Analytical method for cyproconazole in water

Reports:	ECM: EPA MRID No.: 49863304. Garcia-Alix, M. 2011. Cyproconazole – Cyproconazole – Analytical Method for the Determination of Residues of Cyproconazole in Water. Final Determination by LC-MS/MS – Analytical Method. Syngenta Report No. GRM033.04A and Task No. TK0024772. Report prepared by CEMAS, Berkshire, United Kingdom, sponsored by Syngenta, Berkshire, United Kingdom, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 44 pages. Final report issued May 18, 2011. ILV: EPA MRID No. 50103303. Wang, J. 2016. Cyproconazole – Cyproconazole – Independent Laboratory Validation of Residue Method (GRM033.04A) for the Determination of Cyproconazole in Water – Final Report. Syngenta Report No. SR20160919A, Study No. 00115 and Task No. TK0316555. Report prepared by Symbiotic Research, LLC, Mount Olive, New Jersey, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 110 pages. Final report issued October 12, 2016.
Document No.:	MRIDs 49863304 & 50103303
Guideline:	850.6100
Statements:	ECM: The study was not conducted in accordance Good Laboratory Practice (GLP) standards (p. 3 of MRID 49863304). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity statements were not included. ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (40 CFR Part 160), which are compatible with OECD GLP (p. 3 of MRID 50103303). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A certification of authenticity was not included.
Classification:	This analytical method is classified as **Acceptable**. In the ECM, the purity of the test material was not reported and the linearity could not be determined.
PC Code:	128993
EFED Final Reviewer:	Jerrett Fowler, Physical Scientist — Signature: Jerrett Fowler 2018.09.24 14:46:48 -04'00' Date: 9/24/2018 Stephen P. Wente, Senior Scientist — Signature: STEPHEN WENTE Digitally signed by STEPHEN WENTE Date: 2018.09.24 14:15:21 -04'00' Date: 9/24/2018
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist — Signature: Lisa Muto Date: 10/23/17 Kathleen Ferguson, Ph.D., Environmental Scientist — Signature: Kathleen P. Ferguson Date: 10/23/17

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

Executive Summary

This analytical method, Syngenta Residue Method GRM033.04A, is designed for the quantitative determination of cyproconazole in water at the stated LOQ of 0.05 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water. The ECM validated the method using characterized drinking, surface, and ground water matrices. The ILV validated the method using characterized surface and ground water matrices with the second analysis of the first trial with minor modifications to the sample processing and analytical method. Two ion transitions were monitored. In the ECM, the purity of the test material was not reported, the linearity could not be determined because correlation coefficients were not reported, and 10×LOQ chromatograms were not provided. The LOD was not reported in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide[1]	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Cyproconazole	49863304	50103303		Water[2,3]	18/05/2011	Syngenta Crop Protection, LLC	LC/MS/MS	0.05 µg/L

1 Cyproconazole = (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol; 2-(4-Chloro-phenyl)-3-cyclopropyl-1-[1,2,4]triazol-1-yl-butan-2-ol.

2 In the ECM, the drinking water (pH 7.4, 276.2 mg/L total hardness, 1.90 mg/L dissolved organic carbon, 40 mg/L silt content), surface water (pH 7.9, 127.1 mg/L total hardness, 3.16 mg/L dissolved organic carbon, 20 mg/L silt content) and ground water (pH 7.4, 139.1 mg/L total hardness, 0.6 mg/L dissolved organic carbon, <100 mg/L silt content) were fully characterized (Table 1, p. 23 of MRID 49863304). The water sources were not reported.

3 In the ILV, the surface water (pH 8.0, 142 mg/L total hardness, 4.4 mg/L dissolved organic carbon, 6 mg/L silt content) obtained from Musconetcong River in Saxton Falls, New Jersey, and ground water (pH 7.9, 322 mg/L total hardness, 0.7 mg/L dissolved organic carbon, 8 mg/L silt content) obtained from Evian bottled mineral water purchased from a local grocer in Chester, New Jersey, were fully characterized (pp. 13-14; Tables 1-3, pp. 25-27 of MRID 50103303). The water characterization was performed at Agvise Laboratories located in Northwood, North Dakota.

I. Principle of the Method

Samples of water (10 mL) were transferred to 15-mL polypropylene centrifuge tubes and fortified, as necessary (p. 13; Appendix 4, p. 44 of MRID 49863304). An aliquot of the water sample was transferred to an autosampler vial for LC/MS/MS analysis.

Samples were analyzed for cyproconazole using an Agilent 1100 LC coupled to an Applied Biosciences API 4000 triple quadrupole mass spectrometer (pp. 14-15 of MRID 49863304). The following LC conditions were used: Hichrom KR100 5 C18 column (50 x 3.2 mm, 5 µm; column temperature 40°C), mobile phase of (A) acetonitrile and (B) 0.1% acetic acid in Ultra pure water [percent A:B (v:v) at 0.01 min. 30:70, 3.00-3.50 min. 80:20, 3.60-5.00 min. 30:70], and injection volume of 10-50 µL. The following MS/MS conditions were used: TEM 450°C, ESI positive ion polarity, and multiple reaction monitoring (MRM). Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 292.19 → 70.2 and *m/z* 292.19 → 125.0. Observed retention time was *ca*. 2.23 minutes (Figure 4, p. 30).

In the ILV, the method was performed as written, except that surface water was filtered (Whatman Qualitative Circles, Grade 1, 110 mm filter paper) after collection, the LC needle was washed with acetonitrile to prevent carry-over, and the post-column eluate was split to reduce material into the MS source (pp. 17-19; Appendix 1, pp. 65-67 of MIRD 50103303). A Hewlett Packard Series 1100 Modular HPLC coupled to an Applied Biosciences API 4000 triple quadrupole mass spectrometer was used. The following analytical parameters were used in the ILV: Kromasil 100 C18 column (3.0 x 50 mm, 5 µm; column temperature 40°C), mobile phase composition and gradient the same as that of the ECM, TEM 650°C, and injection volume of 50 µL. The same ion pair transitions were monitored as in the ECM. Observed retention time was *ca*. 2.58 minutes (Figure 1, p. 30). The minor modifications of the ILV did not require an Updated ECM.

The Limit of Quantification (LOQ) for cyproconazole was reported as 0.05 µg/L in the ECM and the ILV (pp. 10, 19 of MRID 49863304; p. 10 of MRID 50103303). The Limit of Detection (LOD) was reported as 0.005-0.012 µg/L and 0.004-0.01 µg/L for the quantitation and confirmation transitions, respectively, in the ECM. The LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 49863304): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of cyproconazole at the LOQ (0.05 µg/L) and 10×LOQ (0.5 µg/L) in three water matrices (Tables 2-3, pp. 23-24; DER Attachment 2). Two ion pair transitions were monitored; performance data (results) of the quantitation and confirmation ion analyses were comparable. The drinking water (pH 7.4, 276.2 mg/L total hardness, 1.90 mg/L dissolved organic carbon, 40 mg/L silt content), surface water (pH 7.9, 127.1 mg/L total hardness, 3.16 mg/L dissolved organic carbon, 20 mg/L silt content) and ground water (pH 7.4, 139.1 mg/L total hardness, 0.6 mg/L dissolved organic carbon, <100 mg/L silt content) were fully characterized (Table 1, p. 23). The water sources were not reported.

ILV (MRID 50103303): Mean recoveries and RSDs were within guideline requirements for analysis of cyproconazole at the LOQ (0.05 µg/L) and 10×LOQ (0.5 µg/L) in two water matrices (p.

21; Tables 4-5, p. 28). Two ion pair transitions were monitored; performance data (results) of the quantitation and confirmation ion analyses were comparable. The surface water (pH 8.0, 142 mg/L total hardness, 4.4 mg/L dissolved organic carbon, 6 mg/L silt content) obtained from Musconetcong River in Saxton Falls, New Jersey, and ground water (pH 7.9, 322 mg/L total hardness, 0.7 mg/L dissolved organic carbon, 8 mg/L silt content) obtained from Evian bottled mineral water purchased from a local grocer in Chester, New Jersey, were fully characterized (pp. 13-14; Tables 1-3, pp. 25-27). The water characterization was performed at Agvise Laboratories located in Northwood, North Dakota. The method was validated with the first trial (second analysis) with minor modifications to the sample processing and analytical method; however, the first analysis of the first trial was unsuccessful due to insufficient mixing prior to analysis (pp. 21-22).

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

Analyte[3]	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[4]	Relative Standard Deviation (%)
	Drinking Water					
	Quantitation ion					
Cyproconazole	0.05 (LOQ)	5	94-114	100	8	8.4
	0.5	5	88-99	92	6	6.4
	Confirmation ion					
Cyproconazole	0.05 (LOQ)	5	93-103	98	5	5.0
	0.5	5	85-98	94	5	5.3
	Surface Water					
	Quantitation ion					
Cyproconazole	0.05 (LOQ)	5	79-96	85	7	7.5
	0.5	5	75-100	92	10	11.0
	Confirmation ion					
Cyproconazole	0.05 (LOQ)	5	74-102	88	10	11.8
	0.5	5	80-106	95	9	10.0
	Ground Water					
	Quantitation ion					
Cyproconazole	0.05 (LOQ)	5	101-108	104	3	2.6
	0.5	5	95-108	101	5	4.6
	Confirmation ion					
Cyproconazole	0.05 (LOQ)	5	102-110	106	4	3.5
	0.5	5	103-108	105	2	2.2

Data (uncorrected recovery results; pp. 17-18) were obtained from Tables 2-3, pp. 23-24 of MRID 49863304 and DER Attachment 2.

1 The drinking water (pH 7.4, 276.2 mg/L total hardness, 1.90 mg/L dissolved organic carbon, 40 mg/L silt content), surface water (pH 7.9, 127.1 mg/L total hardness, 3.16 mg/L dissolved organic carbon, 20 mg/L silt content) and ground water (pH 7.4, 139.1 mg/L total hardness, 0.6 mg/L dissolved organic carbon, <100 mg/L silt content) were fully characterized (Table 1, p. 23). The water sources were not reported.

2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 292.19 → 70.2 and *m/z* 292.19 → 125.0.

3 Cyproconazole = (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol; 2-(4-Chloro-phenyl)-3-cyclopropyl-1-[1,2,4]triazol-1-yl-butan-2-ol.

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

Table 3. Independent Validation Method Recoveries for Cyproconazole in Water

Analyte[3]	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface Water					
	Quantitation ion					
Cyproconazole	0.05 (LOQ)	5	91-113	104	8	8
	0.5	5	99-102	100	1	1
	Confirmation ion					
Cyproconazole	0.05 (LOQ)	5	92-107	97	5	5
	0.5	5	101-104	102	1	1
	Ground Water					
	Quantitation ion					
Cyproconazole	0.05 (LOQ)	5	94-108	101	5	5
	0.5	5	98-103	101	2	2
	Confirmation ion					
Cyproconazole	0.05 (LOQ)	5	102-123	107	8	7
	0.5	5	99-104	102	2	2

Data (uncorrected recovery results, Appendix 3, pp. 98-105) were obtained from p. 21; Tables 4-5, p. 28 of MRID 50103303.

1 The surface water (pH 8.0, 142 mg/L total hardness, 4.4 mg/L dissolved organic carbon, 6 mg/L silt content) obtained from Musconetcong River in Saxton Falls, New Jersey, and ground water (pH 7.9, 322 mg/L total hardness, 0.7 mg/L dissolved organic carbon, 8 mg/L silt content) obtained from Evian bottled mineral water purchased from a local grocer in Chester, New Jersey, were fully characterized (pp. 13-14; Tables 1-3, pp. 25-27). The water characterization was performed at Agvise Laboratories located in Northwood, North Dakota.

2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 292.19 → 70.2 and *m/z* 292.19 → 125.0.

3 Cyproconazole = (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol; 2-(4-Chloro-phenyl)-3-cyclopropyl-1-[1,2,4]triazol-1-yl-butan-2-ol.

III. Method Characteristics

The LOQ for cyproconazole was reported as 0.05 µg/L in the ECM and the ILV (pp. 10, 19 of MRID 49863304; p. 10 of MRID 50103303). 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 70-120% and relative standard deviation of ≤20%. Additionally, the ECM noted that the response of the LOQ analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. No LOQ calculations were provided in the ECM. No justifications of the LOQ were provided in the ILV. The LOD was reported as 0.005-0.012 µg/L and 0.004-0.01 µg/L for the quantitation and confirmation transitions, respectively, in the ECM. In the ECM, the LOD 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 background noise. The ECM study author noted that the LOD may vary from instrument to instrument depending on the injection volume and concentrations needed to obtain adequate analyte response. The LOD was not reported in the ILV.

Table 4. Method Characteristics

Analyte			**Cyproconazole[1]**
Limit of Quantitation (LOQ)			0.05 µg/L
Limit of Detection (LOD)	ECM		0.005-0.012 µg/L (Q) 0.004-0.01 µg/L (C)
	ILV		Not reported
Linearity (calibration curve r^2 and concentration range)	ECM		**Not reported**[2]
			(0.02-1.0 ng/mL)
	ILV[3]	Surface	r^2 = 0.9994 (Q & C)
		Ground	r^2 = 0.9988 (Q) r^2 = 0.9992 (C)
			(0.02-1.0 ng/mL)
Repeatable	ECM[4]		Yes at LOQ and 10×LOQ using three characterized water matrices.
	ILV[5,6]		Yes at LOQ and 10×LOQ using two characterized water matrices.
Reproducible			Yes at LOQ and 10×LOQ.
Specific	ECM		Yes, no matrix interferences were observed. **No** 10×LOQ chromatograms were provided. Some minor baseline interference with peak integration was noted, especially in the C chromatograms.
	ILV		Yes, no matrix interferences were observed.

Data were obtained from pp. 10, 19; Tables 2-3, pp. 23-24 (recovery results); Figures 3-9, pp. 29-35 (chromatograms); Figures 10-11, pp. 36-37 (calibration curves) of MRID 49863304; pp. 10, 16, 21; Tables 4-5, p. 28 (recovery results); Figures 13-40, pp. 36-49 (chromatograms); Figures 41-44, pp. 50-51 (calibration curves) of MRID 50103303; DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition.

1 Cyproconazole = (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol; 2-(4-Chloro-phenyl)-3-cyclopropyl-1-[1,2,4]triazol-1-yl-butan-2-ol.

2 Only calibration curves without statistics were provided.

3 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Figures 41-44, pp. 50-51 of MRID 50103303; see DER Attachment 2).

4 In the ECM, the drinking water (pH 7.4, 276.2 mg/L total hardness, 1.90 mg/L dissolved organic carbon, 40 mg/L silt content), surface water (pH 7.9, 127.1 mg/L total hardness, 3.16 mg/L dissolved organic carbon, 20 mg/L silt content) and ground water (pH 7.4, 139.1 mg/L total hardness, 0.6 mg/L dissolved organic carbon, <100 mg/L silt content) were fully characterized (Table 1, p. 23 of MRID 49863304). The water sources were not reported.

5 In the ILV, the surface water (pH 8.0, 142 mg/L total hardness, 4.4 mg/L dissolved organic carbon, 6 mg/L silt content) obtained from Musconetcong River in Saxton Falls, New Jersey, and ground water (pH 7.9, 322 mg/L total hardness, 0.7 mg/L dissolved organic carbon, 8 mg/L silt content) obtained from Evian bottled mineral water purchased from a local grocer in Chester, New Jersey, were fully characterized (pp. 13-14; Tables 1-3, pp. 25-27 of MRID 50103303). The water characterization was performed at Agvise Laboratories located in Northwood, North Dakota.

6 The ILV validated the method with the first trial (second analysis) with minor modifications to the sample processing and analytical method; however, the first analysis of the first trial was unsuccessful due to insufficient mixing prior to analysis (pp. 21-22 of MRID 50103303).

IV. Method Deficiencies and Reviewer's Comments

1. The following deficiencies were noted in the ECM:

The purity of the test material was not reported in the ECM (Figure 1, p. 27 of MRID 49863304).

No calibration coefficients were reported; only calibration curves without statistics were provided (Figures 10-11, pp. 36-37 of MRID 49863304). No summary of the linearity was provided in the study report. The linearity of the method could not be determined.

No 10×LOQ chromatograms were provided. Representative chromatograms should be provided for all fortifications and matrices for review.

The sources of the water matrices were not reported (Table 1, p. 23 of MRID 49863304).

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. 10, 19 of MRID 49863304; p. 10 of MRID 50103303). 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 70-120% and relative standard deviation of ≤20%. Additionally, the ECM noted that the response of the LOQ analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. No LOQ calculations were provided in the ECM. No justifications of the LOQ were provided in the ILV. In the ECM, the LOD 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 background noise. The ECM study author noted that the LOD may vary from instrument to instrument depending on the injection volume and concentrations needed to obtain adequate analyte response. No LOD calculations were reported in ECM. The LOD was not reported in the ILV.

3. The ECM method was validated by the ILV with the second analysis of the extracts of the first trial; the first analysis of the first trial was unsuccessful due to insufficient mixing prior to analysis (pp. 21-22 of MRID 50103303). The reviewer believed that the failed analysis was due to the ILV laboratory error, as opposed to a need for ECM modification, since the ECM method stated that the mixture was to be mixed thoroughly prior to transfer to an autosampler vial.

4. In the ECM, matrix effects were assessed, and no significant suppression or enhancement of the instrument (<10%) of the detector response was observed in the presence of the water matrix (p. 20; Table 4, p. 24 of MRID 49863304). Solvent standards were used for calibration. No significant matrix suppression or enhancement was also noted in the ILV (p. 22 of MRID 50103303).

5. In ECM, the final water extracts with cyproconazole were found to be stable for up to 7 days at < 7°C (p. 20; Table 5, p. 25 of MRID 49863304).

6. The ILV study author provided communication details between the ILV laboratory personnel and the Study Sponsor (p. 21; Appendix 4, pp. 106-110 of MRID 50103303). These

communications included the discussion of the insignificant modifications to the sample processing and the failure of the first analysis of first trial due to insufficient mixing prior to analysis.

7. It was reported for the ILV that a sample set consisting of 13 samples required 8 hours (1 working days) to complete (p. 22 of MRID 50103303).

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

Cyproconazole (SAN619; CCZ)

IUPAC Name: (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol.
2-(4-Chloro-phenyl)-3-cyclopropyl-1-[1,2,4]triazol-1-yl-butan-2-ol.

CAS Name: α-(4-Chlorophenyl)-α-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol.

CAS Number: 94361-06-5 (2RS, 3RS)-isomers.
94361-07-6 (2RS, 3SR)-isomers.
113096-99-4 (unstated stereo chemistry).

SMILES String: Clc1ccc(cc1)C(O)(C(C1CC1)C)Cn1ncnc1 (ISIS v2.3/Universal SMILES).
c1cc(Cl)ccc1C(O)(C(C)C2CC2)Cn3ncnc3 (EPI Suite, v3.12 SMILES).