

Analytical method for sodium chlorate in soil

Reports: ECM: EPA MRID No.: 50888901. Rebstock, M. 2019. Determination of Sodium Chlorate in Soil by LC-MS/MS. Eurofins Method No.: 87636-M. Report prepared by Eurofins EAG Agrosience, LLC, Columbia, Missouri, and sponsored and submitted by Drexel Chemical Company, Memphis, Tennessee; 27 pages. Final report issued June 18, 2019.

ILV: EPA MRID No. 50943001. Keenan, D. 2019. Independent Laboratory Validation of the Analytical Method for the Determination of Sodium Chlorate in Soil by LC-MS/MS. EAG Project No.: 3210W. Report prepared by Eurofins EAG Agrosience, LLC, Hercules, California, and sponsored and submitted by Drexel Chemical Company, Memphis, Tennessee; 46 pages. Final report issued September 11, 2019.

Document No.: MRIDs 50888901 & 50943001

Guideline: 850.6100

Statements: ECM: The study was not required to be conducted in accordance with US EPA FIFRA (40 CFR Part 160) and OECD Good Laboratory Practice (GLP) standards but was conducted in a GLP compliant facility (p. 3 of MRID 50888901). Signed and dated Data Confidentiality and GLP statements were provided (pp. 2-3). Authenticity and Quality Assurance statements were not provided. A signed and dated Method Approval page was provided (p. 4).
ILV: The study was conducted in accordance with US EPA FIFRA (40 CFR Part 160) GLP, with the exception that the reference standards were commercial product which did not specify that characterization was performed according to GLP (p. 3 of MRID 50943001). Signed and dated Data Confidentiality, GLP, Quality Assurance, Authenticity statements were provided (pp. 2-4).

Classification: This analytical method is classified as **supplemental**. Lower level ILV performance data (0.01 mg/kg) was not within guideline requirements and the specificity of the method was marginally not supported by ILV representative chromatograms. The higher level ILV performance data (0.1 mg/kg) is within guideline requirements and accepted as the lowest level of method validation (LLMV).

PC Code: 073301

EFED Final Reviewer: Andrew Shelby,
Physical Scientist

Signature: 
Date: December 2, 2020

CDM/CSS-Dynamac JV Lisa Muto,
Environmental Scientist

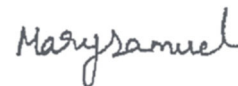
Signature: 

Reviewers:

Mary Samuel, M.S.,
Environmental Scientist

Date: 11/12/2019

Signature:



Date: 11/12/2019

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, Eurofins Method No. 87636-M, is designed for the quantitative determination of sodium chlorate in soil at 0.01 mg/kg using LC/MS/MS though only the higher level of 0.1 mg/kg is validated. The lowest level of method validation (LLMV) of 0.1 mg/kg is **less than** the lowest toxicological level of concern in soil for sodium chlorate. The ECM and ILV validated the method using one uncharacterized soil matrix each. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method or if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. The ILV validated the method for sodium chlorate in soil in the first trial as written, except for minor LC/MS modifications to instrument and parameters. The ILV LLMV performance data was acceptable; however, ILV 0.01 mg/kg performance data was not within guideline requirements. ILV linearity data was acceptable; however, the specificity of the method was not supported by ILV representative chromatograms since matrix interferences were *ca.* 6-34% of the 0.01 mg/kg level. Matrix interference would need to be less than 30% to meet guideline requirements for specificity. All submitted ECM data pertaining to precision, repeatability, reproducibility, linearity, and specificity was acceptable.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Attempted Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Sodium chlorate	50888901 ¹	50943001 ³	Supple mental	Soil	18/06/2019	Drexel Chemical Company	LC/MS/ MS	0.01 mg/kg

¹ In ECM, the soil was obtained from an in-progress terrestrial field dissipation study located in Georgia (Eurofins Study No. S18-05007; pp. 6, 14 of MRID 50888901). The soil characterization was not provided.

² In the ILV, the soil (3210W-001) was not characterized or described in the study (p. 12 of MRID 50943001). The soil was provided to the ILV by the ECM.

I. Principle of the Method

Soil samples (5.00 ± 0.01 g) in 50-mL polypropylene centrifuge tubes were fortified (0.100 and 0.0100 mg/L sodium chlorate fortification solution in water), as necessary (pp. 9-10; Appendix I, p. 16 of MRID 50888901). The sample was extracted twice with 25 mL of water via Geno/Grinder® for 3 minutes at 1200 strokes per minute using two ¼ inch steel bearings. After centrifugation (*ca.* 3500 rpm for *ca.* 3 minutes), the supernatants were transferred to 50-mL centrifuge tubes. The volume was adjusted to 50 mL with water, then *ca.* 1.5 mL of the extract was filtered using a 0.45 µm PTFE syringe filter into a HPLC autosampler vial. The samples were diluted further with water, if necessary, and analyzed by LC/MS/MS.

Sodium chlorate was identified and quantified by LC/MS/MS using an Applied Biosystems/Sciex API 5000 LC/MS/MS (p. 11 of MRID 50888901). The following conditions were employed: Intakt Scherzo SM-C18 MF analytical column (50 mm x 2 mm, 3-µ particle size; column temperature 40°C) eluted with a gradient mobile phase of (A) 0.15% aqueous formic acid and (B) 20mM aqueous ammonium formate [time, percent A:B; time 0.00 min. 95:5, 3.00-4.00 min. 5:95, 4.01-5.00 min. 95:5] using an injection volume of 10 µL and negative TIS (Turbo Ion Spray) ionization MRM scan mode (source temperature 600°C). Sodium chlorate was identified using two ion transitions (quantitation and confirmation, respectively): m/z 83→67 and m/z 83→51. Expected retention time was *ca.* 2.2 minutes for sodium chlorate.

In the ILV, the ECM was performed as written, except for minor LC/MS modifications to instrument and parameters (pp. 12-17, 22; Figure 1, p. 25 of MRID 50943001). The sample filtration prior to HPLC analysis was not reported in the extraction method list but was listed in the procedure scheme. Sodium chlorate was identified and quantified by LC/MS/MS using a Dionex UltiMate 3000 HPLC coupled with either an Applied Biosystems API 4000 or 5500 tandem mass spectrometer. The following HPLC conditions were employed for ILV analysis: Phenosphere 5 µ SAX analytical column (150 mm x 2 mm, 5 µm particle size; column temperature 40°C) eluted with a gradient mobile phase of (A) 0.15% aqueous formic acid and (B) 20mM aqueous ammonium carbonate [time, percent A:B; 0 min. 95:5, 1.0-6.0 min. 5:95, 6.1-8.1 min. 95:5] using an injection volume of 20 µL and negative ESI (electrospray ionization) MRM scan mode (source temperature 700°C for API4000 or 550°C for API5500). Sodium chlorate was identified using two ion transitions (quantitation and confirmation, respectively): m/z 83.0→67.0 and m/z 83.0→51.0; the MS transitions were the same as those of ECM. Expected retention time was *ca.* 3.5-4.2 minutes for sodium chlorate. For matrix assessment, the following HPLC conditions were used: Shimadzu LC-30AD HPLC system equipped with a Phenosphere 5 µ SAX analytical column (150 mm x 2 mm, 5 µm particle size; column temperature 40°C) eluted with a gradient mobile phase of (A) 0.15% aqueous formic acid and (B) 20mM aqueous ammonium carbonate [time, percent A:B; 0.02 min. 95:5, 1.02-5.02 min. 5:95, 5.12-7.02 min. 95:5] using an injection volume of 20 µL. Retention time, MS conditions, and MS monitored transitions were the same as those for the ILV analysis.

The attempted Limit of Quantification (LOQ) and Limit of Determination (LOD) for sodium chlorate in soil were 0.01 mg/kg and 0.003 mg/kg, respectively, in the ECM and ILV (p. 6 of MRID 50888901; p. 9 of MRID 50943001).

II. Recovery Findings

ECM (MRID 50888901): Mean recoveries and RSDs were within guideline requirements [means between 70% and 120% and relative standard deviations (RSD) $\leq 20\%$] for analysis of sodium chlorate at fortification levels of 0.01 mg/kg and 0.1 mg/kg in one soil matrix (p. 6). Sodium chlorate was identified using two ion transitions; performance data (recovery results) for the quantitation and confirmation ion analyses were comparable. The soil was obtained from an in-progress terrestrial field dissipation study located in Georgia (Eurofins Study No. S18-05007; pp. 6, 14). The soil characterization is available in MRID 50954001.

ILV (MRID 50943001): Mean recoveries and RSDs were within guideline requirements for analysis of sodium chlorate at the fortification level 0.1 mg/kg in one soil matrix; mean recoveries and RSDs were not within guideline requirements for analysis of sodium chlorate at 0.01 mg/kg [mean 133% (Q) and 134% (C), RSD 66% (Q) and 59% (C); Table 1, p. 24; Figure 12, p. 37; DER Attachment 2]. Means, standard deviations, and relative standard deviations at the 0.01 mg/kg were reviewer-calculated since the study report excluded the recovery value of one sample due to identification as an outlier. Study-reported means and RSDs at the 0.01 mg/kg level based on four samples were as follows: mean 94% (Q) and 99% (C); and RSD 1% (Q) and 6% (C). Sodium chlorate was identified using two ion transitions; performance data (recovery results) for the quantitation and confirmation ion analyses were comparable. The soil (3210W-001) was not characterized or described in the study (p. 12). The soil was provided to the ILV by the ECM. The ILV validated the method for sodium chlorate in soil in the first trial as written, except for minor LC/MS modifications to instrument and parameters (pp. 12-17, 21-22).

Table 2. Initial Validation Method Recoveries for Sodium Chlorate in Soil^{1,2}

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil						
Quantitation Ion Transition						
Sodium chlorate	0.01	5	98-108	103	3.7	3.6
	0.1 (LLMV)	5	95-104	100	3.2	3.2
Confirmation Ion Transition						
Sodium chlorate	0.01	5	95-102	97	2.7	2.8
	0.1 (LLMV)	5	97-101	99	1.5	1.5

Data (uncorrected recovery results, pp. 12-13) were obtained from p. 6 of MRID 50888901.

1 The soil was obtained from an in-progress terrestrial field dissipation study located in Georgia (Eurofins Study No. S18-05007; pp. 6, 14). The soil characterization was not provided.

2 Sodium chlorate was identified using two ion transitions (quantitation and confirmation, respectively): m/z 83→67 and m/z 83→51.

Table 3. Independent Validation Method Recoveries for Sodium Chlorate in Soil^{1,2}

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil						
Quantitation Ion Transition						
Sodium chlorate	0.01	5 ³	93-288	133	87	66
	0.1 (LLMV)	5	89-106	95	6	7
Confirmation Ion Transition						
Sodium chlorate	0.01	5 ³	92-276	134	79	59
	0.1 (LLMV)	5	90-110	97	8	8

Data (uncorrected recovery results, pp. 17-19; Figure 12, p. 37) were obtained from Table 1, p. 24 and Figure 12, p. 37 of MRID 50943001 and DER Attachment 2.

1 The soil (3210W-001) was not characterized or described in the study (p. 12). The soil was provided to the ILV by the ECM.

2 Sodium chlorate was identified using two ion transitions (quantitation and confirmation, respectively): m/z 83.0→67.0 and m/z 83.0→51.0; the MS transitions were the same as those of ECM.

3 Means, standard deviations, and relative standard deviations were reviewer-calculated since the study report excluded the recovery value of one sample due to identification as an outlier (see DER Attachment 2).

III. Method Characteristics

The attempted LOQ and LOD for sodium chlorate in soil were 0.01 mg/kg and 0.003 mg/kg, respectively, in the ECM and ILV (p. 6 of MRID 50888901; pp. 9, 19-20, 22 of MRID 50943001). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated with a mean recovery of 70-120% and a relative standard deviation of ≤20%. In the ILV, the LOQ was reported from the ECM without justification. The LOD for sodium chlorate in soil was 0.003 mg/kg (30% of the LOQ) in the ECM and ILV. No calculations were provided in support of the LOQ or LOD in the ECM and ILV.

Table 4. Method Characteristics

Analyte		Sodium chlorate
Attempted Limit of Quantitation (LOQ)	ECM	0.01 mg/kg
	ILV	
Limit of Detection (LOD)	ECM	0.003 mg/kg
	ILV	
Linearity (calibration curve r^2 and concentration range) ¹	ECM	$r^2 = 0.9970$ (Q) $r^2 = 0.9976$ (C)
	ILV	$r^2 = 0.9994$ (Q) $r^2 = 1.0000$ (C)
	Range	0.3000-10.00 ng/mL
Repeatable	ECM ²	Yes at 0.01 mg/kg and 0.1 mg/kg (one uncharacterized soil).
	ILV ^{3,4,5}	Yes at 0.1 mg/kg, but No at 0.01 mg/kg (one uncharacterized soil).
Reproducible		Yes at 0.1 mg/kg; No at 0.01 mg/kg.
Specificity	ECM	Yes, no matrix interferences were observed. Insignificant baseline noise interfered with the 0.01 mg/kg analyte peak.
	ILV	No , matrix interferences were <i>ca.</i> 6-34% of the 0.01 mg/kg level (based on quantified concentration). Matrix interference would need to be less than 30% to meet guideline requirements for specificity ⁶

Data were obtained from p. 6 (LOQ/LOD); p. 6 (recovery data); p. 28 (calibration coefficients); Figures 1-2, pp. 18-19 (calibration curve); Figures 6-8, pp. 23-25 (chromatograms) of MRID 50888901; pp. 9, 19-20, 22 (LOQ/LOD); Table 1, p. 24; Figure 12, p. 37 (recovery data); pp. 19, 21; Figures 7-8, pp. 31-32 (calibration curves); Figures 8-11, pp. 33-36 (chromatograms) of MRID 50943001; DER Attachment 2. Q = Quantitation ion transition; C = Confirmatory ion transition.

- 1 Reported ECM and ILV correlation coefficients were reviewer-calculated from r values reported in the study report (pp. 9-10; Figures 1-2, pp. 18-19 of MRID 50888901; pp. 19, 21; Figures 7-8, pp. 31-32 of MRID 50943001; DER Attachment 2). Solvent-based calibration standards were used.
- 2 In ECM, the soil was obtained from an in-progress terrestrial field dissipation study located in Georgia (Eurofins Study No. S18-05007; pp. 6, 14 of MRID 50888901).
- 3 In the ILV, the soil (3210W-001) was not characterized or described in the study (p. 12 of MRID 50943001). The soil was provided to the ILV by the ECM.
- 4 The ILV validated the method for sodium chlorate in soil in the first trial as written, except for minor LC/MS modifications to instrument and parameters (pp. 12-17, 21-22 of MRID 50943001).
- 5 For the ILV, means, standard deviations, and relative standard deviations at the LOQ were reviewer-calculated since the study report excluded the recovery value of one sample due to identification as an outlier (Table 1, p. 24; Figure 12, p. 37 of MRID 50943001). Study-reported means and RSDs at the LOQ based on four samples were as follows: mean 94% (Q) and 99% (C); and RSD 1% (Q) and 6% (C).
- 6 Based on concentration values reported by ILV study authors (Figure 12, p. 37 of MRID 50943001).

IV. Method Deficiencies and Reviewer's Comments

1. The ILV 0.01 mg/kg level performance data was not within guideline requirements: mean 133% (Q) and 134% (C), RSD 66% (Q) and 59% (C; Table 1, p. 24; Figure 12, p. 37 of MRID 50943001; DER Attachment 2). Means, standard deviations, and relative standard deviations at the LOQ were reviewer-calculated since the study report excluded the recovery value of one sample due to identification as an outlier. Study-reported means and RSDs at the LOQ based on four samples were as follows: mean 94% (Q) and 99% (C); and RSD 1% (Q) and 6% (C). OCSPP guidelines state that mean recoveries are between 70% and 120% and RSDs are $\leq 20\%$.
2. The specificity of the method was not supported by ILV representative chromatograms since matrix interferences were *ca.* 6-34% of the 0.01 mg/kg level (based on quantified concentration; Figures 8-12, pp. 33-37 of MRID 50943001). Matrix interferences should not exceed the LOD. Matrix interference would need to be less than 30% to meet guideline requirements for specificity
3. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method since the soil matrix was uncharacterized (p. 12 of MRID 50943001). The reviewer noted that the ILV soil was provided by the ECM.

The ECM soil matrix was not characterized but was described as obtained from an in-progress terrestrial field dissipation study located in Georgia (Eurofins Study No. S18-05007; pp. 6, 14 of MRID 50888901).

4. In the ECM, no reagent blank was present (p. 12 of MRID 50888901).
5. Although the ECM and ILV were conducted by Eurofins EAG Agrosience, LLC, the ECM was conducted at the Missouri laboratory location, and the ILV was conducted at the California laboratory location (p. 1 of MRID 50888901; pp. 1, 5 of MRID 50943001). No personnel overlapped between the ECM and ILV. Laboratory equipment differed between the ECM and ILV. Therefore, it was determined that the ILV was conducted independently of the ECM.
6. The ILV stated that no communication occurred between the ILV and the method developers during the study (p. 22 of MRID 50943001).
7. Matrix effects were studied in the ILV and determined to be insignificant for sodium chlorate (pp. 13-14, 20-21 of MRID 50943001). Solvent-based calibration standards were used for all analyses.
8. The estimations of LOQ and LOD in the ECM and ILV by the study authors were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 6 of MRID 50888901; pp. 9, 19-20, 22 of MRID 50943001). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated with a mean recovery of 70-120% and a relative standard deviation of

≤20%. In the ILV, the LOQ was reported from the ECM without justification. The LOD for sodium chlorate in soil was defined as 30% of the LOQ in the ECM and ILV. No calculations were provided in support of the LOQ or LOD in the ECM and ILV. Detection limits should not be based on arbitrary values. Based on this, the reviewer as determined 0.1 mg/kg to be the lowest level of method validation (LLMV).

9. It reported for the ILV that one sample set of 14 specimens required *ca.* 4 hours for standard solution preparation, *ca.* 4 hours for sample preparation, and *ca.* 2 hours for LC/MS/MS analysis and evaluation (p. 20 of MRID 50943001). Therefore, performing the method for one sample set of 14 specimens required *ca.* 10 hours for one analyst to complete.

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**Sodium chlorate****IUPAC Name:** Sodium chlorate**CAS Name:** Sodium chlorate**CAS Number:** 7775-09-9**SMILES String:** Not found