Analytical method for triticonazole (BAS 595 F; Reg.No. 4378513) and its Z-isomer (M595F014; Reg.No. 5079359) in water

Reports: ECM: EPA MRID No. 50420301. Downs, C. 2017. Validation of Method D1612/01: Method for the determination of BAS 595 F (Reg. No. 4378513) and Its Z-isomer (Reg. No. 5079359) in Surface and Drinking Water by LC-MS/MS. Report prepared, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 118 pages. BASF Study No.: 819169. BASF Registration Document No.: 2016/7011309. Final report issued May 2, 2017.

ILV: EPA MRID No. 50420302. Budgeon, Jr., A.D. 2017. Independent Laboratory Validation of BASF Analytical Method D1612/01: "Method for the determination of BAS 595 F (Reg. No. 4378513) and Its Z-isomer (Reg. No. 5079359) in Surface and Drinking Water by LC-MS/MS". BASF Study No.: 808757. Report prepared by JRF America, Audubon, Pennsylvania, sponsored and submitted by BASF Crop Protection, Research Triangle Park, North Carolina; 87 pages. BASF Registration Document No.: 2016/7011310. JRFA Study No.: AU-2016-30. Final report issued March 15, 2017.

Document No.: MRIDs 50420301 & 50420302

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA (40 CFR 160) Good Laboratory Practice (GLP) standards (p. 3 of MRID 50420301). Signed and dated No Data Confidentiality, GLP, Quality Assurance, Authenticity statements were provided (pp. 2-5).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 50420302). Signed and dated No Data Confidentiality, GLP, Quality Assurance, Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as ACCEPTABLE. The communication should have been detailed in the study report since the BASF Study Monitor for the ILV was the ECM study author/director. ECM and ILV linearity was not satisfactory for the surface water analysis. The ILV water matrices were not characterized.

PC Code: 125620

Final EPA Reviewer: Sheng Lin, Ph.D., Physical Scientist

Signature: SHENG LIN Digitally signed by SHENG LIN Date: 2019.12.19 16:59:29 -05'00'

Date: 12/19/18

CDM/CSS-Dynamac JV Reviewers:

Lisa Muto, Environmental Scientist

Signature: Lisa Muto

Date: 6/21/18

Kathleen Ferguson, Ph.D., Environmental Scientist

Signature: Kathleen P. Ferguson

Date: 6/21/18

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, BASF Analytical Method D1612/01, is designed for the quantitative determination of triticonazole (BAS 595 F; Reg. No. 4378513) and its Z-isomer (M595F014; Reg. No. 5079359) in water at the LOQ of 30 ng/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water for the two analytes. The ECM and ILV validated the method using drinking and surface water matrices; however, the ILV matrices were not characterized. The ILV validated the ECM method for the quantitation and confirmation analyses of both analytes in two water matrices in the first trial with insignificant modifications to the analytical instruments and parameters. Communication between the ILV and BASF Study Monitor should have been detailed in the study report since the BASF Study Monitor for the ILV was the ECM study author/director. All ECM and ILV data regarding repeatability, accuracy, precision, and specificity were satisfactory for both isomers of triticonazole. ECM and ILV linearity was unsatisfactory for the surface water analysis.

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						
Triticonazole (BAS 595 F; Reg.No. 4378513)	50420301	50420302		Water[1,2]	02/05/2017	BASF Corporation	LC/MS/MS	30 ng/L
Z-isomer (M595F014; Reg.No. 5079359)								

[1] In the ECM, surface water (Sample ID# LAKE CRABTREE SURF.H2O; Trial ID# CM16-016; pH 7.3, hardness 24 mg equivalent $CaCO_3$/L, 7.6 ppm total organic carbon), obtained from Lake Crabtree, and drinking water (Sample ID# CM15-030; pH 7.5, hardness 116 mg equivalent $CaCO_3$/L, 0.8 ppm total organic carbon), obtained from a well, were used. Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. Water matrices were provided by the Sponsor; the water sources were not further described.

[2] In the ILV, surface and drinking water were provided by the Sponsor, but characterization data was not reported.

I. Principle of the Method

Samples (10 mL) were transferred to culture tubes and fortified, as necessary, with mixed fortification solutions of triticonazole (BAS 595 F; Reg. No. 4378513) and Z-isomer (M595F014; REG.NO. 5079359) in methanol (p. 15; Table 5, p. 24; Appendix B, pp. 33-35, 39 of MRID 50420301). The sample volumes were adjusted to 10 mL with acetonitrile. The samples were filtered (13 mm, 0.45 μm PTFE) and analyzed by LC/MS/MS.

Samples were analyzed for both analytes using an Acquity UPLC system coupled to an AB Sciex API 6500 mass spectrometer with an ESI Turbo source (p. 15; Tables 5-6, pp. 24-25; Appendix B, pp. 32, 37 of MRID 50420301). The LC/MS conditions consisted of a XSelect HSS T3 C18 column (2.1 x 150 mm, 2.5-μm; column temperature 40°C), a mobile phase of (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid [percent A:B (v:v) at 0.00-0.02 min. 85:15, 0.63 min.

60:40, 3.35 min. 35:65, 4.10-5.45 min. 5:95, 5.50-6.00 min. 85:15] and MS/MS detection in positive ion mode (ionization temperature 550°C). Injection volume was 20 µL. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 318→70 and *m/z* 320→70 for BAS 595 F and its Z-isomer. Retention times were *ca*. 4.95 minutes for BAS 595 F and *ca*. 5.01 minutes for its Z-isomer.

In the ILV, the ECM was performed as written, except for a few minor modifications of analytical instruments and parameters (pp. 13, 15, 19; Tables 10-11, pp. 31-32; Appendix A, p. 74 of MRID 50420302). An Agilent 1290 HPLC System coupled to an AB Sciex API 6500 QTrap Turbo Spray mass spectrometer was used. The LC/MS conditions consisted of a XSelect HSS T3 C18 column (2.1 x 100 mm, 1.8-µm; column temperature 40°C), a mobile phase of (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid [percent A:B (v:v) at 0.00-0.02 min. 85:15, 0.40 min. 60:40, 2.20 min. 35:65, 3.80-5.50 min. 10:90, 6.50-10.00 min. 85:15] and MS/MS detection in positive ion mode (ionization temperature 600°C). Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 318.259→69.900 and *m/z* 320.072→69.900 for BAS 595 F and its Z-isomer. Retention times were *ca*. 3.73 minutes for BAS 595 F and *ca*. 3.87 minutes for its Z-isomer. No significant modifications were made by the ILV.

The Limit of Quantification (LOQ) was 30 ng/L for triticonazole (BAS 595 F; Reg.No. 4378513) and Z-isomer (M595F014; Reg.No. 5079359) in water in the ECM and ILV (p. 7 of MRID 50420301; p. 19 of MRID 50420302). The Limit of Detection (LOD) was 6 ng/L for both analytes in the ECM and ILV.

II. Recovery Findings

ECM (MRID 50420301): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of triticonazole (BAS 595 F; Reg.No. 4378513) and its Z-Isomer (M595F014; Reg.No. 5079359) in two water matrices at fortification levels of 30 ng/L (LOQ) and 300 ng/L (10×LOQ; Table 1, p. 20). Performance data (recovery results) from primary and confirmatory analyses were comparable. Recoveries at 10×LOQ were consistently higher than those at the LOQ. Surface water (Sample ID# LAKE CRABTREE SURF.H2O; Trial ID# CM16-016; pH 7.3, hardness 24 mg equivalent $CaCO_3$/L, 7.6 ppm total organic carbon), obtained from Lake Crabtree, and drinking water (Sample ID# CM15-030; pH 7.5, hardness 116 mg equivalent $CaCO_3$/L, 0.8 ppm total organic carbon), obtained from a well, were used (p. 13; Appendix L, pp. 115-116). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. Water matrices were provided by the Sponsor; the water sources were not further described.

ILV (MRID 50420302): Mean recoveries and RSDs were within guideline requirements for analysis of triticonazole (BAS 595 F; Reg.No. 4378513) and its Z-Isomer (M595F014; Reg.No. 5079359) in two water matrices at fortification levels of 30 ng/L (LOQ) and 300 ng/L (10×LOQ; Tables 1-8, pp. 22-29). Performance data (recovery results) from primary and confirmatory analyses were comparable. Recoveries at 10×LOQ were consistently lower than those at the LOQ. Surface and drinking water matrices were provided by the Sponsor, but characterization data was not reported (p. 14). The ECM method for the quantitation and confirmation analyses of both analytes in two water matrices was validated in the first trial with insignificant modifications to the analytical instruments and parameters (p. 19; Appendix A, p. 74).

Table 2. Initial Validation Method Recoveries for Triticonazole (BAS 595 F; Reg.No. 4378513) and Z-Isomer (M595F014; Reg.No. 5079359) in Water[1,2]

Analyte	Fortification Level (ng/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)[2]
	Surface (Lake) Water					
	Quantitation ion					
Triticonazole E-Isomer (BAS 595 F)	30 (LOQ)	5	88-116	102	12	11
	300	5	116-124	118	4	3
Triticonazole Z-Isomer (M595F014)	30 (LOQ)	5	102-116	109	5	5
	300	5	116-125	120	4	4
	Confirmation ion					
Triticonazole E-Isomer (BAS 595 F)	30 (LOQ)	5	103-120	110	8	7
	300	5	115-125	120	4	4
Triticonazole Z-Isomer (M595F014)	30 (LOQ)	5	72-123	105	21	20
	300	5	116-125	120	4	3
	Drinking (Well) Water					
	Quantitation ion					
Triticonazole E-Isomer (BAS 595 F)	30 (LOQ)	5	97-119	110	9	8
	300	5	112-117	115	2	2
Triticonazole Z-Isomer (M595F014)	30 (LOQ)	5	94-114	108	8	8
	300	5	112-115	113	1	1
	Confirmation ion					
Triticonazole E-Isomer (BAS 595 F)	30 (LOQ)	5	94-119	109	10	9
	300	5	112-121	116	4	3
Triticonazole Z-Isomer (M595F014)	30 (LOQ)	5	105-114	109	4	4
	300	5	111-120	116	3	3

Data (uncorrected recovery results, Appendix C, p. 42; Appendix D, pp. 44-51) were obtained from Table 1, p. 20 of MRID 50420301.

[1] The surface water (Sample ID# LAKE CRABTREE SURF.H2O; Trial ID# CM16-016; pH 7.3, hardness 24 mg equivalent $CaCO_3$/L, 7.6 ppm total organic carbon), obtained from Lake Crabtree, and drinking water (Sample ID# CM15-030; pH 7.5, hardness 116 mg equivalent $CaCO_3$/L, 0.8 ppm total organic carbon), obtained from a well, were used (p. 13; Appendix L, pp. 115-116). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. Water matrices were provided by the Sponsor; the water sources were not further described.

[2] Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 318→70 and *m/z* 320→70 for BAS 595 F and its Z-isomer. Isomers were identified by retention times.

Table 3. Independent Validation Method Recoveries for Triticonazole (BAS 595 F; Reg.No. 4378513) and Z- Isomer (M595F014; Reg.No. 5079359) in Water[1,2]

Analyte	Fortification Level (ng/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)[2]
	Surface Water					
	Quantitation ion					
Triticonazole E-Isomer (BAS 595 F)	30 (LOQ)	5	90.3-96.3	92.3	2.4	2.6
	300	5	70.7-78.7	74.0	3.0	4.0
Triticonazole Z-Isomer (M595F014)	30 (LOQ)	5	102-105	103	1.5	1.5
	300	5	74.7-82.7	78.0	2.9	3.8
	Confirmation ion					
Triticonazole E-Isomer (BAS 595 F)	30 (LOQ)	5	84.7-90.0	87.4	1.9	2.2
	300	5	70.0-78.7	73.1	3.5	4.8
Triticonazole Z-Isomer (M595F014)	30 (LOQ)	5	95.3-98.7	97.3	1.2	1.3
	300	5	78.7-87.3	81.6	3.4	4.1
	Drinking Water					
	Quantitation ion					
Triticonazole E-Isomer (BAS 595 F)	30 (LOQ)	5	89.0-94.0	91.7	1.8	2.0
	300	5	71.3-76.0	73.6	2.0	2.8
Triticonazole Z-Isomer (M595F014)	30 (LOQ)	5	90.3-94.0	92.2	1.5	1.6
	300	5	71.3-75.3	73.5	1.8	2.5
	Confirmation ion					
Triticonazole E-Isomer (BAS 595 F)	30 (LOQ)	5	81.0-93.0	86.8	4.3	5.0
	300	5	70.0-72.7	71.2	1.0	1.4
Triticonazole Z-Isomer (M595F014)	30 (LOQ)	5	80.0-91.3	80.1	4.7	5.5
	300	5	70.0-77.3	73.6	2.8	3.8

Data (uncorrected recovery results, Figure 19, p. 72; Tables 1-8, pp. 22-29) were obtained from Tables 1-8, pp. 22-29 of MRID 50420302.

1 The surface and drinking water were provided by the Sponsor, but characterization data was not reported (p. 14).

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 318.259→69.900 and *m/z* 320.072→69.900 for BAS 595 F and its Z-isomer. Isomers were identified by retention times.

III. Method Characteristics

The LOQ was 30 ng/L for triticonazole (BAS 595 F; Reg.No. 4378513) and Z-isomer (M595F014; Reg.No. 5079359) in water in the ECM and ILV (pp. 7, 18 of MRID 50420301; p. 19 of MRID 50420302). The LOD was 6 ng/L for both analytes in the ECM and ILV. In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested, and the LOD was set to 20% of the LOQ. The LOD was also reported to be the absolute amount of analyte injected into the LC/MS/MS when the lowest calibration standard was analyzed with an acceptable signal to noise ratio of greater than three to one. No calculations were reported to justify the LOQ and LOD for the method in the ECM and ILV.

Table 4. Method Characteristics

Analyte[1]			**Triticonazole (BAS 595 F; Reg.No. 4378513)**	**Z-Isomer (M595F014; Reg.No. 5079359)**
Limit of Quantitation (LOQ)	ECM		30 ng/L	
	ILV			
Limit of Detection (LOD)	ECM		6 ng/L (20% of the LOQ)	
	ILV			
Linearity (calibration curve r^2 and concentration range)[1]	ECM	Surface	r^2 = **0.9938** (Q) r^2 = **0.9914** (C)	r^2 = **0.9928** (Q) r^2 = **0.9948** (C)
		Drinking	r^2 = 0.9990 (Q & C)	r^2 = 0.9992 (Q) r^2 = 0.9982 (C)
		Range	0.006-0.6 ng/mL	
	ILV	Surface	r^2 = **0.9908** (Q) r^2 = 0.9953 (C)	r^2 = **0.9931** (Q) r^2 = **0.9942** (C)
		Drinking	r^2 = 0.9951 (Q) r^2 = 0.9966 (C)	r^2 = 0.9965 (Q) r^2 = 0.9977 (C)
		Range	0.006-0.3 ng/mL	
Repeatable	ECM[2]		Yes at LOQ and 10×LOQ (characterized surface and drinking water matrices).	
	ILV[3,4]		Yes at LOQ and 10×LOQ (uncharacterized surface and drinking water matrices).	
Reproducible			Yes at LOQ and 10×LOQ	
Specific	ECM		Yes, no matrix interferences were observed. Minor baseline noise was noted at the LOQ which was more prominent in the C analysis.	
	ILV		Yes, matrix interferences were < 1% (Q) and <10% (C) of the LOQ (based on peak area). Minor peak shouldering was noted in the C analysis.	Yes, matrix interferences were < 4% (Q) and <10% (C) of the LOQ (based on peak area). Minor peak shouldering was noted in the C analysis.

Data were obtained from pp. 7, 18 (LOQ/LOD); Table 1, p. 20 (recovery data); Appendix D, pp. 44-51 (correlation coefficients); Appendix I, pp. 73-108 (chromatograms) of MRID 50420301; p. 19 (LOQ); Tables 1-8, pp. 22-29 (recovery data); Figures 1-4, pp. 34-37 (calibration curves); Figures 9-18, pp. 58-71 (chromatograms) of MRID 50420302; and DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition.

1 Correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (Appendix D, pp. 44-51 of MRID 50420301; Figures 1-4, pp. 34-37 of MRID 50420302; DER Attachment 2). For the ILV, the reviewer limited the calculated r^2 to 4 significant figures although 10-11 significant figures were reported in the ILV for r. Matrix-matched standards were used, although matrix effects were not observed (pp. 16-17; Table 2, p. 21; Appendix B, pp. 33-34; Appendix I, p. 71 of MRID 50420301).

2 In the ECM, surface water (Sample ID# LAKE CRABTREE SURF.H2O; Trial ID# CM16-016; pH 7.3, hardness 24 mg equivalent $CaCO_3$/L, 7.6 ppm total organic carbon), obtained from Lake Crabtree, and drinking water (Sample ID# CM15-030; pH 7.5, hardness 116 mg equivalent $CaCO_3$/L, 0.8 ppm total organic carbon), obtained from a well, were used (p. 13; Appendix L, pp. 115-116 of MRID 50420301). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. Water matrices were provided by the Sponsor; the water sources were not further described.

3 In the ILV, surface and drinking water were provided by the Sponsor, but characterization data was not reported (p. 14 of MRID 50420302).

4 The ILV validated the ECM method for the quantitation and confirmation analyses of both analytes in two water matrices in the first trial with insignificant modifications to the analytical instruments and parameters (p. 19; Appendix A, p. 74 of MRID 50420302).

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

IV. Method Deficiencies and Reviewer's Comments

1. Communications between the ILV Study Director and BASF Study Monitor (Carlan Downs) and personnel were not described; the ILV reported that no one from BASF visited the testing facility during the course of the study (pp. 6, 19 of MRID 50420302). This communication should have been detailed in the study report to ensure that collusion did not take place since the BASF Study Monitor for the ILV was the ECM study author/director (Carlan Downs; pp. 1, 3 of MRID 50420301).

2. Linearity was not satisfactory for surface water analysis. In the ILV, the linearity was not satisfactory for BAS 595 F, $r^2 = 0.9908$ (Q), and its Z-isomer, $r^2 = 0.9931$ (Q) and 0.9942 (C; Appendix D, pp. 44-51 of MRID 50420301; DER Attachment 2). In the ECM, the linearity was not satisfactory for BAS 595 F, $r^2 = 0.9938$ (Q) and 0.9914 (C), and its Z-isomer, $r^2 = 0.9928$ (Q) and 0.9948 (C; Figures 1-4, pp. 34-37 of MRID 50420302; DER Attachment 2). Linearity is satisfactory when $r^2 \geq 0.995$. However, in the case of the ECM and ILV confirmation ion analysis, the reviewer noted that a confirmatory method is not typically required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data.

3. In the ILV, the water matrices were not characterized. Surface and drinking water were provided by the Sponsor, but characterization data was not reported (p. 14 of MRID 50420302).

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. 7, 18 of MRID 50420301; p. 19 of MRID 50420302). In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested, and the LOD was set to 20% of the LOQ. The LOD was also reported to be the absolute amount of analyte injected into the LC/MS/MS when the lowest calibration standard was analyzed with an acceptable signal to noise ratio of greater than three to one. No calculations were reported to justify the LOQ and LOD for the method in the ECM and ILV. Detection limits should not be based on arbitrary values.

5. The storage stability was investigated by the ECM (p. 16; Tables 3-4, pp. 22-23; Appendices E-F, pp. 52-58 of MRID 50420301). The fortification standards and calibration standards were determined to be stable for at least 3-4 months when stored under refrigeration. The sample extracts were determined to be stable for up to 16 days for surface water and up to 14 days for drinking water when stored under refrigeration.

6. It was reported for the ILV that one sample set of 13 samples required *ca*. 8 working hours including LC/MS/MS analysis time and calculation of results (p. 18 of MRID 50420302).

7. The reviewer noted that the ECM reported the CAS No. of triticonazole as 138182-18-0 (p. 14 of MRID 50420301). The CAS No. reported in the attached structure table (131983-72-7) was verified through the on-line EPA pesticide registry. The reviewer assumed that CAS No. 138182-18-0 was outdated or for the racemic product.

V. References

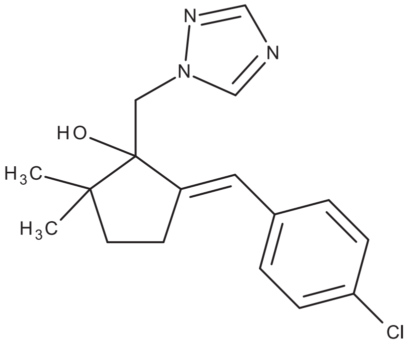
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

DER Attachment 1: Chemical Names and Structures

Triticonazole (BAS 595 F; Reg.No. 4378513)

IUPAC Name:	(RS)-(E)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol
CAS Name:	(5E)-5-[(4-chlorophenyl)methylene]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol
CAS Number:	131983-72-7
SMILES String:	c1cc(Cl)ccc1C=C2CCC(C)(C)C2(O)Cn3ncnc3



Triticonazole Z-Isomer (M595F014; Reg.No. 5079359)

IUPAC Name:	(1RS)-(5Z)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol
CAS Name:	Not reported
CAS Number:	None assigned
SMILES String:	Not found