

Analytical method for tebuconazole in water

Reports: ECM: EPA MRID No. 50670806 (Appendix 4, pp. 53-70). Beck, D. 2017. An Analytical Method for the Determination of Residues of Tebuconazole in Drinking and Surface Water Using LC/MS/MS. Analytical Method. Bayer Method No.: HW-005-W17-01. Activity ID: RAHW0038. Report prepared, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 18 pages. Final report issued February 28, 2017.

ILV: EPA MRID No. 50670806. Wooler, G. 2017. Independent Laboratory Validation of Bayer Method HW-005-W17-01: An Analytical Method for the Determination of Residues of Tebuconazole in Drinking and Surface Water Using LC/MS/MS. Final Report. Sponsor Study ID: RAHW0038. Laboratory Project ID: 107009. Report prepared by Critical Path Service, LLC (CPS), Garnet Valley, Pennsylvania, and sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 72 pages. Final report issued May 12, 2017.

Document No.: MRID 50670806

Guideline: 850.6100

Statements: ECM: 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 4, p. 55 of MRID 50670806). Signed and dated No Data Confidentiality and GLP statements were provided; Quality Assurance and Authenticity statements were not provided (pp. 54-55).


ILV: The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (p. 3 of MRID 50670806). 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. The reproducibility of the method could not be determined since only one set of performance data was submitted, an ILV. The ILV water matrix was not characterized, and the water source was not reported. ECM representative chromatograms were not identified. The LOD was not reported in the ECM.

PC Code: 128997

EFED Final

Reviewer: Andrew Shelby,
Physical Scientist

Signature: 
Date: 3/22/2021

**CDM/CSS-
Dynamac JV**
Reviewers: Lisa Muto, M.S.,
Environmental Scientist

Signature: 

Date: 03/21/2019

Mary Samuel, M.S.,
Environmental Scientist

Signature: 

Date: 03/25/2018

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-005-W17-01, is designed for the quantitative determination of tebuconazole in water at the LOQ of 0.05 ng/mL using LC/MS. The LOQ is less than the lowest toxicological level of concern in water. **The reproducibility of the method could not be determined since only one set of performance data was submitted, an ILV. The ECM was a method only; no performance data was provided.** The ECM and ILV were performed using one uncharacterized water, and the water source was not reported. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. The ILV validated the ECM method in the second trial with insignificant modifications to the analytical instrumentation and parameters. The first trial yielded acceptable recoveries; however, contamination was observed at the retention time of tebuconazole due to contamination in the stock solution. All ILV data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for tebuconazole. ECM representative chromatograms were not identified as those of a method validation. 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	50670806 Appendix 4	50670806		Water ^{1,2}	28/02/2017	Bayer CropScience	LC/MS/MS	0.05 ng/mL

1 The ECM (Appendix 4 of MRID 50670806) was a method only; no performance data was provided.

2 In the ILV, the test water (Sample #031115-W; CPS ID GS-17-28-1) was provided by the Sponsor (p. 12 of MRID 50670806). No characterization data was provided. Water source not described.

I. Principle of the Method

Water samples (10 mL) were fortified, if necessary, in stoppered containers (Appendix 4, p. 61; Appendix 4, Appendix 1, pp. 65-66 of MRID 50670806). The sample was mixed with 200 µL of 0.005 µg/mL internal standard solution (isotopic tebuconazole ¹⁵N₃). The sample was mixed with 0.1 mL of 1% formic acid in water then transferred to a 2 mL HPLC vial for analysis.

Samples were analyzed for tebuconazole using a Shimadzu HPLC system coupled to an Applied Biosystems API 5500 mass spectrometer (Appendix 4, pp. 58, 62-63; Appendix 1, pp. of MRID 50670806). The LC/MS conditions consisted of an Phenomenex Luna C18(2) 100 Å column (50 x 2 mm, 2.5 µm particle size; oven temperature 40°C) with a mobile phase gradient of A) 0.1% formic acid in water and B) 0.1% formic acid in acetonitrile [percent A:B (v:v) at 0.00-0.5 min. 50:50, 2.0 min. 20:80, 2.1-2.5 min. 0:100, 2.6 min. 50:50] and electrospray ionization (ESI) interface MS detection in positive ion mode with MRM (TEM 150°C). Injection volume was 50 µL (adjusted if necessary). The primary ion transition was m/z 308→70 for tebuconazole and m/z 313→75 for tebuconazole-¹⁵N₃. The confirmatory ion transition was m/z 308→125 for tebuconazole. Expected retention time was *ca.* 1.45 minutes for tebuconazole and tebuconazole-¹⁴N₃.

In the ILV, the ECM was performed as written, except for the use of a different LC/MS system (pp. 11, 16-17; Tables 3-4, pp. 22-23 of MRID 50670806). An Agilent 1200 HPLC with Sciex[®] Triple Quad[™] 4000 mass spectrometer was used. All LC/MS conditions were the same, except that Phenomenex[®] Luna[®] C18(2)-HST column (50 x 2 mm, 2.5 µm particle size; column temperature, 40°C), MS system temperature was 450°C and injection volume was 50.0 µL. The primary ion transition was m/z 308.2→70.0 for tebuconazole and m/z 313.1→75.0 for tebuconazole-¹⁵N₃. The confirmatory ion transition was m/z 308.2→125.0 for tebuconazole. These were similar to those of the ECM. Expected retention time was 1.97 minutes for tebuconazole.

The Limit of Quantification (LOQ) for tebuconazole in water was 0.05 ng/mL in the ECM and ILV (pp. 11, 18; Tables 1-2, pp. 20-21; Appendix 4, p. 58 of MRID 50670806). The Limit of Detection (LOD) for tebuconazole in water was 0.025 ng/mL in the ILV; the LOD was not reported in ECM.

II. Recovery Findings

ECM (MRID 50670806 - Appendix 4): The study was a method only; no performance data was provided.

ILV (MRID 50670806): Mean recoveries and RSDs were within guideline requirements for analysis of tebuconazole in one water matrix at fortification levels of 0.05 ng/mL (LOQ) and 0.5 ng/mL (10×LOQ; Table 2, p. 21; DER Attachment 2). Tebuconazole was identified using two ion transitions; primary and confirmatory recovery results were comparable. The test water (Sample #031115-W; CPS ID GS-17-28-1) was provided by the Sponsor (p. 12). No characterization data was provided. Water source not described. The ILV validated the ECM method in the second trial with insignificant modifications to the analytical instrumentation and parameters (pp. 11, 16-17; Tables 3-4, pp. 22-23). The first trial yielded acceptable recoveries; however, contamination was observed at the retention time of tebuconazole due to contamination in the stock solution.

Table 2. Initial Validation Method Recoveries for Tebuconazole in Water^{1,2}

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%) ³	Standard Deviation (%) ³	Relative Standard Deviation (%) ³
Water						
Tebuconazole	0.05 (LOQ)	No performance data submitted				
	0.5					

Data from Appendix 4 of MRID 50670806.

Table 3. Independent Validation Method Recoveries for Tebuconazole in Water^{1,2}

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%) ³	Standard Deviation (%) ³	Relative Standard Deviation (%) ³
Water						
Quantitation ion transition						
Tebuconazole	0.05 (LOQ)	5	90.2-96.2	93.2	2.5	2.7
	0.5	5	98.0-101	99	1	1
Confirmation ion transition						
Tebuconazole	0.05 (LOQ)	5	84.4-110	96	9	10
	0.5	5	96.0-103	100	3	3

Data (uncorrected recovery results, Appendix 2, p. 46) were obtained from Table 2, p. 21 of MRID 50670806 and DER Attachment 2.

- 1 The water (Sample #031115-W; CPS ID GS-17-28-1) was provided by the Sponsor (p. 12). No characterization data was provided. Water source not described.
- 2 The primary ion transition was m/z 308.15→70.16 for tebuconazole and m/z 313.15→75.16 for tebuconazole-¹⁵N₃. The confirmatory ion transition was m/z 308.15→125.05 for tebuconazole. These were similar to those of the ECM.
- 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.

III. Method Characteristics

The LOQ for tebuconazole in water was 0.05 ng/mL in the ECM 1 and ILV (pp. 11, 18; Tables 1-2, pp. 20-21; Appendix 4, p. 58 of MRID 50670806). No justifications, calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM and ILV. In the ILV, the LOD for tebuconazole in water was 0.025 ng/mL; the LOD was not reported in ECM. No calculations or comparisons to background levels were reported to justify the LOD for the method in the ILV.

Table 4. Method Characteristics

Analyte		Tebuconazole
Limit of Quantitation (LOQ)	ECM	0.05 ng/mL
	ILV	
Limit of Detection (LOD)	ECM	Not reported
	ILV	0.025 ng/mL
Linearity (calibration curve r^2 and concentration range)	ECM ¹	$r^2 = 0.9996$ (Q)
	ILV ²	$r^2 = 0.9992$ (Q) $r^2 = 0.9982$ (C)
	Range	0.025-1.00 ng/mL
Repeatable	ECM ³	No performance data submitted
	ILV ^{3,4,5}	Yes at LOQ and 10×LOQ (one uncharacterized water)
Reproducible		Could not be determined; only one set of performance data was submitted.
Specific	ECM	Could not be determined; representative chromatograms were not identified.
	ILV	Yes, no matrix interferences were observed.

Data were obtained from pp. 13, 15-17; Appendix 4, p. 49; Appendix 4, Appendix 1, p. 59 (LOQ/LOD); Table 2, p. 21 (recovery data); Appendix 1, pp. 44-45; Appendix 4, Appendix 2, p. 67 (calibration curves); Figures 5-8, pp. 29-32; Figures 13-16, pp. 37-40; Appendix 4, Appendix 3, p. 68 (chromatograms) of MRID 50670806; 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 4, Appendix 2, p. 67 of MRID 50670806; DER Attachment 2). Only one calibration curve was provided. It was assumed to correspond to the quantitation ion transition.

2 ILV Trial 2 data reported.

3 The ECM (Appendix 4 of MRID 50670806) was a method only; no performance data was provided.

4 In the ILV, the test water (Sample #031115-W; CPS ID GS-17-28-1) was provided by the Sponsor (p. 12 of MRID 50670806). No characterization data was provided. Water source not described.

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 50670806). 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. The reproducibility of Bayer CropScience Analytical Method No. HW-005-W17-01 could not be determined since only one set of performance data was submitted. ECM (Appendix 4 of MRID 50670806) was a method only; no performance data was provided. OCSPP guidelines state that two sets of performance data should be submitted, one for the initial or other internal validation and one for the ILV, and each set of performance data should contain a minimum of five spiked replicates which were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.
2. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method since the water characterization data was not provided in the study report, and the water source (ground, surface, tap, etc.) was not reported (p. 12 of MRID 50670806).
3. The specificity of the method was not supported by ECM representative chromatograms since the representative chromatograms were not identified as those of a method validation.
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. 11, 18; Tables 1-2, pp. 20-21; Appendix 4, p. 58 of MRID 50670806). No justifications, calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM and ILV. In the ILV, the LOD for tebuconazole in water was 0.025 ng/mL; the LOD was not reported in ECM. No calculations or comparisons to background levels were reported to justify the LOD for the method in the ILV. Detection limits should not be based on arbitrary values.
5. Communications between the Study Monitor (Chung Lam) and ILV reportedly involved in clarification of the method only (pp. 6, 17 of MRID 50670806). Details of communications were not reported.
6. The matrix effects were not assessed in the ECM or ILV; solvent-based standards were used in the ECM (p. 15; Appendix 4, p. 61 of MRID 50670806).
7. It was reported for the ILV that one sample set of 13 samples required *ca.* 1 hour for sample processing and 3 hours for LC/MS/MS analysis (p. 17 of MRID 50670806). Overall time required was reported as *ca.* 1 day.

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

