Analytical method for difenoconazole and its metabolites, CGA205375, CGA142856 and CGA71019, in water

Reports:	ECM: EPA MRID No.: 49862302. Manuli, M., and S.-B. Huang. 2014. Difenoconazole - Difenoconazole – Residue Method for the Determination of Difenoconazole, CGA205375, CGA142856 and CGA71019 in Water - Method. Syngenta Report No.: GRM066.01A and Task No.: TK0180148. Report prepared, sponsored, and submitted by Syngenta Crop Protection LLC, Greensboro, North Carolina; 128 pages. Final report issued April 11, 2014. ILV: EPA MRID No. 49862301. Perez, R., D. Patel and S. Perez. 2014. Difenoconazole – Difenoconazole - Independent Laboratory Validation of Residue Method (GRM066.01A) for the Determination of Difenoconazole, CGA205375, CGA142856 and CGA71019 in Water by LC-MS/MS. Report and Task No.: TK0180143. Study No.: ADPEN-2K13-901-TK0180143. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, sponsored and submitted by Syngenta Crop Protection LLC, Greensboro, North Carolina; 272 pages. Final report issued March 20, 2014.
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Guideline:	850.6100
Statements:	ECM: The study was conducted with no claim of compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (p. 3 of MRID 49673102). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). A statement of Quality Assurance and Authenticity was not included. ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (p. 3 of MRID 49862301). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5, 7 of MRID 49862301). An authenticity statement was not included.
Classification:	This analytical method is classified as Supplemental. Representative chromatograms did not validate the method for CGA71019 in one or both matrices and recoveries of CGA71019 were corrected for residues quantified in the controls. The specificity of the method was not validated in the ILV for CGA205375 in surface and ground water and CGA71019 in surface water and was not validated in the ECM for CGA142856 in surface water and CGA71019 in surface and ground water.
PC Code:	128847
Reviewer:	Lewis Ross Brown, III Env. Biologist
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Date:	01-05-17

Executive Summary

This analytical method, Syngenta Residue Method GRM066.01A, is designed for the quantitative determination of difenoconazole and its metabolites, CGA205375, CGA142856 and CGA71019, in water using LC/MS/MS. The method is quantitative for all four analytes at the stated LOQ of 0.10 µg/L (0.10 ppb). The LOQ is less than the lowest toxicological level of concern in water for all four analytes. The ECM validated the method using surface and ground waters; the ILV validated the method using the same water matrices as the ECM. The ILV validated the method with the first trial with insignificant modifications to the analytical instrumentation. The ILV validated the method for CGA71019 with SPE clean up and direct injection; the ECM only performed CGA71019 validation with SPE clean up due to analytical instrument sensitivity. In the ECM, the number of samples was insufficient (n = 3) for all analyses and chromatograms ECM and ILV representative chromatograms did not validate the method for CGA71019 in one or both matrices due to matrix interferences, nearby contaminants or significant baseline noise. The specificity of the method was not validated for CGA205375 in the ILV due to significant baseline noise and for CGA142856 in the ECM/surface water due to significant matrix interferences.

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						
Difenoconazole (CGA169374)	49862302	49862301		Water[2,3]	11/04/2014	Syngenta Crop Protection, LLC.	LC/MS/MS	0.10 µg/L; 0.10 ppb
CGA205375								
CGA142856								
CGA71019								

1 Difenoconazole (CGA169374) = 3-Chloro-4-[(2RS,4RS;2RS,4SR)-4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether. CGA205375 = 1-[2-Chloro-4-(4-chlorophenoxy)phenyl]-2-(1,2,4-triazol-1-yl)ethanol. CGA142856 = 1,2,4-Triazol-1-yl-acetic acid. CGA71019 = 1,2,4-Triazole.

2 In the ECM, characterized surface water (Sample ID: RIMV00312-0001; pH 7.3, calcium 6.0 ppm, magnesium 2.9 ppm, total dissolved solids 58 ppm, hardness 27 mg equiv. $CaCO_3$/L) and ground water (Sample ID: RIMV00312-0002; pH 7.5, calcium 16 ppm, magnesium 4.5 ppm, total dissolved solids 122 ppm, hardness 59 mg equiv. $CaCO_3$/L) were used (Table 1, p. 27 of MRID 49862302). The waters used in the ECM were the same as the waters used in the ILV.

3 The waters used in the ILV were the same as the waters used in the ECM; the waters were supplied by the sponsor, Syngenta (pp. 14-15; Appendix 4, pp. 212-214 of MRID 49862301).

I. Principle of the Method

For analysis of all analytes, water (10 mL) was transferred to a 20-mL glass scintillation vial and fortified, as necessary (p. 14 of MRID 49862302). After shaking gently to mix, 0.8 mL of the water sample was transferred to a 2-mL injection vial containing 0.2 mL of acetonitrile. After mixing the samples with a vortex mixer, the sample was either analyzed immediately by LC/MS/MS or further diluted in acetonitrile:water (20:80, v:v) prior to LC/MS/MS analysis. The Method Flow Chart was provided in the study report (Appendix 4, p. 124).

For analysis of CGA71019 using solid phase extraction (SPE) clean-up, if needed, water (10 mL) was transferred to a 20-mL glass scintillation vial and fortified, as necessary (p. 15; Appendix 1, p. 120 of MRID 49862302). After shaking gently to mix, the sample was transferred to a prepared solid phase extraction column (Bond Elut C-18 cartridge; 100 mg, 3 cc). The column was conditioned with methanol (2xs column volumes), 2 mL of 0.5% ammonium hydroxide in methanol:water (90:10, v:v; 2xs), 2 mL of HPLC water (2xs), 2 mL of 5% formic acid in methanol (2xs), and 2 mL of 2% formic acid (1xs). After 5 mL of the sample was transferred to the prepared cation exchange column, the sample load was allowed to drip through the column under gravity. After the cartridge was washed with 2 mL HPLC water (2xs) and 1 mL of methanol (2xs), the analytes were eluted using three portions each of 2 mL of 0.5% ammonium hydroxide in methanol:water (90:10, v:v). Eluates in 15-mL glass centrifuge tubes and evaporated under nitrogen steam at 40°C to approximately 0.5 mL. methanol:concentrated ammonium (75:25, v:v) under gravity into a 125-mL round bottom flask. The residue was reconstituted in 0.2 mL acetonitrile and brought to a final volume of 1.0 mL using HPLC water. After sonication, the sample was transferred to an autosampler vial for analysis via LC/MS/MS. The Method Flow Chart was provided in the study report (Appendix 4, p. 124).

The method contained the following precautions: 1) the SPE elution profile should be checked prior to validation if a different SPE column is used; 2) bottled Optima grade ultra pure water should be used for the LC mobile phase; 3) difenoconazole has a strong tendency to adhere to the injection needle in some types of autosamplers without flow-through cleaning, so solutions for needle washing must be determined; and 4) to minimize carry-over, blank controls should be injected between high level recovery samples (p. 16 of MRID 49862302).

Samples were analyzed for all analytes using Waters Acquity UPLC coupled to an Applied Biosystems Sciex API 4000 triple quadrupole MS (550°C; pp. 16-18; Appendix 1, p. 120 of MRID 49862302; Appendix 2, p. 103; Appendix 2, Appendix 1, p. 202 of MRID 49862301). The following LC conditions were used: Agilent SB-AQ column (75 x 4.6 mm, 3.5 µm; column temperature 40°C), mobile phase of (A) 0.3% formic acid in water and (B) acetonitrile:methanol (70:30, v:v) [percent A:B (v:v) at 0.0-0.5 min. 95.0:5.0, 1.5-5.0 min. 5.0:95., 5.1-7.0 min. 95.0:5.0], and injection volume of 20 µL. The following MS/MS conditions were used: positive ion polarity and multiple reaction monitoring (MRM). For difenoconazole and CGA205375, two ion pair transitions were monitored (quantification and confirmation, respectively): *m/z* 406.2 → 251.0 and *m/z* 406.2 → 187.9 for difenoconazole and *m/z* 350.1 → 69.9 (^{35}Cl) and *m/z* 352.1 → 69.9 (^{37}Cl) for CGA205375. One ion pair transition was monitored for CGA142856 and CGA71019: *m/z* 128.1 → 70.0 for CGA142856 and *m/z* 70.0 → 43.1 for CGA71019. Expected retention times were 3.53, 3.36, 2.11 and 2.08 minutes for difenoconazole, CGA205375,

CGA142856 and CGA71019, respectively. The reviewer noted that the AB Sciex API 4000 triple quadrupole mass spectrometer was reported in the ECM as the analytical instrument of the method development laboratory while the AB Sciex Triple Quad™ 6500 MS was reported in the ECM as the analytical instrument of the independent validation laboratory (p. 14; Appendix 1, p. 120).

In the ILV, the method was performed as written, except for the analytical instrument: an Agilent 1290 Infinity Series UPLC coupled to an AB Sciex Triple Quad™ 6500 MS (pp. 15-18 of MIRD 49862301). The ECM study author noted that the sensitivity of the ILV analytical instrument allowed for direct injection for the CGA71019 analysis (p. 15 of MRID 49862302).

The Limit of Quantification (LOQ) for difenoconazole, CGA205375, CGA142856 and CGA71019 was reported as 0.10 µg/L (0.1 ppb), which is equivalent to 0.0016 ng on column, in the ECM and the ILV (p. 22 of MRID 49862302; p. 21 of MRID 49862301). The Limit of Detection (LOD) for all analytes was reported as 0.025 µg/L (0.025 ppb), which is equivalent to 0.0005 ng on column, in the ECM and ILV.

II. Recovery Findings

ECM (MRID 49862302): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of difenoconazole, CGA205375, CGA142856 and CGA71019 at the LOQ and 10×LOQ in two water matrices (Appendix 2, Tables 2-7, pp. 142-144 of MRID 49862301; DER Attachment 2). The number of samples was insufficient for all analyses (n = 3). Two ion transitions were monitored for difenoconazole and CGA205375; procedural recoveries from the quantification ion and confirmation ion were comparable. Only one ion transition was monitored for CGA142856 and CGA71019; a confirmatory method is not usually required when LC/MS and GC/MS is the primary method. Procedural recoveries were corrected for residues quantified in the controls; however, raw data for the control samples was not provided (pp. 19-20). Surface water (Sample ID: RIMV00312-0001; pH 7.3, calcium 6.0 ppm, magnesium 2.9 ppm, total dissolved solids 58 ppm, hardness 27 mg equiv. $CaCO_3$/L) and ground water (Sample ID: RIMV00312-0002; pH 7.5, calcium 16 ppm, magnesium 4.5 ppm, total dissolved solids 122 ppm, hardness 59 mg equiv. $CaCO_3$/L) were used (Table 1, p. 27). The waters used in the ECM were the same as the waters used in the ILV.

ILV (MRID 49862301): Mean recoveries and RSDs were within guideline requirements for analysis of difenoconazole, CGA205375, CGA142856 and CGA71019 at the LOQ and 10×LOQ in two water matrices (Tables 2-8, pp. 26-32 and Tables 13-19, pp. 37-43 of MRID 49862301). Two ion transitions were monitored for difenoconazole and CGA205375; procedural recoveries from the quantification ion and confirmation ion were comparable. Only one ion transition was monitored for CGA142856 and CGA71019. Procedural recoveries were corrected when residues were quantified in the controls; residues were only quantified in the controls for CGA71019, both direct injection and SPE clean-up (Appendix 6, pp. 216-244). Surface water (Sample ID: RIMV00312-0001; pH 7.3, calcium 6.0 ppm, magnesium 2.9 ppm, total dissolved solids 58 ppm, hardness 27 mg equiv. $CaCO_3$/L) and ground water (Sample ID: RIMV00312-0002; pH 7.5,

calcium 16 ppm, magnesium 4.5 ppm, total dissolved solids 122 ppm, hardness 59 mg equiv. $CaCO_3$/L) were used; waters were supplied by the sponsor, Syngenta (pp. 14-15; Appendix 4, pp. 212-214). The waters used in the ILV were the same as the waters used in the ECM. The method was validated with the first trial with insignificant modifications to the analytical instrumentation (p. 22). The ILV validated the method for CGA71019 with SPE clean up and direct injection.

Table 2. Initial Validation Method Recoveries for Difenoconazole, CGA205375, CGA142856 and CGA71019 in Water

Analyte[1]	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[2]	Relative Standard Deviation (%)
Surface Water[3]						
	Quantification ion					
Difenoconazole (CGA169374)	0.1 (LOQ)	3	81-90	87	5	5.7
	1.0	3	95-97	96	1	1.2
CGA205375	0.1 (LOQ)	3	91-95	92	2	2.5
	1.0	3	93-98	96	3	2.6
CGA142856	0.1 (LOQ)	3	81-102	90	11	12
	1.0	3	87-92	90	3	2.8
CGA71019	0.1 (LOQ)	3	85-101	90	10	11
	1.0	3	76-83	81	4	5.0
	Confirmation ion					
Difenoconazole (CGA169374)	0.1 (LOQ)	3	85-92	89	4	4.1
	1.0	3	93-94	94	1	0.6
CGA205375	0.1 (LOQ)	3	92-100	96	4	4.2
	1.0	3	97-98	97	2	1.6
Ground Water[3]						
	Quantification ion					
Difenoconazole (CGA169374)	0.1 (LOQ)	3	84-86	85	1	1.4
	1.0	3	89-100	94	6	5.9
CGA205375	0.1 (LOQ)	3	89-99	93	5	5.7
	1.0	3	96-103	99	4	3.8
CGA142856	0.1 (LOQ)	3	99-107	102	4	4.3
	1.0	3	100-106	103	3	2.9
CGA71019	0.1 (LOQ)	3	79-87	82	5	5.7
	1.0	3	80-82	81	1	1.4
	Confirmation ion					
Difenoconazole (CGA169374)	0.1 (LOQ)	3	81-87	84	3	3.6
	1.0	3	89-103	94	8	8.6
CGA205375	0.1 (LOQ)	3	92-96	94	2	2.2
	1.0	3	98-105	101	4	3.6

Data (uncorrected recovery results; pp. 19-20) were obtained from Appendix 2, Tables 2-7, pp. 142-144 of MRID 49862301 and DER Attachment 2.

1 Difenoconazole (CGA169374) = 3-Chloro-4-[(2RS,4RS;2RS,4SR)-4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether. CGA205375 = 1-[2-Chloro-4-(4-chlorophenoxy)phenyl]-2-(1,2,4-triazol-1-yl)ethanol. CGA142856 = 1,2,4-Triazol-1-yl-acetic acid. CGA71019 = 1,2,4-Triazole.

2 Standard deviations for the analytes in the waters were reviewer-calculated based on data provided in Appendix 2, Tables 2-7, pp. 142-144 of MRID 49862301 since the study author did not provide the s.d. values (see DER Attachment 2).

3 Surface water (Sample ID: RIMV00312-0001; pH 7.3, calcium 6.0 ppm, magnesium 2.9 ppm, total dissolved solids 58 ppm, hardness 27 mg equiv. $CaCO_3$/L) and ground water (Sample ID: RIMV00312-0002; pH 7.5, calcium 16 ppm, magnesium 4.5 ppm, total dissolved solids 122 ppm, hardness 59 mg equiv. $CaCO_3$/L) were used (Table 1, p. 27). The waters used in the ECM were the same as the waters used in the ILV.

Table 3. Independent Validation Method Recoveries for Difenoconazole, CGA205375, CGA142856 and CGA71019 in Water

Analyte1	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)2	Relative Standard Deviation (%)
Surface Water[3]						
	Quantification ion					
Difenoconazole (CGA169374)	0.1 (LOQ)	5	93-98	96	2.0	2.1
	1.0	5	91-94	93	1.1	1.2
CGA205375	0.1 (LOQ)	5	99-103	101	1.2	1.2
	1.0	5	93-99	95	2.4	2.5
CGA142856	0.1 (LOQ)	5	84-89	87	1.8	2.1
	1.0	5	92-96	94	1.8	1.9
CGA71019 (SPE clean-up)	0.1 (LOQ)	5	87-109	96	9.1	9.5
	1.0	5	95-102	98	2.4	2.5
CGA71019 (Direct injection)	0.1 (LOQ)	5	99-111	104	5.2	5.0
	1.0	5	99-102	100	1.0	1.0
	Confirmation ion					
Difenoconazole (CGA169374)	0.1 (LOQ)	5	96-103	100	2.8	2.8
	1.0	5	91-95	93	1.8	1.9
CGA205375	0.1 (LOQ)	5	103-104	104	0.8	0.8
	1.0	5	94-96	95	0.9	0.9
Ground Water[3]						
	Quantification ion					
Difenoconazole (CGA169374)	0.1 (LOQ)	5	91-99	96	3.0	3.2
	1.0	5	89-97	93	3.1	3.3
CGA205375	0.1 (LOQ)	5	108-109	108	0.7	0.7
	1.0	5	99-106	102	2.8	2.8
CGA142856	0.1 (LOQ)	5	106-113	110	2.5	2.3
	1.0	5	100-105	103	2.1	2.0
CGA71019 (SPE clean-up)	0.1 (LOQ)	5	103-114	110	4.5	4.1
	1.0	5	91-104	97	4.8	5.0
CGA71019 (Direct injection)	0.1 (LOQ)	5	94-103	99	3.3	3.4
	1.0	5	99-104	101	2.1	2.1
	Confirmation ion					
Difenoconazole (CGA169374)	0.1 (LOQ)	5	96-100	98	2.0	2.1
	1.0	5	92-98	95	2.6	2.7
CGA205375	0.1 (LOQ)	5	108-113	109	2.4	2.2
	1.0	5	101-103	102	1.1	1.0

Data (recovery results were corrected when residues were quantified in the controls, Appendix 6, pp. 216-244) were obtained from Tables 2-8, pp. 26-32 and Tables 13-19, pp. 37-43 of MRID 49862301.

1 Difenoconazole (CGA169374) = 3-Chloro-4-[(2RS,4RS;2RS,4SR)-4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether. CGA205375 = 1-[2-Chloro-4-(4-chlorophenoxy)phenyl]-2-(1,2,4-triazol-1-yl)ethanol. CGA142856 = 1,2,4-Triazol-1-yl-acetic acid. CGA71019 = 1,2,4-Triazole.

2 Surface water (Sample ID: RIMV00312-0001; pH 7.3, calcium 6.0 ppm, magnesium 2.9 ppm, total dissolved solids 58 ppm, hardness 27 mg equiv. $CaCO_3$/L) and ground water (Sample ID: RIMV00312-0002; pH 7.5, calcium 16 ppm, magnesium 4.5 ppm, total dissolved solids 122 ppm, hardness 59 mg equiv. $CaCO_3$/L) were used; waters were supplied by the sponsor, Syngenta (pp. 14-15; Appendix 4, pp. 212-214). The waters used in the ILV were the same as the waters used in the ECM.

III. Method Characteristics

The LOQ for difenoconazole, CGA205375, CGA142856 and CGA71019 was reported as 0.10 µg/L (0.1 ppb), which is equivalent to 0.0016 ng on column, in the ECM and the ILV (p. 22 of MRID 49862302; p. 21 of MRID 49862301). In the ECM, the LOQ was defined as the lowest analyte concentration which yielded a mean recovery of 70-110% and relative standard deviation of ≤20%. Additionally, the method stated that the response of the LOQ should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. No justifications of the LOQ were provided in the ILV. The LOD for all analytes was reported as 0.025 µg/L (0.025 ppb), which is equivalent to 0.0005 ng on column, in the ECM and 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. The method also noted that an estimate of the LOD can be taken as three times the background noise and that the LOD can vary between runs and from instrument to instrument.

Table 4. Method Characteristics

Analyte[1]		**Difenoconazole**	**CGA205375**	**CGA142856**	**CGA71019**
Limit of Quantitation (LOQ)		0.10 µg/L (0.1 ppb) equivalent to 0.0016 ng on column			
Limit of Detection (LOD)		0.025 µg/L (0.025 ppb) equivalent to 0.0005 ng on column[2]			
Linearity (calibration curve r^2 and concentration range)	ECM[3,4]	r^2 = 0.9994 (Q) r^2 = 0.9998 (C)	r^2 = 0.9998 (Q & C)	r^2 = 0.9996	r^2 = 0.9986
		(0.02-5.0 ng/mL)			(0.2-5.0 ng/mL)
	ILV[3,4]	r^2 = 0.9994 (Q & C)	r^2 = 0.9998 (Q) r^2 = 0.9996 (C)	r^2 = 0.9998	r^2 = 0.9994 (Direct) r^2 = 0.9998 (SPE)
		(0.0005-0.2 ng)			
Repeatable	ECM[5]	Yes at LOQ and 10×LOQ, but n = **3**.			Yes at LOQ and 10×LOQ, but n = **3**, with SPE clean up.[6]
	ILV[7]	Yes at LOQ and 10×LOQ.			Yes at LOQ and 10×LOQ with SPE clean up or direct injection.
Reproducible		Yes at LOQ and 10×LOQ.			Yes at LOQ and 10×LOQ with SPE clean up or direct injection.
Specific	ECM	Yes. Interferences were quantified as *ca.* 25% of LOQ.[8] Some non-uniform peak integration was noted in the C chromatogram.	Yes. Interferences were quantified as *ca.* 10% of LOQ. Some peak tailing was observed in the Q chromatogram.	Yes, for ground water (interferences were *ca.* 6% of LOQ). **No**, for surface water where interferences were quantified as *ca.* 32% of LOQ.[9] Some non-uniform peak integration was noted.	**No**. Interferences were quantified as *ca.* 15% of LOQ; however, significant baseline noise around the analyte peak interfered with peak attenuation and integration at the LOQ.[10]
	ILV	Yes. Interferences were quantified as *ca.* 2% of LOQ. Some baseline noise interfered with peak integration.	**No**. Interferences were quantified as *ca.* 1% of LOQ; however, significant baseline noise around the analyte peak interfered with peak attenuation and integration at the LOQ and 10×LOQ.[11]	Yes, no matrix interferences were noted.	Yes, in ground water; interferences were quantified as *ca.* 3% of LOQ with direct injection or SPE clean up. **No**, in surface water, interferences were quantified as *ca.* 22% of LOQ with SPE clean up[12]; no matrix interferences observed with direct injection. A nearby peak (RT 2.17 min.; height = analyte at LOQ) interfered with analyte

Analyte[1]		Difenoconazole	CGA205375	CGA142856	CGA71019
					integration and identification.[13] In ground water, interferences were quantified as <3% of LOQ with direct injection or SPE clean up.

Data were obtained from p. 22 of MRID 49862302; p. 21; Tables 2-8, pp. 26-32 and Tables 13-19, pp. 37-43 (ILV recovery results); Figures 1-5, pp. 50-56 (ILV calibration curves); Figures 11-50, pp. 72-111 (ILV chromatograms); Appendix 2, Tables 2-7, pp. 142-144 (ECM recovery results); Appendix 2, Figures 6-11, pp. 162-167 (ECM calibration curves); Appendix 2, Figures 12-19, pp. 168-197 (ECM chromatograms) of MRID 49862301; DER Attachment 2. Q = Quantification ion; C = Confirmation ion.

1 Difenoconazole (CGA169374) = 3-Chloro-4-[(2RS,4RS;2RS,4SR)-4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether. CGA205375 = 1-[2-Chloro-4-(4-chlorophenoxy)phenyl]-2-(1,2,4-triazol-1-yl)ethanol. CGA142856 = 1,2,4-Triazol-1-yl-acetic acid. CGA71019 = 1,2,4-Triazole.

2 The method LOD was based on the AB Sciex Triple Quad™ 6500 MS instrument (p. 22 of MRID 49862302).

3 Two ion transitions were monitored for difenoconazole and CGA205375. Only one ion transition was monitored for CGA142856 and CGA71019. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

4 ECM and ILV correlation coefficients for all analytes were reviewer-calculated from r values provided in the study reports (Figures 1-5, pp. 50-56; Appendix 2, Figures 6-11, pp. 162-167 of MRID 49862301; DER Attachment 2).

5 In the ECM, characterized surface water (Sample ID: RIMV00312-0001; pH 7.3, calcium 6.0 ppm, magnesium 2.9 ppm, total dissolved solids 58 ppm, hardness 27 mg equiv. $CaCO_3$/L) and ground water (Sample ID: RIMV00312-0002; pH 7.5, calcium 16 ppm, magnesium 4.5 ppm, total dissolved solids 122 ppm, hardness 59 mg equiv. $CaCO_3$/L) were used (Table 1, p. 27 of MRID 49862302). The waters used in the ECM were the same as the waters used in the ILV.

6 Validation of CGA71019 was not possible in the ECM using direct injection due to the sensitivity of the analytical instrument, AB Sciex API 4000 triple quadrupole mass spectrometer (p. 14; Appendix 1, p. 120 of MRID 49862302).

7 The waters used in the ILV were the same as the waters used in the ECM; the waters were supplied by the sponsor, Syngenta (pp. 14-15; Appendix 4, pp. 212-214 of MRID 49862301).

8 Based on Appendix 2, Figure 12, p. 168-170 and Figure 13, pp. 172-174 of MRID 49862301.

9 Based on Appendix 2, Figure 16, pp. 184-189 of MRID 49862301.

10 Based on Appendix 2, Figure 18, p. 193 and Figure 19, p. 196 of MRID 49862301.

11 Based on Figures 17-18, pp. 78-79 and Figures 37-38, pp. 98-99 of MRID 49862301.

12 Based on Figures 28-29, pp. 89-90 of MRID 49862301.

13 Based on Figures 24-26, pp. 85-87; Figures 28-29, pp. 89-90 of MRID 49862301. Mainly seen in surface water matrix, although was noted in 10×LOQ chromatogram of CGA71019 direct injection with ground water (Figure 46, p. 107).

IV. Method Deficiencies and Reviewer's Comments

1. The ECM MRID 49862302 was a finalized report which contained the results, as well as conclusions, of the ILV. The recovery data, calibration curves and chromatograms presented in ECM MRID 49862302 were those generated in the ILV; the data tables contained the footnote: "All data obtained from validation Syngenta Study TK 0180143" (Tables 2-8, pp. 28-34 and Figures 2-51, pp. 54-115 of MRID 49862302). Data from extractability and matrix studies were also generated in the ILV (Tables 9-12, pp. 35-38; Tables 20-24, pp. 46-50). ECM MRID 49862302 was dated after ILV MRID 49862301. The recovery data and chromatograms for the internal validation by Syngenta were found in the Appendix 2 of the ILV, where the entire "Draft" ECM was provided, not in ECM MRID 49862302.

2. The method calculations reported that procedural recoveries were corrected for residues quantified in the controls; however, raw data for the control samples was not provided (pp. 19-20 of MRID 49862302). In the ILV, recoveries were also corrected when residues were quantified in the controls; residues were only quantified in the controls for CGA71019, both direct injection and SPE clean-up (Appendix 6, pp. 216-244 of MRID 49862301).

3. In the ILV, the specificity of the method was not validated for CGA205375 in surface and ground water and CGA71019 in surface water. For CGA205375, significant baseline noise around the analyte peak interfered with peak attenuation and integration at the LOQ and 10×LOQ (Figures 17-18, pp. 78-79; Figures 37-38, pp. 98-99 of MRID 49862301). For CGA71019 in surface water, interferences were quantified as <22% of LOQ with SPE clean up; no matrix interferences observed with direct injection (Figures 28-29, pp. 89-90). A nearby peak (RT 2.17 min.; height = analyte at LOQ) interfered with analyte integration and identification (Figures 24-26, pp. 85-87; Figures 28-29, pp. 89-90). It was mainly seen in the surface water matrix, although it was noted in the 10×LOQ chromatogram of CGA71019 direct injection with ground water (Figure 46, p. 107). This contaminant was not seen in the ECM with the AB Sciex 4000. At the request of the study monitor, the source of the contaminant was determined in the ILV (p. 21). Via injection of single and mixed-analyte standards of CGA142856 and CGA71019 and full product ion scans, the ILV determined that the contaminant peak was generated by an in-source fragmentation of the parent ion of CGA142856 to parent ion of CGA71019 and a fragment ion (mass 43).

   In the ECM, the specificity of the method was not validated for CGA142856 in surface water and CGA71019 in surface and ground water. For CGA142856 in surface water, matrix interferences were quantified as *ca*. 32% of the LOQ, which was >LOD (Appendix 2, Figure 12, p. 168-170 and Figure 13, pp. 172-174 of MRID 49862301). Additionally, some non-uniform peak integration was noted. For CGA71019, significant baseline noise around the analyte peak interfered with peak attenuation and integration at the LOQ (Appendix 2, Figure 18, p. 193 and Figure 19, p. 196 of MRID 4986230).

4. In the ECM analysis, the number of samples was insufficient (n = 3) for all analyses at the LOQ and 10×LOQ (Appendix 2, Tables 2-7, pp. 142-144 of MRID 49862301). OCSPP guidelines recommend that a minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

5. The estimations of the LOQ in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 22 of MRID 49862302; p. 21 of MRID 49862301). No calculations were reported in ECM or ILV to support the method LOQ. In the ECM, the LOQ was defined as the lowest analyte concentration which yielded a mean recovery of 70-110% and relative standard deviation of ≤20%. Additionally, the method stated that the response of the LOQ should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. 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. The method also noted that an estimate of the LOD can be taken as three times the background noise and that the LOD can vary between runs and from instrument to instrument.

   Additionally, the lowest toxicological level of concern in water for the analytes was not reported in the ECM and ILV. An LOQ above toxicological levels of concern results in an unacceptable method classification.

6. The reviewer noted that the same water matrices were reported in the ECM and ILV (Table 1, p. 27 of MRID 49862302; pp. 14-15; Appendix 4, pp. 212-214 of MRID 49862301). The ECM water matrix characterization was not reported in the "Draft" ECM provided in the Appendix 2 of the ILV, but the reviewer determined that the water characterization of the ILV (which was reported in the ECM MRID 49862302 and ILV MRID 49862301) corresponded to the water matrices of both validations since the sponsor supplied the water matrices to the ILV.

7. Communications between the sponsor and the ILV were provided (p. 21; Appendix 7, p. 267 of MRID 49862301).

8. In the ILV, the stability of final extracts was investigated (p. 23 of MRID 49862302; p. 20; Tables 9-12, pp. 33-36; Tables 20-24, pp. 44-48 of MRID 49862301). Final extracts of all analytes in surface and ground water were found to be stable for up to 19 days when stored at *ca*. 5 ± 2°. It was recommended that solutions are analyzed as soon as possible.

9. In the ILV, matrix effects were studied and determined to be minimal for all analytes in surface and ground water samples, with the exception of CGA142856 which showed a suppression of *ca*. 26% (p. 21; Table 24, p. 48 of MRID 49862301). Non-matrix matched standards were used (p. 22 of MRID 49862302).

10. The reviewer noted the following typographical errors in the ECM recovery data: the ranges were incorrectly reported for the ground water as 81-87% for the LOQ and 89-

103% for 10×LOQ, instead of 99-107% for the LOQ and 100-106% for 10×LOQ (Appendix 2, Table 6, p. 144 of MRID 49862301).

11. It was reported for the ILV that one batch of 13 samples required less than one working day to complete (p. 21 of MRID 49862301). Instrument analysis of water samples was performed overnight.

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

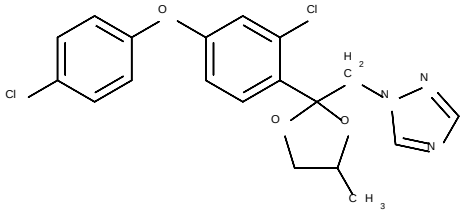
Difenoconazole (CGA169374)

IUPAC Name: 3-Chloro-4-[(2RS,4RS;2RS,4SR)-4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether

CAS Name: 1-[[2-[2-Chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxan-2-yl]methyl-1H-1,2,4-triazole

CAS Number: 119446-68-3

SMILES String: O1CC(C)OC1(Cn2ncnc2)c3c(Cl)cc(Oc4ccc(Cl)cc4)cc3



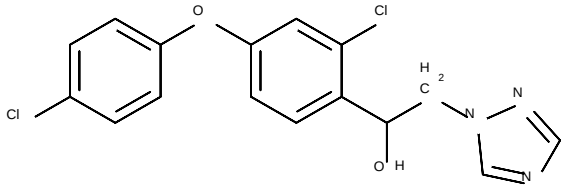
CGA205375

IUPAC Name: 1-[2-Chloro-4-(4-chlorophenoxy)phenyl]-2-(1,2,4-triazol-1-yl)ethanol

CAS Name: Alpha-[2-chloro-4-(4-chlorophenoxy)phenyl]-1H-1,2,4-triazole-1-ethanol

CAS Number: 117018-19-6

SMILES String: OC(Cn1cncn1)c2ccc(Oc3ccc(Cl)cc3)cc2Cl



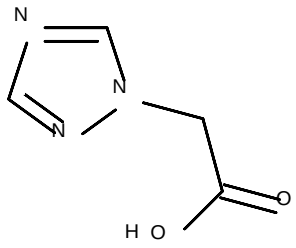
CGA142856

IUPAC Name: 1,2,4-Triazol-1-yl-acetic acid

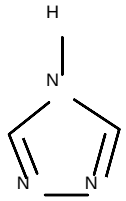
CAS Name: 1H-1,2,4-Triazole-1-acetic acid

CAS Number: 110964-79-9

SMILES String: OC(=O)Cn1cncn1



CGA71019
IUPAC Name: 1,2,4-Triazole
CAS Name: 1H-1,2,4-Triazole
CAS Number: 288-88-0
SMILES String: c1nnc[nH]1



Test Material:	Difenoconazole
MRID:	49862302
Title:	Difenoconazole - Difenoconazole – Residue Method for the Determination of Difenoconazole, CGA205375, CGA142856 and CGA71019 in Water - Method
MRID:	49862301
Title:	Difenoconazole – Difenoconazole - Independent Laboratory Validation of Residue Method (GRM066.01A) for the Determination of Difenoconazole, CGA205375, CGA142856 and CGA71019 in Water by LC-MS/MS
EPA PC Code:	128847
OCSPP Guideline:	850.6100

For CDM/CSS-Dynamac JV

Primary Reviewer: Lisa Muto **Signature:** 

Date: 11/8/16

Secondary Reviewer: Kathleen Ferguson **Signature:** 

Date: 11/8/16

Quality Assurance Manager: Joan Gaidos **Signature:** 

Date: 11/8/16

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