

**Test Material:** Acifluorfen

**MRID:**

**Title:** BASF Analytical Method A9206. Determination of Acifluorfen, Acifluorfen-Amine, Acifluorfen-Acetamide, and Des-Carboxy-Acifluorfen in Water.

**MRID:** 49085302

**Title:** Acifluorfen. Independent Laboratory Validation of BASF A 9206 for the Analysis of Acifluorfen in Water. 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 water**

**Reports:** ECM: MRID 49085303. United Phosphorus, Inc. 2013. BASF Analytical Method A9206; Determination of Acifluorfen, Acifluorfen-Amine, Acifluorfen-Acetamide, and Des-Carboxy-Acifluorfen in Water. Project ID: UPI-2013-002. Completion Date: February 26, 2013. 49 pages.

ILV: MRID 49085302. Li, N., Li, F., and Patel, D. 2012. Acifluorfen; Independent Laboratory Validation of BASF A 9206 for the Analysis of Acifluorfen in Water; Final Report. Performing Laboratory: JRF America, Audubon, Pennsylvania. Laboratory Project ID: AU-2012-02. Sponsor: United Phosphorus, Inc., King of Prussia, Pennsylvania. Study Completion Date: February 20, 2012. 67 pages.

**Document No.:** MRIDs 49085303 and 49085302

**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 were provided (pp. 2-5).


**Classification:** This analytical method is classified as supplemental – upgradeable. The ILV did not successfully repeat the method at the LOQ in all water samples and 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 water used in the ECM was not characterized. Chromatograms were not provided for reagent blanks or for spiked samples at 10×LOQ for des-carboxy-acifluorfen, acifluorfen, and acifluorfen-acetamide.

**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 A9206, is designed for the quantitative determination of acifluorfen and its metabolites acifluorfen-amine, acifluorfen-acetamide, and des-carboxy-acifluorfen in water 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 1.0 µg/L. The ILV was successful at validating the method for acifluorfen using groundwater at the LOQ of 1.0 µg/L (mean recovery of  $102 \pm 5.4\%$ ); however, was not successful at validating the method for surface water at the LOQ (mean recovery of  $58.4 \pm 13.4\%$ ). Metabolites were not included in the ILV analysis. Lowest toxicological levels of concern in water were not reported.

Following the attempted validation of the original method, the ILV modified the method at the request of the sponsor, eliminating the derivatization step with subsequent analysis for acifluorfen by LC/MS/MS. This modification extended the LOQ for acifluorfen in water to 0.1 µg/L, with acceptable recoveries in surface water and groundwater at the LOQ and 10xLOQ (0.1 and 1.0 µg/L). 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 <sup>1</sup>	49085303	49085302	Water	Unknown <sup>2</sup>	United Phosphorus, Inc.	HPLC with fluorescence detection (for acifluorfen-amine) or GC/ECD (for all other compounds)	1 µg/L

The ILV modified the method (LC/MS/MS) and extended the LOQ to 0.1 µg/L.

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

<sup>2</sup> The final date of the updated ECM was 2/26/2013; the original date of BASF Method A9206 was not reported.

## I. Principle of the Method

BASF analytical method A9206 was developed for the analysis of acifluorfen and its metabolites (acifluorfen-amine, acifluorfen-acetamide, and des-carboxy-acifluorfen) in water (pp. 11-12; 15). Water samples are acidified using 1N HCl and partitioned three times with dichloromethane:acetonitrile (1:1) by shaking for 30 seconds. Following each partition, the organic phase is passed through a phase separation paper to remove all traces of water; the extracts were combined, concentrated to <20 mL using a RapidVap at 40°C, and reconstituted in 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 (1 hour). 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 descarboxy-acifluorfen, methylated acifluorfen as acifluorfen-methyl-ester and acifluorfen-acetamide as acifluorfen acid amide methyl ester (pp. 17-18). The limit of quantification (LOQ) is 1.0 µg/L for all analytes.

The BASF method A9206 was followed as written in the ILV by JRF America using surface water collected from a creek in Delaware and ground water obtained from a well in Pennsylvania (MRID 49085302, pp. 11, 13). At the request of the sponsor, the method was then modified to

extend the LOQ to 0.1 µg/L. The modified method eliminated the derivatization procedure, diluted the final volume to 10 mL instead of 25 mL with acetonitrile, and analyzed for acifluorfen directly by UPLC (Acquity BEH C18 column, 5.0 cm x 2.1 mm, 1.7 µm) 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. 11-15). The LOQ is 0.1 µg/L for acifluorfen (metabolites were not included in the ILV analysis).

## II. Recovery Findings

Mean recoveries and relative standard deviations for all analytes and matrices were generally within guideline requirements (mean 70-120% and RSD ≤ 20%) with exceptions shown in red and italicized in Table 2 below (ECM, pp. 27-28; ILV, p. 18). Only 3 or 4 tests were performed for the 10× LOQ fortification level. Fewer than 5 tests were also performed for the 200× and 500×LOQ fortification levels.

**Table 2. Initial Validation Method Recoveries for Analytes in Water**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Des-Carboxy-Acifluorfen	1 (LOQ)	8	62.2-82.6	70.5	7.4	10.5
	10	3	70.8-84.9	77.2	7.1	9.2
	50	5	88.9-96.5	92.1	2.9	3.2
	100	10	79.5-96.6	91.0	5.4	6.0
	200	1	82.8	82.8	NA	NA
	500	3	97-100.5	98.4	1.8	1.9
	1000	5	91.9-101.3	94.8	3.8	4.0
Acifluorfen (as Acifluorfen Methyl Ester) <sup>1</sup>	1 (LOQ)	8	52.9-96.6	70.7	14.0	19.8
	10	3	60.8-86.2	<i>69.7</i>	14.3	<i>20.5</i>
	50	5	73.4-81.6	77.6	3.1	4.0
	100	10	71.4-105.2	84.8	12.3	14.6
	200	1	72.2	72.2	NA	NA
	500	3	89.6-96.1	91.9	3.6	4.0
	1000	5	83.2-102.1	91.9	8.5	9.2
Acifluorfen Acetamide (as Acifluorfen Acid Amide Methyl Ester) <sup>2</sup>	1 (LOQ)	8	65.5-110.5	80.8	14.5	18.0
	10	3	64.3-105.4	80.5	21.9	<i>27.2</i>
	50	5	69.9-81.0	75.5	4.5	5.9
	100	10	72.0-106.4	83.4	9.4	11.3
	200	1	87.0	87.0	NA	NA
	500	3	77.0-86.3	82.3	4.8	5.8
	1000	5	81.9-93.1	87.0	5.3	6.1
Acifluorfen Amine	1 (LOQ)	6	84.3-119.0	102.8	14.1	13.7
	10	4	106.3-123.5	113.9	7.9	6.9
	50	5	109.0-129.0	<i>120.1</i>	8.7	7.2
	100	10	91.3-120.5	110.0	10.9	9.9
	200	1	107.8	107.8	NA	NA
	500	3	115.8-134.3	<i>124.7</i>	9.3	7.4

	1000	5	71.5-117.5	102.9	18.8	18.3
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Data were obtained from MRID 49085303, Appendix 3, pp. 27-28 (see reviewer Excel file).

<sup>1</sup> Identified as methyl acifluorfen in Appendix 3.

<sup>2</sup> Identified as methyl acifluorfen acetamide in Appendix 3.

**Table 3. Independent Validation Method Recoveries for Analytes in Water-Original Method**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water						
Acifluorfen	1 (LOQ)	5	48.7-66.5	58.4	7.8	13.4
	10	5	69.9-104	80.4	13.6	16.9
Groundwater						
Acifluorfen	1 (LOQ)	5	95.3-107	102	5.5	5.4
	10	5	99.0-109	103	4.0	3.8

Data were obtained from MRID 49085302, Tables 1-2, p. 18.

**Table 4. Independent Validation Method Recoveries for Analytes in Water-Modified Method**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water						
Acifluorfen	0.1 (LOQ)	5	72.7-95.4	81.0	9.5	11.8
	1.0	5	89.8-104	96.4	5.4	5.6
Groundwater						
Acifluorfen	0.1 (LOQ)	5	82.4-120	101	15.4	15.3
	1.0	5	83.0-109	94.9	10.3	10.8

Data were obtained from MRID 49085302, Tables 3-4, p. 19.

### III. Method Characteristics

The LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, which is 1.0 µg/L for all analytes in the ECM and 0.1 µg/L for acifluorfen in the ILV modified method. 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	ILV-Modified Method	ECM			
	Acifluorfen	Acifluorfen	Acifluorfen	Acifluorfen amine	Acifluorfen acetamide	Des-carboxy-acifluorfen
Limit of Quantitation (LOQ)	1 µg/L	0.1 µg/L	1 µg/L	1 µg/L	1 µg/L	1 µg/L
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	Estimated as 4 times the background noise
Linearity (calibration curve $r^2$ and concentration range)	$r^2=0.9993$ 10-500 µg/L	$r^2 = 0.9968$ 0.25-25 µg/L	$r^2 = 0.994$ 20 – 400 µg/L	$r^2 = 0.993$ 2 – 40 µg/L	$r^2 = 0.990$ 20 – 400 µg/L	$r^2 = 0.998$ 20 – 400 µg/L
Repeatable	No <sup>2</sup>	Yes	Yes <sup>1</sup>	Yes <sup>1</sup>	Yes <sup>1</sup>	Yes
Reproducible	NA	N/A	No <sup>2</sup>	NA	NA	NA
Specific	Yes	Yes GC/ECD (no MS)	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 49085302, p. 15; Tables 3-4, p. 19; Figure 1, p. 21; Figure 12, p. 33 and from the ECM, pp. 13, 21; Figures 1-4, pp. 31-32).

<sup>1</sup> Mean recovery and RSDs across all fortification levels were within the acceptable range; however, mean recovery and RSD were not within the acceptable range at all fortification levels (see DER Table 2).

<sup>2</sup> The average recovery at the LOQ for surface water in the ILV was 58.4%. The validation succeeded for groundwater and for surface water at 10x LOQ.

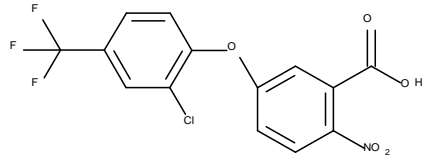
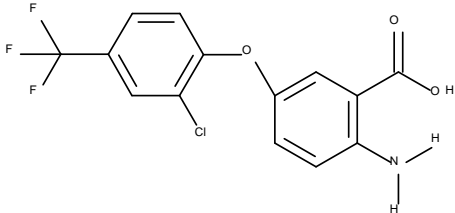
#### IV. Method Deficiencies and Reviewer's Comments

1. The ILV did not successfully repeat the method at the LOQ in all water samples and did not analyze for the metabolites acifluorfen-amine, acifluorfen-acetamide, and des-carboxy-acifluorfen which are included in the ECM. At the LOQ of 1.0 µg/L, mean recovery from surface water samples were 58.4% with a range of 48.7-66.5% (mean recovery was 102%, range of 95.3-107% from groundwater samples; MRID 49085302, Tables 1-2, p. 18). Following the attempted validation of the original method, at the request of the sponsor the ILV modified the method, eliminating the derivatization procedure and analyzing for acifluorfen directly by UPLC with MS/MS detection. This modified method produced acceptable recoveries in surface and groundwater samples at the extended LOQ of 0.1 µg/L and 10xLOQ at 1.0 µg/L. However, an internal validation in the ECM was not performed on the updated method.
2. The water used in the ECM was not characterized.
3. No chromatograms are provided for reagent blanks in the ECM. No chromatograms are provided in the ECM for spiked samples at 10× LOQ for des-carboxy acifluorfen, acifluorfen, and acifluorfen acetamide.



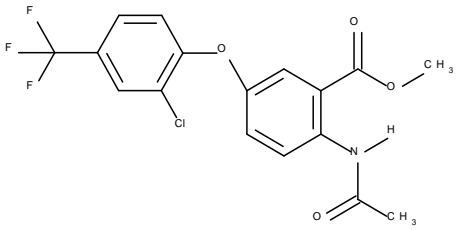
4. The LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, which is not a scientifically acceptable practice (LOQ is typically calculated as the mean matrix blank value plus ten times the standard deviation). Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.
5. The lowest toxicological level of concern in water is not reported. The established LOQ must be less than toxicological levels of concern.
6. The required minimum of five spiked replicates was not analyzed at 10× LOQ in the ECM. Only three (des-carboxy acifluorfen, acifluorfen, and acifluorfen acetamide) or four (acifluorfen amine) replicates were analyzed.
7. ECM performance data for acifluorfen at 10× LOQ were not within guideline criteria (mean recovery of 69.7% and RSD of 20.5%; reviewer-calculated). ECM performance data for acifluorfen acetamide at 10× LOQ were not within guideline criteria (RSD 27.2%). ECM performance data for acifluorfen amine at 50× LOQ and 500× LOQ were not within guideline criteria (recoveries of 120.1% and 124.7%). ILV performance data for acifluorfen in surface water at the LOQ were not within guideline criteria (mean recovery of 58.4%). All other analytes in water 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 (MRID 49085303, Figures 6-8, pp. 34-35; Figures 13-15, pp. 38-39). Calibration curves were reconstructed for the ILV.
9. ILV recoveries for acifluorfen in groundwater were corrected for acifluorfen measured in the untreated controls (MRID 49085302, p. 65).
10. The linearity correlation coefficients ( $r^2$ ) for acifluorfen, acifluorfen amine, and acifluorfen acetamide in the ECM were less than 0.995, indicating that linearity is not satisfactory (MRID 49085303, Figure 2-4, pp. 31-32).

DER ATTACHMENT 1. Acifluorfen and Its Environmental Transformation Products. <sup>A</sup>

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 <sub>14</sub> H <sub>7</sub> ClF <sub>3</sub> NO <sub>5</sub> MW: 361.5 g/mol SMILES: <chem>O=C(O)c(c(N(=O)=O))ccc1Oc(c(cc2)C(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 <sub>14</sub> H <sub>9</sub> ClF <sub>3</sub> NO <sub>3</sub> 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 <sub>16</sub> H <sub>11</sub> ClF <sub>3</sub> NO <sub>4</sub> 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>		<b>850.6100 ECM soil</b>	<b>49085304</b>		
Des-carboxy- Acifluorfen (BH 9048-DC)	IUPAC: <b>4-[2-Chloro-4-(trifluoromethyl)phenoxy]-nitrobenzene</b>  Formula: $C_{13}H_7ClF_3NO_3$ MW: <b>317.65 g/mol</b> SMILES: <chem>c1cc(ccc1[N+](=O)[O-])Oc2ccc(cc2Cl)C(F)(F)F</chem>		<b>850.6100 ECM water</b>	<b>49085303</b>	NA	NA
			<b>850.6100 ECM soil</b>	<b>49085304</b>		
MAJOR (>10%) TRANSFORMATION PRODUCTS						
<b>No major transformation products were identified.</b>						
MINOR (<10%) TRANSFORMATION PRODUCTS						
<b>No minor transformation products were identified.</b>						
REFERENCE COMPOUNDS NOT IDENTIFIED						
Acifluorfen Methyl Ester (BH 9048-ME)	IUPAC: <b>Methyl-5-(2-Chloro-4-trifluoromethylphenoxy)-2-nitrobenzoate</b>  CAS #: <b>50594-67-7</b>  Formula: $C_{15}H_9ClF_3NO_5$ MW: <b>375.7 g/mol</b> SMILES: <chem>COC(=O)c1cc(ccc1[N+](=O)[O-])Oc2ccc(cc2Cl)C(F)(F)F</chem>		<b>850.6100 ECM water</b>	<b>49085303</b>	NA	NA
			<b>850.6100 ECM soil</b>	<b>49085304</b>		

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: <b>Methyl-5-(2-Chloro-4-trifluoromethylphenoxy)-2-acetylamino benzoate</b>  Formula: <b>C<sub>17</sub>H<sub>13</sub>ClF<sub>3</sub>NO<sub>4</sub></b> MW: <b>387.7 g/mol</b> SMILES: <chem>CC(=O)Nc1ccc(cc1C(=O)OC)Oc2ccc(cc2Cl)C(F)(F)F</chem>		<b>850.6100 ECM water</b>	<b>49085303</b>	NA	NA
			<b>850.6100 ECM soil</b>	<b>49085304</b>		

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