Analytical method for thiabendazole in water

Reports:	ECM: EPA MRID No.: 49393802. Hargreaves, S.L. Thiabendazole – GRM046.01A - Thiabendazole – Residue Method for the Determination of Residues in Water. Final Determination by LC-MS/MS – Analytical Method. Syngenta Report No. GRM046.01A and Task No. T001439-08. Report prepared, sponsored, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 66 pages. Final report issued December 11, 2008. ILV: EPA MRID No. 50103005. Wang, J. 2016. Thiabendazole – Thiabendazole – Independent Laboratory Validation of Residue Method (GRM046.01A) for the Determination of Thiabendazole in Water – Final Report. Syngenta Report No. SR20160912A, Study No. 00114, and Task No. TK0235334. Report prepared by Symbiotic Research, LLC, Mount Olive, New Jersey, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 133 pages. Final report issued September 22, 2016.
Document No.:	MRIDs 49393802 & 50103005
Guideline:	850.6100
Statements:	ECM: The study was not conducted in accordance Good Laboratory Practice (GLP) standards (p. 3 of MRID 49393802). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity statements were not included. A signed and dated Summary of Revisions to Previous Versions was included (p. 5). ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 50103005). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A certification of authenticity was included with the QA statement (p. 4).
Classification:	This analytical method is classified as acceptable.
PC Code:	060101
EFED Final Reviewer:	[Provide final reviewer(s)'s name and title]
	Signature: SHENG LIN Digitally signed by SHENG LIN Date: 2019.05.13 16:08:31 -04'00'
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Executive Summary

This analytical method, Syngenta Residue Method GRM046.01A, is designed for the quantitative determination of thiabendazole in water at the LOQ of 0.05 µg/L using LC/MS/MS. The LOQ of Syngenta Residue Method GRM046.01A is less than the lowest toxicological level of concern in water. The ECM used three characterized water matrices; the ILV used two characterized water matrices. Two ion transitions were monitored. The ILV validated the method in the first trial with insignificant modifications of the LC column and the use of filter paper instead of the column reservoir frit to filter water samples prior to SPE. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory; however, in the ECM, the purity of the test material was not reported. The LOD was not reported in the ILV.

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						
Thiabendazole	49393802 (GRM046.01A)	50103005		Water[1,2]	11/12/2008	Syngenta Crop Protection, LLC	LC/MS/MS	0.05 µg/L

1 In the ECM, ground water [pH 7.2, 0.032% silt content (w/w), <1 mg/L dissolved organic carbon, 366 mg/L total hardness as $CaCO_3$], obtained from a store as Evian bottled mineral water, surface water [pH 7.79, 0.033% silt content (w/w), 6.3 mg/L dissolved organic carbon, 633 mg/L total hardness as $CaCO_3$], obtained from LeRhony River, Vergèze, and drinking water [pH 7.3, 0.057% silt content (w/w), <1 mg/L dissolved organic carbon, 354 mg/L total hardness as $CaCO_3$], obtained from a tap in the Eurofins building, were used. The water characterization facility was not reported.

2 In the ILV, the ground water [pH 7.8, 6 ppm silt content, 0.6 ppm dissolved organic carbon, 323 mg/L total hardness as $CaCO_3$], obtained from a local grocer (Chester, New Jersey) as bottled natural spring water, and surface water [pH 7.9, 10 ppm silt content, 4.7 ppm dissolved organic carbon, 138 mg/L total hardness as $CaCO_3$], obtained from Musconetcong River in Saxton Falls, New Jersey, were used. Water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

Page citations in this review refer to those written in the bottom-most, right-handed corner of the document pages.

I. Principle of the Method

Syngenta Residue Method GRM046.01A

Water (50 mL) in polypropylene centrifuge tubes was fortified with thiabendazole in methanol for procedural recoveries (pp. 11-15; Appendix 7, p. 66 of MRID 49393802). The samples were acidified to pH 2 to 2.5 using 50 µL of concentrated HCl. A Waters Oasis HLB solid phase extraction (SPE) cartridge (200 mg, 6 mL) was prepared by conditioning with 1 x 2 mL of methanol followed by 1 x 2 mL of water. The fortified sample (50 mL) were applied to the column at a rate of *ca*. 1-2 mL/min. via a column reservoir (70 mL capacity) fitted with a frit. The column should not be allowed to become dry. After completion of the sample load, 1 mL of ultra-pure water was passed through the column, and the column was dried under high vacuum for 15 minutes. The sample was eluted with 2 mL of methanol; positive pressure or vacuum was applied to collect all of the column eluate. The eluate was concentrated to dryness using a stream of air and a heating block set at 40°C. The residue was reconstituted in 2 mL of acetonitrile:10 mM ammonium acetate (60:40, v:v) for LC/MS/MS analysis.

Samples are analyzed using a Perkin Elmer Series 200 HPLC coupled to an Applied Biosystems 4000 Triple Quadrupole Mass Spectrometer (pp. 16-17; Appendix 1, p. 26; Appendix 4, p. 33 of MRID 49393802). The following HPLC conditions were used: Partisil SCX column (4.6 mm x 150 mm, 10 µm; column temperature 40°C), isocratic mobile phase of acetonitrile:10 mM ammonium acetate (60:40, v:v), TurboIonSpray in positive mode (TEM 500°C), and MRM. Injection volume was 10 µL. Expected retention time for thiabendazole is *ca*. 3.26 minutes. Thiabendazole was identified using two ion transitions (primary and confirmatory, respectively): *m/z* 202→175 and *m/z* 202→131.

The ECM noted that bottled HPLC grade ultra-pure water was used for the LC mobile phase to reduced background noise (p. 14 of MRID 49393802).

ILV

The ILV performed Syngenta Residue Method GRM046.01A as written, except for insignificant modifications of the LC column and the use of filter paper instead of the column reservoir frit to filter water samples prior to SPE (pp. 14, 16, 18-19 of MRID 50103005). Samples were analyzed using a Hewlett Packard Series 1100 Modular HPLC system [Agilent Zorbax 300-SCX column (4.6 mm x 150 mm, 5 µm, column temperature 40°C)] and Applied Biosystems 4000 Triple Quadrupole Mass Spectrometer (TEM 600°C). All other analytical parameters were the same as the ECM, including MS/MS conditions. Expected retention time was *ca*. 3.67 minutes (Figure 1, p. 30).

In the ECM and ILV, the Limit of Quantification (LOQ) for thiabendazole in Syngenta Residue Method GRM046.01A was reported as 0.05 µg/L (ppm; pp. 12, 21 of MRID 49393802; pp. 21-22 of MRID 50103005). The Limit of Detection (LOD) for thiabendazole was 0.001 µg/L in the ECM; the LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 49393802): For Syngenta Residue Method GRM046.01A, mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of thiabendazole at the LOQ (0.05 µg/L) and 10×LOQ (0.5 µg/L) in three water matrices (Appendix 3, Tables 2-3, p. 28; DER Attachment 2). Two ion pair transitions were monitored; performance data (results) of the quantitation and confirmation ion analyses were comparable. The ground water [pH 7.2, 0.032% silt content (w/w), <1 mg/L dissolved organic carbon, 366 mg/L total hardness as $CaCO_3$], obtained from a store as Evian bottled mineral water, surface water [pH 7.79, 0.033% silt content (w/w), 6.3 mg/L dissolved organic carbon, 633 mg/L total hardness as $CaCO_3$], obtained from LeRhony River, Vergèze, and drinking water [pH 7.3, 0.057% silt content (w/w), <1 mg/L dissolved organic carbon, 354 mg/L total hardness as $CaCO_3$], obtained from a tap in the Eurofins building, were used in the study (Appendix 3, Table 1, p. 28). The water characterization facility was not reported.

ILV (MRID 50103005): For Syngenta Residue Method GRM046.01A, mean recoveries and RSDs were within guidelines for analysis of thiabendazole at the LOQ (0.05 µg/L) and 10×LOQ (0.5 µg/L) in two water matrices (p. 21). Two ion pair transitions were monitored; performance data (results) of the quantitation and confirmation ion analyses were comparable. The ground water [pH 7.8, 6 ppm silt content, 0.6 ppm dissolved organic carbon, 323 mg/L total hardness as $CaCO_3$], obtained from a local grocer (Chester, New Jersey) as bottled natural spring water, and surface water [pH 7.9, 10 ppm silt content, 4.7 ppm dissolved organic carbon, 138 mg/L total hardness as $CaCO_3$], obtained from Musconetcong River in Saxton Falls, New Jersey, were used in the study (pp. 13-14; Tables 1-3, pp. 25-27). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. Syngenta Residue Method GRM046.01A was validated in the first trial with insignificant modifications of the LC column and the use of filter paper instead of the column reservoir frit to filter water samples prior to SPE (pp. 14, 16, 18-19, 21).

Table 2. Initial Validation Method Recoveries for Thiabendazole in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Ground Water					
	Quantitation ion					
Thiabendazole	0.05	5	84-92	88	3	4
	0.5	5	90-104	97	6	6
	Confirmation ion					
Thiabendazole	0.05	5	87-96	92	3	4
	0.5	5	90-104	97	7	7
	Surface Water					
	Quantitation ion					
Thiabendazole	0.05	5	94-96	95	1	1
	0.5	5	84-94	90	4	5
	Confirmation ion					
Thiabendazole	0.05	5	91-97	94	3	3
	0.5	5	84-92	90	4	4
	Drinking Water					
	Quantitation ion					
Thiabendazole	0.05	5	84-88	87	2	2
	0.5	5	87-89	88	1	1
	Confirmation ion					
Thiabendazole	0.05	5	90-95	92	2	2
	0.5	5	85-89	87	1	2

Data (uncorrected recovery results; pp. 18-19) were obtained from Appendix 3, Tables 2-3, p. 28 of MRID 49393802 and DER Attachment 2.

1 The ground water [pH 7.2, 0.032% silt content (w/w), <1 mg/L dissolved organic carbon, 366 mg/L total hardness as $CaCO_3$], obtained from a store as Evian bottled mineral water, surface water [pH 7.79, 0.033% silt content (w/w), 6.3 mg/L dissolved organic carbon, 633 mg/L total hardness as $CaCO_3$], obtained from LeRhony River, Vergèze, and drinking water [pH 7.3, 0.057% silt content (w/w), <1 mg/L dissolved organic carbon, 354 mg/L total hardness as $CaCO_3$], obtained from a tap in the Eurofins building, were used in the study (Appendix 3, Table 1, p. 28). The water characterization facility was not reported.

2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 202→175 and *m/z* 202→131.

3 Standard deviations were reviewer-calculated based on data provided in the study report since the study author did not report these values (see DER Attachment 2). Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for Thiabendazole in Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[4]	Relative Standard Deviation (%)[4]
	Ground Water					
	Quantitation ion					
Thiabendazole	0.05	5	69-101	92	12	13
	0.5	5	84-93	89	3	3
	Confirmation ion					
Thiabendazole	0.05	5	70-100	87	10	11
	0.5	5	85-94	89	3	3
	Surface Water					
	Quantitation ion					
Thiabendazole	0.05	5	103-108	105	2	2
	0.5	5	77-103	95	10	11
	Confirmation ion					
Thiabendazole	0.05	5	95-113	107	6	6
	0.5	5	76-102	93	10	10

Data (uncorrected recovery results) were obtained from p. 21 of MRID 50103005.

1 The ground water [pH 7.8, 6 ppm silt content, 0.6 ppm dissolved organic carbon, 323 mg/L total hardness as $CaCO_3$], obtained from a local grocer (Chester, New Jersey) as bottled natural spring water, and surface water [pH 7.9, 10 ppm silt content, 4.7 ppm dissolved organic carbon, 138 mg/L total hardness as $CaCO_3$], obtained from Musconetcong River in Saxton Falls, New Jersey, were used in the study (pp. 13-14; Tables 1-3, pp. 25-27). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 202→175 and *m/z* 202→131.

III. Method Characteristics

In the ECM and ILV, the LOQ for thiabendazole in Syngenta Residue Method GRM046.01A was 0.05 µg/L (ppm; pp. 12, 21 of MRID 49393802; pp. 21-22 of MRID 50103005). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, i.e. which yielded a mean recovery of 70-110% and relative standard deviation of ≤20%. The analyte peak also should not be lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. The LOD for thiabendazole was 0.001 µg/L in the ECM. In the ECM, the LOQ was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times the mean amplitude of the background noise The ECM study author noted that the LOD may vary between runs and from instrument to instrument. No justifications of the LOQ were provided in the ILV. The LOD was not reported in the ILV.

Table 4. Method Characteristics for Thiabendazole[1] in Water

			Thiabendazole
Limit of Quantitation (LOQ)	ECM		0.05 µg/L
	ILV		
Limit of Detection (LOD)	ECM		0.001 µg/L
	ILV		**Not reported**
Linearity (calibration curve r^2 and concentration range)	ECM		r^2 = 0.9998-0.9999 (Q & C)
	ILV[1]	Ground	r^2 = 0.9994 (Q) r^2 = 0.9996 (C)
		Surface	r^2 = 0.9994 (Q & C)
	Concentration range		0.5-20 µg/L
Repeatable	ECM[2]		Yes at LOQ and 10×LOQ.
	ILV[3,4]		Yes at LOQ and 10×LOQ.
Reproducible			Yes at LOQ and 10×LOQ.
Specific	ECM		Yes, no matrix interferences were observed.
	ILV		

Data were obtained from pp. 12, 21; Appendix 3, Tables 2-3, p. 28 (recovery results); Table 6, p. 32; Figures 31-32, pp. 63-64 (calibration curves); Figures 3-30, pp. 35-62 (chromatograms) of MRID 49393802; pp. 21-22; p. 21 (recovery results); Figures 41-44, pp. 50-51 (calibration curves); Figures 13-40, pp. 36-49 (chromatograms) of MRID 50103005; DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition.

1 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Figures 41-44, pp. 50-51 of MRID 50103005; see DER Attachment 2).

2 In the ECM, ground water [pH 7.2, 0.032% silt content (w/w), <1 mg/L dissolved organic carbon, 366 mg/L total hardness as $CaCO_3$], obtained from a store as Evian bottled mineral water, surface water [pH 7.79, 0.033% silt content (w/w), 6.3 mg/L dissolved organic carbon, 633 mg/L total hardness as $CaCO_3$], obtained from LeRhony River, Vergèze, and drinking water [pH 7.3, 0.057% silt content (w/w), <1 mg/L dissolved organic carbon, 354 mg/L total hardness as $CaCO_3$], obtained from a tap in the Eurofins building, were used (Appendix 3, Table 1, p. 28 of MRID 49393802). The water characterization facility was not reported.

3 In the ILV, the ground water [pH 7.8, 6 ppm silt content, 0.6 ppm dissolved organic carbon, 323 mg/L total hardness as $CaCO_3$], obtained from a local grocer (Chester, New Jersey) as bottled natural spring water, and surface water [pH 7.9, 10 ppm silt content, 4.7 ppm dissolved organic carbon, 138 mg/L total hardness as $CaCO_3$], obtained from Musconetcong River in Saxton Falls, New Jersey, were used (pp. 13-14; Tables 1-3, pp. 25-27 of MRID 50103005). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

4 The ILV validated the method in the first trial with insignificant modifications of the LC column and the use of filter paper instead of the column reservoir frit to filter water samples prior to SPE (pp. 14, 16, 18-19, 21 of MRID 50103005).

IV. Method Deficiencies

1. The purity of the test material was not reported in the ECM (p. 11 of MRID 49393802).

2. The estimations of the LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 12, 21 of MRID 49393802; pp. 21-22 of MRID 50103005). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, i.e. which yielded a mean recovery of 70-110% and relative standard deviation of ≤20%. The analyte peak also should not be lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. In the

ECM, the LOQ was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times the mean amplitude of the background noise The ECM study author noted that the LOD may vary between runs and from instrument to instrument. No justifications of the LOQ were provided in the ILV. The LOD was not reported in the ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

V. Reviewer's Comments

1. The ILV study author provided communication details between the ILV laboratory personnel and the Study Monitor, Louis Mayer of Syngenta (p. 21; Appendix 4, pp. 125-133 of MRID 50103005). These communications included approvals for modifications of the method, exchange of protocols and notifications of successful trials. The reviewer also noted that the ILV was charged with the validations of thiabendazole and cyproconazole simultaneously, and the communications discussed both chemicals.

2. In the ECM, the matrix effects were determined to be insignificant (<±20%; pp. 20-21, 30; Appendix 3, Table 4, p. 30 of MRID 49393802).

3. In the ECM, the final water extracts were found to be stable for up to 11 days at *ca*. 4°C (pp. 22, 31; Table 5, p. 31 of MRID 49393802).

4. It was reported for the ILV that a single analyst can complete a set of thirteen samples (one reagent blank, two matrix controls, and ten fortified samples) in one working day (8 hours; p. 22 of MRID 50103005).

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

Thiabendazole

IUPAC Name: 2-Thiazol-4-yl-1H-benzimidazole

CAS Name: 2-(4-Thiazolyl)-1H-benzimidazole

CAS Number: 148-79-8

SMILES String: Not found