirements (mean 70-120%) (ECM, pp. 28-31; ILV, p. 17). Relative standard deviations were generally within guideline requirements ($RSD \leq 20\%$) with exceptions shown in red and italicized in Table 2 below. Only 3 or 4 tests were performed for the 5 \times LOQ and 100 \times LOQ fortification levels.

Table 2. Initial Validation Method Recoveries for Analytes in Soil

Analyte	Fortification Level (μ g/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Des-Carboxy-Acifluorfen	10 (LOQ)	17	58.3-86.1	70.3	9.6	13.6
	50	4	71.1-101.1	82.7	13.9	16.8
	100	24	56.7-85.6	73.1	7.5	10.2
	1000	3	67.8-78.1	73.2	5.2	7.1
Acifluorfen (as Acifluorfen Methyl Ester) ¹	10 (LOQ)	17	50.8-115.1	74.0	14.9	<i>20.2</i>
	50	4	54.2-94.4	75.2	17.2	<i>22.8</i>
	100	24	53.9-87.2	71.9	9.0	12.5
	1000	3	69.8-82.4	74.3	7.0	9.4
Acifluorfen Acetamide (as Acifluorfen Acid Amide Methyl Ester)	10 (LOQ)	17	57.8-134.9	103.3	18.0	17.4
	50	4	81.4-129.9	104.5	21.9	<i>20.9</i>
	100	24	60.0-117.8	97.7	15.3	15.7
	1000	3	64.7-81.2	74.3	8.6	11.5
Acifluorfen Amine	10 (LOQ)	17	60.0-116.0	75.8	14.5	19.1
	50	4	68.0-83.5	73.0	7.1	9.8
	100	24	60.6-106.4	78.9	11.7	14.8
	1000	3	78.9-120.6	98.6	21.0	<i>21.3</i>

Data were obtained from MRID 49085304, Appendix 3, pp. 28-31 (see reviewer Excel file).

¹ Identified as methyl acifluorfen in Appendix 3.

² Identified as methyl acifluorfen acetamide in Appendix 3.

Table 3. Independent Validation Method Recoveries for Analytes in Soil

Analyte	Fortification Level ($\mu\text{g}/\text{kg}$)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Soil						
Acifluorfen	5 (LOQ)	5	70.0-81.3	75.2	4.8	6.4
	50	5	86.4-92.9	89.5	2.9	3.2
Clay Soil						
Acifluorfen	5 (LOQ)	5	75.1-110	93.0	13.5	14.5
	50	5	82.6-93.7	86.5	5.1	5.9

Data were obtained from MRID 49085301, Tables 2-3, p. 17.

III. Method Characteristics

The LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, which is 10 $\mu\text{g}/\text{kg}$ for all analytes in the ECM and 5 $\mu\text{g}/\text{kg}$ for acifluorfen in the ILV (p. 23). The LOD was not reported in the ECM or ILV but was defined as four times background noise. The LOQ was not established using scientifically acceptable procedures.

Table 4. Method Characteristics

	ILV	ECM			
	Acifluorfen	Acifluorfen	Acifluorfen amine	Acifluorfen acetamide	Des-carboxy-acifluorfen
Limit of Quantitation (LOQ)	5 $\mu\text{g}/\text{kg}$	10 $\mu\text{g}/\text{kg}$	10 $\mu\text{g}/\text{kg}$	10 $\mu\text{g}/\text{kg}$	10 $\mu\text{g}/\text{kg}$
Limit of Detection (LOD)	Estimated as 4 times the background noise	Estimated as 4 times the background noise	Estimated as 4 times the background noise	Estimated as 4 times the background noise	Estimated as 4 times the background noise
Linearity (calibration curve r^2 and concentration range)	$r^2=0.9989$ (sandy) $r^2=0.9972$ (clay) 0.1 – 25 $\mu\text{g}/\text{L}$	$r^2 = 0.998$ 20 – 300 $\mu\text{g}/\text{L}$	$r^2 = 0.998$ 1 – 40 $\mu\text{g}/\text{L}$	$r^2 = 0.993$ 20 – 300 $\mu\text{g}/\text{L}$	$r^2 = 0.998$ 20 – 300 $\mu\text{g}/\text{L}$
Repeatable	Yes	Yes ¹	Yes ¹	Yes ¹	Yes
Reproducible	NA	NA	NA	NA	NA
Specific	Yes	Yes GC/ECD (no MS)	Yes HPLC/FD (no MS)	Yes GC/ECD (no MS)	Yes GC/ECD (no MS)

Data were obtained from the ILV MRID 49085301, p. 12; Tables 1-2, p. 17; Figures 1-2, p. 19 and from the ECM, p. 23; Figures 1-8 & 13-15, pp. 33-37 & 40-41). The results were not reproducible because changes were made to the method.

¹ RSDs for acifluorfen, acifluorfen amine, and acifluorfen acetamide were between 20 and 23% for one or more fortification levels, but the overall RSD was $\leq 20\%$.

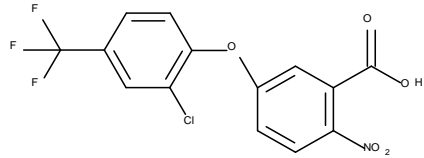
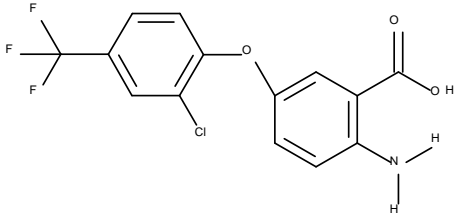
IV. Method Deficiencies and Reviewer's Comments

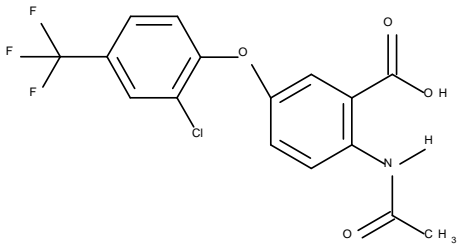
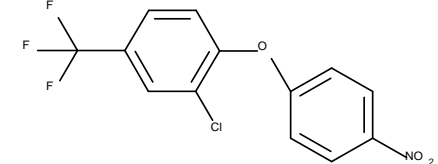
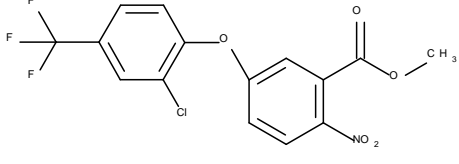
1. Modifications to the method by the ILV were not implemented in the ECM and the ILV did not analyze for the metabolites acifluorfen-amine, acifluorfen-acetamide, and des-carboxy-acifluorfen which are included in the ECM. The modified method added a centrifugation step to the partition step between dichloromethane and petroleum ether,

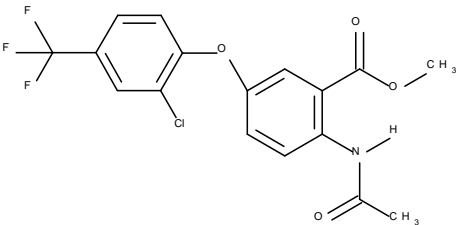
modified the transfer step to use methanol followed by dilution with methanol:water (10:90, v:v) rather than acetonitrile, eliminated the derivatization procedure, and analyzed for acifluorfen directly by UPLC/MS/MS, whereas the ECM analyzed for acifluorfen (as acifluorfen methyl ester) by GC/ECD. It was stated in the ILV that the method was modified from GC/ECD to LC/MS/MS as a result of a request by the sponsor to change the LOQ from 0.01 ppm to 0.005 ppm (p. 41). An internal validation in the ECM was not performed on the updated method.

2. The soil used in the ECM was not characterized.
3. No chromatograms are provided for reagent blanks in the ECM.
4. The LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, which is not a scientifically acceptable practice (LOQ is typically calculated as the mean matrix blank value plus ten times the standard deviation). Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.
5. The lowest toxicological level of concern in soil is not reported. The established LOQ must be less than toxicological levels of concern.
6. The required minimum of five spiked replicates was analyzed at the LOQ and 10× LOQ in both the ECM and ILV. At 5× LOQ and 100× LOQ in the ECM, however, only four replicates and three replicates, respectively, were analyzed.
7. ECM performance data for acifluorfen at the LOQ and at 5× LOQ were not within guideline criteria (RSDs of 20.2% and 22.8%). ECM performance data for acifluorfen acetamide at 5× LOQ were not within guideline criteria (RSD 20.9%). ECM performance data for acifluorfen amide at 100× LOQ were not within guideline criteria (RSD 21.3%). However, all other ECM and ILV performance data for all analytes in soil met guideline criteria.
8. Calibration curves could not be reconstructed for the ECM because complete data were not provided. Calibration chromatograms were provided for only a subset of calibration standards (Figures 6-8, pp. 36-37; Figures 13-15, pp. 40-41). Calibration curves were reconstructed for the ILV.
9. The linearity correlation coefficient (r^2) for methyl acifluorfen acetamide in the ECM was 0.993. Linearity is satisfactory when $r^2 \geq 0.995$ (Figure 3, p. 34).
10. The ILV stated that matrix suppression or enhancement should be investigated by fortifying acifluorfen standard into a control extract and that for soils used in the ILV it was found that 25 times dilution was necessary to eliminate the ion suppression (p. 14).

DER ATTACHMENT 1. Acifluorfen and Its Environmental Transformation Products. ^A

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)	Final %AR (study length)
PARENT						
Acifluorfen (BAS 9048 H)	IUPAC: 5-(2-Chloro- <i>α,α,α</i> -trifluoro- <i>p</i> -toloxy)-2-nitrobenzoic acid CAS: 5-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid CAS #: 50594-66-6 Formula: C ₁₄ H ₇ ClF ₃ NO ₅ MW: 361.5 g/mol SMILES: <chem>O=C(O)c(c(N(=O)=O)ccc1Oc(c(cc2C(F)(F)F)Cl)c2)c1</chem>		850.6100 ILV soil	49085301	NA	NA
			850.6100 ILV water	49085302		
			850.6100 ECM water	49085303		
			850.6100 ECM soil	49085304		
Acifluorfen Amine (BH 9048-A)	IUPAC: 5-(2-Chloro-4-trifluoromethylphenoxy)-2-aminobenzoic acid Formula: C ₁₄ H ₉ ClF ₃ NO ₃ MW: 331.7 g/mol SMILES: <chem>c1cc(c(cc1C(F)(F)F)Cl)Oc2ccc(c2)C(=O)O)N</chem>		850.6100 ECM water	49085303	NA	NA
			850.6100 ECM soil	49085304		
Acifluorfen Acetamide (BH 9048-AA)	IUPAC: 5-(2-Chloro-4-trifluoromethylphenoxy)-2-acetylamino benzoic acid Formula: C ₁₆ H ₁₁ ClF ₃ NO ₄ MW: 373.7 g/mol		850.6100 ECM water	49085303	NA	NA

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)	Final %AR (study length)
	SMILES: <chem>CC(=O)Nc1ccc(cc1C(=O)O)Oc2ccc(cc2Cl)C(F)(F)F</chem>		850.6100 ECM soil	49085304		
Des-carboxy- Acifluorfen (BH 9048-DC)	IUPAC: 4-[2-Chloro-4-(trifluoromethyl)phenoxy]-nitrobenzene Formula: $C_{13}H_7ClF_3NO_3$ MW: 317.65 g/mol SMILES: <chem>c1cc(ccc1[N+](=O)[O-])Oc2ccc(cc2Cl)C(F)(F)F</chem>		850.6100 ECM water	49085303	NA	NA
			850.6100 ECM soil	49085304		
MAJOR (>10%) TRANSFORMATION PRODUCTS						
No major transformation products were identified.						
MINOR (<10%) TRANSFORMATION PRODUCTS						
No minor transformation products were identified.						
REFERENCE COMPOUNDS NOT IDENTIFIED						
Acifluorfen Methyl Ester (BH 9048-ME)	IUPAC: Methyl-5-(2-Chloro-4-trifluoromethylphenoxy)-2-nitrobenzoate CAS #: 50594-67-7 Formula: $C_{15}H_9ClF_3NO_5$ MW: 375.7 g/mol SMILES: <chem>COC(=O)c1cc(ccc1[N+](=O)[O-])Oc2ccc(cc2Cl)C(F)(F)F</chem>		850.6100 ECM water	49085303	NA	NA
			850.6100 ECM soil	49085304		

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)	Final %AR (study length)
	<chem>COc1ccc(cc1Cl)C(F)(F)F</chem>					
Acifluorfen Acid Methyl Ester (BH 9048-AAME)	IUPAC: Methyl-5-(2-Chloro-4-trifluoromethylphenoxy)-2-acetylamino benzoate Formula: C₁₇H₁₃ClF₃NO₄ MW: 387.7 g/mol SMILES: <chem>CC(=O)Nc1ccc(cc1C(=O)OC)Oc2ccc(cc2Cl)C(F)(F)F</chem>		850.6100 ECM water	49085303	NA	NA
			850.6100 ECM soil	49085304		

^A AR means "applied radioactivity". MW means "molecular weight". NA means "not applicable". ECM means "environmental chemical methods". ILV means "independent laboratory validation".