Analytical method for dicamba in water

Reports:	ECM: EPA MRID No. 51052504. Hargreaves, S.L. 2007. Dicamba. GRM022.02A. Dicamba – Residue Method for the Determination of Residues in Water. Analytical Method. Syngenta Report No.: GRM022.02A and Task No.: TK0537723. Report prepared and sponsored by Syngenta, Berkshire, United Kingdom, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 64 pages. Final report issued October 2, 2007. ILV: EPA MRID No. 51052503. Kotthoff, M. 2016. Dicamba. Dicamba - Independent Laboratory Validation of Analytical Method GRM022.02A for the Determination of Residues of Dicamba (SAN837) in Water. Method Validation. Report and Study No.: SYN-037/6-22. Task No.: TK0207487. Report prepared by Fraunhofer Institute for Molecular Biology and Applied Ecology, IME, Schmallenberg, Germany, sponsored by Syngenta, Berkshire, United Kingdom, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 47 pages. Final report issued January 20, 2016.
Document No.:	MRIDs 51052504 & 51052503
Guideline:	850.6100
Statements:	ECM: The study was not claimed to be in accordance with USEPA FIFRA Good Laboratory Practices (GLP; 1989), and there was no GLP study director for the study (p. 3 of MRID 51052504). 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 version was provided (p. 5). ILV: The study was conducted in accordance with German GLP standards which are based on the OECD GLP, which are accepted by the European Community, the US (FDA and EPA) and Japanese (MHLW, MAFF, and METI) regulatory authorities (p. 3; Appendix 3, pp. 46-47 of MRID 51052503). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5; Appendix 3, pp. 46-47). A statement of the authenticity of the study report was also included with the QA statement.
Classification:	This analytical method is classified as **supplemental**. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is considered the lowest level of method validation (LLMV) rather than LOQ. The ILV only validated the method in drinking water. The number of trials was not reported. Calibration curves and correlation coefficients were not provided for all matrices in the ECM. The LOD was not reported in the ILV.
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This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. The CDM/CSS-Dynamac JV role does not include establishing Agency policies.

Executive Summary

This analytical method, Syngenta Analytical Method GRM022.02A, is designed for the quantitative determination of the dicamba at 0.05 µg/L in water using GC/MS. The LOQ is less than the lowest toxicological levels of concern for dicamba in water (5 µg/L). Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the reported method LOQ for dicamba in water; however, three types of water matrices (uncharacterized river, drinking, and ground) were used in the ECM, while one water matrix (characterized drinking water) was used in the ILV. The number of ILV trials was not reported; however, the reviewer believed that the ILV validated Syngenta Analytical Method GRM022.02A with the first trial for dicamba in drinking water at both fortification levels since no method modifications were reported, except for the use of different analytical equipment and modified analytical parameters. All ILV data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for dicamba. All ECM data regarding repeatability, accuracy, precision, and specificity were satisfactory for dicamba, except for performance data of the 10×LOQ confirmation ion 2 analysis in groundwater. ECM linearity data was satisfactory for dicamba; however, calibration curves and correlation coefficients were not provided for all matrices in the ECM. The LOD was not reported in the ILV.

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						
Dicamba	51052504[1]	51052503[2]	Supplemental	Water	02/10/2007	Syngenta Crop Protection, LLC	GC/MS	0.05 µg/L

1 The water matrices were not characterized or described aside from designation as river water, groundwater, and drinking water (Appendix 3, Tables 1-3, pp. 27-28 of MRID 51052504).

2 The drinking water (pH 6.89, hardness 6.34°dH, 0.5519 mg/L total organic carbon) was taken from the ILV kitchen tap (p. 10; Table 1, p. 15 of MRID 51052503). The water originates from a spring of the water board Eslohe/Schmallenberg, in the surrounding of the institute. Water characterization laboratory was not reported.

I. Principle of the Method

Samples (50.0 mL) of water were fortified with dicamba fortification solutions in acetone (10, 1.0, 0.1, or 0.01 µL/mL), as necessary, then acidified with 200 µL of concentrated HCl (pp. 11, 13-15; Appendices 1-2, pp. 25-26; Appendix 7, p. 64 of MRID 51052504). The samples were applied to an IST solid phase extraction (SPE) cartridge (100 mg, 10 mL) which was pre-conditioned with 2 mL each of methanol then ultra-pure water. The cartridge was not allowed to become dry. After the samples were applied under gravity or low vacuum, the cartridge was dried under high vacuum (<500 mbar) for 15 minutes, and water was wiped off the sides of the SPE cartridge. The method noted that it was important that all water was removed from the sample prior to the derivatization procedure. The acidified analyte was eluted using 2 mL of acetonitrile with low vacuum then high vacuum (for *ca*. 5 seconds). The method noted that the SPE procedure should be optimized if another SPE column is used. The final volume of the extract was adjusted to 2 mL using acetonitrile, then 1-mL aliquots were transferred to autosampler vials for derivatization. N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA; 100 µL) was added to each autosampler vial with shaking then heating at 60°C for 15 minutes using a heating block then submitted to GC/MS analysis.

Samples were analyzed for dicamba using an Agilent 6890 GC coupled to an Agilent 5973 mass spectrometer operating in the negative ion chemical ionization mode (pp. 11, 16-17; Appendix 1, p. 24 of MRID 51052504). The following GC conditions were used: Varian CPSIL-8 CB (5% phenyl 95%, dimethylpolysiloxane) column (30.0 m x 0.25 mm i.d., df = 0.25 µm); helium GC carrier gas; injector temperature 275°C; detector temperature 300°C; temperature program: 60°C (hold for 1 min.), ramp 20°C/min. to 300°C then hold for 1 min.; methane MS reagent gas; and injection volume of 1 µL. Expected retention time was 10.2 minutes for dicamba. Three ions were monitored (primary, confirmatory 1, and confirmatory 2, respectively): *m/z* 184, *m/z* 185, and *m/z* 186.

The ILV performed the ECM method as written, except for the use of different analytical equipment and modified analytical parameters (p. 11; Appendix 1, pp. 41-43 of MRID 51052503). Samples were analyzed for dicamba using a Varian 450-GC system coupled to a Varian 320-MS detector operating in the negative ion chemical ionization mode. The following GC conditions were used: Restex Rxi-5Sil MS column (20 m x 0.18 mm i.d., df = 0.18 µm); helium GC carrier gas; injector temperature 275°C; temperature program: 100°C (hold for 1 min.), ramp 20°C/min. to 300°C then hold for 1 min.; and injection volume of 1 µL. Expected retention time was 6.97 minutes for dicamba. Three ions were monitored which were the same as those in the ECM. The reviewer noted that an IST ISOLUTE (100 mg/10 mL) SPE cartridge was used. No other ILV modifications were reported.

The Limit of Quantification (LOQ) for dicamba in water was reported as 0.05 µg/L in the ECM and ILV (pp. 11, 20-21 of MRID 51052504; pp. 9, 10, 12 of MRID 51052503). The Limit of Detection (LOD) in water for dicamba was estimated as 0.008-0.014 µg/L, 0.007-0.014 µg/L, and 0.008-0.014 µg/L for *m/z* 184, *m/z* 185, and *m/z* 186, respectively, in the ECM; the LOD was not reported in the ILV. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is considered the lowest level of method validation (LLMV) rather than an LOQ.

II. Recovery Findings

ECM (MRID 51052504): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of dicamba in three water matrices at the LOQ (0.05 µg/L) and 10×LOQ (0.50 µg/L), except for the 10×LOQ confirmation ion 2 analysis in groundwater (mean 69%; Appendix 3, Tables 1-3, pp. 27-28). Recovery results of the quantitative and two confirmatory ions were comparable, except for the confirmation ion 2 analysis in groundwater. The water matrices were not characterized or described aside from designation as river water, groundwater, and drinking water.

ILV (MRID 51052503): Mean recoveries and RSDs met requirements for analysis of dicamba in drinking (tap) water at the LOQ (0.05 µg/L) and 10×LOQ (0.50 µg/L) using matrix-matched calibration standards (Tables 3-5, p. 16). Recovery results of the quantitative and two confirmatory ions were comparable. The drinking water (pH 6.89, hardness 6.34°dH, 0.5519 mg/L total organic carbon) was taken from the ILV kitchen tap (p. 10; Table 1, p. 15). The water originates from a spring of the water board Eslohe/Schmallenberg, in the surrounding of the institute. Water characterization laboratory was not reported. Although the number of trials was not reported, the reviewer believed that the method was validated by the ILV with the first trial for dicamba in drinking water at both fortification levels without method modifications, except for the use of different analytical equipment and modified analytical parameters (pp. 11-12; Appendix 1, pp. 41-43). No updated ECM was required.

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

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
Surface (River) Water						
Quantitation ion						
Dicamba	0.05 (LOQ)	5	85-102	92	7	8
	0.50	5	84-101	94	7	8
Confirmation ion 1						
Dicamba	0.05 (LOQ)	5	82-96	89	7	7
	0.50	5	83-103	94	9	10
Confirmation ion 2						
Dicamba	0.05 (LOQ)	5	77-94	85	8	9
	0.50	5	81-95	88	6	7
Groundwater						
Quantitation ion transition						
Dicamba	0.05 (LOQ)	5	91-125	105	14	13
	0.50	5	75-94	83	7	9
Confirmation ion 1						
Dicamba	0.05 (LOQ)	5	86-120	105	14	14
	0.50	5	78-88	80	4	5
Confirmation ion 2						
Dicamba	0.05 (LOQ)	5	81-104	93	11	12
	0.50	5	64-81	**69**	7	10
Drinking Water						
Quantitation ion transition						
Dicamba	0.05 (LOQ)	5	89-110	99	8	8
	0.50	5	88-102	97	6	6
Confirmation ion 1						
Dicamba	0.05 (LOQ)	5	79-103	95	10	10
	0.50	5	78-91	85	5	6
Confirmation ion 2						
Dicamba	0.05 (LOQ)	5	86-110	94	10	11
	0.50	5	74-87	78	6	7

Data (uncorrected results, pp. 18-19) were obtained from Appendix 3, Tables 1-3, pp. 27-28 of MRID 51052504; DER Attachment 2. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is considered the lowest level of method validation (LLMV) rather than an LOQ.

1 The water matrices were not characterized or described aside from designation as river water, groundwater, and drinking water (Appendix 3, Tables 1-3, pp. 27-28 of MRID 51052504).

2 Three ions were monitored (primary, confirmatory 1, and confirmatory 2, respectively): *m/z* 184, *m/z* 185, and *m/z* 186.

3 Standard deviations were reviewer-calculated since the values were not reported in the study report. Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for Dicamba in Water[1, 2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
Drinking (Tap) Water						
Quantitation ion transition						
Dicamba	0.05 (LOQ)	5	94-102	97	4	4
	0.50	5	94-102	99	3	3
Confirmation ion 1						
Dicamba	0.05 (LOQ)	5	87-93	90	3	3
	0.50	5	92-101	97	3	3
Confirmation ion 2						
Dicamba	0.05 (LOQ)	5	94-102	98	4	3
	0.50	5	94-103	99	3	3

Data (uncorrected results, Appendix 1, p. 44) were obtained from Tables 3-5, p. 16 of MRID 51052503; DER Attachment 2. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is considered the lowest level of method validation (LLMV) rather than an LOQ.

1 The drinking water (pH 6.89, hardness 6.34 °dH, 0.5519 mg/L total organic carbon) was taken from the ILV kitchen tap (p. 10; Table 1, p. 15 of MRID 51052503). The water originates from a spring of the water board Eslohe/Schmallenberg, in the surrounding of the institute. Water characterization laboratory was not reported.

2 Three ions were monitored (primary, confirmatory 1, and confirmatory 2, respectively): *m/z* 184, *m/z* 185, and *m/z* 186. The monitored ions of the ILV were the same as those of the ECM.

3 Standard deviations were reviewer-calculated since the values were not reported in the study report. Rules of significant figures were followed.

III. Method Characteristics

The LOQ for dicamba and DCSA in water was reported as 0.05 µg/L in the ECM and ILV (pp. 11, 20-21 of MRID 51052504; pp. 9, 10, 12 of MRID 51052503). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-110% with a relative standard deviation of 20% has been obtained. The LOQ was also defined as when the response of the analyte peak is no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. No calculations for the LOQ were reported in the ILV or ECM. The LOQ was reported in the ILV without justification. The LOD in water was estimated as 0.008-0.014 µg/L, 0.007-0.014 µg/L, and 0.008-0.014 µg/L for *m/z* 184, *m/z* 185, and *m/z* 186, 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 and estimated as three times the background noise. The LOD was not reported in the ILV.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is considered the lowest level of method validation (LLMV) rather than an LOQ.

Table 4. Method Characteristics

		Dicamba
Limit of Quantitation (LOQ)*	ECM	0.05 µg/L
	ILV	
Limit of Detection (LOD)	ECM	0.008-0.014 µg/L (*m/z* 184) 0.007-0.014 µg/L (*m/z* 185) 0.008-0.014 µg/L (*m/z* 186)
	ILV	**Not reported**
Linearity (calibration curve r and concentration range)[1]	ECM[2]	r = 1.0000 (*m/z* 184, *m/z* 185, & *m/z* 186)
	ILV	r = 0.9998 (*m/z* 184, *m/z* 185, & *m/z* 186)
	Range	0.625-50 ng/mL
Repeatable	ECM[3]	Yes for LOQ and 10×LOQ in uncharacterized surface (river) water, groundwater, and drinking water. (No for C2 10×LOQ in groundwater)[4]
	ILV[5,6]	Yes for LOQ and 10×LOQ in characterized drinking (tap) water.
Reproducible		Yes for 0.05 µg/L (LLMV)* and 0.50 µg/L in water matrices.
Specific	ECM	Yes, matrix interferences were <6-8% (based on peak area) in river and drinking water matrices and <15% (based on peak area).in groundwater matrix. Minor contamination/baseline noise was observed near the analyte peak.
	ILV	Yes, no matrix interferences were observed.

Data were obtained from pp. 11, 20-21 (LOQ/LOD); p. 21 (linearity data); Appendix 3, Tables 1-3, pp. 27-28 (recovery data); Appendix 4, Figures 3-32, pp. 30-59 (chromatograms); Appendix 5, Figures 33-35, pp. 60-62 (calibration curves) of MRID 51052504; 9, 10, 12 (LOQ/LOD); p. 26 (linearity coefficients); Tables 3-5, p. 16 (recovery data); Figures 2-16, pp. 20-34 (chromatograms); Figures 17-19, pp. 35-37 (calibration curves) of MRID 51052503; DER Attachment 2. Q = quantitative ion; C1 = confirmatory ion 1; C2 = confirmatory ion 2.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 Reported r values were reviewer-calculated from r^2 values reported in the study reports (Figures 33-35, pp. 60-62 of MRID 51052504; Figures 17-19, pp. 35-37 of MRID 51052503; DER Attachment 2). Values were reported to four significant figures.

2 Only one set of calibration curves and correlation coefficients were provided; however, solvent-based calibration standards were used for quantitation of river water and groundwater samples, while matrix-matched standards were used for quantitation of drinking water samples (p. 28; Table 4, pp. 29; Appendix 5, Figures 33-35, pp. 60-62 of MRID 51052504). The calibration curves were only specified for the monitored ion not matrix.

3 In the ECM, the water matrices were not characterized or described aside from designation as river water, groundwater, and drinking water (Appendix 3, Tables 1-3, pp. 27-28 of MRID 51052504).

4 Deficiencies in the confirmation ion do not affect the repeatability of the method since a confirmatory method is not usually required when GC/MS or GC/MS is used as the primary method to generate study data.

5 In the ILV, the drinking water (pH 6.89, hardness 6.34°dH, 0.5519 mg/L total organic carbon) was taken from the ILV kitchen tap (p. 10; Table 1, p. 15 of MRID 51052503). The water originates from a spring of the water board Eslohe/Schmallenberg, in the surrounding of the institute. Water characterization laboratory was not reported.

6 Although the number of trials was not reported, the reviewer believed that the method was validated by the ILV with the first trial for dicamba in drinking water at both fortification levels without method modifications, except for the use of different analytical equipment and modified analytical parameters (pp. 11-12; Appendix 1, pp. 41-43 of MRID 51052503). No updated ECM was required.

IV. Method Deficiencies and Reviewer's Comments

1. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 11, 20-21 of MRID 51052504; pp. 9, 10, 12 of MRID 51052503). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-110% with a relative standard deviation of 20% has been obtained. The LOQ was also defined as when the response of the analyte peak is no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. No calculations for the LOQ were reported in the ILV or ECM. The LOQ was reported in the ILV without justification. 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 and estimated as three times the background noise. The LOD was not reported in the ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

   Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is considered the lowest level of method validation (LLMV) rather than an LOQ.

2. The ILV only validated the method in drinking water (p. 10; Table 1, p. 15 of MRID 51052503). The ECM validated the method using river (surface) water, groundwater, and drinking water; however, the matrices were not characterized (Appendix 3, Tables 1-3, pp. 27-28 of MRID 51052504).

3. The number of trials was not reported; however, the reviewer believed that the method was validated by the ILV with the first trial for dicamba in drinking water at both fortification levels since no method modifications were reported, except for the use of different analytical equipment and modified analytical parameters (pp. 11-12; Appendix 1, pp. 41-43 of MRID 51052503).

4. Only one set of calibration curves and correlation coefficients were provided in the ECM; however, solvent-based calibration standards were used for quantitation of river water and groundwater samples, while matrix-matched standards were used for quantitation of drinking water samples (p. 28; Table 4, pp. 29; Appendix 5, Figures 33-35, pp. 60-62 of MRID 51052504). The calibration curves were only specified for the monitored ion not matrix.

5. The ECM performance data was not acceptable for the 10×LOQ confirmation ion 2 analysis in groundwater (mean 69%; Appendix 3, Tables 1-3, pp. 27-28 of MRID 51052504). The reviewer noted that deficiencies in the confirmation ion do not affect the repeatability of the method since a confirmatory method is not usually required when GC/MS or GC/MS is used as the primary method to generate study data. OCSPP guidelines state that acceptable mean recoveries are 70-120%.

6. The ILV reported that no communications occurred between the ILV laboratory and Syngenta Study Sponsor (Simon Emburey) during the study (pp. 5, 11 of MRID 51052503).

7. No ECM representative chromatograms of the calibration standards were included in the study report.

8. In the ECM, no significant matrix effects (<20%) were observed for river water and groundwater samples, so solvent-based calibration standards were used for quantitation (p. 28; Table 4, pp. 29 of MRID 51052504). For drinking water, significant matrix effects (<20%; confirmation ion 2 only) were observed, so matrix-matched standards were used. Matrix effects were not studied in the ILV. No interferences were noted in ILV representative chromatograms of control samples.

9. In the ECM, it was stated that extract stability has not been performed, and it was recommended that analysis occur on the day of preparation/derivatization (p. 21 of MRID 51052504).

10. The time requirement for the method in the ECM was reported as 0.5 day (8-hour working period) for the analysis of a batch of 20 samples (p. 16 of MRID 51052504). or ILV.

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

Dicamba (SAN837)

IUPAC Name: 3,6-Dichloro-o-anisic acid
CAS Name: 3,6-Dichloro-2-methoxybenzoic acid
CAS Number: 1918-00-9
SMILES String: COc1c(Cl)ccc(Cl)c1C(O)=O