Analytical method for triticonazole (BAS 595 F) and its metabolites M595F001, M595F002, and M595F014 in soil

Reports: ECM: EPA MRID No. 50420303 (Appendix D, pp. 110-149). Obermann, M., and S. Langenbach. 2016. Validation of Analytical Method L0353/01 for the Determination of BAS 595 F (Triticonazole) and its Metabolites M595F001, M595F002 and M595F014 in Soil by LC-MS/MS. Report prepared by BASF SE Crop Protection, Limburgerhof, Germany, and sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 50 pages (excerpt of full 169-page study report). BASF Study No.: 819529. BASF Registration Document No.: 2016/1190727. Final report issued September 27, 2016.

ILV: EPA MRID No. 50420303. Perez, R. 2017. Independent Laboratory Validation of BASF Analytical Method L0353/01: "Method for the Determination of BAS 595 F (Triticonazole) and its Metabolites M595F001, M595F002 and M595F014 in Soil by LC-MS/MS". Report prepared by ADPEN, Jacksonville, Florida, and sponsored and submitted by BASF Crop Protection, Research Triangle Park, North Carolina; 151 pages. BASF Study No.: 808754. BASF Registration Document No.: 2016/7015898. ADPEN Study No.: 17G0704. Final report issued October 9, 2017.

Document No.: MRID 50420303

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with OECD and German Good Laboratory Practice (GLP) standards (Appendix D, pp. 112, 114). Signed and dated GLP and Quality Assurance statements were provided (Appendix D, pp. 112-114). An Authenticity statement was included with the GLP statement. No Data Confidentiality statement was provided, but it was not signed and dated.

ILV: The study was conducted in accordance with USEPA FIFRA GLP (40 CFR Part 160) standards (p. 3 of MRID 50420303). Signed and dated No Data Confidentiality, GLP, Quality Assurance, Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as UPGRADEABLE. The ECM was incomplete, lacking the recovery raw data, calibration data, representative chromatograms, and soil characterization to support the method. An updated ECM should be submitted which incorporates the ILV recommendations. The specificity of the method for BAS 595 F and its Z-isomer M595F014 was not supported by ILV representative chromatograms since the isomer peaks were not fully resolved.

PC Code: 125620

Final EPA Reviewer:	Sheng Lin, Ph.D., Physical Scientist	Signature: SHENG LIN Digitally signed by SHENG LIN Date: 2019.12.19 16:57:46 -05'00'	
		Date: 12/19/19	
CDM/CSS-Dynamac JV	Lisa Muto, Environmental Scientist	Signature: Lisa Muto	
		Date:	6/21/18

Reviewers:

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 L0353/01, is designed for the quantitative determination of triticonazole (BAS 595 F) and its metabolites M595F001, M595F002, and M595F014 in soil at the LOQ of 0.002 mg/kg using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil for the four analytes. The ECM validated the method using two uncharacterized soil matrices; however, the ECM was incomplete, lacking the recovery raw data, calibration data, and representative chromatograms to support the method. The ILV validated the method using one silt loam soil matrix; however; it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and that the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. The ILV validated the ECM method for the quantitation and confirmation analyses of all four analytes in one soil matrix in the first trial with insignificant modifications to the analytical instruments. However, the ILV reported three recommendations which should be incorporated into the ECM sample processing procedure to prevent loss of test material. An updated ECM should be submitted which incorporates the ILV recommendations. All ILV data regarding repeatability, accuracy, precision, and linearity were satisfactory for all four analytes. The specificity of the method for BAS 595 F and its Z-isomer M595F014 was not supported by ILV representative chromatograms since the isomer peaks were not fully resolved, but ILV representative chromatograms of M595F001 and M595F002 were acceptable. ECM summarized recovery and linearity data was acceptable.

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)	Appendix D of MRID 50420303[1]	50420303		Soil[2,3]	27/09/2016	BASF Corporation	LC/MS/MS	0.002 mg/kg
M595F001								
M595F002								
M595F014								

1

2 In the ECM, LUFA 2.4 and LUFA 2.2 soil matrices were used. Soil characterization was performed and included in the ECM, but not included in the excerpt of the ECM which was included in the ILV. Soil sources were not described.

3 In the ILV, silt loam soil (Sample ID: PA.NY.T.CHAR 18-24.A.; 21% sand 72% silt 7% clay, pH 5.1 in saturated paste, 0.18% organic carbon) was provided by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota.

All page references refer to page numbers written in the upper right-handed corner of MRID 50420303.

I. Principle of the Method

A 5 g soil samples was transferred to a flask and fortified, as necessary, with a mixed fortification solution prepared in water:acetonitrile (80:20, v:v; Appendix D, pp. 133-138). The sample was extracted by solid-liquid extraction using sonication and shaking with 2.5 mL of 0.1 M ammonium hydroxide for 10 minutes. Then, the sample was extracted twice with 12.5 mL of acetone for 1 hour (300 rpm) and 10 minutes (ultra-sonication). After each extraction, the suspension was centrifuged (10 minutes at 3000 rpm) and decanted over cotton wool into a graduated cylinder. The volume of the combined extracts was adjusted to 30 mL with acetone, and a 15-mL aliquot was evaporated (rotary evaporator at 40°C) until the aqueous phase remained. The sample was transferred to a centrifuge tube, and the volume was adjusted to 2 mL with water. A Bond Elut C_{18}-solid phase extraction (SPE) cartridge (3 mL/500 mg) was pre-conditioned with 1.5 mL each of acetonitrile and water. The sample was applied to the cartridge with vacuum; the flask was rinsed with 0.4 mL of water to ensure complete transfer. The cartridge was washed with 1.5 mL of water. The residues were eluted with acetonitrile/methanol (95:5, v:v). Afterwards, the liquid phase was evaporated to nearly dryness using a nitrogen evaporator at 40°C, and the remaining liquid phase was transferred to a 5 mL culture tube and redissolved in 4 mL of water and 1 mL of acetonitrile. Final determination of the residues was conducted by LC-MS/MS.

Samples were analyzed for both analytes using a Waters Acquity LC system coupled to an AB Sciex Triple Quad 5500 mass spectrometer with an ESI Turbo source (p. 15; Tables 5-6, pp. 24-25; Appendix B, pp. 32, 37 of MRID Appendix D of MRID 50420303). The LC/MS conditions consisted of a Phenomenex Luna Phenyl Hexyl column (4.6 x 100 mm, 5-µm; column temperature 25°C), Phenomenex SecurityGuard C18 (4 x 3.0 mm), a isocratic mobile phase of (A) water:formic acid (1000:2, v:v) and (B) methanol:formic acid (1000:2, v:v) [percent A:B (v:v) at 0.0-10.0 min. 30:70] and MS/MS detection in positive ion mode (ionization temperature not reported). Injection volume was 35 µL. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 318→70 and *m/z* 318→125 for BAS 595 F and M595F014; *m/z* 334→70 and *m/z* 334→125 for M595F001 and M595F002. Retention times were *ca*. 5.75, 2.45, 3.13, and 6.15 minutes for BAS 595 F, M595F001, M595F002, and M595F014, respectively.

In the ILV, the ECM was performed as written, except for a few minor modifications of analytical instruments (p. 17; Table 10, pp. 34). An Agilent 1290 HPLC System coupled to an AB Sciex Triple Quad 5500 mass spectrometer with an ESI Turbo source was used. The LC/MS conditions were the same as those of the ECM, but the ionization temperature was reported as 550°C. The same two ion transitions were monitored for each analyte. Retention times were *ca*. 5.9, 2.5, 3.2, and 6.2 minutes for BAS 595 F, M595F001, M595F002, and M595F014, respectively. No significant modifications were made by the ILV; however, the ILV made three recommendations to the sample processing procedure which should be incorporated in the ECM.

The Limit of Quantification (LOQ) was 0.002 mg/kg for triticonazole (BAS 595 F) and M595F001, M595F002, and M595F014 in soil in the ECM and ILV (p. 7; Appendix D, p. 115). The Limit of Detection (LOD) was 0.0004 mg/kg for all four analytes in the ECM and ILV.

II. Recovery Findings

ECM (Appendix D of MRID 50420303): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of triticonazole (BAS 595 F) and its metabolites M595F001, M595F002, and M595F014 in two soil matrices at fortification levels of 0.002 mg/kg (LOQ) and 0.02 mg/kg (10×LOQ; Appendix D, pp. 116-117). Performance data (recovery results) from primary and confirmatory analyses were comparable; however, individual recovery values were not reported, only means and RSDs (See Reviewer's Comment #1). The calculation method prescribed for recovery results to be corrected if residues were quantified in the controls; however, it could not be determined if the recovery results were corrected since raw data was not provided in the excerpt (Appendix D, p. 139). LUFA 2.4 and LUFA 2.2 soil matrices were used (Appendix D, 131). Soil characterization was performed and included in the ECM (Figure A 90-91), but not included in the excerpt of the ECM which was included in the ILV. Soil sources were not described.

ILV (MRID 50420303): Mean recoveries and RSDs were within guideline requirements for analysis of triticonazole (BAS 595 F) and its metabolites M595F001, M595F002, and M595F014 in one soil matrix at fortification levels of 0.002 mg/kg (LOQ) and 0.02 mg/kg (10×LOQ; pp. 9-10; Tables 1-8, pp. 24-31). Performance data (recovery results) from primary and confirmatory analyses were comparable. Silt loam soil (Sample ID: PA.NY.T.CHAR 18-24.A.; 21% sand 72% silt 7% clay, pH 5.1 in saturated paste, 0.18% organic carbon) was provided by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota (p. 15; Appendix E, p. 151). The ECM method for the quantitation and confirmation analyses of all four analytes in one soil matrix was validated in the first trial with insignificant modifications to the analytical instruments (p. 8, 17; Table 10, p. 34). However, the ILV reported three recommendations which should be incorporated into the ECM: the cotton wool filtration should be replaced with a different filtration method to create a cleaner extract; the amount of water used to rinse the flasks after transfer to the SPE cartridge should be increased to ensure complete transfer; and the reduced extract, after nitrogen evaporation, should be reconstituted in the concentration flask (p. 22; Appendix A, p. 86). An updated ECM should be submitted which incorporates the ILV recommendations.

Table 2. Initial Validation Method Recoveries for Triticonazole (BAS 595 F) and Its Metabolites M595F001, M595F002, and M595F014 in Soil[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)[2]
	LUFA 2.4 Soil					
	Quantitation ion					
Triticonazole (BAS 595 F)	0.002 (LOQ)	5	--[3]	104	--	6.9
	0.02	5	--	101	--	7.8
M595F001	0.002 (LOQ)	5	--	106	--	2.5
	0.02	5	--	99	--	6.2
M595F002	0.002 (LOQ)	5	--	108	--	8.1
	0.02	5	--	101	--	6.3
M595F014	0.002 (LOQ)	5	--	94	--	7.5
	0.02	5	--	100	--	7.1
	Confirmation ion					
Triticonazole (BAS 595 F)	0.002 (LOQ)	5	--	105	--	7.3
	0.02	5	--	100	--	7.8
M595F001	0.002 (LOQ)	5	--	107	--	2.2
	0.02	5	--	97	--	5.9
M595F002	0.002 (LOQ)	5	--	108	--	9.2
	0.02	5	--	99	--	6.6
M595F014	0.002 (LOQ)	5	--	100	--	8.3
	0.02	5	--	99	--	7.1
	LUFA 2.2 Soil					
	Quantitation ion					
Triticonazole (BAS 595 F)	0.002 (LOQ)	5	--	103	--	3.7
	0.02	5	--	101	--	9.3
M595F001	0.002 (LOQ)	5	--	99	--	2.2
	0.02	5	--	94	--	8.4
M595F002	0.002 (LOQ)	5	--	98	--	2.3
	0.02	5	--	95	--	8.5
M595F014	0.002 (LOQ)	5	--	98	--	2.6
	0.02	5	--	100	--	8.8
	Confirmation ion					
Triticonazole (BAS 595 F)	0.002 (LOQ)	5	--	102	--	3.4
	0.02	5	--	99	--	9.2
M595F001	0.002 (LOQ)	5	--	102	--	1.6
	0.02	5	--	94	--	8.3
M595F002	0.002 (LOQ)	5	--	98	--	4.4
	0.02	5	--	94	--	8.9
M595F014	0.002 (LOQ)	5	--	106	--	7.6
	0.02	5	--	99	--	9.2

Data (recovery results were corrected if residues were quantified in the controls; Appendix D, p. 139) were obtained from Appendix D, pp. 116-117.

1 LUFA 2.4 and LUFA 2.2 soil matrices were used (Appendix D, 131). Soil characterization was performed and included in the ECM (Figure A 90-91), but not included in the excerpt of the ECM which was included in the ILV. Soil sources were not described.

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 318→70 and *m/z* 318→125 for BAS 595 F and M595F014; *m/z* 334→70 and *m/z* 334→125 for M595F001 and M595F002. Isomers were identified by retention times.

3 Individual recovery values were not reported, only means and RSDs (See Reviewer's Comment #1).

Table 3. Independent Validation Method Recoveries for Triticonazole (BAS 595 F) and Its Metabolites M595F001, M595F002, and M595F014 in Soil[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)[2]
	Silt Loam Soil					
	Quantitation ion					
Triticonazole (BAS 595 F)	0.002 (LOQ)	5	71.3-76.0	74.7	1.9	2.5
	0.02	5	67.4-74.2	71.9	2.7	3.7
M595F001	0.002 (LOQ)	5	79.3-87.0	83.7	3.3	3.9
	0.02	5	72.6-80.8	78.0	3.3	4.2
M595F002	0.002 (LOQ)	5	78.1-83.1	80.3	1.9	2.3
	0.02	5	71.8-80.9	75.7	3.7	4.9
M595F014	0.002 (LOQ)	5	75.9-80.1	78.6	1.8	2.3
	0.02	5	69.4-79.7	74.5	3.7	5.0
	Confirmation ion					
Triticonazole (BAS 595 F)	0.002 (LOQ)	5	74.1-81.8	77.2	3.7	4.7
	0.02	5	68.7-82.0	75.4	6.2	8.3
M595F001	0.002 (LOQ)	5	84.5-93.7	87.7	5.2	6.0
	0.02	5	70.5-82.4	77.6	5.4	7.0
M595F002	0.002 (LOQ)	5	79.9-89.7	82.9	3.9	4.7
	0.02	5	73.8-89.0	78.1	6.2	8.0
M595F014	0.002 (LOQ)	5	78.3-84.3	81.1	2.3	2.8
	0.02	5	71.1-81.3	77.6	3.9	5.1

Data (corrected recovery results; Tables 1-8, pp. 24-31; Appendix D, p. 139) were obtained from pp. 9-10 and Tables 1-8, pp. 24-31.

1 The silt loam soil (Sample ID: PA.NY.T.CHAR 18-24.A.; 21% sand 72% silt 7% clay, pH 5.1 in saturated paste, 0.18% organic carbon) was provided by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota (p. 15; Appendix E, p. 151).

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 318→70 and *m/z* 318→125 for BAS 595 F and M595F014; *m/z* 334→70 and *m/z* 334→125 for M595F001 and M595F002. Isomers were identified by retention times.

III. Method Characteristics

The LOQ was 0.002 mg/kg for triticonazole (BAS 595 F) and its metabolites M595F001, M595F002, and M595F014 in soil in the ECM and ILV (p. 7; Appendix D, p. 115). The LOD was 0.0004 mg/kg for all four 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. In the ECM, the LOD was also reported to be the lowest calibration level used. In the ILV, 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)**	**M595F001**	**M595F002**	**M595F014**
Limit of Quantitation (LOQ)	ECM	0.002 mg/kg			
	ILV				
Limit of Detection (LOD)	ECM	0.0004 mg/kg (20% of the LOQ)			
	ILV				
Linearity (calibration curve r^2 and concentration range)	ECM[1]	r^2 = > 0.9980			
	ILV[2]	r^2 = 0.9990 (Q) r^2 = 0.9993 (C)	r^2 = 0.9995 (Q) r^2 = 0.9983 (C)	r^2 = 0.9997 (Q) r^2 = 0.9994 (C)	r^2 = 0.9997 (Q) r^2 = 0.9995 (C)
	Range	0.2-20 ng/mL			
Repeatable	ECM[3,4]	Yes at LOQ and 10×LOQ (two uncharacterized soil matrices).			
	ILV[5,6]	Yes at LOQ and 10×LOQ (one characterized soil matrix).			
Reproducible		Yes at LOQ and 10×LOQ			
Specific	ECM	**Could not be determined**. No representative chromatograms were included.[4]			
	ILV	**No**, no matrix interferences were observed; however, isomer peaks overlapped at *ca*. 30% peak height which interfered with peak integration.[7]	Yes, no matrix interferences were observed. Some minor baseline noise interfered with peak attenuation.		**No**, no matrix interferences were observed; however, isomer peaks overlapped at *ca*. 20% peak height which interfered with peak integration.[8]

Data were obtained from p. 7 (ILV LOQ); pp. 9-10 and Tables 1-8, pp. 24-31 (ILV recovery data); Figures 1-4, pp. 36-39 (ILV calibration curves); Figures 9-20, pp. 68-83 (ILV chromatograms); Appendix D, p. 115 (ECM LOQ/LOD); Appendix D, pp. 116-117 (ECM recovery data); Appendix D, p. 115 (ECM correlation coefficient summary) of MRID 50420303; and DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition.

1 Summary correlation coefficient (r^2) value was reviewer-calculated from r summary value provided in the study report (Appendix D, p. 115; DER Attachment 2). Individual r values were not reported. Solvent standards were used.

2 Correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (Figures 1-4, pp. 36-39; DER Attachment 2). The reviewer limited the calculated r^2 to 4 significant figures although 4-7 significant figures were reported in the ILV for r. Solvent standards were used.

3 In the ECM, LUFA 2.4 and LUFA 2.2 soil matrices were used (Appendix D, 131). Soil characterization was performed and included in the ECM (Figure A 90-91), but not included in the excerpt of the ECM which was included in the ILV. Soil sources were not described.

4 ECM was provided in Appendix D of the ILV; however, only a 50-page excerpt of the full 169-page study report was included. The ECM was not provided separately.
5 In the ILV, silt loam soil (Sample ID: PA.NY.T.CHAR 18-24.A.; 21% sand 72% silt 7% clay, pH 5.1 in saturated paste, 0.18% organic carbon) was provided by the Sponsor and characterized by Agvise Laboratories, Northwood, North Dakota (p. 15; Appendix E, p. 151).
6 The ILV validated the ECM method for the quantitation and confirmation analyses of all four analytes in one soil matrix in the first trial with insignificant modifications to the analytical instruments (p. 8, 17; Table 10, p. 34). However, the ILV reported three recommendations which should be incorporated into the ECM: the cotton wool filtration should be replaced with a different filtration method to create a cleaner extract; the amount of water used to rinse the flasks after transfer to the SPE cartridge should be increased to ensure complete transfer; and the reduced extract, after nitrogen evaporation, should be reconstituted in the concentration flask (p. 22; Appendix A, p. 86). An updated ECM should be submitted which incorporates the ILV recommendations.
7 Based on Figure 11, pp. 70-71.
8 Based on Figure 20, pp. 82-83.

IV. Method Deficiencies and Reviewer's Comments

1. The ECM was incomplete, lacking the recovery raw data, calibration data, and representative chromatograms to support the method. The ECM for BASF Analytical Method L0353/01 was submitted as Appendix D of the ILV MRID 50420303; however, only a 50-page excerpt of the full 169-page study report was included. The ECM was not provided separately. In the Abstract of the ECM report, linearity, selectivity and specificity were addressed based on calibration curve and chromatogram raw data, but this raw data was not included in the ECM excerpt. Additionally, the Abstract of the ECM report contained summarized recovery data for the study, but the detailed recovery tables and raw recovery data were not included in the ECM excerpt so recovery ranges and standard deviations were not provided or calculable.

   Additionally, soil characterization was performed and included in the full ECM (Figure A 90-91), but not included in the excerpt of the ECM which was included in the ILV (Appendix D, p. 131).

2. An updated ECM should be submitted which incorporates the three ILV recommendations: the cotton wool filtration should be replaced with a different filtration method to create a cleaner extract; the amount of water used to rinse the flasks after transfer to the SPE cartridge should be increased to ensure complete transfer; and the reduced extract, after nitrogen evaporation, should be reconstituted in the concentration flask (p. 22; Appendix A, p. 86).

3. The specificity of the method for BAS 595 F and its Z-isomer M595F014 was not supported by ILV representative chromatograms since the isomer peaks were not fully resolved (Figure 11, pp. 70-71 and Figure 20, pp. 82-83). The overlap of the isomer peaks occurred at *ca*. 30% peak height for BAS 595 F and at *ca*. 20% peak height for M595F014. Analytes should be fully resolved from any interferences to allow for accurate peak integration/quantitation.

4. The ILV matrix was characterized (silt loam soil); however, it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method since the ECM soils were not characterized (p. 15; Appendix E, p. 151). Also, since no terrestrial field dissipation (TFD) studies were provided for review, it could not be determined if the ILV

soil matrices covered the range of soils used in the TFD studies.

5. Communications between the ILV Study Director and BASF Study Monitor (John E. Jones III) 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, 22 of MRID 50420303). This communication should have been detailed in the study report.

6. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 7; Appendix D, p. 115). 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. In the ECM, the LOD was also reported to be the lowest calibration level used. In the ILV, 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.

7. The storage stability was investigated by the ECM (Appendix D, pp. 116, 141-149). The fortification standards and calibration standards were determined to be stable for up to 22 days when stored under refrigeration (4°C) in the dark. The sample extracts were determined to be stable for up to 6 days for final extracts and up to 5 days for raw extracts (0.1 M ammonium hydroxide and acetone) when stored under refrigeration (4°C) in the dark.

8. Matrix effects were determined to be insignificant by the ECM, and solvent standards were used for calibration curves (Appendix D, pp. 116, 135-136, 139).

9. 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. 21 of MRID 50420303).

10. The reviewer noted that the ECM reported the CAS No. of triticonazole as 138182-18-0 (Appendix D, p. 131). 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

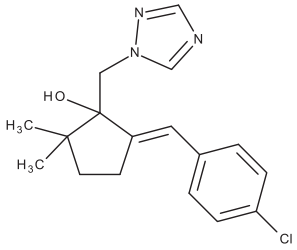
Triticonazole (BAS 595 F; Reg. No. 4378513)

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



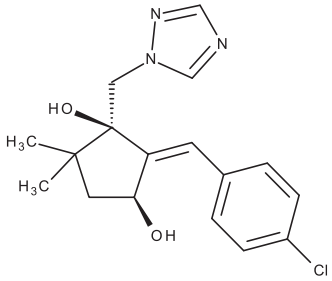
M595F001 (Reg.No. 5079285)

IUPAC Name: (1RS,2E,3SR)-2-(4-chlorobenzylidene)-5,5-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-1,3-cyclopentanediol

CAS Name: Not reported

CAS Number: None assigned

SMILES String: CC1(C[C@@H](/C(=C\c2ccc(cc2)Cl)/C1(Cn3cncn3)O)O)C



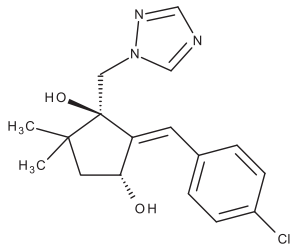
M595F002 (Reg.No. 5079144)

IUPAC Name: (1R,2E,3RS)-2-(4-chlorobenzylidene)-5,5-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-1,3-cyclopentanediol

CAS Name: Not reported

CAS Number: None assigned

SMILES String: CC1(C[C@H](/C(=C\c2ccc(cc2)Cl)/C1(Cn3cncn3)O)O)C



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

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