Analytical method for tebuconazole in soil

Reports: ECM 1: EPA MRID No. 50670805 (Appendix 6, pp. 43-73). Netzband, D.J. 2009. Analytical Method for the Determination of Residues of Tebuconazole in Soil Using LC/MS/MS. Residue Analytical Method No.: HW-001-S09-01. Report prepared by Bayer CropScience, Stilwell, Kansas, and sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 31 pages. Final report issued May 14, 2009.

ECM 2: EPA MRID No. 50670805 (Appendix 7, pp. 74-79). Netzband, D. 2017. Method Modification HW-001-S09-RTP 01 - Analytical Method for the Determination of Residues of Tebuconazole in Soil Using LC/MS/MS. Residue Analytical Method No.: HW-001-S09-01. Report prepared by Bayer CropScience, Stilwell, Kansas, and sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 6 pages. Final report issued April 19, 2017.

ILV: EPA MRID No. 50670805. Davidson, J.D., and J. Shepherd. 2017. Independent Laboratory Validation Of Analytical Method for the Determination of Residues of Tebuconazole in Soil Using LC/MS/MS. Final Report. Study and Activity ID: RAHW0037 and Study ID: 007SRUS17R0077. Report prepared by SynTech Research Laboratory Services, LLC, Stilwell, Kansas, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 79 pages. Final report issued May 25, 2017.

Document No.: MRID 50670805

Guideline: 850.6100

Statements: ECM 1: The study was not conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, 40 CFR, Part 160, since it was not a study (Appendix 6, p. 45 of MRID 50670805). Signed and dated No Data Confidentiality and GLP statements were provided; Quality Assurance and Authenticity statements were not provided (pp. 44-45).
ECM 2: No adherence to GLP standards was reported. No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were not provided.
ILV: The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (p. 3 of MRID 50670805). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3, 5). An authenticity statement was not included.

Classification: This analytical method is classified as unacceptable. ECM linearity was not satisfactory for tebuconazole. The ILV soil matrix was not able to be compared to Terrestrial Field Dissipation (TFD) matrices. No ECM 10×LOQ representative chromatograms were provided for review; ECM soil matrix was not characterized or described. The LOD was not reported in the ECM.

PC Code: 128997

EFED Final Reviewer:	Andrew Shelby, Physical Scientist	**Signature:**  **Date:** 3/23/2021
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature:  Date: 03/21/2019
	Mary Samuel, M.S., Environmental Scientist	Signature:  Date: 03/25/ 2019

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Bayer CropScience Analytical Method No. HW-001-S09-01, including the Method Modification HW-001-S09-01-RTP 01, is designed for the quantitative determination of tebuconazole in soil at the LOQ of 10 µg/kg using LC/MS. The LOQ is less than the lowest toxicological level of concern in soil. In the original ECM, only one ion transition was monitored; the Method Modification of the original ECM only contained the addition of a confirmation ion transition without recovery data. The ECM and ILV were performed using one soil each; however, the ECM soil matrix was not characterized or described in texture. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and that the matrices covered the range of soils used in the Terrestrial Field Dissipation (TFD) studies since no TFD studies were referenced. The ILV validated the ECM method in the second trial with insignificant modifications to the analytical instrumentation and parameters, including validation of the confirmation ion transition. The first trial failed with low recoveries due to low temperatures and improper apparatus sealing during the microwave extraction. All ECM and ILV data regarding repeatability, accuracy, precision, and specificity were satisfactory for tebuconazole; however, no ECM 10×LOQ representative chromatograms were provided for review. ILV linearity was satisfactory; ECM linearity was not. The LOD was not reported in the ECM.

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						
Tebuconazole	50670805 Appendices 6 & 7	50670805		Soil[1,2]	14/05/2009 (Original Report) 19/04/2017 (Method Modification)[3]	Bayer CropScience	LC/MS/MS	10 µg/kg

1 In the ECM 1 (Appendix 6 of MRID 50670805), the soil was not characterized in the study report, and the soil texture was not reported.

2 In the ILV, Kansas silt loam soil [29% sand, 57% silt, 14% clay; pH 5.5 (1: soil:water ratio); 0.99% organic carbon] was collected from a test field at SynTech Research Laboratory Services, LLC (p. 12 of MRID 50670805).

3 The Method Modification (ECM 2; Appendix 7 of MRID 50670805) only contained the addition of a confirmation ion transition to the existing method. No additional recovery data was provided.

I. Principle of the Method

Soil samples (20 ± 0.05 g) were fortified, if necessary, in a Milestone Ethos E Teflon pressure reactor vessel and allowed to sit for about 5 minutes (Appendix 6, pp. 50, 53-55 of MRID 50670805). The sample was mixed with *ca.* 50 mL of methanol:water (70:30, v:v). If the sample was originally measured in a centrifuge tube, the tube was rinsed with the 50 mL of methanol:water (70:30, v:v) before adding it to the sample. Microwave extraction with rotor system was performed for 10 minutes at 100°C with 800 Watts Max (ramp from ambient to 90°C) then for 15 minutes at 100°C with 350 Watts Max (maintain 90°C). After cooling, the samples were depressurized and 500 µL of 1.0 µg/mL internal standard solution was added (isotopic tebuconazole $^{15}N_3$). After shaking, an aliquot (*ca*. 1.5 mL) of the sample was transferred to a 2 mL HPLC vial. After centrifugation (at least 10 minutes at *ca*. 3500 rpm), an aliquot (*ca*. 1 mL) of the supernatant was transferred to a 2 mL HPLC vial and diluted with *ca*. 1 mL of methanol:water (70:30, v:v) for analysis.

Samples were analyzed for tebuconazole using a Shimadzu LC-10AD VP HPLC system coupled to an Applied Biosystems API 4000 mass spectrometer (Appendix 6, Appendix 1, pp. 58-63; Appendix 7, pp. 76-77 of MRID 50670805). The LC/MS conditions consisted of an Phenomenex Prodigy column (50 x 2 mm, 5 µm particle size; column temperature ambient) with a mobile phase gradient of A) water with 5mM ammonium acetate and B) methanol [percent A:B (v:v) at 0.00-0.50 min. 80:20, 2.00-4.00 min. 5:95, 4.01-6.00 min. 80:20] and Turbo Spray MS detection in positive ion mode with MRM (TEM 500°C). Injection volume was 1 µL. The primary ion transition was *m/z* 308.00→70.00 for tebuconazole and *m/z* 311.00→73.00 for tebuconazole-$^{15}N_3$. The confirmatory ion transition was *m/z* 308.00→125.00 for tebuconazole. Expected retention time was *ca*. 3.6 minutes for tebuconazole and tebuconazole-$^{14}N_3$.

In the ILV, the ECM 1 and ECM 2 were performed as written, except for the use of a different LC/MS system (pp. 8, 12-14, 17; Appendix 3, pp. 38-39 of MRID 50670805). A Thermo UltiMate 3000 XRS HPLC system coupled to a TSQ Quantiva mass spectrometer was used. All LC/MS conditions were the same, except that Phenomenex Gemini column (50 x 2 mm, 5 µm particle size; column temperature ambient, *ca*. 19°C) was used, MS system temperature was 325°C, and injection volume was 10 µL. The primary ion transition was *m/z* 308.15→70.16 for tebuconazole and *m/z* 313.15→75.16 for tebuconazole-$^{14}N_3$. The confirmatory ion transition was *m/z* 308.15→125.05 for tebuconazole. These were similar to those of the ECM. Expected retention time was *ca*. 4.07 minutes for tebuconazole.

The Limit of Quantification (LOQ) for tebuconazole in soil was 10 µg/kg in the ECM 1 and ILV (pp. 13, 15-17; Appendix 6, p. 49; Appendix 6, Appendix 1, p. 59 of MRID 50670805). The Limit of Detection (LOD) for tebuconazole in soil was 3.33 µg/kg in the ILV; the LOD was not reported in ECM 1. The Minimum Detection Limit (MDL) was calculated as 0.741-0.838 µg/kg in the ILV. ECM 2 only contained the addition of a confirmation ion transition to the existing method; no LOQ or LOD was reported in ECM 2.

II. Recovery Findings

ECM 1 & 2 (MRID 50670805 - Appendices 6 and 7): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of tebuconazole in one soil matrix at fortification levels of 10 µg/kg (LOQ) and 100 µg/kg (10×LOQ; Appendix 6, Appendix 3, p. 66; DER Attachment 2). Tebuconazole was identified using one ion transition; a confirmatory method is not usually required when LC/MS or GC/MS is the primary method used to generate study data. Method Modification of the original ECM contained the addition of a confirmation ion transition without recovery data. The soil characterization data was not provided in the study report, and the soil texture was not reported.

ILV (MRID 50670805): Mean recoveries and RSDs were within guideline requirements for analysis of tebuconazole in one soil matrix at fortification levels of 10 µg/kg (LOQ) and 100 µg/kg (10×LOQ; Tables 1-2, p. 19). Tebuconazole was identified using two ion transitions; primary and confirmatory recovery results were comparable. Kansas silt loam soil [29% sand, 57% silt, 14% clay; pH 5.5 (1: soil:water ratio); 0.99% organic carbon] was collected from a test field at SynTech Research Laboratory Services, LLC (p. 12). The ILV validated the ECM method in the second trial with insignificant modifications to the analytical instrumentation and parameters, including validation of the confirmation ion transition (pp. 8, 12-14, 17; Appendix 3, pp. 38-39). The first trial failed with low recoveries due to low temperatures and improper apparatus sealing during the microwave extraction.

Table 2. Initial Validation Method Recoveries for Tebuconazole in Soil[1,2]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)[3]	Standard Deviation (%)[3]	Relative Standard Deviation (%)[3]
	Soil					
Tebuconazole	10 (LOQ)	**3**	94-101	97	4	4
	100	**3**	98-99	98	1	1

Data (uncorrected recovery results, Appendix 6, Appendix 2, p. 64) were obtained from Appendix 6, Appendix 3, p. 66 of MRID 50670805 and DER Attachment 2.

1 The soil was not characterized in the study report, and the soil texture was not reported.

2 The primary ion transition was *m/z* 308.00→70.00 for tebuconazole and *m/z* 311.00→73.00 for tebuconazole-$^{15}N_3$. The confirmatory ion transition was *m/z* 308.00→125.00 for tebuconazole. Recovery data was only provided for the primary ion transition.

3 Means, relative standard deviations, and standard deviations were reviewer-calculated since these values were not provided in the study report. Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for Tebuconazole in Soil[1,2]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Kansas Silt Loam Soil					
	Quantitation ion transition					
Tebuconazole	10 (LOQ)	5	95-101	97	2.2	2.3
	100	5	97-99	98	0.9	0.9
	Confirmation ion transition					
Tebuconazole	10 (LOQ)	5	91-96	93	2.0	2.1
	100	5	96-98	97	0.7	0.7

Data (uncorrected recovery results, pp. 14-15) were obtained from Tables 1-2, p. 19 of MRID 50670805.

1 The Kansas silt loam soil [29% sand, 57% silt, 14% clay; pH 5.5 (1: soil:water ratio); 0.99% organic carbon] was collected from a test field at SynTech Research Laboratory Services, LLC (p. 12).

2 The primary ion transition was *m/z* 308.15→70.16 for tebuconazole and *m/z* 313.15→75.16 for tebuconazole-$^{14}N_3$. The confirmatory ion transition was *m/z* 308.15→125.05 for tebuconazole. These were similar to those of the ECM.

III. Method Characteristics

The LOQ for tebuconazole in soil was 10 µg/kg in the ECM 1 and ILV (pp. 13, 15-17; Appendix 6, p. 49; Appendix 6, Appendix 1, p. 59 of MRID 50670805). No justifications, calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM 1 and ILV. In the ILV, the LOD for tebuconazole in soil was 3.33 µg/kg, one-third of the LOQ; the LOD was not reported in ECM 1. The MDL was calculated as 0.741-0.838 µg/kg in the ILV using the following equation:

$MDL = (t_{0.99} \times SD)$

Where, $t_{0.99}$ is the one-tailed t statistic at the 99% confidence level for n-1 replicates (for n = 5, $t_{0.99}$ = 3.747), and SD is the standard deviation of the analyte recovery measurements at the target LOQ. No calculations or comparisons to background levels were reported to justify the LOD for the method in the ILV. ECM 2 only contained the addition of a confirmation ion transition to the existing method; no LOQ or LOD was reported in ECM 2.

Table 4. Method Characteristics

Analyte		Tebuconazole
Limit of Quantitation (LOQ)	ECM	10 µg/kg
	ILV	
Limit of Detection (LOD)	ECM	**Not reported**
	ILV	3.33 µg/kg
Linearity (calibration curve r^2 and concentration range)	ECM[1,2]	r^2 = **0.9923** (Q)
	ILV	r^2 = 1.0000 (Q) r^2 = 0.9989 (C)
	Range	0-200 µg/mL (0-1000 µg/kg)
Repeatable	ECM[3]	Yes at LOQ and 10×LOQ (one uncharacterized soil)
	ILV[4,5]	Yes at LOQ and 10×LOQ (one characterized soil)
Reproducible		Yes at LOQ and 10×LOQ
Specific	ECM	For the LOQ: Yes, matrix interferences were <10% of the LOQ (based on peak area) and only minor baseline noise was observed. **No** 10×LOQ representative chromatograms were provided for review.
	ILV	Yes, matrix interferences were <13% of the LOQ (based on peak area).

Data were obtained from pp. 13, 15-17; Appendix 6, p. 49; Appendix 6, Appendix 1, p. 59 (LOQ/LOD); Tables 1-2, p. 19; Appendix 6, Appendix 3, p. 66 (recovery data); Appendix 1, pp. 22-23; Appendix 6, Appendix 2, p. 65 (calibration curves); Appendix 2, pp. 34-37; Appendix 6, Appendix 4, pp. 26-30 (chromatograms) of MRID 50670805; DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition.

1 ECM correlation coefficients (r^2) value was reviewer-calculated from r value provided in the study report (Appendix 6, Appendix 2, p. 65 of MRID 50670805; DER Attachment 2). Although r values were reported to five significant figures, the reviewer only reported correlation coefficients to four significant figures.

2 Tebuconazole was identified using one ion transition; a confirmatory method is not usually required when LC/MS or GC/MS is the primary method used to generate study data. Method Modification of the original ECM contained the addition of a confirmation ion transition without recovery data.

3 In the ECM, the soil was not characterized in the study report, and the soil texture was not reported.

4 In the ILV, Kansas silt loam soil [29% sand, 57% silt, 14% clay; pH 5.5 (1: soil:water ratio); 0.99% organic carbon] was collected from a test field at SynTech Research Laboratory Services, LLC (p. 12 of MRID 50670805).

5 The ILV validated the ECM method in the second trial with insignificant modifications to the analytical instrumentation and parameters, including validation of the confirmation ion transition (pp. 8, 12-14, 17; Appendix 3, pp. 38-39 of MRID 50670805). The first trial failed with low recoveries due to low temperatures and improper apparatus sealing during the microwave extraction.

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. ECM linearity was not satisfactory for tebuconazole ($r^2 = 0.9923$; Appendix 6, Appendix 2, p. 65 of MRID 50670805; DER Attachment 2). Linearity is satisfactory when $r^2 \geq 0.995$.

2. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method since only one soil matrix was tested. OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs. Even though a certain number of soil matrices is not specified in the OCSPP guidelines, more than one soil/soil matrix would need to be included in an ILV in order to cover the range of soils used in the terrestrial field dissipation (TFD) studies. Additionally, no TFD studies accompanied the Method Validation for soil texture comparison.

   The ECM soil characterization data was not provided in the study report, and the soil texture was not reported.

3. No ECM 10×LOQ representative chromatograms were provided for review. Representative chromatograms from all fortifications should be provided for review to assess the specificity of the method.

4. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 13, 15-17; Appendix 6, p. 49; Appendix 6, Appendix 1, p. 59 of MRID 50670805). No justifications, calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM 1 and ILV. In the ILV, the LOD was defined as one-third of the LOQ; the LOD was not reported in ECM 1. No calculations or comparisons to background levels were reported to justify the LOD for the method in the ILV. ECM 2 only contained the addition of a confirmation ion transition to the existing method; no LOQ or LOD was reported in ECM 2. Detection limits should not be based on arbitrary values.

   The MDL was calculated in the ILV using the following equation: MDL = ($t_{0.99}$ x SD), where, $t_{0.99}$ is the one-tailed t statistic at the 99% confidence level for n-1 replicates (for n = 5, $t_{0.99} = 3.747$), and SD is the standard deviation of the analyte recovery measurements at the target LOQ (pp. 13, 15-17 of MRID 50670805). The reviewer noted that the calculation for MDL is equivalent to the equation used for LOD in other studies.

5. Communications between the Client and ILV involved a clarification of the study protocol, approval for equipment substitutions, and discussion of first trial study results (p. 16; Appendix 5, p. 42 of MRID 50670805).

6. The matrix effects were not assessed in the ECM or ILV; solvent-based standards were used in the ECM (Appendix 6, p. 52 of MRID 50670805).

7. It was reported for the ILV that one sample set of 13 samples required *ca*. 2-3 hours for sample processing and 6 hours for LC/MS/MS analysis (p. 17 of MRID 50670805).

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

Tebuconazole (HWG 1608)

IUPAC Name: (RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol
CAS Name: α-[2-(4-Chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol
CAS Number: 107534-96-3
SMILES String: c1cc(Cl)ccc1CCC(O)(C(C)(C)C)Cn2ncnc2