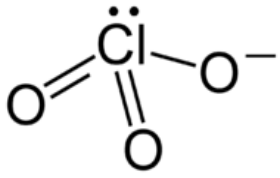
INTRODUCTION

The purpose of this study was to conduct an independent laboratory validation (ILV) for the determination of sodium chlorate in soil. The analysis of sodium chlorate was performed by Liquid Chromatography with tandem mass-spectrometric detection (LC-MS/MS), based on the method described in "Determination of Sodium Chlorate in Soil by LC-MS/MS ", Eurofins Method No. 87636-M, June 18, 2019. (Reference 1).

This study was designed to satisfy data requirements outlined in the U.S. EPA Residue Test Guideline, OPPTS 850.6100 (Reference 2). The study was initiated on July 23, 2019. The experimental work was conducted from July 26, 2019 through August 13, 2019 at Eurofins EAG Agroscience LLC., 625-B Alfred Nobel Drive, Hercules, CA 94547, under the approved protocol provided in Appendix A, according to the US EPA FIFRA Good Laboratory Practice Standards, 40 CFR §160.

MATERIAL AND METHODS

Test/Reference Substances

Common Name:	**Chlorate**
Lot Number:	4-184CLO3-2Y
Inventory Number:	3210W-002
Chemical Structure:	
Molecular Formula:	ClO_3-
Purity:	1000 µg/mL ± 5 µg/mL
Date of Expiry:	July 20, 2020
Storage Conditions:	Refrigerated

A copy of the Certificate of Analysis for the test/reference substance is provided in Appendix B.

Other Chemicals

HPLC grade water, was obtained from Burdick & Jackson. Optima grade formic acid and Optima grade water was obtained from Fisher Scientific. LC-MS grade ammonium formate was obtained from Sigma-Aldrich.

Equipment List

Laboratory balances
graduated cylinders
Hamilton® syringes
Microfilterfuge
GenoGrinder®
Centrifuge
Polypropylene bottles and tubes
Volumetric flask
HPLC vials
AB Sciex API 4000 Series tandem mass spectrometer with Agilent 1260 HPLC system (LC-MS/MS)
AB Sciex API 5500 Series tandem mass spectrometer with Agilent 1260 HPLC system (LC-MS/MS)

Test System

Source of Test System

The soil test system was used in this study was procured by Eurofins-Hercules from Eurofins – Columbia. Upon receipt the soil was assigned an inventory number (3210W-001) and stored in a freezer while not in use.

Test Method

The analytical method for the analysis of sodium chlorate was independently validated at Eurofins EAG Agroscience LLC - Hercules by liquid chromatography with tandem mass-spectrometric detection (LC-MS/MS) for sodium chlorate and is described in "Determination of Sodium Chlorate in Soil by LC-MS/MS ", Eurofins Method No. 87636-M, June 18, 2019. (Reference 1).

The soil samples were spiked with known concentrations of sodium chlorate and extracted with water by shaking. The extracts were centrifuged to separate solids and the supernatant was transferred to a temporary storage container. Aliquots of the supernatant were filtered following extraction, and samples were diluted as necessary to fit the standard curve. The final sample solutions were aliquoted into autosampler vials prior to high-performance liquid chromatograph and subjected to reversed-phase chromatography

coupled with tandem mass spectrometry (MS/MS) with negative electrospray ionization for sodium chlorate. The percent method recovery was determined using an external standardization where a linear curve for solvent based calibration standards was generated along with the samples. Details of the extraction and analysis procedures are described below.

Stock Solution

A single chlorate stock solution was purchased from Fisher Scientific at a concentration of 1000 µg/mL. Upon receipt it was assigned an inventory number (3210W-002) and stored in the refrigerator when not in use.

Preparation of Fortification Solutions

Fortification solutions were prepared by measuring appropriate volumes of stock solution (adjusted for sodium chlorate equivalents) using Hamilton® syringes into 50-mL volumetric flasks. The final solutions were diluted to the mark with water. Recommended preparations are provided in the table below.

Sodium Chlorate Fortification Solutions			
Solution Identification	**Amount removed (mL)**	**Final Volume (mL)***	**Final Solution**
**stock solution (1276 µg/mL)	3.915	50	100 µg/mL
100 µg/mL	5	50	10 µg/mL
100 µg/mL	0.5	50	1.0 µg/mL Fort Solution
10 µg/mL Fort Solution	0.5	50	0.1 µg/mL Fort Solution
1.0 µg/mL Fort Solution	0.5	50	0.01 µg/mL Fort Solution

*diluted with acetonitrile **concentration presented as sodium chlorate equivalents

Preparation of Solvent-Based Calibration Solutions

Sodium chlorate solvent-based calibration standards were prepared from the 1.0 µg/ml and 0.1 µg/mL standard solutions, by adding an appropriate volume via Hamilton® syringe in a 50-mL volumetric flask and diluting to volume with water. Recommended preparations are described below.

Solvent Based Calibration Solutions			
Solution Used	**Volume (mL)**	**Final Volume (mL)***	**Concentration (ng/mL)**
1.0 µg/mL	0.5	50	10
1.0 µg/mL	0.25	50	5
1.0 µg/mL	0.1	50	2
0.1 µg/mL	0.5	50	1
0.1 µg/mL	0.25	50	0.5
0.1 µg/mL	0.15	50	0.3

*diluted with water

Preparation of Matrix-Matched Calibrant Solution

Duplicate matrix-matched and solvent based calibration standards were prepared to assess matrix effect, by mixing 10 µL of the 0.1 µg/mL fortification solution with 990 µL of the final control sample solution (matrix matched) or with 990 µL of HPLC water (solvent based). The matrix matched and solvent based calibrants were prepared directly into an autosampler vial prior to analysis and analyzed in the same sequence to assess matrix.

Fortification Procedure

Fortification of untreated soil samples was conducted at the following fortification levels as shown below using a Hamilton® syringe:

Fortification Procedure				
Identification	Fortification Solution (µg/mL)	Volume Delivered (µL)	Sample weight (g)	Concentration (µg/g)
LOQ Fort	1	50	5	0.01
10X LOQ Fort	1	500	5	0.1

Fortification was conducted to determine the percent recovery and accuracy within the independent laboratory validation. This procedure was performed in quintuplicate at each fortification level.

Extraction Method: Soil Matrix

1. Weigh 5 g of soil into a 50-mL plastic centrifuge tube.
2. Fortify the samples as previously described.
3. Add 25 mL of water to each tube plus two (2) 4 mm steel balls.
4. Place on GenoGrinder® for 3 minutes @ 1200 rpm
5. Centrifuge samples for 3 minutes @ 3500 rpm.
6. Decant supernatant into a clean 50-mL plastic centrifuge tube.
7. Add additional 25 mL of water to each tube.
8. Place on GenoGrinder® for 3 minutes @ 1200 rpm
9. Centrifuge samples for 3 minutes @ 3500 rpm.
10. Combine supernatants in the clean 50-mL centrifuge tube.
11. Bring to final volume of 50 mL with water and shake samples well.
12. Dilute samples if necessary with water and transfer samples to autosampler vials.
13. Analyze samples and calibrants by LC-MS/MS analysis.

A schematic diagram of the procedure is presented in Figure 1.

Liquid Chromatography with Tandem Mass Spectrometry Analytical Method (LC-MS/MS)

LC conditions (Matrix Assessment)
Shimadzu LC-30AD HPLC system (LC-MS/MS)
Column: Phenosphere 5 µ SAX 150 x 2 mm column
Column Temperature: 40ºC
Injection volume: 20 µL
Flow rate: 0.5 mL/min
Retention Time: Ranged from 3.5 – 4.2 minutes.
Mobile Phase:
A: 0.15% Formic Acid in Water
B: 20 mM ammonium carbonate in water
Gradient:

Time (minute)	Mobile Phase A (%)	Mobile Phase B (%)
0.02	95	5
1.02	5	95
5.02	5	95
5.12	95	5
7.02	95	5

LC conditions (ILV Analysis)
Dionex UltiMate 3000 HPLC system (LC-MS/MS)
Column: Phenosphere 5 µ SAX 150 x 2 mm column
Column Temperature: 40ºC
Injection volume: 20 µL
Flow rate: 0.5 mL/min
Retention Time: Ranged from 3.5 – 4.2 minutes.
Mobile Phase:
A: 0.15% Formic Acid in Water
B: 20 mM ammonium carbonate in water
Gradient:

Time (minute)	Mobile Phase A (%)	Mobile Phase B (%)
0	95	5
1.0	5	95
6.0	5	95
6.1	95	5
8.1	95	5

MS/MS conditions

An Applied Biosystems API 4000 or 5500 tandem mass spectrometer was used with electrospray ionization (ESI) in negative polarity mode to acquire data by multiple-reaction monitoring (MRM).

API 4000:

Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	CE	CXP
Sodium chlorate				
83.0	67.0	300	-32.0	-10
83.0	51.0	300	-42.0	-25.0

CE = Collision Energy
CXP = Collision Cell Exit Potential

Source-Dependent Settings:

Temperature (TEM):	700°C
Nebulizer Gas (GS1):	67.0
Ion Spray Gas (GS2):	70.0
Curtain Gas (CUR):	25.0
Collision Activated Dissociation Gas (CAD):	12.0
Ionization Spray (IS)	-1200.0
Entrance Potential (EP)	-10.0
Declustering Potential (DP)	-27.0

Post-column effluent was diverted into the mass spectrometer between 3.1 and 4.7 minutes.

API 5500:

Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	CE
Sodium chlorate			
83.0	67.0	300	-32.0
83.0	51.0	300	-42.0

CE = Collision Energy

Source-Dependent Settings:

Temperature (TEM):	550°C
Nebulizer Gas (GS1):	50.0
Ion Spray Gas (GS2):	50.0
Curtain Gas (CUR):	20.0
Collision Activated Dissociation Gas (CAD):	6.0
Ionization Spray (IS)	-4500.0
Entrance Potential (EP)	-10.0
Declustering Potential (DP)	-30.0
Collision Cell Exit Potential (CXP)	-20.0

Post-column effluent was diverted into the mass spectrometer between 2.3 and 3.8 minutes.

LC-MS/MS Analysis

Soil samples were analyzed in an analytical set, consisting of a solvent blank, reagent blank, two control extracts, five 0.01 ppm fortified controls and five 0.1 ppm fortified controls. In addition, to ensure accuracy was maintained over the course of the analytical set, quality control (QC) calibrants were added to the sequence. Calibrants were interspersed between samples and analyzed in a single sequence of injections.

Methods of Calculation

Quantitation

The quantitation of sodium chlorate was conducted using peak area relative to the theoretical concentrations of the solvent based calibrants. The content of sodium chlorate

in samples was quantitated against a 1/x weighted linear curve of solvent based calibrants. An example for the quantitation of sodium chlorate in a fortified control is presented below:

$$X \text{ (ng/mL sodium chlorate)} = \frac{y - b}{m}, \text{ where:}$$

y = peak area sodium chlorate
x = concentration of sodium chlorate (ng/mL)
m = slope
b = intercept

Weighting of the calibration curve was applied to provide better curve fit at the lower concentration levels of sodium chlorate.

The calculation of weighted curve equations (linear regression) and concentrations (ng/mL) present in samples and calibrants was conducted using Analyst® software.

The Percent Recovery of a fortified sample is determined as follows:

Residue (ppm) ÷ Fortification level (ppm) × 100

Note: Control residues are only subtracted if the average concentration is above the lowest celebrant (0.3 ng/mL).

An example calculation for the recovery of sodium chlorate (m/z 83/67 ion transition) in soil fortified at 0.01 ppm (sample designated Low Fort 1) is given in following:

Linear regression equation: $y = (2.69e + 4)\, x + (8.83)$ $(r = 0.9997)$

The calculated sodium chlorate concentration (ng/mL) in Low Fort 1 final extract (solving for x):

Sodium chlorate ng/mL = (2.51e + 4 – 8.83) ÷ 2.69e +4 = 0.933 ng/mL

Where:2.51e +4 is the peak area of sodium chlorate (m/z 83/67) for Low Fort 1
8.83 is the y-intercept from the linear regression equation
2.69 e + 4 is the slope from the linear regression equation

The residue (ppm) of fortified sample Low Fort 1 (sodium chlorate):

Where,

Concentration (ng/mL) =	0.933
Extraction Volume (mL) =	50
Sample Weight (g) =	5
Dilution Factor =	1
Conversion = 1 µg/1000 ng	

Sodium chlorate residue (ppm) = 0.933 ng/mL × 50 mL × 1 × 1 µg/1000 ng ÷5 g

= 0.00933 ppm

The percent recovery of the fortified sample Low Fort 1 (Sodium chlorate):

= {0.00933 ppm ÷ 0.01 ppm (fort. level)} × 100%

= 93%

Note: values were rounded for presentation and may differ slightly from reported values.

Calibration Range

The calibration curve ranged from 0.3 ng/mL to 10 ng/mL for sodium chlorate in soil, and was generated by Analyst® software for the independent laboratory validation.

Limit of Quantitation

Based on current validated methodology, the limit of quantitation (LOQ) was set at 0.01 ppm in soil.

Limit of Detection

Based on current validated methodology, the limit of detection (LOD) was set at 0.003 ppm or 30% LOQ in soil.

Matrix Effect

No matrix effect was observed during the ILV sample set analysis; to confirm, duplicate matrix-matched calibrants were prepared at 1.0 ng/mL (LOQ level) to assess matrix effect. A percent difference was calculated by comparing the 1.0 ng/mL matrix-matched calibrant and the 1.0 ng/mL solvent based calibrant, the calculated percent difference was < 5% matrix enhancement indicating no matrix effect.

Time Required for Completion of a Sample Set

A soil sample set consisted of one solvent blank, one reagent blank, two controls (untreated samples), and five fortified samples at each level (i.e. LOQ and 10X LOQ). The time required for one sample set from preparation of standard solutions, initiation of extraction, until the completion of instrumental analysis and data evaluation is as follows:

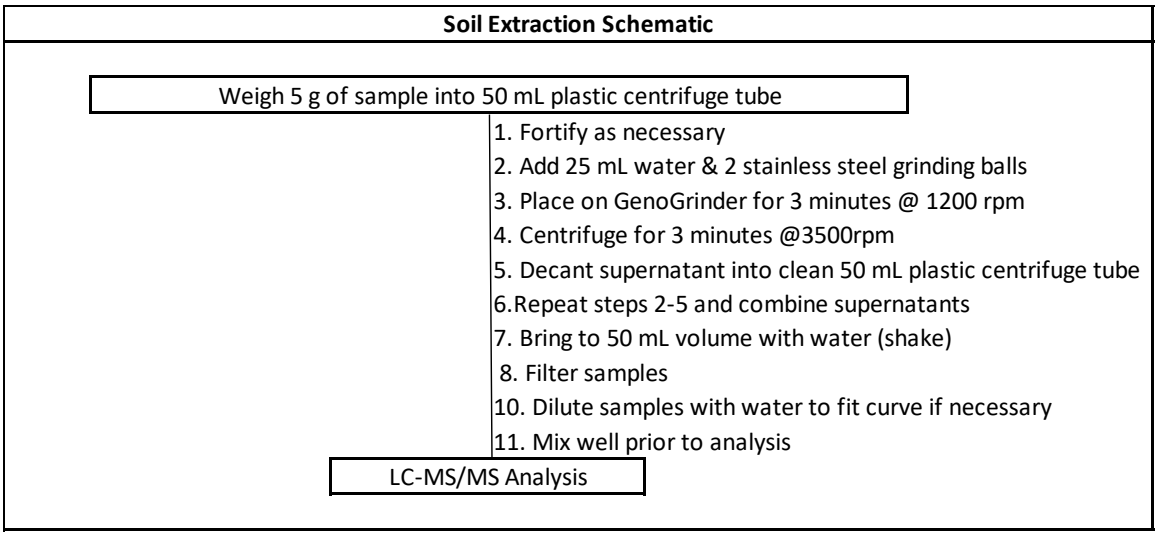
- Preparation of standard solutions takes approximately 4 hours
- Sample preparation takes approximately 4 hours
- LC-MS/MS and data processing (quantitation and confirmation) take approximately 2 hours (does not include automated analysis)

TOTAL = approximately 10 hours for one analyst to complete a sample set.

Statistical Methods

Means, standard deviation, relative standard deviation, and 1/x weighted linear regression fit were the only statistical methods employed in this study.

Figure 1. Extraction Schematic: Soil



Appendix A. Protocol

7.0 Test/Reference Substance

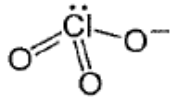
7.1 Test/Reference Substance:

The reference method determines residues of sodium chlorate equivalents in soil. The reference standard used is an aqueous solution of chlorate ion.

Chlorate:

Concentration: ~1000 µg/mL
Matrix: Water

Molecular Weight: 83.447 g/mol
Molecular Formula: ClO_3^-
Molecular Structure:
Structure:



Lot number, purity, and expiration date of the test/reference substance will be provided in the final report.

7.2 Test/Reference Substance Documentation: Assurance that the test, control and reference substances are properly characterized and stored is the responsibility of the test facility management. The sponsor or commercial source will maintain information as to location of chemical synthesis (e.g., method of synthesis, spectral analysis, etc.) of the test, control and reference substances. The substance will be stored throughout the study period under the conditions specified by the Sponsor or supplier, to insure their stability. Stability information may be provided to Eurofins EAG Agroscience LLC by the Sponsor to be included in the Final Report, as per 40 CFR Part 160.105. Storage conditions used will be documented in the study and/or facility records.

7.3 Safety: Material Precautions normally applied to the handling of pesticides. Safety Data Sheet (SDS) will be supplied by Sponsor or commercial source if available.

7.4 Receipt: Documentation in compliance with GLP regulations will be maintained for shipment, storage and use of test and reference standards.

7.5 Storage: The test/reference substance will be stored as indicated on the label/shipping instructions or certificate of analysis, when not in use. Storage conditions used will be documented in the study and/or facility records.

7.6 Route of Administration: Known volumes of diluted test/reference substance of known concentration in solution will be spiked in the control matrices.

7.7 Justification of Route of Administration: To assess known quantity of residues introduced to the control matrices and recovered by the analytical method(s).

8.0 Test System (Matrix):

Soil: The soil used in this study was provided by the originating method validation laboratory (Eurofins EAG Agroscience, LLC, 7200 E. ABC Lane, Columbia, MO 65202). The soil was characterized using USDA classification with regard to texture (percent sand, percent silt, percent clay), moisture, pH and percent organic matter in the original method study. The characterization report will be provided in the final report of this study. Soil will be stored in a freezer (usually < 0°C) when not in use.

8.1 Justification: Registration requires the analytical method for the determination of sodium chlorate in soil to be validated independently.

8.2 Identification: An untreated control soil sample will be labeled with a Eurofins EAG Agroscience Services-Hercules Inventory number assigned by Compound Control. The Sample will be labeled with, at a minimum, the following information: Eurofins EAG Agroscience Services-Hercules sample identification number and date/initials.

9.0 Study Design:

9.1 Analytical Method: Chlorate ion will be extracted and analyzed from soil by a method provided by the Sponsor as described in the report "Determination of Sodium Chlorate in Soil by LC-MS/MS", Eurofins [Method No. 87636-M, June 13, 2019, author Mathew Rebstock.] The method is based on liquid extraction prior to LC-MS/MS approach.

9.2 Linearity Curves: For each analyte, a minimum of six (6) data points, using either solvent based (water) or matrix matched calibrants for the soil matrix. The linearity calibration curves should have a correlation coefficient >0.99.

9.3 Confirmation of Interferences: Signals in blank control samples should be <30% LOQ.

9.4 Matrix effect: calibrants will be prepared in solvent and analyzed by LC-MS/MS. At least one matrix matched calibrant will also be prepared. The response of each calibrant type (matrix or solvent based) at the same concentration level will be compared to assess matrix effect. Solvent based calibrants will be used during the study if matrix effects are not significant (ie. signal enhancement < 120% recovery and signal suppression > 80%

recovery). Matrix calibrants may be used if the signal enhancement or suppression is significant (> 20% deviation from solvent based calibrant response). It is noted that matrix-matched calibrants were not used in the original method.

9.5 Recoveries: Method validation will be conducted for each matrix as follows:

1 regeant blank
2 untreated control samples
5 recoveries at the LOQ
5 recoveries at 100X LOQ

Acceptable mean recoveries of each compound will be between 70% and 120% for each fortification level and each matrix. Acceptable %RSD at each fortification level will be no more than 20%. Lower or higher recovery ranges may be deemed acceptable upon approval of the Sponsor.

The LOQ will be 0.01 mg/kg sodium chlorate equivalence.

The limit of detection (LOD) is defined as approximately 30% of the LOQ and is estimated to be 3.0 ppb sodium chlorate equivalents in soil.

9.6 Quantitation: Quantitation will be conducted against a calibration curve of the reference substance. Quantitation will be assessed on one MS/MS ion transition for each analyte using LC-MS/MS.

9.7 Confirmation: Confirmation will be conducted by monitoring one additional MS/MS ion transition for each analyte using LC-MS/MS.

9.8 Proposed Statistical Methods: The following statistical methods will be applied to data generated for the independent validation study: linear regression, quadratic regression (may include 1/x weighting factor), standard deviation, relative standard deviation, confidence limits and averages.

9.9 Methods for Control of Bias: Methods to control bias will include analysis of untreated control matrices, interspersing calibration standards during analysis and assaying multiple replicates at each fortification level.

10.0 Quality Assurance:

At Eurofins-Hercules, each project is assigned by its management to a member of the scientific staff with the appropriate education, training, and experience to accomplish the designed task. The QA Unit monitors the study to ensure that facilities, equipment, personnel, practices, procedures, records, and controls are designed to function in compliance with U.S. EPA FIFRA GLP Standards in 40 CFR Part 160, the protocol and applicable SOPs. The Quality Assurance Unit

(QAU) will conduct inspections of this study, including at least one critical phase inspection, the study protocol and a draft of the final report, and will provide the same written documentation of findings to management and the Study Director. Appropriate corrective action, if needed, will be instituted immediately. The QAU will issue a final report signed statement listing the date's inspections or audits were made and the date's findings were reported to management and the Study Director.

11.0 Records to be Maintained:

All original data records and original final report will be the property of the Sponsor and will be temporarily archived at Eurofins-Hercules and will be transferred to the Sponsor upon authorization. An exact copy of the Final Report and facility records (such as equipment use/calibration logs, etc.) will be retained in the archives at Eurofins-Hercules for the period required by the EPA.

12.0 Final Report:

A final report will be written for this study in accordance with PR 2011-3 format. The report will include, but is not limited to:

- The name and address of the Study Director and of the testing facility
- A statement of GLP compliance
- A description of the exact analytical conditions employed in the study
- Description of the instrumentation used and operating conditions
- Calibrant data and curves
- All results from all validation sets analyzed
- Representative chromatograms from all ILV trials analyzed, including chromatograms of a standard and a control sample, and a chromatogram at each fortification level. Method (reagent) blanks should be included, if applicable
- The total number of person-hours required for analysis of one set of samples for each compound. The time period desired here is from the initiation of extraction until the completion of the instrumental analysis
- Description of any problems encountered
- A complete summary of any contact (communication) between the Study Director and the method developers or others familiar with the method, including the reasons for the contact, any changes in the method that resulted, and the time of this communication with respect to the progress of the study
- Recommended changes to improve the method, where necessary
- The final protocol and any amendments