Test Material:	Cyprodinil
MRID:	49570202
Title:	Cyprodinil - Analytical Method (GRM010.04A) for Determination of CGA219417 and CGA249287 in Water by LC-MS/MS
MRID:	49570203
Title:	Cyprodinil - Independent Laboratory Validation of Residue Method (GRM010.04A) for the Determination of CGA219417 and CGA249287 in Water by LC-MS/MS
EPA PC Code:	288202
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lynne Binari **Signature:** 

Date: 6/29/15

Secondary Reviewer: Lisa Muto **Signature:** 

Date: 6/29/15

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 6/29/15

Analytical method for cyprodinil (CGA219417) and its transformation product CGA249287 in water

Reports:	ECM: EPA MRID No. 49570202. Lin, K. 2015. Cyprodinil - Analytical Method (GRM010.04A) for Determination of CGA219417 and CGA249287 in Water by LC-MS/MS. Report prepared, sponsored, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 53 pages. Syngenta Report No.: GRM010.04A and Task No.: TK0211147. Final report issued January 13, 2015. ILV: EPA MRID No. 49570203. Perez, R., S. Perez, and A. Ratliff. 2015. Cyprodinil - Independent Laboratory Validation of Residue Method (GRM010.04A) for the Determination of CGA219417 and CGA249287 in Water by LC-MS/MS. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 132 pages. Report No.: 2K14-901-TK0211149-001, Study No.: 2K14-901-TK0211149, and Task No.: TK0211149. Final report issued January 7, 2015.
Document No.:	MRIDs 49570202 & 49570203
Guideline:	850.6100
Statements:	ECM: There was no claim of compliance with USEPA Good Laboratory Practice (GLP) standards for this study (p. 3 of MRID 49570202). Signed and dated Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity Certification statements were not provided. ILV: The study was conducted in compliance with USEPA GLP standards (p. 3 of MRID 49570203). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). The Quality Assurance statement specified that the quality assurance unit "inspected this study and the report to assure the integrity of the data", but does not specify that the study report provides a true and accurate record of the results obtained.
Classification:	This analytical method is classified as **Acceptable**. Reviewer comments include that the LOQ and LOD encompassed the toxicological level of concern for cyprodinil, but were determined via the method evaluation at the lowest spike level subjectively selected by the study investigators. For the ILV, recovery results were corrected for residues detected in the matrix controls. The ILV chromatograms could not be adequately evaluated because full traces were not provided.
PC Code:	288202
Reviewer:	Gabe Rothman Environmental Scientist
	Signature:  **Date:** November 1, 2016

For both MRIDs, page citations in this review refer to the bottommost set of page numbers located in the lower right corner of each page of the MRID.

Executive Summary

The analytical method, Syngenta Residue Method GRM010.04A, is designed for the quantitative determination of cyprodinil (CGA219417) and its transformation product CGA249287 in water using LC/MS/MS. The method is quantitative for the analytes at the stated LOQ of 0.1 µg/L (ppb). The LOQ is less than the lowest toxicological level of concern in water. The independent laboratory validated the method for analysis of cyprodinil and CGA249287 at the LOQ and 10x LOQ in surface and ground water after one trial. However, the ILV chromatograms, especially the method blank and matrix control sample chromatograms, could not be adequately evaluated because full traces were not provided. No major modifications were made by the independent laboratory.

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						
Cyprodinil (CGA219417)	49570202	49570203		Water[1]	13/01/2015	Syngenta	LC/MS/MS	0.1 µg/L (ppb)
CGA249287								

1 Characterized surface (ECM validation and ILV) and ground (ILV) water matrices were used (Appendix 2, Table 1, p. 102; Appendix 4, pp. 116-117 of MRID 49570203). Syngenta provided the water matrices for the ILV, and the surface water used in the ECM validation and ILV appeared to be of the same source.

I. Principle of the Method

Field water samples should be stored in darkness in plastic containers, rather than glass, to prevent losses of cyprodinil and CGA249287 due to photodegradation or adsorption (p. 13).

Water (10 mL) was fortified with 0.01 µg/mL (ultrapure water:acetonitrile, 99:1, v:v) and 0.1 µg/mL (ultrapure water:acetonitrile, 90:10, v:v) mixed standard solutions of cyprodinil (CGA219417) and CGA249287 for procedural recoveries (pp. 11, 14; Appendix 3, p. 51 of MRID 49570202). The volume of the fortification solution added should not be <0.1 mL or >0.2 mL. Surface water was used for the validation; the water matrix was partially characterized, and the source was only described as "Local Lake" (Appendix 2, p. 102 of MRID 49570203). Water samples (10 mL) are transferred to a 50-mL polypropylene centrifuge tube, manually shaken vigorously for 10 seconds, then an aliquot (*ca*. 1.5 mL) is transferred into an autosampler vial and analyzed directly by LC/MS/MS.

Samples are analyzed using a Waters Acquity UPLC® system (I Class) and an Applied Biosystems Sciex API 4000 triple quadrupole MS with TurboIonSpray interface (pp. 15-16 of MRID 49570202). The following LC conditions were used: Phenomenex Synergi 4µ Hydro-RP column (4.6 mm x 75 mm, 4 µm, column temperature 40°C), mobile phase of (A) ultrapure water and (B) methanol [percent A:B (v:v) at 0-1 min. 50:50, 5-7.5 min. 0:100, 7.6-10 min. 50:50], and injection volume of 10 µL. The following MS/MS conditions were used: positive ion mode and multiple reaction monitoring (MRM). Analytes are identified using two ion pair transitions; one for quantitation (Q, "primary") and one for confirmation (C). Ion transitions monitored were as follows: *m/z* 226.1→93.1 (Q) and *m/z* 226.1→77.0 (C) for cyprodinil (CGA219417), and *m/z* 150.1→133.0 (Q) and *m/z* 150.1→66.9 (C) for CGA249287. Expected retention times are *ca*. 6.0 and 4.0 minutes for cyprodinil (CGA219417) and CGA249287, respectively.

ILV: Test compounds and two water matrices (ground, surface) were supplied by Syngenta (p. 12 of MRID 49570203). The water matrices were characterized, but source locations were not reported (Appendix 4, pp. 116-117). The independent laboratory performed the sample preparation method as written, with the following changes: 8-mL aliquots of water were fortified then diluted to 10 mL, and the samples were vortexed to mix in addition to the manual shaking step (p. 14; Appendix 1, p. 64). An Agilent 1200 SL HPLC system and Agilent 6490 Series QQQ MS with Agilent Jet Stream electrospray ionization were used (p. 15). The following LC/MS/MS conditions were also modified: a Synergi 4µ Hydro-RP column (2 mm x 75 mm) was used, the mobile phase ratios were adjusted to [percent A:B (v:v) at 0-0.5 min. 99:1, 3.5-4.5 min. 1:99, 4.6-5.5 min. 99:1], and ions monitored were *m/z* 226.14→93.00 (Q) and *m/z* 226.14→77.00 (C) for cyprodinil (CGA219417), and *m/z* 150.11→66.90 (Q) and *m/z* 150.11→118.00 (C) for CGA249287. Expected retention times are *ca*. 3.8 and 2.9 minutes for cyprodinil (CGA219417) and CGA249287, respectively.

LOQ and LOD: In the ECM and ILV, the LOQ and LOD for both analytes were 0.1 µg/L (ppb) and 0.02 µg/L (ng/mL, ppb), respectively (pp. 10, 20 of MRID 49570202; pp. 10, 17 of MRID 49570203).

II. Recovery Findings

ECM (MRID 49570202): Recovery results presented in Tables 1-8 (pp. 40-47) of the study report are data generated by ADPEN Laboratories for the ILV (Tables 2-9, pp. 22-29 of MRID 49570203) and are discussed in the following paragraph. Recovery results generated by Syngenta were obtained from the draft ECM (Appendix 2, Table 2, pp. 103-104 of MRID 49570203). Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of cyprodinil (CGA219417) and its transformation product CGA249287 in surface (lake) water at fortification levels of 0.1 µg/L (ppb) and 1 µg/L (10x LOQ; Appendix 2, Table 2, pp. 103-104 of MRID 49570203 and DER Attachment 2). Analytes were identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The lake water matrix was partially characterized, and appears to be the same matrix as that used in the ILV (Appendix 2, Table 1, p. 102; Appendix 4, p. 116 of MRID 49570203).

ILV (MRID 49570203): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of cyprodinil (CGA219417) and its transformation product CGA249287 in surface water and ground water at fortification levels of 0.1 µg/L (ppb, LOQ) and 1.0 µg/L (10x LOQ; p. 17; Tables 2-9, pp. 22-29). Analytes were identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The method was validated for both analytes at both fortification levels in surface and ground water after one trial, with alternate LC/MS/MS parameters and MRM transitions (pp. 10, 14-16). The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota (Appendix 4, pp. 116-117). The surface water matrix appears to be the same as that used for the ECM validation (Appendix 2, Table 1, p. 102).

Table 2. Initial Validation Method Recoveries for Cyprodinil (CGA219417) and Its Transformation Product CGA249287 in Surface Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Quantitation Ion					
Cyprodinil	0.1 (LOQ)	5	81-89	84	3	4
	1	5	92-96	94	2	2
CGA249287	0.1 (LOQ)	5	93-100	97	3	3
	1	5	98-107	102	4	4
	Confirmation Ion					
Cyprodinil	0.1 (LOQ)	5	83-89	87	2	3
	1	5	90-96	94	2	2
CGA249287	0.1 (LOQ)	5	93-102	96	3	4
	1	5	97-108	102	5	5

Data (recovery results) were obtained from Appendix 2, Table 2, pp. 103-104 of MRID 49570203 and DER Attachment 2 (mean, SD, RSD). Insufficient information was provided to determine whether or not recovery results were corrected; example calculations allow for correction of recoveries for residues detected in matrix control samples (pp. 17-18 of MRID 49570202; Appendix 2, pp. 80-81 of MRID 49570203). Data presented in Tables 1-8 of MRID 49570202 (pp. 40-47) are recovery results generated by the ILV (Tables 2-9, pp. 22-29 of MRID 4949570203).

1 Partially characterized surface water with source location described as "Local Lake" (Appendix 2, p. 102 of MRID 49570203).

Table 3. Independent Validation Method Recoveries for Cyprodinil (CGA219417) and Its Transformation Product CGA249287 in Surface and Ground Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface Water					
	Quantitation Ion					
Cyprodinil	0.1 (LOQ)	5	78-81	79	1.6	2.0
	1.0	5	87-89	88	0.5	0.6
CGA249287	0.1 (LOQ)	5	93-97	95	2.1	2.2
	1.0	5	94-99	97	1.5	1.6
	Confirmation Ion					
Cyprodinil	0.1 (LOQ)	5	76-80	79	1.7	2.2
	1.0	5	89-91	90	0.8	0.9
CGA249287	0.1 (LOQ)	5	87-93	91	2.6	2.8
	1.0	5	95-99	97	1.5	1.5
	Ground Water					
	Quantitation Ion					
Cyprodinil	0.1 (LOQ)	5	78-83	81	1.9	2.4
	1.0	5	89-91	90	0.8	0.9
CGA249287	0.1 (LOQ)	5	98-101	99	1.0	1.0
	1.0	5	100-101	100	0.5	0.5
	Confirmation Ion					
Cyprodinil	0.1 (LOQ)	5	74-82	79	3.2	4.0
	1.0	5	85-89	88	1.5	1.7
CGA249287	0.1 (LOQ)	5	93-96	94	1.3	1.3
	1.0	5	100	100	0.3	0.3

Data (recovery results, corrected for residues found in matrix control samples) were obtained from Tables 2-9, pp. 22-29 of MRID 49570203.

1 The water matrices, supplied by Syngenta, were characterized, but source locations were not reported (p. 12; Appendix 4, pp. 116-117).

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD for cyprodinil (CGA219417) and its transformation product CGA249287 in water were 0.1 and 0.02 µg/L (ppb, ng/mL), respectively (p. 20 of MRID 49570202; p. 17 of MRID 49570203). The ECM defined the LOQ as the lowest analyte concentration at which the methodology has been validated and a mean recovery of 70-110% and RSD of ≤20% has been obtained. The ECM defined the LOD as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated matrix control sample at the corresponding retention time, and an estimate of the LOD can be taken as three times the background noise.

Table 4. Method Characteristics for Cyprodinil (CGA219417) and Its Transformation Product CGA249287 in Water

		Cyprodinil (CGA219417)	CGA249287
Limit of Quantitation (LOQ)		0.1 µg/L (ppb)	
Limit of Detection (LOD)		0.02 µg/L	
Linearity (calibration curve r^2 and concentration range)[1]	ECM:	Q ion: r^2 = **0.9916** C ion: r^2 = **0.9922**	Q ion: r^2 = 0.9998 C ion: r^2 = 0.9998
	ILV:	Q ion: r^2 = 0.9982-0.9992 C ion: r^2 = 0.9980-0.9984	Q ion: r^2 = 0.9986-0.9998 C ion: r^2 = 0.9986-0.9996
	Range:	0.2-20.0 pg (0.00020-0.02000 ng)	
Repeatable	ECM:	Yes at LOQ and 10x LOQ.	
	ILV:	Yes at LOQ and 10x LOQ.	
Reproducible		Yes.	
Specific	ECM:	Interferences (based on ppb found) were detected at 30% and 55% of LOD at analyte retention time for Q and C ions, respectively, in matrix controls (Figure 6, p. 33 of MRID 49570202).	Interferences (based on ppb found) were detected at 9% and 23% of LOD at analyte retention time for Q and C ions, respectively, in matrix controls (Figure 7, p. 36).
		Matrix was surface (lake) water.[2]	
	ILV:	Interferences (based on ppb found) were <50% of the LOD at analyte retention times for the Q and C ions, respectively, in matrix controls (Tables 2-9, pp. 22-29 of MRID 49570203). Method blank and matrix control sample chromatograms could not be adequately evaluated because full traces were not provided.	
		Matrices were surface and ground water; source locations were not reported.[2]	

Data were obtained from pp. 10, 20; Figures 4-5, pp. 31-32 of MRID 49570202; p. 17; Tables 2-9, pp. 22-29; Figures 1-2, pp. 31-32; Figures 17-18, pp. 47-48; Figures 21-22, pp. 51-52; Figures 25-26, pp. 55-56; Figures 29-30, pp. 59-60; Appendix 2, Tables 1-2, pp. 102-104; Appendix 4, pp. 116-117; Appendix 6, pp. 120-131 of MRID 49570203, and DER Attachment 2.

Linearity is satisfactory when r^2 ≥0.995.

1 1/x weighting (Figures 4-5, pp. 31-32 of MRID 49570202; Appendix 6, pp. 120-131 of MRID 49570203). ECM and ILV coefficient of determination (r^2) values are reviewer-generated from reported correlation coefficient (r) values (DER Attachment 2).

2 Characterizations were provided (Appendix 2, Table 1, p. 102; Appendix 4, pp. 116-117 of MRID 49570203). The surface water matrix used in the ECM and ILV appear to be the same.

IV. Method Deficiencies and Reviewer's Comments

1. The ECM validation performance data were only presented in the draft ECM report (Appendix 2, Table 2, pp. 103-104 of MRID 49570203). All of the recovery results presented in the tables of the final ECM report (Tables 1-8, pp. 40-47 of MRID 49570202) are the data generated by ADPEN Laboratories during the ILV of the method (Tables 2-9, pp. 22-29 of MRID 49570203).

2. LOQ and LOD encompassed the toxicological level of concern for cyprodinil, but were determined via the method evaluation at the lowest spike level subjectively selected by the study investigators. This is inconsistent with objective procedures as defined in 40 CFR Part 136, Appendix B. The ECM defined the LOQ as the lowest analyte concentration at which the methodology has been validated and a mean recovery of 70-110% and RSD of ≤20% has been obtained (p. 20 of MRID 49570202). The ECM defined the LOD as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated matrix control sample at the corresponding retention time and an estimate of the LOD can be taken as three times the background noise. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in water was not reported. A LOQ above toxicological levels of concern results in an unacceptable method classification.

3. For the ILV, recovery results were corrected when residues were found in the matrix control samples (Tables 2-9, pp. 22-29 of MRID 49570203).

   It could not be determined if recovery results were corrected for the ECM validation because insufficient information was provided. The example calculations allow for correction of recoveries for residues detected in matrix control samples (pp. 17-18 of MRID 49570202).

4. For the ILV, the chromatograms, especially the method blank and matrix control sample chromatograms, could not be adequately evaluated because full traces were not provided Figures 3-32, pp. 33-62 of MRID 49570203).

5. For the ILV, calibration curve linearity values were identified as "R^2" in the study report text (p. 16 of MRID 49570203), but as "Corr (r)" in the Analyte Residue Reports (Appendix 6, pp. 120-131). Linearity values appear to be presented on the plotted calibration curves, but were not identified as either correlation coefficient (r) or coefficient of determination (r^2; Figures 1-2, pp. 31-32). The reviewer interpreted the designation of "Corr (r)" to indicate correlation coefficient (r) and generated coefficient of determination (r^2) values (DER Attachment 2).

6. For the ECM validation, chromatograms for reagent blank samples were not provided (Figures 2-3, pp. 25-30; Figures 6-7, pp. 33-38 of MRID 49570202). Standard curve plots with regression curve analyses were provided, but the individual calibration standard data were not provided (Figures 4-5, pp. 31-32). For cyprodinil, linearity was not satisfactory (r^2 ≥0.995) for either the quantitation ion or confirmation ion standard curve plots.

7. All communications prior to running the samples between the independent laboratory and the developers or previous users of the ECM were not provided. The independent laboratory provided "all pertinent communications" (p. 18; Appendix 7, p. 132 of MRID 49570203).

8. For the ECM validation, the water matrix characterization was not provided in the final report (MRID 49570202). In the draft ECM report (Appendix 2, pp. 65-113 of MRID 49570203), the surface water was partially characterized and the source location was reported as "Local Lake" (Appendix 2, Table 1, p. 102 of MRID 49570203). It appears that the surface water matrix used in the ILV is the same as that used for the ECM, but this was not specified (Appendix 4, p. 116 of MRID 49570203).

9. For the ECM validation, the purities of the test compounds used were not reported, only described as "analytical standards", "GLP certified" (Appendix 2, p. 50 of MRID 49570202).

10. The equipment substitutions and method modifications implemented by the independent laboratory (see section **I. Principle of the Method**, ILV: above for details) are not considered substantial changes to the ECM.

11. A typographical error in the draft ECM report title, "Primisulfuron - Analytical Method (GRM010.04A) for Determination of CGA219417 and CGA249287 in Water by LC-MS/MS" (Appendix 2, p. 65 of MRID 49570203) was corrected in the ECM final report title to specify cyprodinil.

12. It was reported for the ILV that a single analyst could complete a set of thirteen samples (one reagent blank, two matrix controls, and ten fortified samples) in less than three hours with LC/MS/MS analysis performed overnight (p. 18 of MRID 49570203).

V. References

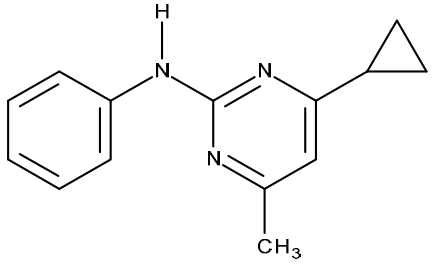
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

Cyprodinil (CGA219417)

IUPAC Name: 4-Cyclopropyl-6-methyl-N-phenylpyrimidin-2-amine.
(4-Cyclopropyl-6-methyl-pyrimidin-2-yl)-phenyl-amine.
N-(4-Cyclopropyl-6-methyl-pyrimidi-2-yl)-aniline.
CAS Name: 4-Cyclopropyl-6-methyl-N-phenyl-2-pyrimidinamine.
CAS Number: 121552-61-2
SMILES String: c1ccccc1Nc2nc(C3CC3)cc(C)n2



CGA249287

IUPAC Name: 4-Cyclopropyl-6-methyl-pyrimidin-2-ylamine.
4-Cyclopropyl-6-methyl-pyridin-2-ylamine.
CAS Name: Not available.
CAS Number: 92238-61-4
SMILES String: Cc1cc(nc(n1)N)C2CC2