

Test Material: Ethalfluralin
EPA PC Code: 113101
OCSPP Guideline: 850.6100

MRID: 48915801

Title: Validation report for Method GRM 07.07 – determination of residues of benfluralin, ethalfluralin, and trifluralin in soil by gas chromatography with electron-impact mass spectrometry detection.

For CDM Smith

Primary Reviewer: Lisa Muto

Signature: 

Date: 3/31/14

Secondary Reviewer: Dan Hunt

Signature: 

Date: 3/31/14

QC/QA Manager: Joan Gaidos

Signature: 

Date: 3/31/14

Test Material: Ethalfluralin
MRID: 49385901

Title: Independent Laboratory Validation of an Analytical Method for the Determination of Ethalfluralin in Soil.

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 soil

Reports: ECM: EPA MRID No. 48915801. Dial, Jr., G.E. 2007. Validation report for Method GRM 07.07 – determination of residues of benfluralin, ethalfluralin, and trifluralin in soil by gas chromatography with electron-impact mass spectrometry detection. Laboratory Study ID: 071040. Report prepared, sponsored and submitted by Regulatory Laboratories – Indianapolis Lab, Dow AgroSciences LLC, Indianapolis, Indiana; 51 pages. Final report issued May 11, 2007.

ILV: EPA MRID 49385901. Garia-Alix, M. 2012. Independent Laboratory Validation of an Analytical Method for the Determination of Ethalfluralin in Soil. Dow AgroSciences Study Reference No.: 120138. CEMAS Study No.: CEMS-5394. 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; 70 pages. Final report issued December 17, 2012.

Document No.: MRID 49385901

Guideline: 850.6100


Statements: ECM: Previously submitted and reviewed. Data Confidentiality, GLP, Quality Assurance, and Certification of Authenticity statements were not included in this submission.

ILV: The study was conducted in accordance with OECD and UK Good Laboratory Practices (GLP; p. 3; Appendix C, p. 70). 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).

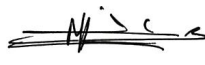
Classification: This analytical method is classified as Supplemental. The ECM included an insufficient number of samples spiked at the LOQ, did not determine performance data at 10x LOQ and the soil matrices were not well characterized. The LOQ is greater than the current lowest toxicological level of concern and approximately equal to the toxicological level of concern associated with the current lowest EC25.

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

This analytical method, Method GRM 07.07, is designed for the quantitative determination of ethalfluralin in soil at the LOQ of 0.010 µg/g using GC/MS. The LOQ is greater than the current

lowest toxicological level of concern in soil, but maybe used to measure down to the EC25. The ILV was successful in the first trial.

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 | 48915801 | 49385901 | | Soil | 5/11/2007 | Dow AgroSciences LLC | GC/MS | 0.010 µg/g |

I. Principle of the Method

Fortified samples (10.0 ± 0.05 g) were extracted by shaking on a reciprocating shaker for a minimum of 30 minutes (180 excursions/minute) with 20 mL of acetonitrile:water solution (99:1, v:v; pp. 10-11; Appendix A, pp. 26-27 of 48915801). After centrifugation (2000 rpm for 5 minutes), an aliquot (5.0 mL) of the extract solution was transferred to a 30-mL vial and diluted with 10 mL of water. The mixture was purified using a 300-mg multi-mode (C18, SAX, SCX) solid phase extraction (SPE) cartridge. The SPE cartridge was conditioned with methanol and HPLC water. The extract was applied then the analytes were eluted with hexane (2 x 2 mL). The eluate was mixed with 3 mg of peanut oil then diluted to 5 mL with hexane. The samples were vortexed prior to GC/MS analysis.

Samples were analyzed for ethalfluralin by gas chromatography (Durabond-5, 30 m x 0.25 mm, 0.25-µm column, initial column temperature 100°C) with electron impact tandem mass spectrometry (splitless mode; carrier gas, helium; Appendix A, Appendix 1, pp. 58-59 of 49385901; pp. 10-11; Appendix A, pp. 24-25, 27-28; Appendix A, Figure 11, p. 50 of 48915801). Injection volume was 5 µL. Three parent-to-daughter ions were monitored: the 333→276 was the quantitative transition; the 333→316 was the confirmatory transition; and the 333→292 was monitored for additional identification. The relative ratios of the three transitions were quantified for confirmation of ethalfluralin (Appendix A, Appendix 1, pp. 66-67 of 49385901).

In the ILV, the extraction procedure and GC/MS analysis was performed according to the ECM, without modification (pp. 14-16). The only noted minor change was the 30-mL vial for a 22-mL vial.

In the ECM and ILV, the LOQ and LOD were 0.010 µg/g and 0.003 µg/g, respectively (p. 13 of 48915801; p. 10; Appendix A, Appendix 1, p. 66 of 49385901).

II. Recovery Findings

ECM (MRID 48915801): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of ethalfluralin in silt loam and clay loam soils; however, fortifications were performed at the LOQ and 200 \times LOQ (pp. 7, 13; Appendix A, Table 3, p. 37 of 48915801). 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 (provided for the silt loam soil; Appendix A, Figure 11, p. 50 of 48915801).

ILV (MRID 49385901): Mean recoveries and relative standard deviations (RSD) were within requirements (mean 70-120%; RSD \leq 20%) for analysis of ethalfluralin in silty clay loam and clay loam soils at the LOQ and 10 \times LOQ (p. 18; Tables 1-8, pp. 24-30 of 49385901). 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 soil matrices were fully characterized at CEMAS (UK textural classifications; p. 12 of 49385901). The reviewer assumed that the method validation was successful on the first trial, although the number of trials was not specifically reported (pp. 10, 18, 21 of 49385901).

Table 2. Initial Validation Method Recoveries for Ethalfluralin in Soil

| Analyte | Fortification Level ($\mu\text{g/g}$) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|--|---|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| Silt loam soil (M641)¹ | | | | | | |
| Ethalfluralin | 0.010 (LOQ) | 4 | 87-98 | 94 | 5 | 5 |
| | 2.000 | 5 | 82-105 | 94 | 10 | 10 |
| Clay loam soil (M649)¹ | | | | | | |
| Ethalfluralin | 0.010 (LOQ) | 4 | 79-94 | 86 | 7 | 8 |
| | 2.000 | 2 | 77-83 | 80 | 4 | 5 |
| Both soils² | | | | | | |
| Ethalfluralin | 0.010 (LOQ) | 8 | 79-98 | 90 | 7.0 | 7.8 |
| | 2.000 | 7 | 77-105 | 90 | 10.6 | 11.7 |

Data were obtained from pp. 7, 13; Appendix A, Table 3, p. 37 of MRID 48915801.

1 Reviewer-calculated using data from Appendix A, Table 3, p. 37 of MRID 48915801 (see DER Attachment 2).

2 Provided by study author.

Table 3. Independent Validation Method Recoveries for Ethalfluralin in Soil¹

| Analyte | Fortification Level (mg/kg) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|-------------------------------------|-----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| Silty Clay loam | | | | | | |
| <i>m/z</i> 276 (quantitation ion) | | | | | | |
| Ethalfluralin | 0.003 (LOD) | 1 | -- ² | -- | -- | -- |
| | 0.01 (LOQ) | 5 | 85-98 | 89 | 5.4 | 6.1 |
| | 0.1 | 5 | 92-105 | 97 | 5.4 | 5.6 |
| <i>m/z</i> 316 (confirmation ion 1) | | | | | | |
| Ethalfluralin | 0.003 (LOD) | 1 | -- ² | -- | -- | -- |
| | 0.01 (LOQ) | 5 | 86-95 | 89 | 3.6 | 4.0 |
| | 0.1 | 5 | 91-105 | 97 | 5.4 | 5.6 |
| <i>m/z</i> 292 (confirmation ion 2) | | | | | | |
| Ethalfluralin | 0.003 (LOD) | 1 | -- ² | -- | -- | -- |
| | 0.01 (LOQ) | 5 | 87-98 | 91 | 4.4 | 4.8 |
| | 0.1 | 5 | 88-104 | 94 | 6.1 | 6.5 |
| Clay loam | | | | | | |
| <i>m/z</i> 276 (quantitation ion) | | | | | | |
| Ethalfluralin | 0.003 (LOD) | 1 | -- ² | -- | -- | -- |
| | 0.01 (LOQ) | 5 | 78-87 | 81 | 3.9 | 4.9 |
| | 0.1 | 5 | 75-88 | 83 | 6.0 | 7.3 |
| <i>m/z</i> 316 (confirmation ion 1) | | | | | | |
| Ethalfluralin | 0.003 (LOD) | 1 | -- ² | -- | -- | -- |
| | 0.01 (LOQ) | 5 | 75-87 | 80 | 4.8 | 6.0 |
| | 0.1 | 5 | 73-87 | 81 | 6.5 | 8.0 |
| <i>m/z</i> 292 (confirmation ion 2) | | | | | | |
| Ethalfluralin | 0.003 (LOD) | 1 | -- ² | -- | -- | -- |
| | 0.01 (LOQ) | 5 | 76-86 | 81 | 4.1 | 5.0 |
| | 0.1 | 5 | 74-87 | 82 | 5.9 | 7.2 |

Data (uncorrected recoveries) obtained from Tables 7-8, p. 30 of MRID 49385901.

1 Soils were fully characterized (UK textural classifications; p. 12).

2 Ethalfluralin recovered ranged 0.0023-0.0027 mg/kg in the silty clay loam soil and 0.0023-0.0025 mg/kg in the clay loam soil. 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 reported as 0.010 µg/g and 0.003 µg/g, respectively (p. 13 of 48915801; p. 10; Appendix A, Appendix 1, pp. 65-66, 68 of 49385901). The LOQ and LOD were calculated from the standard deviation (*s*) of the 0.010 µg/g recovery results of the combined soil data. The LOQ and LOD were calculated as 10*s* and 3*s*, respectively (based on the method of Keith, L.H. *et al.* 1983). The calculated LOQ and LOD values for ethalfluralin, benfluralin and trifluralin ranged 0.0067 to 0.0082 µg/g and 0.0020 to 0.0025 µg/g, respectively (0.0071 µg/g and 0.0021 µg/g, respectively, for ethalfluralin - from Table 6, p. 39 of MRID 48915801). These values supported the LOQ and LOD 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 | |
|--|-------------|--|-------------------------------|
| | | Silty clay soil | Clay loam soil |
| Limit of Quantitation (LOQ) | | 0.010 g µg/g | |
| Limit of Detection (LOD) | | 0.003 µg/g | |
| Linearity (calibration curve r^2 and concentration range) ² | ECM | $r^2 = 0.9970^1$ | No data reported ¹ |
| | ILV | $r^2 = 0.9989$ (<i>m/z</i> 276) $r^2 = 0.9993$ (<i>m/z</i> 316) $r^2 = 0.9994$ (<i>m/z</i> 292) | |
| | Conc. range | (0.001-0.1 µg/mL) | |
| Repeatable | | Yes | |
| Reproducible | | Yes | |
| Specific | | Yes | |

Data were obtained from p. 13; Appendix A, Figure 6, p. 45 of 48915801 and p. 18; Tables 1-8, pp. 24-30; Figures 1-3, pp. 33-35; Figure 7, p. 39; Figure 11, p. 43 of MRID 49385901.

1 Only one representative calibration curve was included in the ECM report. The soil was identified as Samples Set 071040 S03, which was indicated as M641, Silt loam soil, in Appendix A, Figure 8, p. 47 of 48915801

2 ILV calibration curves were confirmed by the reviewer ($r^2 = 0.9988-0.9995$) using data obtained from Figures 1-3, pp. 33-35 of MRID 49385901 (see DER Attachment 2).

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM, an insufficient number of samples were spiked at the LOQ. OCSPP Guideline 850.6100 requires that a minimum of five spiked replicates are analyzed at each concentration. At the LOQ, only four replicates were analyzed in each soil.

In the ECM, performance data were not determined at 10xLOQ. Performance data should be determined at the LOQ and 10xLOQ. In this ECM, performance data were determined at the LOQ and 200xLOQ.

2. The estimation of the LOQ and LOD in the ECM was based on scientifically acceptable procedures as defined in 40 CFR Part 136. The LOQ is greater than the current lowest toxicological level of concern in soil. However, this method may provide some utility because the LOQ is approximately equal to the toxicological level of concern associated with the lowest EC25.
3. The soils utilized in the ECM were not fully characterized, with only texture, pH and percent organic carbon reported (p. 9 of 48915801).
4. In the ECM, Method GRM 07.07 directed the correction of sample recoveries for analyte concentration in the control samples (Section 10.2; Appendix A, pp. 29-30 of 48915801). The ECM reportedly followed Section 10.2 of Method GRM 07.07 for the calculation of ethalfluralin recovery in the samples. The calculations of the ILV reported the correction for background in the controls (p. 17 of 49385901). 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 0.003 mg/kg limit of detection”, so the control values were not necessarily valued at 0.000 µg/g (Appendix A, Table 3, p. 37 of 48915801; Tables 1-6, pp. 24-29 of 49385901). In Section 10.3, Method GRM 07.07 also provided calculations in order to correct recoveries for method efficiency and to determine the dry weight concentrations of the analytes; however, this correction was not shown in the procedure or results of the ECM or the ILV (p. 17; Appendix A, Appendix 1, pp. 64-65 of 49385901).

5. The reviewer calculated the ECM recovery values for the individual soils since the study author only reported overall recovery values for the combined data of both soils.
6. The acceptable recoveries of benfluralin and trifluralin at 0.010 µg/g and 2.000 µg/g (LOQ and 200×LOQ) from the silt loam and clay loam soils were also reported using the Method GRM 07.07 in the ECM study report. The LOQ and LOD for benfluralin and trifluralin were the same as those for ethalfluralin.
7. The matrix effects were assessed for each matrix by the ILV (p. 19; Table 9, p. 31 of 49385901). No significant suppression or enhancement of the instrument (<10%) was observed for all ions monitored.
8. The storage stability of the final extracts and standard solutions were assessed in the ILV (pp. 19-20; Tables 10-12, pp. 31-32 of 49385901). The final extracts were found to be stable up to 7 days at 2-8°C (all three ions measured). Mean recoveries, s.d. and RSDs ranged 92%, 5.0-7.7% and 6.3-8.4%, respectively, for the silty clay loam soil and 78-80%, 6.1-7.3% and 7.8-9.2%, respectively, for the clay loam soil. The standard solutions were found to be stable up to 37 days at 2-8°C (*m/z* 276 quantitation ion measured).
9. In the ECM, the timeframe required for sample set analysis was reported as *ca.* 6 hours (Appendix A, p. 33 of 48915801). It was reported for the ILV that one set of thirteen samples required *ca.* eight person hours to complete (p. 20 of 49385901). Subsequent GC/MS analysis was performed overnight. Evaluation of results required *ca.* two 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.
- EPA MRID No. 48915801. Dial, Jr., G.E. 2007. Determination of Residues of Benfluralin,

Ethalfluralin, and Trifluralin in Soil by Gas Chromatography with Electron-Impact Mass Spectrometry Detection. Method GRM 07.07. Laboratory Study ID: 071040. Report prepared, sponsored and submitted by Regulatory Laboratories – Indianapolis Lab, Dow AgroSciences LLC, Indianapolis, Indiana; 51 pages. Final report issued May 11, 2007.

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

IUPAC Name: N-Ethyl- α, α, α -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

