

**Test Material:** Ethalfluralin

**MRID:** 49385902

**Title:** Validation of an Analytical Method for the Determination of Ethalfluralin in Water

**MRID:** 49385903

**Title:** Independent Laboratory Validation of an Analytical Method for the Determination of Ethalfluralin in Water by Gas Chromatography with Electron-impact Mass Spectrometry Detection (GC/MS)

**EPA PC Code:** 113101

**OCSPP Guideline:** 850.6100

**For CDM Smith**

**Primary Reviewer:** Lisa Muto

**Signature:**



**Date:** 1/9/15

**Secondary Reviewer:** Lynne Binari

**Signature:**



**Date:** 1/9/15

**QC/QA Manager:** Joan Gaidos

**Signature:**



**Date:** 1/9/15

**Analytical method for ethalfluralin in drinking, surface and ground water**

**Reports:** ECM: EPA MRID No. 49385902. Betson, S. 2012. Validation of an Analytical Method for the Determination of Ethalfluralin in Water. Dow AgroSciences Study Reference No.: 120814. CEMAS Study No.: CEMS-5608. Report prepared by CEM Analytical Services (CEMAS), Berkshire, United Kingdom, sponsored and submitted by Regulatory Sciences and Government Affairs- Indianapolis Lab, Dow AgroSciences LLC, Indianapolis, Indiana; 123 pages. Final report issued December 21, 2012.  
ILV: EPA MRID 49385903. Amic, S. 2013. Independent Laboratory Validation of an Analytical Method for the Determination of Ethalfluralin in Water by Gas Chromatography with Electron-impact Mass Spectrometry Detection (GC/MS). Dow AgroSciences Study Reference No.: 130633. EAS Chem SAS Study No.: S13-01932. Report prepared by Eurofins Agrosience Services Chem SAS, Vergèze, France, sponsored and submitted by Regulatory Sciences and Government Affairs- Indianapolis Lab, Dow AgroSciences LLC, Indianapolis, Indiana; 71 pages. Final report issued October 4, 2013.

**Document No.:** MRIDs 49385902 & 49385903


**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in accordance with OECD and UK Good Laboratory Practices (GLP; p. 3; Appendix C, p. 123). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were included (pp. 2-4). An authenticity statement was not included; however, a signature page was included (p. 5).  
ILV: The study was conducted in accordance with OECD GLP (p. 3). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were included (pp. 2-4). A statement of the authenticity of the study report was included as part of the Quality Assurance Statement (p. 4). A signature page was also included (p. 5).

**Classification:** This analytical method is classified as Acceptable. Representative chromatograms in the ECM and ILV were faint and difficult to interpret. It will be helpful if clear copies are provided by the registrant.


**PC Code:** 113101

**Reviewer:** Patricia Engel  
Physical Scientist

**Signature:**   
**Date:** 10/16/2018

Reviewer:

**Mohammed Ruhman**  
**Ph.D., Senior Scientist**

**Signature:**   
**Date:** 10/16/2018

**Executive Summary**

The analytical method, Dow AgroSciences Study Reference No. 120814, is designed for the quantitative determination of ethalfluralin in ground, surface and drinking water at the LOQ of 5.0 ng/L using GC/MS. The LOQ is less than the current lowest toxicological level of concern in

water. The ILV was successful in the first trial. The supporting chromatograms were decipherable, but not clearly readable with normal, expected resolution.

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Ethalfluralin	49385902	49385903		Drinking, Surface and Ground Water	21/12/2012	Dow AgroSciences LLC	GC/MS	5.0 ng/L

## I. Principle of the Method

Samples (500 mL) were mixed with 4 mL of methanol then fortified as necessary (pp. 16, 19 of MRID 49385902). Samples were manually mixed then left to stand for 5 minutes. The mixture was purified using a Strata C18 solid phase extraction (SPE) cartridge (500 mg, 6 mL). The SPE cartridge was conditioned with ethyl acetate, methanol and ultrapure water (4 mL each). The extract was applied then the analyte was eluted with ethyl acetate (6 mL) without vacuum initially. The eluate was reduced under a stream of nitrogen with a Techne Dri-block concentrator (temperature not specified; p. 20 of MRID 49385902). The residue was reconstituted in 1 mL of ethyl acetate prior to GC/MS analysis.

Samples were analyzed for ethalfluralin by gas chromatography (Agilent Model 6890A gas chromatograph) using a Durabond-5MS, 30 m x 0.25 mm, 0.25- $\mu$ m column (initial oven temperature 100°C) with electron impact tandem mass spectrometry (splitless mode; carrier gas, helium; pp. 20-21 of MRID 49385902). Injection volume was not specified. Ions monitored were  $m/z$  276 (quantitation ion),  $m/z$  292 (confirmation ion 1) and  $m/z$  316 (confirmation ion 2). The relative ratios of the three transitions were quantified for confirmation of ethalfluralin (p. 23 of MRID 49385902).

In the ILV, the extraction procedure and GC/MS analysis was performed according to the ECM, without modification (pp. 14-16 of MRID 49385903). The only noted differences were the specification of the evaporation temperature (room temperature) and GC/MS injection volume of 4  $\mu$ L.

In the ECM and ILV, the Limit of Quantification (LOQ) and Limit of Detection (LOD) were 5 ng/L and 1.5 ng/L, respectively (p. 26 of MRID 49385902; p. 11; Tables 1-18, pp. 24-35 of MRID 49385903).

## II. Recovery Findings

ECM (MRID 49385902): Mean recoveries and relative standard deviations (RSD) were within requirements (mean 70-120%; RSD  $\leq$ 20%) for analysis of ethalfluralin in drinking, ground and surface waters at the LOQ and 10 $\times$ LOQ (p. 24; Tables 1-12, pp. 30-40). One sample per matrix was also dosed at the LOD. Confirmation of the identified peaks was based on the observation of its three parent-to-daughter ion transitions and the quantification of the relative ratios of those transitions. The water matrices were fully characterized at CEMAS (pp. 13-16). The drinking water was obtained from the test facility (CEMAS). The ground water was obtained from a well near Henley-on-Thames, United Kingdom. The surface water was obtained from the Cut River, Bracknell, United Kingdom.

ILV (MRID 49385903): Mean recoveries and relative standard deviations (RSD) were within requirements (mean 70-120%; RSD  $\leq$ 20%) for analysis of ethalfluralin in drinking, ground and surface waters at the LOQ and 10 $\times$ LOQ (pp. 18-19; Tables 1-18, pp. 24-35). One sample per matrix was also dosed at the LOD. Confirmation of the identified peaks was based on the observation of its three parent-to-daughter ion transitions and the quantification of the relative ratios of those transitions. The water matrices were fully characterized at Eurofins IPL Sud, Vergèze, France (non-GLP facility; ISO certified facility; p. 12). The drinking water was obtained from a tap in Vergèze, France. The ground water was obtained from Evian, France. The surface water was obtained from Rhône, Gard, France. The method validation was successful on the first trial (p. 18).

**Table 2. Initial Validation Method Recoveries for Ethalfluralin in Water<sup>1</sup>**

Analyte	Fortification Level (ng/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Drinking Water</b>						
<i>m/z</i> 276 (quantitation ion)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	72-107	83	14.0	16.9
	50	5	75-115	87	16.3	18.6
<i>m/z</i> 292 (confirmation ion 1)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	77-94	86	7.4	8.6
	50	5	75-114	87	15.7	17.9
<i>m/z</i> 316 (confirmation ion 2)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	69-106	86	13.5	15.8
	50	5	75-118	88	17.5	19.8
<b>Ground Water</b>						
<i>m/z</i> 276 (quantitation ion)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	74-92	82	7.6	9.3
	50	5	65-103	85	16.3	19.2
<i>m/z</i> 292 (confirmation ion 1)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	76-95	85	7.1	8.3
	50	5	66-102	85	16.0	18.7
<i>m/z</i> 316 (confirmation ion 2)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	77-101	86	10.4	12.1
	50	5	66-102	85	15.6	18.3
<b>Surface Water</b>						
<i>m/z</i> 276 (quantitation ion)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	74-101	90	10.1	11.2
	50	5	87-102	95	5.9	6.2
<i>m/z</i> 292 (confirmation ion 1)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	71-106	91	15.5	17.1
	50	5	86-101	94	5.7	6.0
<i>m/z</i> 316 (confirmation ion 2)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	71-97	89	10.7	12.0
	50	5	88-102	95	8.7	9.5

Data (uncorrected recoveries) obtained from Tables 1-12, pp. 30-40 of MRID 49385902.

<sup>1</sup> Water matrices were fully characterized (pp. 13-16).

<sup>2</sup> Ethalfluralin recovered ranged 1.160-1.323 ng/L, 1.360-1.466 ng/L and 1.154-1.345 ng/L in the drinking water, ground water and surface water, respectively. The % recovery was reported as below the LOQ in the study report.

**Table 3. Independent Validation Method Recoveries for Ethalfluralin in Water<sup>1</sup>**

Analyte	Fortification Level (ng/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Drinking Water</b>						
<i>m/z</i> 276 (quantitation ion)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	73-89	80	80	8
	50	5	77-89	84	84	7
<i>m/z</i> 292 (confirmation ion 1)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	75-93	81	81	9
	50	5	79-91	86	86	6
<i>m/z</i> 316 (confirmation ion 2)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	74-88	79	79	7
	50	5	79-92	87	87	7
<b>Surface Water</b>						
<i>m/z</i> 276 (quantitation ion)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	77-86	80	80	5
	50	5	70-85	76	76	7
<i>m/z</i> 292 (confirmation ion 1)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	77-88	82	82	5
	50	5	70-85	77	77	7
<i>m/z</i> 316 (confirmation ion 2)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	75-86	81	81	6
	50	5	71-88	78	78	8
<b>Ground Water</b>						
<i>m/z</i> 276 (quantitation ion)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	80-86	83	83	3
	50	5	86-89	88	88	2
<i>m/z</i> 292 (confirmation ion 1)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	74-89	80	80	8
	50	5	88-91	89	89	1
<i>m/z</i> 316 (confirmation ion 2)						
Ethalfluralin	1.5 (LOD)	1	-- <sup>2</sup>	--	--	--
	5 (LOQ)	5	76-85	80	80	4
	50	5	89-91	90	90	1

Data (uncorrected recoveries) obtained from Tables 1-18, pp. 24-35 of MRID 49385903.

1 Water matrices were fully characterized (p. 12).

2 Ethalfluralin recovered ranged 1.36-1.53 ng/L, 1.29-1.46 ng/L and 1.20-1.62 ng/L in the drinking water, ground water and surface water, respectively. The % recovery was reported as below the LOQ in the study report.

### III. Method Characteristics

In the ECM and ILV, the LOQ and LOD were 5 ng/L and 1.5 ng/L, respectively (p. 26; Tables 14-16, pp. 41-42 of MRID 49385902; p. 11; Tables 1-18, pp. 24-35 of MRID 49385903). In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise at the retention time of the analyte in the control samples. The LOD values were estimated as three times the background noise. This value was noted to vary between runs and instruments. LOQ values were estimated by the equation  $LOQ = LOD \div 0.3$ . The results for the calculated LOD and LOQ values for each ion/matrix supported the designated LOQ and LOD values for the method. The LOQ and LOD values of the ILV were based on the ECM; the ILV justified the LOQ with the successful validation results of the ILV.

**Table 4. Method Characteristics**

		Ethalfluralin		
		Drinking water	Ground water	Surface water
Limit of Quantitation (LOQ)		5 ng/L		
Limit of Detection (LOD)		1.5 ng/L		
Linearity (calibration curve $r^2$ and concentration range) <sup>1,2</sup>	ECM	$r^2 = 0.9989$ ( <i>m/z</i> 276) $r^2 = 0.9984$ ( <i>m/z</i> 292) $r^2 = 0.9983$ ( <i>m/z</i> 316)		
	ILV	$r^2 = 0.9968$ ( <i>m/z</i> 276) $r^2 = 0.9972$ ( <i>m/z</i> 292) $r^2 = 0.9967$ ( <i>m/z</i> 316)		
	Conc. range	(0.75-100 $\mu\text{g/L}$ or $\text{ng/mL}$ )		
Repeatable		Yes		
Reproducible		Yes		
Specific		Yes <sup>3</sup>		

Data were obtained from p. 26; Tables 14-16, pp. 41-42; Figures 1-3, pp. 43-45; Figures 5-7, pp. 47-49; Figures 9-11, pp. 51-53; Figures 13-15, pp. 55-57 of MRID 49385902; p. 11; Tables 1-18, pp. 24-35; Figures 1-3, pp. 38-40; Figures 6-8, pp. 43-45; Figures 12-14, pp. 49-51; Figures 18-20, pp. 55-57 of MRID 49385903.

1 Not matrix-specific.

2 ECM calibration curves were confirmed by the reviewer ( $r^2 = 0.9979$ - $0.9982$ ) using data obtained from Figures 1-3, pp. 43-45 of MRID 49385902 (see DER Attachment 2). ILV calibration curves were confirmed by the reviewer ( $r^2 = 0.9974$ - $0.9978$ ) using data obtained from Figures 1-3, pp. 38-40 of MRID 49385903 (see DER Attachment 2).

3 The chromatograms depicting the analyte peaks and control matrices were displayed in the ECM and ILV Figures with faint to light resolution. The chromatograms were decipherable, but not clearly readable with normal, expected resolution.

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

1. The chromatograms depicting the analyte peaks and control matrices were displayed in the ECM and ILV Figures with faint to light resolution. The chromatograms were decipherable, but not clearly readable with normal, expected resolution. The reviewer determined that there was no interference with the analyte peak based on the provided chromatograms; however, the reviewer noted a margin of error in this evaluation based on poor quality of resolution in the submitted chromatograms. In the ECM and ILV, the study authors noted that there were no interferences present in the chromatograms of the

untreated control sample at the retention times of the analyte that were greater than 30% of the LOQ (quantitative and confirmatory ions; p. 24 of MRID 49385902; p. 19 of MRID 49385903).

2. This ECM (MRID 49385902) was conducted to update the ChemService residue analytical method number 19/2002, Garofani, S. 2003. "Determination of Ethalfuralin Residues in Drinking, Ground and Surface waters by Gas Chromatography with Electron Capture Detector" and achieve a lower limit of quantitation, 5.0 ng/L (p. 10 of MRID 49385902). ChemService residue analytical method number 19/2002 (ChemService 19/2002) was included as Appendix B, pp. 62-121 of MRID 49385902. The LOQ for ChemService 19/2002 is 0.05 µg/L. Water samples were passed through a pre-conditioned SPE C18 cartridge, eluted with ethyl acetate, then analyzed using gas chromatography with electron capture detection (GC/ECD; Appendix B, p. 72; Appendix B, Appendix 1, pp. 92-95). The sample extraction procedure was similar to that of this ECM; however, the initial sample volume and ethyl acetate elution volume differed. The use of GC/MS was described for confirmation of ethalfuralin (Appendix B, p. 74; Appendix B, Appendix 1, p. 101). The GC/MS column and chromatographic conditions differed greatly from those of this ECM.

For ChemService residue analytical method number 19/2002, GC/ECD recovery results of ethalfuralin in drinking water, ground water and surface water ranged 73-109%, 69-108% and 72-111%, respectively, for all fortification levels (LOQ, 10×LOQ, 100×LOQ and 1000×LOQ, n = 6 for each fortification level; Appendix B, Tables 3-26, pp. 78-89; Appendix B, Appendix 1, Tables 5-8, pp. 99-100 of MRID 49385902). The nominal LOQ was 0.05 µg/L; however, the actual fortification rates for the LOQ and 10×LOQ samples were 0.06 µg/L and 0.60 µg/L, respectively, in drinking and ground water. The reviewer noted that the overall recovery range for ground water was reported as "70-108%" in the study report (Appendix B, p. 76; Appendix B, Appendix 1, Table 6, p. 99), even though the recovery of Spike D5 was 68.54% (Appendix B, Table 18, p. 85; Appendix B, Appendix 1, Table 8, p. 100). This value was treated as an outlier in the study report (Appendix B, Appendix 1, Table 6, p. 99). No GC/MS recovery results were reported.

Representative GC/ECD and GC/MS chromatograms for ChemService residue analytical method number 19/2002 were faint and/or difficult to interpret (Appendix B, Appendix 1, Figures 1-9, pp. 103-111 of MRID 49385902).

3. The determinations of the LOD and LOQ in the ECM and ILV were acceptable, based on scientifically procedures as defined in 40 CFR Part 136. In the ECM, the LOD values were estimated as three times the background noise (p. 26; Tables 14-16, pp. 41-42 of MRID 49385902). This value was noted to vary between runs and instruments. LOQ values were estimated by the equation  $LOQ = LOD \div 0.3$ . The results for the calculated LOD and LOQ values for each ion/matrix supported the designated LOQ and LOD values for the method. The LOQ and LOD values of the ILV were based on the ECM; the ILV justified the LOQ with the successful validation results of the ILV (p. 11; Tables 1-18, pp. 24-35 of MRID 49385903).



4. In the ECM, the method directed the correction of sample recoveries for analyte concentration in the control samples (p. 22 of MRID 49385902). Generally, since analyte found in the controls was reported as “ND”, the reviewer considered the ethalfluralin samples to be uncorrected for matrix blanks; however, the reviewer noted that “ND” was defined as “residue...below the 1.5 ng/L, the limit of detection”, so the control values were not necessarily valued at 0.000 ng/L (Tables 1-12, pp. 30-40). The calculations of the ILV did not report the correction for background in the controls (pp. 17-18 of MRID 49385903).
5. The matrix effects were assessed for each matrix in the ECM and ILV (Table 13, p. 40 of MRID 49385902; Table 22, p. 37 of MRID 49385903). No significant suppression or enhancement of the instrument (<20%) was observed for all ions monitored (p. 25 of MRID 49385902; p. 19 of MRID 49385903).
6. The storage stability of the final extracts were assessed in the ECM and ILV; the standard solutions were also assessed in the ECM. In the ECM, the final extracts of the surface water samples were found to be stable up to 7 days under refrigeration; however, those of the ground and drinking water were not found to be stable (primary transition ion measured; pp. 25-26; Tables 17-18, p. 42 of MRID 49385902). Based on this data, the study author recommended that extracts are analyzed immediately. Mean recoveries were 65%, 65% and 87%, respectively, for the drinking water, ground water and surface water. The standard solutions were found to be stable up to 11 days under refrigeration (primary transition ion measured; standard curves were compared).  
  
In the ILV, the final extracts were found to be stable at 4°C up to 8 days for the surface and ground water samples and up to 10 days for the drinking water samples (primary and confirmatory transition ions measured; pp. 19-20; Tables 19-21, pp. 36-37 of MRID 49385903). Mean recoveries and RSDs after storage were 80% and 5% (*m/z* 276) and 77% and 6% (*m/z* 292), respectively, for the drinking water; 77% and 6% (*m/z* 276) and 77% and 6% (*m/z* 292), respectively, for the surface water; and 81% and 3% (*m/z* 276) and 80% and 6% (*m/z* 292), respectively, for the ground water.
7. No communication occurred between the ILV and ECM study directors (p. 21 of MRID 49385903).
8. It was reported for the ILV that one set of thirteen samples required *ca.* four person hours to complete (p. 20 of MRID 49385903). Subsequent GC/MS analysis was performed unattended (14 hours). Evaluation of results required *ca.* three hours. The overall time for a sample set was *ca.* 1.5 calendar days.

## **V. References**

U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

**Attachment 1: Chemical Names and Structures****Ethalfluralin (pp. 61, 71 of MRID 49385902)**

**IUPAC Name:** N-Ethyl- $\alpha, \alpha, \alpha$ -trifluoro-N-(2-methylallyl)-2,6-dinitro-*p*-toluidine

**CAS Name:** N-Ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)benzamine

**CAS Number:** 55283-68-6

**SMILES String:** Not reported

