

Analytical method for cycloate in water

Reports: ECM: EPA MRID No. 50730601. DeVellis, S.R. 2018. Validation of an Environmental Chemistry Method for the Determination of Cycloate in Groundwater and Surface Water by LC-MS/MS. Smithers Viscient Study No.: 14113.6130. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored and submitted by Helm Agro US, Inc., Tampa, Florida; 58 pages. Final report issued April 11, 2018.

ILV: EPA MRID No. 50730603. Cashmore, A. 2018. Independent Laboratory Validation of Analytical Method 14113.6130 for the Determination of Cycloate in Ground Water and Surface Water. Laboratory Project ID: 3202260. Report prepared by Smithers Viscient (ESG) Ltd., North Yorkshire, United Kingdom, and sponsored and submitted by Helm Agro US, Inc., Tampa, Florida; 68 pages (including one blank page). Final report issued October 26, 2018.

Document No.: MRID 50730601 & 50730603

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, 40 CFR, Part 160, as well as OECD GLP (p. 3 of MRID 50730601). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An authenticity statement was included with the QA statement.

ILV: The study was conducted in accordance with UK Health Department GLP Regulations, and OECD GLP standards, which are also accepted by the USEPA and Japanese authorities, except for the characterization of the surface water (p. 3; Appendix 3, p. 51 of MRID 50730603). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5; Appendix 3, p. 51).

Classification: This analytical method is classified as acceptable. The ILV surface water was sourced from the same place as the ECM surface water.

PC Code: 041301


**EFED Final
Reviewer:**

James Lin
Environmental Engineer


Signature: 
Date: 03/25/2020

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Reviewers:**

Lisa Muto, M.S.,
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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 JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Smithers Viscient Study No. 14113.6130, is designed for the quantitative determination of cycloate in water at the LOQ of 0.100 µg/L using LC/MS. The LOQ is less than the lowest toxicological level of concern in water. The ECM and ILV were performed using two water matrices, groundwater and surface water. The ECM groundwater was not characterized. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method since the ILV surface water was sourced from the same place as the ECM surface water. The ILV validated the ECM method in the first trial with insignificant modifications to the analytical instrumentation and parameters. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for cycloate in both water matrices.

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						
Cycloate	50730601	50730603		Water ^{1,2}	11/04/2018	Helm Agro US, Inc.	LC/MS/MS	0.100 µg/L

1 In the ECM, the groundwater (not characterized) was collected as filtered well water from Smithers Viscient, Wareham, Massachusetts (pp. 11-12 of MRID 50730601). The surface water (SMV Lot No.12 Jul 17 Water-A; pH 6.2, dissolved oxygen concentration 5.92 mg/L) was collected from the Weweantic River in Taunton, Massachusetts.

2 In the ILV, the groundwater (CS 13/18; pH 8.0, hardness 349 mg/L CaCO₃, 0.00 mg/L dissolved organic carbon, electrical conductivity 436 µS/cm) was collected from a bore hole at Smithers Rapra, United Kingdom by Smithers Viscient Harrogate (p. 12; Appendix 2, p. 50 of MRID 50730603). The surface water (ID# 19Mar18 WAT-B; 18/0000000/18; pH 6.2, dissolved oxygen concentration 5.92 mg/L) was collected from the Weweantic River in Taunton, Massachusetts and provided by Smithers Viscient, Wareham, Massachusetts. The surface water source was the same as that of the ECM.

I. Principle of the Method

Water samples (8 mL) were fortified, if necessary, with 0.08 or 0.80 mL of 0.01 mg/L fortification solution (pp. 14-16 of MRID 50730601). The sample was diluted to 10 mL with methanol. The high fortification samples were further diluted by taking 4 mL of the sample and diluting to 10 mL using methanol:test water (20:80, v:v).

Samples were analyzed for cycloate using a Shimadzu LC-20ADXR HPLC system coupled to an Applied Biosystems API 5000 mass spectrometer (pp. 11, 16-17 of MRID 50730601). The LC/MS conditions consisted of a Waters Atlantis T3 column (100 x 4.6 mm, 3.0 µm particle

size; oven temperature 40°C) with a mobile phase gradient of A) 0.1% formic acid in water and B) 0.1% formic acid in acetonitrile [percent A:B (v:v) at 0.01-0.50 min. 95.0:5.00, 0.60 min. 15.0:85.0, 4.50-5.00 min. 0.00:100, 5.10-6.00 min. 95.0:5.00] and electrospray ionization (ESI) interface MS detection in positive ion mode with MRM (source temperature 600°C). Injection volume was 50.0 µL. The primary and confirmatory ion transitions were m/z 216.18→82.97 and m/z 216.18→54.95, respectively. Expected retention time was *ca.* 4.1 minutes for cycloate.

In the ILV, the ECM was performed as written, except for the use of a different LC/MS system (pp. 13-16, 20 of MRID 50730603). A Shimadzu Nexera series HPLC system with AB Sciex API 5000 MS/MS detector was used. All LC/MS conditions were the same, except that MS temperature was 550°C. The primary and confirmatory ion transitions were m/z 215.90→83.00 and m/z 215.90→54.95, respectively. Expected retention time was *ca.* 3.8 minutes for cycloate.

The Limit of Quantification (LOQ) for cycloate in water was 0.100 µg/L in the ECM and ILV (pp. 11, 18-21 of MRID 50730601; pp. 18-20 of MRID 50730603). The Limit of Detection (LOD) for cycloate in water was calculated to be 0.0105-0.0149 µg/L for ground water and 0.0293-0.0297 µg/L for surface water in the ECM; the LOD was calculated to be 0.00367-0.00587 µg/L for ground water and 0.0133-0.0206 µg/L for surface water in the ILV.

II. Recovery Findings

ECM (MRID 50730601): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of cycloate in two water matrices at fortification levels of 0.100 µg/L (LOQ) and 1.00 µg/L (10×LOQ; p. 21; Tables 1-4, pp. 27-30). Cycloate was identified using two ion transitions; primary and confirmatory recovery results were comparable. The groundwater (not characterized) was collected as filtered well water from Smithers Viscient, Wareham, Massachusetts (pp. 11-12). The surface water (SMV Lot No.12 Jul 17 Water-A; pH 6.2, dissolved oxygen concentration 5.92 mg/L) was collected from the Weweantic River in Taunton, Massachusetts.

ILV (MRID 50730603): Mean recoveries and RSDs were within guideline requirements for analysis of cycloate in two water matrices at fortification levels of 0.100 µg/L (LOQ) and 1.00 µg/L (10×LOQ; p. 19; Tables 1-4, pp. 24-27). Cycloate was identified using two ion transitions; primary and confirmatory recovery results were comparable. The groundwater (CS 13/18; pH 8.0, hardness 349 mg/L CaCO₃, 0.00 mg/L dissolved organic carbon, electrical conductivity 436 µS/cm) was collected from a bore hole at Smithers Rapra, United Kingdom by Smithers Viscient Harrogate (p. 12; Appendix 2, p. 50). The surface water (ID# 19Mar18 WAT-B; 18/0000000/18; pH 6.2, dissolved oxygen concentration 5.92 mg/L) was collected from the Weweantic River in Taunton, Massachusetts and provided by Smithers Viscient, Wareham, Massachusetts. The surface water source was the same as that of the ECM. The ILV validated the ECM method in the first trial with insignificant modifications to the analytical instrumentation and parameters (pp. 13-16, 20; Appendix 4, p. 52).

Table 2. Initial Validation Method Recoveries for Cycloate in Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Groundwater						
Quantitation ion transition						
Cycloate	0.100 (LOQ)	7	99.7-114	107	4.75	4.43
	1.00	5	104-110	108	2.13	1.98
Confirmation ion transition						
Cycloate	0.100 (LOQ)	7	104-114	108	3.33	3.09
	1.00	5	103-111	108	3.02	2.80
Surface Water						
Quantitation ion transition						
Cycloate	0.100 (LOQ)	7	98.4-119	110	6.80	6.20
	1.00	5	95.4-115	111	9.90	8.93
Confirmation ion transition						
Cycloate	0.100 (LOQ)	7	96.1-119	106	8.01	7.54
	1.00	5	95.5-122	110	10.0	9.12

Data (uncorrected recovery results, pp. 19-20) were obtained from p. 21; Tables 1-4, pp. 27-30 of MRID 50730601.

1 The groundwater (not characterized) was collected as filtered well water from Smithers Viscient, Wareham, Massachusetts (pp. 11-12). The surface water (SMV Lot No.12 Jul 17 Water-A; pH 6.2, dissolved oxygen concentration 5.92 mg/L) was collected from the Weweantic River in Taunton, Massachusetts.

2 The primary and confirmatory ion transitions were m/z 216.18→82.97 and m/z 216.18→54.95, respectively.

Table 3. Independent Validation Method Recoveries for Cycloate in Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Groundwater						
Quantitation ion transition						
Cycloate	0.100 (LOQ)	7	99-103	101	1.5	1.5
	1.00	5	94-103	98	3.9	4.0
Confirmation ion transition						
Cycloate	0.100 (LOQ)	7	97-103	101	1.9	1.9
	1.00	5	93-102	98	3.8	3.8
Surface Water						
Quantitation ion transition						
Cycloate	0.100 (LOQ)	7	94-107	102	4.2	4.1
	1.00	5	94-99	96	2.2	2.3
Confirmation ion transition						
Cycloate	0.100 (LOQ)	7	94-112	102	6.7	6.5
	1.00	5	96-100	98	1.5	1.6

Data (uncorrected recovery results, p. 17) were obtained from p. 19; Tables 1-4, pp. 24-27 of MRID 50730603.

1 The groundwater (CS 13/18; pH 8.0, hardness 349 mg/L CaCO₃, 0.00 mg/L dissolved organic carbon, electrical conductivity 436 µS/cm) was collected from a bore hole at Smithers Rapra, United Kingdom by Smithers Viscient Harrogate (p. 12; Appendix 2, p. 50). The surface water (ID# 19Mar18 WAT-B; 18/0000000/18; pH 6.2, dissolved oxygen concentration 5.92 mg/L) was collected from the Weweantic River in Taunton, Massachusetts and provided by Smithers Viscient, Wareham, Massachusetts. The surface water source was the same as that of the ECM.

2 The primary and confirmatory ion transitions were m/z 215.90→83.00 and m/z 215.90→54.95, respectively. These were similar to those of the ECM.

III. Method Characteristics

The LOQ for cycloate in water was 0.100 µg/L in the ECM and ILV (pp. 11, 18-21 of MRID 50730601; pp. 18-20 of MRID 50730603). The LOQ in the ECM was defined as the lowest fortification level; in the ILV, the LOQ was based on the lowest level validated. The LOD for cycloate in water was calculated to be 0.0105-0.0149 µg/L for ground water and 0.0293-0.0297 µg/L for surface water in the ECM; the LOD was calculated to be 0.00367-0.00587 µg/L for ground water and 0.0133-0.0206 µg/L for surface water in the ILV. In the ECM and ILV, the LOD was calculated using the standard deviation of the average recovery in units of concentration of the seven samples fortified at the LOQ, multiplied by one-tailed t-statistic at the 99% confidence level for n-1 replicates. The LOD was calculated for each matrix using the following equation, according to the U.S. EPA, 2016, 1994, Definition and Procedure for the Determination of the Method Detection Limit, Revision 1.11 and Revision 2.:

$$\text{LOD} = t_{0.99} \times \text{SD} + \text{average residue in the untreated controls}$$

Where, $t_{0.99}$ is the one-tailed t statistic for $n = 7$ (3.143) and SD is the standard deviation of the analyte recovery measurements at the target LOQ. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV

Additionally, the Method Detection Limit (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. The MDL was calculated as 0.0625 µg/L in the ECM and ILV.

Table 4. Method Characteristics

Analyte		Cycloate	
Matrix		Groundwater	Surface water
Limit of Quantitation (LOQ)	ECM	0.100 µg/L	
	ILV		
Limit of Detection (LOD)	ECM	0.0149 µg/L (Q) 0.0105 µg/L (C)	0.0297 µg/L (Q) 0.0293 µg/L (C)
	ILV	0.00367 µg/L (Q) 0.00587 µg/L (C)	0.0133 µg/L (Q) 0.0206 µg/L (C)
Linearity (calibration curve r^2 and concentration range)	ECM	$r^2 = 0.995$ (Q) $r^2 = 0.997$ (C)	$r^2 = 0.997$ (Q) $r^2 = 0.996$ (C)
	ILV	$r^2 = 0.9964$ (Q) $r^2 = 0.9953$ (C)	$r^2 = 0.9988$ (Q & C)
	Range	0.05-0.5 µg/L	
Repeatable	ECM ¹	Yes at LOQ and 10×LOQ (uncharacterized water matrix)	Yes at LOQ and 10×LOQ (characterized water matrix)
	ILV ^{2,3}	Yes at LOQ and 10×LOQ (two characterized water matrices)	
Reproducible	Yes at LOQ and 10×LOQ		
Specific	ECM	Yes, matrix interferences were <10% of the LOQ (based on peak area). Minor baseline noise was noted.	
	ILV	Yes, no matrix interferences were observed. Minor baseline noise was noted which interfered with peak attenuation and integration at LOQ.	

Data were obtained from pp. 11, 18-21 (LOQ/LOD); p. 21; Tables 1-4, pp. 27-30 (recovery data); p. 21; Figures 11-14, pp. 45-48 (calibration data & curves); Figures 1-10, pp. 35-44 (chromatograms) of MRID 50730601; pp. 18-20 (LOQ/LOD); p. 19; Tables 1-4, pp. 24-27 (recovery data); p. 19; Figures 1-2, pp. 31-32; Figures 15-16, pp. 39-40 (calibration data & curves); Figures 3-14, pp. 33-38; Figures 17-28, pp. 41-46 (chromatograms) of MRID 50730603. Q = Quantitation ion transition; C = Confirmation ion transition.

1 In the ECM, the groundwater (not characterized) was collected as filtered well water from Smithers Visient, Wareham, Massachusetts (pp. 11-12 of MRID 50730601). The surface water (SMV Lot No.12 Jul 17 Water-A; pH 6.2, dissolved oxygen concentration 5.92 mg/L) was collected from the Weweantic River in Taunton, Massachusetts.

2 In the ILV, the groundwater (CS 13/18; pH 8.0, hardness 349 mg/L CaCO₃, 0.00 mg/L dissolved organic carbon, electrical conductivity 436 µS/cm) was collected from a bore hole at Smithers Rapra, United Kingdom by Smithers Visient Harrogate (p. 12; Appendix 2, p. 50 of MRID 50730603). The surface water (ID# 19Mar18 WAT-B; 18/0000000/18; pH 6.2, dissolved oxygen concentration 5.92 mg/L) was collected from the Weweantic River in Taunton, Massachusetts and provided by Smithers Visient, Wareham, Massachusetts. The surface water source was the same as that of the ECM.

3 The ILV validated the ECM method in the first trial with insignificant modifications to the analytical instrumentation and parameters (pp. 13-16, 20; Appendix 4, p. 52 of MRID 50730603).

IV. Method Deficiencies and Reviewer's Comments

1. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 11, 18-21 of MRID 50730601; pp. 18-20 of MRID 50730603). The LOQ in the ECM was defined as the lowest fortification level; in the ILV, the LOQ was based on the lowest level validated. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM and ILV. Detection limits should not be based on arbitrary values. In the ECM and ILV, the LOD was calculated using the standard deviation of the average recovery in units of concentration of the seven samples fortified at the LOQ, multiplied by one-tailed t-statistic at the 99% confidence level for n-1 replicates. The LOD was calculated for each matrix using the following equation, according to the U.S. EPA, 2016, 1994, Definition and Procedure for the Determination of the Method Detection Limit, Revision 1.11 and Revision 2.
2. Communications between the Study Sponsor from Helm Agro US, Inc. and ILV reportedly involved exchange of study protocol and successful validation results (Appendix 5, p. 53 of MRID 50730603). Details of communications were not reported.
3. The matrix effects were assessed in the ECM or ILV; matrix-based standards were used in the ECM and ILV although no matrix effects were observed (pp. 23-24; Tables 5-8, pp. 31-34 of MRID 50730601; pp. 10, 20; Tables 5-6, pp. 28-29 of MRID 50730603).
4. It was reported for the ILV that one sample set of 15 samples required *ca.* 8 hours for sample processing and 5 hours for automated LC/MS/MS analysis (p. 13 of MRID 50730603).

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**Cycloate**

IUPAC Name: S-ethyl cyclohexyl(ethyl)thiocarbamate
CAS Name: S-ethyl N-cyclohexyl-N-ethylcarbamothioate
CAS Number: 1134-23-2
SMILES String: CCSC(=O)N(CC)C1CCCCC1

