

**Analytical method for acibenzolar-S-methyl and its metabolite CGA210007 in water**

**Reports:** ECM GRM051.08C (Draft): EPA MRID No.: MRID 50424601 (Appendix 2, pp. 78-139). Manuli, M., S. Braid. Not dated. Acibenzolar-S-methyl – Acibenzolar-S-methyl – Analytical Method GRM051.08C for the Determination of Acibenzolar-S-methyl and CGA210007 in Water by Direct Injection LC-MS/MS Analysis. Syngenta Report No. GRM051.08C and Task No. TK0215240. Report prepared, sponsored, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 62 pages. Final report date not reported; chromatograms were generated 07/17/2014 and 07/21/2014 (Appendix 2, p. 78 of MRID 50424601).

ECM GRM051.08C (Updated): EPA MRID No.: 49979901. Lin, K., M. Manuli, S. Braid. 2016. Acibenzolar-S-methyl – Acibenzolar-S-methyl – Analytical Method GRM051.08C for the Determination of Acibenzolar-S-methyl and CGA210007 in Water by Direct Injection LC-MS/MS Analysis. Syngenta Report No. GRM051.08C and Task No. TK0215240. Report prepared, sponsored, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 61 pages. Final report issued September 6, 2016.

ILV: EPA MRID No. 50424601. Perez, R., Y-C., Park, A. Ratliff. 2015. Acibenzolar-S-methyl – Acibenzolar-S-methyl – Independent Laboratory Validation of Residue Method (GRM051.08C) for the Determination of Acibenzolar-S-methyl and CGA210007 in Water by Direct Injection LC-MS/MS Analysis – Final ILV Report. Syngenta Report No. 2K15-0211490-001, Study No. 2K15-0211490 and Task No. TK0211490. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 190 pages. Final report issued September 23, 2015.

**Document No.:** MRIDs 49979901 & 50424601


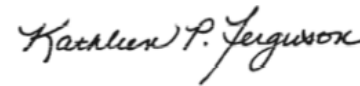
**Guideline:** 850.6100

**Statements:** ECM: The study was not conducted in accordance Good Laboratory Practice (GLP) standards (pp. 3, 6 of MRID 49979901). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity statements were not included. A signed and dated Summary of Revisions to Previous Versions was included (p. 4).

ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 50424601). 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**. Only one set of performance data was submitted for each of two method versions. However, the main difference between the draft and updated method versions was acidification of water samples and solutions. Therefore, independent

validation of each method version is not considered necessary.

<b>PC Code:</b>	061402		
<b>EFED Final Reviewer:</b>	Lewis R. Brown, III Environmental Biologist	Signature: Lewis Ross Brown, III, Environmental Biologist Date: 04/02/2018	
<b>CDM/CSS-Dynamac JV Reviewers:</b>	Lisa Muto, M.S., Environmental Scientist	Signature:  Date: 2/15/18	
	Kathleen Ferguson, Ph.D., Environmental Scientist	Signature:  Date: 2/15/18	

*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, Syngenta Residue Method GRM051.08C, is designed for the quantitative determination of acibenzolar-S-methyl and its metabolite CGA210007 in water at the LOQ of 0.05 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water (14 µg/L; DP 439439). The ECM GRM051.08C (Draft) validated the method using characterized surface and ground water. The ILV validated the method GRM051.08C (Draft) for both analytes using characterized surface and ground water after the second trial, with the addition of 0.1% formic acid to standards and samples to improve analyte stability and the use of matrix-matched calibration standards. The first trial failed due to poor storage stability and matrix effects. MRID 49979901 [ECM GRM051.08C (Updated)] was an updated version of the method of ECM GRM051.08C (Draft) based on ILV findings. While the sample processing and analytical method was updated, the recovery results and chromatograms provided in MRID 49979901 were based on ECM GRM051.08C (Draft) which does not include acidification of water samples. In MRID 49979901, no new performance data was generated for ECM GRM051.08C (Updated); therefore, **only one set of performance data was submitted for each version of the method**. Two ion transitions were monitored for both analytes. All ECM GRM051.08C (Draft) and ILV data was satisfactory regarding accuracy, precision, and specificity at 0.05 µg/L and 0.5 µg/L fortifications. However, the ECM representative calibration data did not support the method. The LOD was not reported in the ILV.

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide <sup>1</sup>	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Acibenzolar-S-methyl (CGA245704)	49979901 <sup>2</sup>	50424601 <sup>3</sup>		Water	06/09/2016	Syngenta Crop Protection, LLC	LC/MS/MS	0.05 µg/L
CGA210007								
Acibenzolar-S-methyl (CGA245704)	Appendix 2 of 50424601 <sup>4</sup>	None submitted		Water	??/??/2014 <sup>5</sup>			
CGA210007								

1 Acibenzolar-S-methyl = CGA245704; Benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester; CGA210007 = Benzo[1,2,3]thiadiazole-7-carboxylic acid.

2 Referred to as GRM051.08C (Updated) in DER. Updated method of GRM051.08C (Draft). No new results were generated for this ECM. Only the sample processing and analytical method were updated.

3 In the ILV, the surface water (Julian Surface Water; RIMV00115-0001; pH 8.6, 84 ppm total dissolved solids, 21 mg/L total hardness as CaCO<sub>3</sub>) and ground water (Summerfield Ground Water; RIMV00115-0002; pH 7.7, 156 ppm total dissolved solids, 54 mg/L total hardness as CaCO<sub>3</sub>) were obtained from Syngenta (Sponsor) and characterized by Agvise Laboratories, Northwood, North Dakota (p. 14; Appendix 4, pp. 142-143 of MRID 50424601).

4 Referred to as GRM051.08C (Draft) in DER (Appendix 2, pp. 78-139 of MRID 50424601). In this ECM, the surface water (CCON/037/010; pH 7.9, 50 mg/L silt content, 6.37 mg/L dissolved organic carbon, 280 mg/L total hardness as CaCO<sub>3</sub>), obtained from River Thames, Windsor, United Kingdom, and ground water (CCON/038/003; pH 7.8, 15 mg/L silt content, 0.4 mg/L dissolved organic carbon, 284 mg/L total hardness as CaCO<sub>3</sub>) obtained from a well at Fawley Lodge, Henley-on-Thames, United Kingdom, were used (Table 1, p. 25 of MRID 49979901; Appendix 2, Table 1, p. 102 of MRID 50424601).

5 Completion date was not reported; chromatograms were generated 07/17/2014 and 07/21/2014 (Appendix 2, p. 78 of MRID 50424601).

Page citations in this review refer to those written in the bottom-most, right-handed corner of the document pages.

ECM GRM051.08C (Draft): Appendix 2 of the ILV MRID 50424601 contained an undated draft version of GRM051.08C (62 pages; Appendix 2, pp. 78-139 of MRID 50424601).

ECM GRM051.08C (Updated): ECM MRID 49979901 was an updated version of the method of ECM GRM051.08C (Draft) based on ILV findings.

## I. Principle of the Method

### Syngenta Residue Method GRM051.08C (Draft) – Appendix 2 of MRID 50424601

Water (50 mL) was fortified, as necessary, with a mixed standard solution of acibenzolar-S-methyl (CGA245704) and CGA210007 in acetonitrile (10.0 µg/mL, 1.0 µg/mL or 0.1 µg/mL) for procedural recoveries (Appendix 2, pp. 90-91; Appendix 2, Appendix 4, p. 139 of MRID 50424601). An aliquot was transferred to an autosampler vial then analyzed directly using LC/MS/MS against matrix-matched calibration standards.

### Syngenta Residue Method GRM051.08C (Updated) – MRID 49979901

Water (20 mL) was transferred into a 50-mL polypropylene centrifuge tube, acidified to pH <5 with 20 µL of concentrated formic acid, and fortified, as necessary, with a mixed standard solution of acibenzolar-S-methyl (CGA245704) and CGA210007 in acetonitrile (10.0 µg/mL, 1.0 µg/mL or 0.1 µg/mL) for procedural recoveries (pp. 11, 13; Appendix 4, p. 61 of MRID 49979901). The samples were shaken vigorously for 10 seconds to mix well. If particles are visible, centrifuge or filter (0.45 µm syringe filter) sample. An aliquot (*ca.* 1.5 mL) was transferred to an autosampler vial then analyzed directly using LC/MS/MS.

### Precautions for Syngenta Residue Method GRM051.08C (Draft & Updated)

The method reported the following experimental precautions: 1) LC/MS/MS mobile phase should be prepared with bottled HPLC-grade water; 2) to prevent carry-over, high concentration fortification solutions should be diluted prior to analysis and blank samples should be run between high concentration samples; and 3) additional needle and valve washed with organic solvents like acetonitrile also can be used to reduce carry-over (p. 14 of MRID 49979901; Appendix 2, p. 91 of MRID 50424601).

### LC/MS/MS Analysis for Syngenta Residue Method GRM051.08C (Draft & Updated)

GRM051.08C (Draft): Samples were analyzed using an Agilent 1200 HPLC system and an AB Sciex 5500 MS with TurboIonSpray interface (Appendix 2, pp. 92-95 of MRID 50424601).

GRM051.08C (Updated): Samples were analyzed using an Agilent 1290 UPLC system (operated as HPLC) and an Applied Biosystems Sciex QTrap 5500 MS with TurboIonSpray interface (pp. 14-17; Appendix 1, p. 58; Appendix 3, p. 60 of MRID 49979901).

The following LC conditions were used in GRM051.08C (Draft & Updated): Waters X-Select CSH column (3.0 mm x 50 mm, 2.5 µm, column temperature 40°C), mobile phase of (A) 00.1% formic acid in HPLC water and (B) methanol [percent A:B (v:v) at 0.0 min. 80:20, 2.0-4.5 min. 10:90, 4.6-6.0 min. 80:20], and injection volume of 100 µL (pp. 14-17; Appendix 1, p. 58; Appendix 3, p. 60 of MRID 49979901; Appendix 2, pp. 92-95 of MRID 50424601). The following MS/MS conditions were used for acibenzolar-S-methyl: positive ion mode and multiple reaction monitoring (MRM; 550°C). The following MS/MS conditions were used for CGA210007: negative ion mode and multiple reaction monitoring (MRM; 550°C). Analytes are

identified using two ion pair transitions; one for quantitation (Q, "primary") and one for confirmation (C). Ion transitions monitored were as follows:  $m/z$  210.97→135.9 (Q) and  $m/z$  210.97→91.00 (C) for acibenzolar-S-methyl (CGA245704),  $m/z$  178.83→107.00 (Q) and  $m/z$  178.83→57.00 (C) for CGA210007.

GRM051.08C (Draft): Expected retention times were *ca.* 3.87 and 3.46 minutes for acibenzolar-S-methyl (CGA245704) and CGA210007, respectively (Appendix 2, pp. 93-95 of MRID 50424601). Analytes are identified using two ion pair transitions; one for quantitation (Q, "primary") and one for confirmation (C). Ion transitions monitored were as follows:  $m/z$  211→136 (Q) and  $m/z$  211→91 (C) for acibenzolar-S-methyl (CGA245704),  $m/z$  179→107 (Q) and  $m/z$  179→57 (C) for CGA210007.

GRM051.08C (Updated): Expected retention times were *ca.* 3.15 and 2.74 minutes for acibenzolar-S-methyl (CGA245704) and CGA210007, respectively (pp. 15-17 of MRID 49979901). Analytes are identified using two ion pair transitions; one for quantitation (Q, "primary") and one for confirmation (C). Ion transitions monitored were as follows:  $m/z$  210.97→135.9 (Q) and  $m/z$  210.97→91.00 (C) for acibenzolar-S-methyl (CGA245704),  $m/z$  178.83→107.00 (Q) and  $m/z$  178.83→57.00 (C) for CGA210007.

### ILV

The independent laboratory performed the ECM GRM051.08C (Draft) as written, with the addition of 0.1% formic acid to standards and samples to improve analyte stability (pp. 16-19; Figures 3-34, pp. 42-73 Appendix 1, p. 77; Appendix 7, pp. 181-182 of MRID 50424601). The LC/MS instrument and conditions were the same models as those used in the ECM GRM051.08C (Updated). Monitored ion transitions and expected retention times were the same, as well. ECM MRID 49979901 [ECM GRM051.08C (Updated)] appeared to be an updated version of the method of ECM GRM051.08C (Draft) based on ILV findings.

### LOQ and LOD

In the ECM and ILV, Limit of Quantification (LOQ) for acibenzolar-S-methyl (CGA245704) and its transformation product CGA210007 in water was 0.05 µg/L (pp. 10, 21 of MRID 49979901; pp. 11, 19, 20, 22; Appendix 2, p. 98; Appendix 2, Tables 11-12, pp. 108-109 of MRID 50424601). The Limit of Detection (LOD) for acibenzolar-S-methyl and CGA210007 was 0.01 µg/L in the ECM GRM051.08C (Updated); the LOD was referenced, but not reported in the ILV. In the ECM GRM051.08C (Draft), the calculate LODs were 1.1242-10.2857 ng/L for acibenzolar-S-methyl and 0.5263-7.9965 ng/L CGA210007.

## **II. Recovery Findings**

ECM (MRID 49979901 & Appendix 2 of MRID 50424601): For GRM051.08C (Draft) which had no acidification of water samples and solutions, mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of acibenzolar-S-methyl and its transformation product CGA210007 in two water matrices at

fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10×LOQ; Tables 2-3, pp. 26-27 of MRID 49979901; Appendix 2, Tables 2-5, pp. 103-104 of MRID 50424601; DER Attachment 2). Two ion pair transitions were monitored; performance data (results) of the quantitation and confirmation ion analyses were comparable. This study, GRM051.08C, noted that the data was obtained from validation study of GRM051.08A (Tables 2-3, pp. 26-27 of MRID 49979901). The surface water (CCON/037/010; pH 7.9, 50 mg/L silt content, 6.37 mg/L dissolved organic carbon, 280 mg/L total hardness as CaCO<sub>3</sub>), obtained from River Thames, Windsor, United Kingdom, and ground water (CCON/038/003; pH 7.8, 15 mg/L silt content, 0.4 mg/L dissolved organic carbon, 284 mg/L total hardness as CaCO<sub>3</sub>) obtained from a well at Fawley Lodge, Henley-on-Thames, United Kingdom, were used (Table 1, p. 25; Appendix 2, Table 1, p. 102 of MRID 50424601). For GRM051.08C (Updated) which had acidification of water samples and solutions, no performance data or chromatograms were reported (see Reviewer's Comment #1).

ILV (MRID 50424601): Mean recoveries and RSDs were within guidelines for analysis for analysis of acibenzolar-S-methyl and its transformation product CGA210007 in two water matrices at fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10×LOQ; p. 20). Two ion pair transitions were monitored; performance data (results) of the quantitation and confirmation ion analyses were comparable. The surface water (Julian Surface Water; RIMV00115-0001; pH 8.6, 84 ppm total dissolved solids, 21 mg/L total hardness as CaCO<sub>3</sub>) and ground water (Summerfield Ground Water; RIMV00115-0002; pH 7.7, 156 ppm total dissolved solids, 54 mg/L total hardness as CaCO<sub>3</sub>) were obtained from Syngenta (Sponsor) and characterized by Agvise Laboratories, Northwood, North Dakota (p. 14; Appendix 4, pp. 142-143). The method was validated for both analytes at both fortification levels in ground and surface water after the second trial, with the addition of 0.1% formic acid to standards and samples to improve analyte stability and the use of matrix-matched calibration standards (pp. 11, 16-18; Appendix 1, p. 77; Appendix 7, pp. 181-182). The first trial failed due to poor storage stability and matrix effects. The ILV was conducted to validate ECM Syngenta Method GRM051.08C (Draft; p. 23). The original ECM Syngenta Method GRM051.08C (Draft) validated by the ILV did not contain the addition of 0.1% formic acid to the samples and standards (Appendix 2, p. 91). ECM MRID 49979901 [ECM GRM051.08C (Updated)] appeared to be an updated version of the method of ECM GRM051.08C (Draft) based on ILV findings.

**Table 2. Initial Validation Method Recoveries for Acibenzolar-S-Methyl (CGA245704) and Its Transformation Product CGA210007 in Water<sup>1</sup>**

Analyte <sup>2</sup>	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>3</sup>	Relative Standard Deviation (%)
<b>GRM051.08C (Draft) – No Acidification of Water Samples and Solutions<sup>4,5</sup></b>						
<b>Surface Water</b>						
Quantitation Ion Transition						
Acibenzolar-S-methyl (CGA245704)	0.05	5	102-105	104	1	1.3
	0.5	5	97-99	98	1	0.7
CGA210007	0.05	5	101-105	103	1	1.4
	0.5	5	100-110	106	4	3.9
Confirmation Ion Transition						
Acibenzolar-S-methyl (CGA245704)	0.05	5	95-107	101	5	5.1
	0.5	5	106-117	111	5	3.9
CGA210007	0.05	5	92-101	97	4	3.8
	0.5	5	108-115	112	3	2.4
<b>Ground Water</b>						
Quantitation Ion Transition						
Acibenzolar-S-methyl (CGA245704)	0.05	5	99-103	101	2	1.9
	0.5	5	93-102	99	4	3.8
CGA210007	0.05	5	94-100	97	2	2.4
	0.5	5	92-98	95	2	2.5
Confirmation Ion Transition						
Acibenzolar-S-methyl (CGA245704)	0.05	5	104-116	109	5	4.1
	0.5	5	93-104	100	4	4.2
CGA210007	0.05	5	90-98	94	3	3.6
	0.5	5	91-98	95	3	3.2
<b>GRM051.08C (Updated) – Acidification of Water Samples and Solutions<sup>6</sup></b>						
<b>No performance data submitted</b>						

Data (uncorrected recovery results; pp. 18-19) were obtained from Tables 2-3, pp. 26-27 of MRID 49979901; Appendix 2, Tables 2-5, pp. 103-104 of MRID 50424601; and DER Attachment 2.

1 The ECM MRID 49979901, GRM051.08C (Updated), noted that the reported data was obtained from validation study of GRM051.08A (Tables 2-3, pp. 26-27 of MRID 49979901). The reviewer noted that this data was also reported in GRM051.08C (Draft); therefore, the reviewer determined that the performance data was based on the sample processing and analytical method detailed in GRM051.08C (Draft).

2 Acibenzolar-S-methyl = CGA245704; Benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester; CGA210007 = Benzo[1,2,3]thiadiazole-7-carboxylic acid.

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

4 The surface water (CCON/037/010; pH 7.9, 50 mg/L silt content, 6.37 mg/L dissolved organic carbon, 280 mg/L total hardness as CaCO<sub>3</sub>), obtained from River Thames, Windsor, United Kingdom, and ground water (CCON/038/003; pH 7.8, 15 mg/L silt content, 0.4 mg/L dissolved organic carbon, 284 mg/L total hardness as CaCO<sub>3</sub>) obtained from a well at Fawley Lodge, Henley-on-Thames, United Kingdom, were used (Table 1, p. 25 of MRID 49979901; Appendix 2, Table 1, p. 102 of MRID 50424601).

5 Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 211→136 (Q) and *m/z* 211→91 (C) for acibenzolar-S-methyl (CGA245704), *m/z* 179→107 (Q) and *m/z* 179→57 (C) for CGA210007.

6 Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 210.97→135.9 (Q) and *m/z* 210.97→91.00 (C) for acibenzolar-S-methyl (CGA245704), *m/z* 178.83→107.00 (Q) and *m/z* 178.83→57.00 (C) for CGA210007.

**Table 3. Independent Validation Method Recoveries for Acibenzolar-S-Methyl (CGA245704) and Its Transformation Product CGA210007 in Water<sup>1,2</sup>**

Analyte <sup>3</sup>	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Acidification of Water Samples and Solutions</b>						
<b>Surface Water</b>						
Quantitation Ion Transition						
Acibenzolar-S-methyl (CGA245704)	0.05	5	84-89	87	1.7	2.0
	0.5	5	84-90	87	2.3	2.7
CGA210007	0.05	5	92-101	97	3.6	3.7
	0.5	5	88-95	91	2.7	3.0
Confirmation Ion Transition						
Acibenzolar-S-methyl (CGA245704)	0.05	5	81-89	86	3.2	3.7
	0.5	5	84-93	87	3.9	4.5
CGA210007	0.05	5	89-96	95	3.5	3.7
	0.5	5	90-94	92	1.9	2.1
<b>Ground Water</b>						
Quantitation Ion Transition						
Acibenzolar-S-methyl (CGA245704)	0.05	5	82-91	86	3.2	3.7
	0.5	5	81-88	83	3.1	3.7
CGA210007	0.05	5	98-99	98	0.4	0.4
	0.5	5	93-98	96	1.9	2.0
Confirmation Ion Transition						
Acibenzolar-S-methyl (CGA245704)	0.05	5	79-84	81	2.3	2.9
	0.5	5	84-91	87	2.6	3.0
CGA210007	0.05	5	95-102	98	2.7	2.7
	0.5	5	96-100	98	1.3	1.4

Data (uncorrected recovery results; Tables 2-9, pp. 26-33; Appendix 5, p. 144) were obtained from p. 20 of MRID 50424601.

1 The surface water (Julian Surface Water; RIMV00115-0001; pH 8.6, 84 ppm total dissolved solids, 21 mg/L total hardness as CaCO<sub>3</sub>) and ground water (Summerfield Ground Water; RIMV00115-0002; pH 7.7, 156 ppm total dissolved solids, 54 mg/L total hardness as CaCO<sub>3</sub>) were obtained from Syngenta (Sponsor) and characterized by Agvise Laboratories, Northwood, North Dakota (p. 14; Appendix 4, pp. 142-143).

2 Two ion pair transitions were monitored (quantitation and confirmation, respectively):  $m/z$  210.97→135.9 (Q) and  $m/z$  210.97→91.00 (C) for acibenzolar-S-methyl (CGA245704),  $m/z$  178.83→107.00 (Q) and  $m/z$  178.83→57.00 (C) for CGA210007.

3 Acibenzolar-S-methyl = CGA245704; Benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester; CGA210007 = Benzo[1,2,3]thiadiazole-7-carboxylic acid.



### III. Method Characteristics

In the ECM and ILV, the LOQ for acibenzolar-S-methyl (CGA245704) and its transformation product CGA210007 in water was 0.05 µg/L (pp. 10, 21 of MRID 49979901; pp. 11, 19, 20, 22; Appendix 2, p. 98; Appendix 2, Tables 11-12, pp. 108-109 of MRID 50424601). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated with acceptable precision, i.e. relative standard deviation of  $\leq 20\%$ . Additionally, the ECM reported that the response of the LOQ 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 justifications of the LOQ were provided in the ILV. The LOD acibenzolar-S-methyl and CGA210007 was 0.01 µg/L in the ECM GRM051.08C (Updated). In the ECM GRM051.08C (Draft), the calculate LODs were 1.1242-10.2857 ng/L for acibenzolar-S-methyl and 0.5263-7.9965 ng/L CGA210007. 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. Additionally, the ECM reported that an estimate of the LOD can be taken as three times the background noise and may vary between runs and from instrument to instrument. The LOD was referenced, but not reported in the ILV.

**Table 4. Method Characteristics for Acibenzolar-S-methyl (CGA245704) and Its Transformation Product CGA210007 in Water**

Analyte <sup>1</sup>		CGA245704	CGA210007		
Limit of Quantitation (LOQ)	ECM	0.05 µg/L			
	ILV				
Limit of Detection (LOD)	ECM GRM051.08C (Draft)	1.1242-10.2857 ng/L (matrices/ions combined)	0.5263-7.9965 ng/L (matrices/ions combined)		
	ECM GRM051.08C (Updated)	0.01 µg/L			
	ILV	<b>Not reported</b>			
Linearity (calibration curve $r^2$ and concentration range)	ECM GRM051.08C (Draft) <sup>2</sup>	Surface	<b>Not reported</b>	$r^2 = 0.9976$ (Q) $r^2 = 0.9987$ (C)	
		Ground	<b>Not shown</b>		
		Solvent	$r^2 = 0.9998$ (Q) $r^2 = 0.9991$ (C)	$r^2 = 0.9966$ (Q) $r^2 = 0.9974$ (C)	
		Range	0.01-1.0 ng/mL		
	ECM GRM051.08C (Updated) <sup>2</sup>	Surface	<b>Not reported</b>		
		Ground	<b>Not shown</b>		
		Range	0.01-1.0 ng/mL		
	ILV <sup>3</sup>	Surface	$r^2 = 0.9990-0.9994$ (Q) $r^2 = 0.9974-0.9996$ (C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9992-0.9998$ (C)	
		Ground	$r^2 = 0.9988-0.9990$ (Q) $r^2 = 0.9962-0.9980$ (C)	$r^2 = 0.9998$ (Q) $r^2 = 0.9992-0.9998$ (C)	
		Range	0.01-1.0 ng/mL		
Repeatable	ECM GRM051.08C (Draft) <sup>4</sup>	Yes at LOQ and 10×LOQ using characterized surface and ground water. ( <b>method</b> - no acidification of water samples and solutions)			
	ECM GRM051.08C (Updated) <sup>5,6</sup>	<b>No performance data submitted</b> ( <b>method</b> - acidification of water samples and solutions)			
	ILV <sup>7,8</sup>	Yes at LOQ and 10×LOQ using characterized surface and ground water. ( <b>method</b> - acidification of water samples and solutions)			
Reproducible		<b>Could not be determined</b> Only one set of performance data was submitted for each method.			
Specific	ECM GRM051.08C (Draft)	Surface	Yes, matrix interferences were insignificant (Q); baseline noise interfered with peak attenuation and integration (C).		
		Ground			
	ECM GRM051.08C (Updated) <sup>6</sup>	Surface	<b>No representative chromatograms submitted</b>		
		Ground			
	ILV	Surface	Yes, matrix interferences were insignificant (Q); significant contaminants ( $\geq$ LOQ peak height) were noted near analyte peak (C).	Yes, matrix interferences were insignificant.	
		Ground			

Data were obtained from pp. 10, 21; Tables 2-3, pp. 26-27 (recovery results); Figures 5-7, pp. 38-40 (calibration curves); Figures 10-16, pp. 43-49 and 2-7, pp. 50-56 (chromatograms) of MRID 49979901; pp. 11, 16, 19, 20, 22; p. 20 (recovery results); Figures 1-2, pp. 38-40 (calibration curves); Figures 19-34, pp. 58-73 (chromatograms); Appendix 6, pp. 154-176 (correlation coefficients); Appendix 2, pp. 78-139 [GRM051.08C (Draft)] of MRID 50424601 of MRID 50424601; DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition.

ECM GRM051.08C (Draft): Appendix 2 of the ILV MRID 50424601 contained an undated draft version of GRM051.08C (62 pages; Appendix 2, pp. 78-139 of MRID 50424601).

ECM GRM051.08C (Updated): It appeared to the reviewer that ECM MRID 49979901 [ECM GRM051.08C (Updated)] was an updated version of the method of ECM GRM051.08C (Draft) based on ILV findings.

ECM: ECM GRM051.08C (Draft) and ECM GRM051.08C (Updated).

1 Acibenzolar-S-methyl = CGA245704; Benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester; CGA210007 = Benzo[1,2,3]thiadiazole-7-carboxylic acid.

2 Only three representative ECM calibration curves were provided for review: two solvent-based and one matrix-based (surface water for CGA210007; Figures 5-7, pp. 38-40 of MRID 49979901; Appendix 2, Figures 21-23, pp. 130-132 of MRID 50424601). Calculated correlation coefficients or generalized correlation coefficients were not reported in the ECM GRM051.08C (Updated). ECM raw data was not provided.

3 ILV coefficient of determination ( $r^2$ ) values are reviewer-generated from reported correlation coefficient ( $r$ ) values (1/x weighting; Appendix 6, pp. 154-176 of 50424601; DER Attachment 2). Matrix-based calibration standards.

4 In the ECM GRM051.08C (Draft), the surface water (CCON/037/010; pH 7.9, 50 mg/L silt content, 6.37 mg/L dissolved organic carbon, 280 mg/L total hardness as  $\text{CaCO}_3$ ), obtained from River Thames, Windsor, United Kingdom, and ground water (CCON/038/003; pH 7.8, 15 mg/L silt content, 0.4 mg/L dissolved organic carbon, 284 mg/L total hardness as  $\text{CaCO}_3$ ) obtained from a well at Fawley Lodge, Henley-on-Thames, United Kingdom, were used (Table 1, p. 25 of MRID 49979901; Appendix 2, Table 1, p. 102 of MRID 50424601).

5 ECM MRID 49979901, GRM051.08C (Updated), noted that the data was obtained from validation study of GRM051.08A (see Reviewer's Comment #5).

6 ECM MRID 49979901, GRM051.08C (Updated), did not contain performance data or chromatograms for the updated method with acidification of the water samples and solutions.

7 In the ILV, the surface water (Julian Surface Water; RIMV00115-0001; pH 8.6, 84 ppm total dissolved solids, 21 mg/L total hardness as  $\text{CaCO}_3$ ) and ground water (Summerfield Ground Water; RIMV00115-0002; pH 7.7, 156 ppm total dissolved solids, 54 mg/L total hardness as  $\text{CaCO}_3$ ) were obtained from Syngenta (Sponsor) and characterized by Agvise Laboratories, Northwood, North Dakota (p. 14; Appendix 4, pp. 142-143 of MRID 50424601).

8 The method was validated for both analytes at both fortification levels in ground and surface water after the second trial, with the addition of 0.1% formic acid to standards and samples to improve analyte stability and the use of matrix-matched calibration standards (pp. 11, 16-18; Appendix 1, p. 77; Appendix 7, pp. 181-182 of MRID 50424601). The first trial failed due to poor storage stability and matrix effects. The ILV was conducted to validate ECM Syngenta Method GRM051.08C (Draft; p. 23). The original ECM Syngenta Method GRM051.08C (Draft) validated by the ILV did not contain the addition of 0.1% formic acid to the samples and standards (Appendix 2, p. 91). ECM MRID 49979901 [ECM GRM051.08C (Updated)] appeared to be an updated version of the method of ECM GRM051.08C (Draft) based on ILV findings.

#### IV. Method Deficiencies and Reviewer's Comments

1. ECM MRID 49979901 [ECM GRM051.08C (Updated)] was an updated version of the method of ECM GRM051.08C (Draft) based on ILV findings. While the method was updated, the recovery results and chromatograms provided in MRID 49979901 were based on ECM GRM051.08C (Draft) which does not include acidification of water samples, not ECM GRM051.08C (Updated) which does include acidification of water samples (pp. 11, 13; Appendix 4, p. 61 of MRID 49979901; Appendix 2, pp. 90-91; Appendix 2, Appendix 4, p. 139 of MRID 50424601). In MRID 49979901, no new results were generated for ECM GRM051.08C (Updated). Only the sample processing and analytical method and instrumentation were updated to exactly match those in the ILV.

The data reported in Tables 2-3, pp. 26-27 of MRID 49979901 exactly matched the data reported in Appendix 2, Tables 2-5, pp. 103-104 of MRID 50424601. Therefore, the

reviewer determined that the performance data was based on the sample processing and analytical method detailed in GRM051.08C (Draft) without acidification of the water samples and solutions.

The representative chromatograms provided in Figures 10-16, pp. 43-49 and 2-7, pp. 50-56 of MRID 49979901 exactly matched the representative chromatograms provided in Appendix 2, Figures 4-20, pp. 113-129 of MRID 50424601. These chromatograms corresponded to GRM051.08C (Draft) since the retention times and specific ion transition values of the analytes match those reported in GRM051.08C (Draft), not GRM051.08C (Updated).

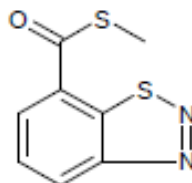
2. Only one set of performance data was submitted for each method: GRM051.08C (Draft) which had no acidification of water samples and solutions; and 2) GRM051.08C (Updated) which had acidification of water samples and solutions. For GRM051.08C (Draft), data from an initial/internal validation was provided, but no independent laboratory validation was performed. For GRM051.08C (Updated), data from an independent laboratory validation was provided, but no initial/internal validation was performed. OCSPP guidelines state that two sets of performance data should be submitted, one for the initial or other internal validation and one for the ILV.
3. The linearity of the method was not sufficiently supported by data provided in ECM GRM051.08C (Draft) and ECM GRM051.08C (Updated). Only three representative ECM calibration curves were provided for review: two solvent-based and one matrix-based (surface water for CGA210007; Figures 5-7, pp. 38-40 of MRID 49979901; Appendix 2, Figures 21-23, pp. 130-132 of MRID 50424601). Although correlation coefficients were reported in GRM051.08C (Draft), correlation coefficients should be reported for both analytes in each test matrix since the analysis relied on matrix-matched calibration standards.
4. Communication occurred between the ILV and ECM. The ILV study report provided communication details between the ILV study author (Rolando Perez) and the Study Monitor (pp. 20-21; Appendix 7, pp. 181-182 of MRID 50424601). One of the authors, Myra E. Manuli, of the GRM051.08C (Draft) and ECM MRID 49979901 GRM051.08C (Updated) was the Study Monitor for the ILV. These communications included trial success or failure, storage stability and matrix effects experimental results, and approval of method modifications.
5. ECM MRID 49979901, GRM051.08C, noted that the data was obtained from validation study of GRM051.08A (Langridge, G. 2014. Validation of Residue Analytical Method GRM051.08A for the Determination of Acibenzolar-S-methyl, CGA210007 and SYN546642 in Water by Direct Injection LC-MS/MS Analysis. CEMAS report number CEMR-6644; p. 23; Tables 2-3, pp. 26-27 of MRID 49979901). GRM051.08C was reported as a revised version of GRM051.08A for the determination of acibenzolar-S-methyl and CGA210007 only (p. 4). It was also noted that the method number GRM051.08B was not applied to any version of this method due to limitations in the method numbering tool. The reviewer noted that GRM051.08A was not included in the

ECM or ILV.

6. The ECM MRID 49979901 [ECM GRM051.08C (Updated)] did not contain a list of amendments/modifications/updates to the method of ECM GRM051.08C (Draft).
7. The reviewer noted that the representative chromatograms reported in ECM MRID 49979901 [ECM GRM051.08C (Updated)] were numbered in a non-sequential manner (Figures 10-16, pp. 43-49 and 2-7, pp. 50-56 of MRID 49979901). The reviewer believed that this was due to the cut-and-paste method used to compile the updated study report.
8. The LOD was referenced, but not reported in the ILV (pp. 11, 19, 20, 22 of MRID 50424601).
9. The stability of the analytes in the water samples was studied. In the ILV, surface and ground water extracts were found to be stable for up to 7 days under refrigerated conditions (*ca.* 1-10°C in the dark; Tables 11-12, pp. 35-36 of MRID 50424601). In the ECM, surface and ground water extracts were found to be unstable up to 7 days under refrigerated conditions (*ca.* 2-8°C in the dark; Tables 5-7, pp. 30-32 of MRID 49979901; Appendix 2, Tables 7-8, pp. 105-106). The storage stability data of GRM051.08C (Draft) matched that of GRM051.08C (Updated). The reviewer noted that the analytes (mainly acibenzolar-S-methyl) would not be expected to be stable in water solutions without acidification (p. 21 of MRID 50424601).
10. The matrix effects were determined to be significant in the ECM and ILV based on GRM051.08A (see Reviewer's Comment #5) or GRM051.08C (Draft; pp. 20-21; Table 4, pp. 28-29 of MRID 49979901; p. 21; Table 10, p. 34; Appendix 2, pp. 97-98; Appendix 2, Table 6, p. 105 of MRID 50424601). The matrix effect data of the ILV matched that of GRM051.08C (Draft), and the matrix effect data of GRM051.08C (Updated) differed from the other two studies. Matrix-matched standards were used for calibration in all methods.
11. It was reported for the ILV that a single analyst can complete a set of twenty samples in eight working-hours, with LC/MS/MS analysis performed overnight (p. 21 of MRID 50424601).

## V. References

1. 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.
2. 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****Acibenzolar-S-methyl (CGA245704)****IUPAC Name:** Benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester**CAS Name:** 1,2,3-Benzothiadiazole-7-carbothioic acid, S-methyl ester**CAS Number:** 135158-54-2**SMILES String:** Not found**CGA210007****IUPAC Name:** Benzo[1,2,3]thiadiazole-7-carboxylic acid**CAS Name:** Not reported**CAS Number:** 35272-27-6**SMILES String:** Not found