Analytical method for dicamba (dicamba acid) and its degradate DCSA in water

Reports:	ECM: EPA MRID No. 50914302. Jutson, J.I. 2019. Validation of the Analytical Method for the Determination of Dicamba Acid and DCSA Degradate in Ground Water and Surface Water by LC-MS/MS. Smithers Viscient Study No.: 14166.6104. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored and submitted by Dicamba Registrants Coalition, c/o Wagner Regulatory Associates, Hockessin, Delaware; 115 pages. Final report issued July 18, 2019.
	ILV: EPA MRID No. 50931310. Cashmore, A. 2019. Independent Laboratory Validation of Analytical Method 14166.6104 for the Determination of Dicamba Acid and DCSA Degradate in Water. Study No.: 3202423. Report prepared by Smithers ERS Limited, Harrogate, North Yorkshire, United Kingdom, and sponsored and submitted by Dicamba Registrants Coalition, c/o Wagner Regulatory Associates, Hockessin, Delaware; 108 pages. Final report issued November 18, 2019 (Sponsor signatures dated December 6, 2019; pp. 2-5).
Document No.:	MRIDs 50914302 & 50931310
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practices (GLP; 1989), which are accepted by the OECD GLP standards (1998), except that the test and reference substances were non-GLP characterized prior to use in the study (p. 3 of MRID 50914302). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the Quality Assurance statement.
	ILV: The study was conducted in accordance with UK GLP standards (1999) as amended by the GLP Regulations (2004) and the OECD GLP (ENV/MC/CHEM(98)17 (Revised 1997, Issued January 1998), except that the test system was GLP characterized in a non-study specific basis (p. 3; Appendix 6, p. 108 of MRID 50931310). Studies are suitable for submission to the US FDA/EPA/Japanese regulatory authorities under OECD mutual acceptance of data treaty. Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5). A statement of the authenticity of the study report was also included with the GLP statement.
Classification:	This analytical method is classified as **supplemental** for surface water and **unacceptable** for groundwater. 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. ILV performance data in ground water samples were not acceptable for dicamba at the LOQ and DCSA at the LOQ and 10×LOQ.

PC Code:	029801		
Reviewer:	Chuck Peck Senior Fate Scientist	Signature: Date:	2021.11.17 13:52:09 -05'00'
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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, Smithers Viscient Analytical Method 14166.6104, is designed for the quantitative determination of the dicamba (dicamba acid) and its DCSA degradate (3,6-dichlorosalicyclic acid) at 0.100 µg/L in water using LC/MS/MS. The LOQ is less than the lowest toxicological levels of concern for dicamba and DCSA in water (5 µg/L and 31 µg/L for dicamba and DCSA, respectively). Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the reported method LOQ for both analytes in surface water; however, the LLMV for dicamba in ground water was 1.00 µg/L. No LLMV was established by the ILV and ECM performance data for DCSA in ground water. The ECM and ILV validated the method using different characterized surface and ground water matrices. The ILV validated the method with the first trial for dicamba and DCSA in surface water at both fortifications and for dicamba and DCSA in ground water at the LOQ fortification and with the second trial for dicamba and DCSA in ground water at the 10×LOQ fortification. The first failed validation attempt for dicamba and DCSA in ground water at the 10×LOQ fortification was presumed to be due to the experimental error of not acidifying the samples prior to extraction. The ILV validated Smithers Viscient Analytical Method 14166.6104 as written with insignificant final dilution changes and analytical instrument and equipment modifications. No updated ECM was required. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for dicamba and DCSA in surface water using matrix-matched calibration standards, but the specificity of the method was not well-supported by ILV representative chromatograms due to significant contamination/baseline noise. All ILV data regarding linearity and specificity were satisfactory for dicamba and DCSA in ground water using solvent-based calibration standards. ILV performance data was not acceptable for dicamba and DCSA at the LOQ and DCSA at 10×LOQ. All ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for dicamba and DCSA in ground water using solvent-based calibration standards. The ECM also successfully validated the method for both analytes in surface water using solvent-based calibration standards.

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	50914302[1]	50931310[2]	Supplemental for surface water Unacceptable for groundwater	Water	18/07/2019	Dicamba Registrants Coalition, c/o Wagner Regulatory Associates	LC/MS/MS	0.100 µg/L
DCSA								

1 In the ECM, the water matrices were ground water and surface water (p. 16 of MRID 50914302). The ground water (not characterized) used in the study was filtered well water collected on site at Smithers Viscient, Wareham, which was prepared by filtering to remove any potential organic contaminants (p. 16 of MRID 50914302). The surface water (pH 5.96, dissolved oxygen concentration 10.3 mg/L) used for this method validation analysis was collected from the Taunton River in Massachusetts (SMV Lot No. 03Jan19 Wat-C, collected with *ca*. 30-60 cm depth of overlying water on 3 January 2019).

2 In the ILV, the Fountains Abbey surface water (sample code CS 14/18; pH 7.44, hardness 86 mg/L $CaCO_3$, conductivity 154 µS/cm, dissolved organic carbon 11.2 mg/L) obtained from The Lake, Studley Royal, Ripon, United Kingdom, and Borehole groundwater (sample code CS 13/18; pH 8.0, hardness 349 mg/L $CaCO_3$, conductivity 436 µS/cm, dissolved organic carbon 0.00 mg/L) obtained from Smithers Rapra were used (p. 16; Appendix 2, pp. 92-93 of MRID 50931310). Water characterization was performed by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom.

I. Principle of the Method

Samples (50.0 mL, final volume) of water were fortified with 250 µL of 20.0 mg/L or 200 mg/L mixed fortification solutions, as necessary, then the pH was adjusted to 1.62 (ground water) and 1.66 (surface water) using 12N HCl (pp. 21-24 of MRID 50914302). The samples were extracted three times with 50 mL of dichloromethane (DCM) via one minute of vigorous shaking. The volume of the combined DCM extracts was reduced to *ca*. 3 mL by rotary evaporation using minimal heating (30°C), transferred to a glass centrifuge using 3-mL DCM rinsings, then reduced to dryness via nitrogen at room temperature. The residue was reconstituted with 2.50 mL (LOQ) or 12.5 mL (10×LOQ) of acetonitrile:purified reagent water (25:75, v:v) and mixed via vortex and sonication for 5 minutes.

Samples were analyzed for dicamba and DCSA using a Shimadzu LC-20AD HPLC coupled to an AB MDS Sciex API 5000 mass spectrometer equipped with an ESI Turbo V interface in the negative ion, multiple reaction monitoring (MRM) mode (pp. 13, 24-25 of MRID 50914302). The following LC conditions were used: Agilent EC-C18 Poroshell 120 column (3.0 mm x 100 mm, 2.7 µm; column temperature 40°C), mobile phase of (A) 0.1% formic acid in reagent grade water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.20 min. 75.0:25.0, 5.50-7.01 min. 5.00:95.0, 7.01-9.00 min. 75.0:25.0], MS temperature 550°C, and injection volume of 15.0 µL. Expected retention times were *ca*. 3.6 and 3.2 minutes for dicamba acid and DCSA, respectively. Two ion pair transitions were monitored (quantitation and confirmatory, respectively): m/z 219.0→175.0 and m/z 221.0→177.0 for dicamba, and m/z 205.0→161.0 and m/z 205.0→125.0 for DCSA.

The ILV performed the ECM method as written, except for the fact that all surface water samples were reconstituted with 2.50 mL of acetonitrile:purified reagent water (25:75, v:v) then the 10×LOQ samples were further diluted 0.2 to 1 with control matrix water prior to analysis and for insignificant modifications to the analytical parameters and equipment (pp. 17-24; Appendix 3, pp. 94-105 of MRID 50931310). Samples were analyzed for dicamba and DCSA using Shimadzu Nexera series HPLC system coupled with an AB Sciex API 5000 triple quadrupole mass spectrometer equipped with an ESI interface in the negative ion, MRM mode. The LC/MS/MS parameters were the same as those of the ECM, except that MS temperature was 400°C. Two ion pair transitions were monitored (quantitation and confirmatory, respectively): m/z 218.9→174.4 and m/z 220.9→176.7 for dicamba, and m/z 205.0→161.0 and m/z 205.0→125.0 for DCSA; the monitored ion transitions of the ILV were similar to those of the ECM. Expected retention times were ca. 3.15 and 2.65 minutes for dicamba and DCSA, respectively. The ILV noted that the LC column and mobile phases and MS ionization type, polarity, and scan type could not be modified.

The Limit of Quantification (LOQ) for dicamba and DCSA in water was reported as 0.100 µg/L in the ECM and ILV (pp. 26-27, 28-29, 31-36 of MRID 50914302; pp. 26-27 of MRID 50931310). The Limit of Detection (LOD) in water was calculated as 0.004-0.01 µg/L and 0.002-0.006 µg/L for dicamba and DCSA, respectively, in the ECM and 0.00442-0.0430 µg/L and 0.00262-0.0613 µg/L for dicamba and DCSA, respectively, 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 50914302): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of dicamba and DCSA in two water matrices at the LOQ (0.100 µg/L) and 10×LOQ (1.00 µg/L; Tables 1-12, pp. 40-51). Surface water recovery results were reported using solvent-based and matrix-matched calibration standards; ground water recovery results were only reported for solvent-based calibration standards. Recovery results of the quantitative and confirmatory ion transitions were comparable. The water matrices were ground water and surface water (p. 16). The ground water (not characterized) used in the study was filtered well water collected on site at Smithers Viscient, Wareham, which was prepared by filtering to remove any potential organic contaminants (p. 16). The surface water (pH 5.96, dissolved oxygen concentration 10.3 mg/L) used for this method validation analysis was collected from the Taunton River in Massachusetts (SMV Lot No. 03Jan19 Wat-C, collected with ca. 30-60 cm depth of overlying water on 3 January 2019).

ILV (MRID 50931310): Mean recoveries and RSDs met requirements for analysis of dicamba and DCSA in surface water at the LOQ (0.100 µg/L) and 10×LOQ (1.00 µg/L) using matrix-matched calibration standards (Tables 1-8, pp. 36-43). For ground water samples using solvent-based calibration standards, mean recoveries met requirements for analysis of dicamba at 10×LOQ (1.00 µg/L); however, RSDs were unacceptable for both analytes/fortifications/ion monitored, except for dicamba at 1.00 µg/L. RSDs in ground water were 56% for dicamba at the LOQ (Quantification (Q) & Confirmation (C) ion transitions), 54% for DCSA at the LOQ (Q &

C), and 24-25% for DCSA at 1.00 µg/L (Q & C). For all analyses with unacceptable results, means, standard deviation, and RSD were reviewer-calculated (n = 5) since the study report statistics excluded one of the recovery values because an error following the method was suspected or the value was designated as an outlier by Grubb's test (n = 4). Recovery results of the quantitative and confirmatory ion transitions were fairly comparable for dicamba in surface water and DCSA in both water matrices, but less comparable for dicamba in ground water. The Fountains Abbey surface water (sample code CS 14/18; pH 7.44, hardness 86 mg/L $CaCO_3$, conductivity 154 µS/cm, dissolved organic carbon 11.2 mg/L) obtained from The Lake, Studley Royal, Ripon, United Kingdom, and Borehole groundwater (sample code CS 13/18; pH 8.0, hardness 349 mg/L $CaCO_3$, conductivity 436 µS/cm, dissolved organic carbon 0.00 mg/L) obtained from Smithers Rapra were used (p. 16; Appendix 2, pp. 92-93). Water characterization was performed by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom. The method was validated by the ILV with the first trial for dicamba and DCSA in surface water at both fortifications and for dicamba and DCSA in ground water at the LOQ fortification and with the second trial for dicamba and DCSA in ground water at the 10×LOQ fortification (p. 28). The first failed validation attempt for dicamba and DCSA in ground water at the 10×LOQ fortification was presumed to be due to the experimental error of not acidifying the samples prior to extraction. The ILV validated Smithers Viscient Analytical Method 14166.6104 as written with insignificant final dilution changes and analytical instrument and equipment modifications (pp. 17-24; Appendix 4, p. 106). No updated ECM was required.

Table 2. Environmental Chemistry Method Recoveries for Dicamba and DCSA in Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water – Matrix-matched calibration standards						
Quantitation ion transition						
Dicamba	0.100 (LOQ)	5	96.2-110	103	5.35	5.19
	1.00	5	100-110	105	3.63	3.45
DCSA	0.100 (LOQ)	5	66.2-86.2	76.8	8.57	11.1
	1.00	5	78.2-88.1	83.0	3.57	4.30
Confirmation ion transition						
Dicamba	0.100 (LOQ)	5	97.3-106	102	4.20	4.11
	1.00	5	97.3-110	105	5.14	4.87
DCSA	0.100 (LOQ)	5	63.6-87.0	77.2	9.74	12.6
	1.00	5	82.1-87.2	84.3	2.12	2.52
Surface Water – Solvent-based calibration standards						
Quantitation ion transition						
Dicamba	0.100 (LOQ)	5	83.6-94.9	91.2	4.67	5.12
	1.00	5	93.1-97.9	95.1	2.04	2.14
DCSA	0.100 (LOQ)	5	68.2-91.0	78.6	9.15	11.6
	1.00	5	80.9-86.6	82.7	2.25	2.72
Confirmation ion transition						
Dicamba	0.100 (LOQ)	5	82.9-100	92.8	7.13	7.69
	1.00	5	94.2-101	97.4	3.15	3.24
DCSA	0.100 (LOQ)	5	67.1-93.4	77.7	10.3	13.3
	1.00	5	80.6-89.1	83.5	3.28	3.93
Ground Water- Solvent-based calibration standards						
Quantitation ion transition						
Dicamba	0.100 (LOQ)	5	101-112	107	4.25	3.96
	1.00	**5**	89.2-99.2	95.9	3.94	4.11
DCSA	0.100 (LOQ)	5	94.3-117	107	8.80	8.25
	1.00	**5**	74.6-99.3	90.6	9.48	10.5
Confirmation ion transition						
Dicamba	0.100 (LOQ)	5	109-113	111	1.76	1.59
	1.00	5	92.5-103	98.5	3.95	4.01
DCSA	0.100 (LOQ)	5	92.5-115	108	9.04	8.38
	1.00	5	75.8-98.6	89.9	8.52	9.47

Data (uncorrected results, p. 27) were obtained from Tables 1-12, pp. 40-51 of MRID 50914302. 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.

1 The water matrices were ground water and surface water (p. 16). The ground water (not characterized) used in the study was filtered well water collected on site at Smithers Viscient, Wareham, which was prepared by filtering to remove any potential organic contaminants (p. 16). The surface water (pH 5.96, dissolved oxygen concentration 10.3 mg/L) used for this method validation analysis was collected from the Taunton River in Massachusetts (SMV Lot No. 03Jan19 Wat-C, collected with *ca*. 30-60 cm depth of overlying water on 3 January 2019).

2 Two ion pair transitions were monitored (quantitation and confirmatory, respectively): *m/z* 219.0→175.0 and *m/z* 221.0→177.0 for dicamba, and *m/z* 205.0→161.0 and *m/z* 205.0→125.0 for DCSA.

Table 3. Independent Validation Method Recoveries for Dicamba and DCSA in Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water – Matrix-matched calibration standards						
Quantitation ion transition						
Dicamba	0.100 (LOQ)	5	91.5-101	95.3	4.26	4.47
	1.00	5	92.9-102	95.8	3.61	3.77
DCSA	0.100 (LOQ)	5	82.8-88.8	85.3	2.24	2.62
	1.00	5	86.4-91.6	88.8	2.18	2.45
Confirmation ion transition						
Dicamba	0.100 (LOQ)	5	95.9-103	100	3.24	3.24
	1.00	5	89.5-99.4	95.2	3.96	4.16
DCSA	0.100 (LOQ)	5	85.6-99.7	92.0	6.36	6.91
	1.00	5	84.9-93.3	89.7	3.13	3.49
Ground Water - Solvent-based calibration standards						
Quantitation ion transition						
Dicamba	0.100 (LOQ)	5[3]	0-90.9	72 (89.7)	40 (0.79)	56 (0.88)
	1.00	5	89.7-101	98.3	4.86	4.94
DCSA	0.100 (LOQ)	5[3]	2.3-90.3	71 (88.5)	39 (1.43)	54 (1.62)
	1.00	5[4]	46.9-98.5	85 (94.3)	21 (3.56)	25 (3.78)
Confirmation ion transition						
Dicamba	0.100 (LOQ)	5[3]	0-107	83 (104)	46 (2.65)	56 (2.56)
	1.00	5	84.2-90.4	88.1	2.59	2.95
DCSA	0.100 (LOQ)	5[3]	3.18-95.5	74 (92.1)	40 (2.34)	54 (2.54)
	1.00	5[4]	49.7-97.6	87 (96.0)	21 (1.54)	24 (1.60)

Data (uncorrected results, p. 24) were obtained from Tables 1-8, pp. 36-43 of MRID 50931310. 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.

1 The Fountains Abbey surface water (sample code CS 14/18; pH 7.44, hardness 86 mg/L $CaCO_3$, conductivity 154 µS/cm, dissolved organic carbon 11.2 mg/L) obtained from The Lake, Studley Royal, Ripon, United Kingdom, and Borehole groundwater (sample code CS 13/18; pH 8.0, hardness 349 mg/L $CaCO_3$, conductivity 436 µS/cm, dissolved organic carbon 0.00 mg/L) obtained from Smithers Rapra were used (p. 16; Appendix 2, pp. 92-93). Water characterization was performed by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom.

2 Two ion pair transitions were monitored (quantitation and confirmatory, respectively): m/z 218.9→174.4 and m/z 220.9→176.7 for dicamba, and m/z 205.0→161.0 and m/z 205.0→125.0 for DCSA; the monitored ion transitions of the ILV were similar to those of the ECM.

3 Mean, standard deviation, and RSD were reviewer-calculated (n = 5) since the study report statistics excluded one of the recovery values because an error following the method was suspected (n = 4). Study reported values are provided in parenthesis. Rules of significant figures were followed.

4 Mean, standard deviation, and RSD were reviewer-calculated (n = 5) since the study report statistics excluded one of the recovery values based on Grubb's Test as a significant outlier (n = 4). Study reported values are provided in parenthesis. Rules of significant figures were followed.

III. Method Characteristics

The LOQ for dicamba and DCSA in water was reported as 0.100 µg/L in the ECM and ILV (pp. 26-29, 31-36 of MRID 50914302; pp. 24-27 of MRID 50931310). No justifications or calculations for the LOQ were reported in the ILV or ECM. The LOD in water was calculated as 0.004-0.01 µg/L and 0.002-0.006 µg/L for dicamba and DCSA, respectively, in the ECM. The LOD was calculated in the ECM using the following equation:

$LOD = (3x(N_{ctl})/(Resp_{LS}) \times Conc_{LS} \times DF_{CNTL}$

Where, LOD is the limit of detection of the analysis, N_{ctl} is the mean noise in height of the control samples (or blanks), $Resp_{LS}$ is the mean response in height of the two low calibration standards, $Conc_{LS}$ is the concentration of the low calibration standard, and DF_{CNTL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 0.0500).

In the ILV, the LOD in water was calculated as 0.00442-0.0430 µg/L and 0.00262-0.0613 µg/L for dicamba and DCSA, respectively, in the ILV. An estimate of the LOD was made at 3 × baseline noise for quantitation and confirmatory transitions for both compounds. The LOD was calculated in the ILV using the following equation:

LOD (µg/L) = 3 × height of control baseline noise × control dilution factor × calibration standard concentration (µg/mL) / height of calibration standard peak.

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	DCSA
Limit of Quantitation (LOQ)*	ECM	0.100 µg/L	
	ILV		
Limit of Detection (LOD)	ECM	0.004-0.008 µg/L (GW/sb calc) 0.008-0.01 µg/L (SW/mm calc) 0.009-0.01 µg/L (SW/sb calc)	0.004 µg/L (GW/sb calc) 0.002-0.006 µg/L (SW/mm calc) 0.002-0.004 µg/L (SW/sb calc)
	ILV	0.0243-0.0309 µg/L (SW/mm calc) 0.00442-0.0430 µg/L (GW/sb calc)	0.0129-0.0613 µg/L (SW/mm calc) 0.00262-0.00420 µg/L (GW/sb calc)
Linearity (calibration curve r and concentration range)	ECM[1]	r = 0.9990 (GW/sb Q & C) r = 0.9950 (SW/mm Q) r = 0.9955 (SW/mm C) r = 0.9995 (SW/sb Q) r = 0.9990 (SW/sb C)	r = 0.9995 (GW/sb Q & C) r = 0.9955 (SW/mm Q & C) r = 0.9995 (SW/sb Q & C)
	ILV[2]	r = 0.9996 (SW/mm Q) r = 0.9980 (SW/mm C) r = 0.9994/0.9997 (GW/sb Q) r = 0.9994/0.9986 (GW/sb C)	r = 0.9995 (SW/mm Q) r = 0.9978 (SW/mm C) r = 0.9997/0.9995 (GW/sb Q) r = 0.9994/0.9990 (GW/sb C)
	Range	0.400-5.00 µg/L	
Repeatable	ECM[3]	Yes for LOQ and 10×LOQ in characterized ground water (sb). Yes for LOQ and 10×LOQ in characterized surface water (sb & mm).	
	ILV[4,5]	Yes for LOQ and 10×LOQ in characterized surface water (mm); surface water (sb) not tested.	
		Yes for 10×LOQ in RSDs in characterized ground water (sb); **No** at LOQ (RSD **56**% Q & C),	**No** at LOQ (RSD **54**% Q & C) and 10×LOQ (RSD **24-25**% Q & C) in characterized ground water (sb).
Reproducible		Yes for 0.100 µg/L (LLMV)* and 1.00 µg/L in surface water (mm).	
		Yes for 1.00 µg/L (LLMV)* in ground water (sb); **No** at 0.100 µg/L.	**No** at 0.100 µg/L and 1.00 µg/L in ground water (sb) – no LLMV.
Specific	ECM	Yes, matrix interferences were <1% (based on peak area). Some contamination/baseline noise was observed.	Yes, matrix interferences were <2% (based on peak area). Some contamination/baseline noise was observed.
		No significant difference was observed between the representative mm and sb LOQ chromatograms.	
	ILV	Yes, matrix interferences were <10% (based on peak area). Significant contamination/baseline noise was observed in surface water.[6] Minor baseline noise affected peak integration in ground water.	Yes, matrix interferences were <1% (Q, based on peak area; 24% based on peak area in C - SW)[7]. Significant contamination/baseline noise was observed in surface water.[8] Minor baseline noise affected peak integration in ground water.

Data were obtained from pp. 26-29, 31-36 (LOQ/LOD); p. 30 (linearity coefficients); Tables 1-12, pp. 40-51 (recovery data); Figures 31-42, pp. 90-101 (calibration curves); Figures 1-30, pp. 60-89 (chromatograms) of MRID 50914302; pp. 24-27 (LOQ/LOD); p. 26 (linearity coefficients); Tables 1-8, pp. 36-43 (recovery data); Figures 1-2, pp. 49-50; Figures 13-14, pp. 56-57; Figures 25-28, pp. 63-66; Figures 45-48, pp. 75-78 (calibration curves); Figures 3-44, pp. 51-74 (chromatograms) of MRID 50931310; DER Attachment 2. Q = quantitative ion transition; C = confirmatory ion transition; SW = Surface water; GW = Ground water; mm = matrix-match calibration standards; sb = solvent-based calibration standards.

* 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. 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 (p. 30 of MRID 50914302; DER Attachment 2). Values were reported to four significant figures.

2 Two correlation coefficients were reported for the ILV ground water validations, one for the LOQ and one for the 10×LOQ validations (p. 26).

3 In the ECM, the water matrices were ground water and surface water (p. 16 of MRID 50914302). The ground water (not characterized) used in the study was filtered well water collected on site at Smithers Viscient, Wareham, which was prepared by filtering to remove any potential organic contaminants (p. 16 of MRID 50914302). The surface water (pH 5.96, dissolved oxygen concentration 10.3 mg/L) used for this method validation analysis was collected from the Taunton River in Massachusetts (SMV Lot No. 03Jan19 Wat-C, collected with *ca*. 30-60 cm depth of overlying water on 3 January 2019).

4 In the ILV, the Fountains Abbey surface water (sample code CS 14/18; pH 7.44, hardness 86 mg/L CaCO₃, conductivity 154 µS/cm, dissolved organic carbon 11.2 mg/L) obtained from The Lake, Studley Royal, Ripon, United Kingdom, and Borehole groundwater (sample code CS 13/18; pH 8.0, hardness 349 mg/L CaCO₃, conductivity 436 µS/cm, dissolved organic carbon 0.00 mg/L) obtained from Smithers Rapra were used (p. 16; Appendix 2, pp. 92-93 of MRID 50931310). Water characterization was performed by Smithers Viscient (ESG) Ltd., Harrogate, United Kingdom.

5 The method was validated by the ILV with the first trial for dicamba and DCSA in surface water at both fortifications and for dicamba and DCSA in ground water at the LOQ fortification and with the second trial for dicamba and DCSA in ground water at the 10×LOQ fortification (p. 28 of MRID 50931310). The first failed validation attempt for dicamba and DCSA in ground water at the 10×LOQ fortification was presumed to be due to the experimental error of not acidifying the samples prior to extraction. The ILV validated Smithers Viscient Analytical Method 14166.6104 as written with insignificant final dilution changes and analytical instrument and equipment modifications (pp. 17-24; Appendix 4, p. 106). No updated ECM was required.

6 Based on Figures 9-12, pp. 54-55 of MRID 50931310.

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

8 Based on Figures 21-24, pp. 61-62 of MRID 50931310.

IV. Method Deficiencies and Reviewer's Comments

1.   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 an LOQ (pp. 26-29, 31-36 of MRID 50914302; pp. 24-27 of MRID 50931310). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the reported method LOQ for both analytes in surface water using matrix-matched calibration standards; however, the LLMV for dicamba in ground water using solvent-based calibration standards was 1.00 µg/L (reported method LOQ = 0.100 µg/L). No LLMV was established by the ILV and ECM performance data for DCSA in ground water using solvent-based calibration standards.

2.   ILV performance data in ground water samples using solvent-based calibration standards was not acceptable for dicamba at the LOQ (56%; Q & C), DCSA at the LOQ (54%; Q & C), and DCSA at 1.00 µg/L (24-25%; Q & C; Tables 1-8, pp. 36-43). The means, standard deviation, and RSDs for these analyses were reviewer-calculated (n = 5) since the study report statistics excluded one of the recovery values because an error following the method was suspected or was designated as an outlier by Grubb's test (n = 4). OCSPP guidelines state that acceptable recovery results are defined as means of 70-120% and RSDs of ≤20%.

3.   The communications between the ILV laboratory and Study Monitor (Rachel Hardie, Wagner Regulatory Associates) were summarized and listed but not detailed (pp. 1, 26; Appendix 5, p. 107 of MRID 50914302). Communications involved protocol issue and informing the Study Monitor about the poor instrument sensitivity for dicamba during linearity checks, improved instrument sensitivity for dicamba after instrument re-optimization, success/results of ILV trial, and request for repeat of ground water trial at 10×LOQ. The reviewer also noted that the ECM laboratory (Smithers Viscient, Wareham) and ILV laboratory (Smithers ERS Limited, Harrogate) were part of the same company, but the reported laboratory personnel differed between the ECM and ILV (p. 5 of MRID 50914302; p. 6 of MRID 50931310). The ILV reported that the method was provided to the ILV via the ECM on behalf of the Sponsor (p. 13 of MRID 50931310).

      OCSPP 850.6100 guidance states that, if the laboratory that conducted the validation belonged to the same organization as the originating laboratory, 1) the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion, and 2) the analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies.

4.   The specificity of the method for surface water was not well-supported by ILV representative chromatograms since significant contamination/baseline noise was observed in surface water (Figures 9-12, pp. 54-55; Figures 21-24, pp. 61-62 of MRID 50931310).

5.   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. 26-29, 31-36 of MRID 50914302; pp. 24-27 of MRID 50931310). No justifications or calculations for the LOQ were reported in the ILV or ECM. The LOD was calculated in the ECM using the following equation: $LOD = (3x(N_{ctl})/(Resp_{LS}) \times Conc_{LS} \times DF_{CNTL}$, where, LOD is the limit of detection of the analysis, N_{ctl} is the mean noise in height of the control samples (or blanks), $Resp_{LS}$ is the mean response in height of the two low calibration standards, $Conc_{LS}$ is the concentration of the low calibration standard, and DF_{CNTL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 0.0500). The LOD was calculated in the ILV using the following equation: LOD (µg/L) = 3 × height of control baseline noise × control dilution factor × calibration standard concentration (µg/L) / height of calibration standard peak. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

      The Method Detection Limit (MDL) was also calculated in the ECM and ILV as 0.01 µg/L (pp. 26-29, 31-36 of MRID 50914302; pp. 24-27 of MRID 50931310). In the ECM, the MDL was defined as the lowest concentration in test samples which can be detected based on the concentration of the low calibration standard and the dilution factor of the control solutions. In the ILV, the MDL was calculated as the initial sample concentration equivalent to the lowest calibration standard (based upon a lowest standard concentration of 0.4 µg/L and a dilution factor of 0.05).

6.   In the ECM, no significant matrix effects were observed (<20%; pp. 29, 31-36; Tables 13-20, pp. 52-59 of MRID 50914302). In the ECM, an interference peak was noticed in the surface water which was resolved chromatographically and did not affect the validation analysis.

In the ILV, no significant matrix effects were observed for the analytes/matrices/ monitored ions, except for the confirmatory ion transition for DCSA in ground water (p. 27; Tables 9-12, pp. 44-47 of MRID 50931310).The ILV noted that the first matrix-assessment attempt was rejected due to poor instrument sensitivity for dicamba; rejected results were not provided. An interfering peak at the retention time if DCSA, which was large enough to cause a significant matrix effect, but small enough not impact the specificity of the method; therefore, solvent-based calibration standards were used for the ground water validation and matrix-matched calibration standards were used for the surface water validation.

7.   In the ILV, it was reported that "On 10 September 2019, the legal name of Smithers Viscient Ltd was changed to Smithers ERS Limited. The legal entity did not change. Smithers ERS Limited is a business unit of The Smithers Group that is engaged in providing contract research services." (p. 13 of MRID 50931310).

8.   The time requirement for the method was not reported in the ECM 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 acid (Dicamba)

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
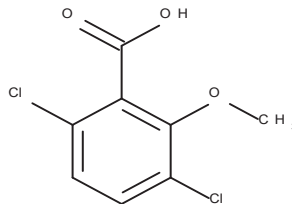
DCSA (3,6-Dichlorosalicyclic acid)

IUPAC Name:	3,6-Dichloro-2-hydroxybenzoic acid
CAS Name:	3,6-Dichlorosalicylic acid
CAS Number:	3401-80-7
SMILES String:	O=C(O)c(c(ccc1Cl)Cl)c1O