3 APPARATUS AND EQUIPMENT

Due to the potential for contamination resulting from low detection limits, disposable equipment should be used when possible. If glassware is used, care should be taken to minimize the potential for contamination due to insufficient cleaning of the glassware.

Top-loading Balance (weighing samples):	Mettler ML3002E (Mettler Instrument Corporation, Hightstown, NJ)
Centrifuge:	Sorvall Legend XTR Benchtop Centrifuge
Sample Processor	Geno/Grinder®, Sample Prep 2010, SPEX
LC-MS/MS System:	Sciex API 5000 LC-MS/MS; Waters Acquity Column Manager UPM, Sample Manager UPA, Binary Solvent Manager UPB, Sample Organizer, and Applied Biosystem (AB) Sciex Analyst Software for data collection and system control (version 1.6.2)
LC Column:	Imtakt Scherzo SM-C18 MF, 50 mm × 2 mm, 3-µm particle size
Pipets (glass):	Graduated, serological, various sizes; Class-A, various sizes
Pipets (automatic):	Gilson – various sizes and tips (Gilson, Inc., Middletown, WI), Eppendorf repeating pipette
Stock Solution Containers:	Volumetric flasks, glass, Class-A, various sizes Amber vials, glass, PTFE-lined closure, various sizes (for storage)
Sample Containers:	50-mL polypropylene centrifuge tubes
Syringe Filter:	0.45 µm, PTFE
Additional glassware:	25-mL tilt measurer 2000-mL graduated cylinder 2-mL glass autosampler vials
Vortexer	Thermo Scientific Model No.: M16715

4 REAGENTS AND MATERIALS

Reagents are HPLC-grade or higher, except where noted. Wear proper personal protective equipment when handling chemicals and reagents. Review each chemical SDS for further safety information.

Acetonitrile:	Fisher Scientific, Fairlawn, NJ
Ammonium Formate:	Fisher Scientific, Fairlawn, NJ
Formic Acid:	>99%, Fisher Scientific, Fairlawn, NJ
Methanol:	Fisher Scientific, Fairlawn, NJ
Water:	Fisher Scientific, Fairlawn, NJ
Chlorate Standard:	Inorganic Ventures, Inc.

4.1 Reagents and Materials to be Prepared

Volumes may be adjusted accordingly for different quantities. Reagent solution stability was not determined. Statements are provided based on routine use of solutions during method

development and validation. Alternate expirations may be assigned as necessary based on individual laboratory standard procedures.

0.15 % Formic Acid (aq)

Combine 2000 mL of water and 3 mL of formic acid and mix well. This solution was assigned an expiration of no greater than 3 months from the date of preparation and stored at room temperature.

1.0 M Ammonium Formate (aq)

Dissolved 6.37 g of ammonium formate into 100 mL of water. This solution was assigned an expiration of no greater than 3 months from the date of preparation and stored at room temperature.

20mM Ammonium Formate (aq)

Combine 2000 mL of water and 40 mL of a 1.0 M ammonium formate (aq) solution and mix well. This solution was assigned an expiration of no greater than 3 months from the date of preparation and stored at room temperature.

1:1:1 Acetonitrile:Methanol:Water

Combine 4 L each of acetonitrile, methanol, and LCMS-grade water and mix well. This solution was assigned an expiration of no greater than 1 year from the date of preparation and stored at room temperature.

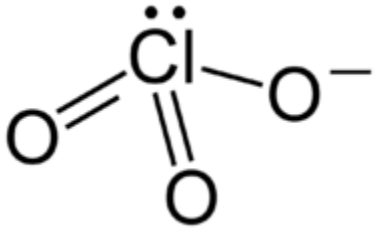
1:1:2 Acetonitrile:Methanol:Water

Combine 4 L each of acetonitrile and methanol with 8 L of LCMS-grade water and mix well. This solution was assigned an expiration of no greater than 1 year from the date of preparation and stored at room temperature.

5 REFERENCE STANDARD

Chlorate:

Concentration:	~1000 µg/mL
Matrix:	Water
Molecular Weight:	83.447 g/mol
Molecular Formula:	ClO_3
Molecular Structure:	



6 STANDARD PREPARATION

Prepare all standard solutions in HPLC-grade solvents using appropriate analytical techniques. Alternative or additional standard concentrations and volumes may be prepared as needed.

6.1 Stock Standard Solutions

Stock standard solutions of chlorate in water can be purchased directly or prepared by dissolving an appropriate chlorate salt in water. If prepared from a dry reagent, appropriate analytical techniques should be employed to ensure standard integrity and accuracy. Based on study needs, the concentration of stock standard solutions may be expressed as sodium chlorate equivalents by multiplying the chlorate concentration by the molecular weight ratio between the two compounds (i.e., 106.44/83.447 = 1.27554).

Example: 1001 µg chlorate/mL × 1.27554 = 1277 µg sodium chlorate equiv./mL

6.2 Fortification and Intermediate Standard Solutions

Typically, the following concentrations of fortification and intermediate standard solutions are prepared. Concentrations are listed as sodium chlorate equivalents in water and preparations will differ dependent on the prepared concentration of the primary chlorate stock solution as described in section 6.1. Alternate solutions may be prepared as needed. All prepared fortification and intermediate standard solutions are expected to be stable for extended periods when stored at room temperature.

100 µg/mL: Transfer 3.916 mL of the 1277 µg/mL stock standard solution to a 50-mL volumetric flask. Bring to volume with water and mix well.

10.0 µg/mL: Transfer 5.0 mL of the 100 µg/mL standard solution to a 50-mL volumetric flask. Bring to volume with water and mix well.

1.00 µg/mL: Transfer 0.50 mL of the 100 µg/mL standard solution to a 50-mL volumetric flask. Bring to volume with water and mix well.

0.100 µg/mL: Transfer 0.50 mL of the 10.0 µg/mL standard solution to a 50-mL volumetric flask. Bring to volume with water and mix well.

0.0100 µg/mL: Transfer 0.50 mL of the 1.00 µg/mL standard solution to a 50-mL volumetric flask. Bring to volume with water and mix well.

6.3 Calibration Standard Solutions

Typically, the following calibration solutions are prepared. The concentration range covers 30% of the LOQ, and samples are diluted as needed to reduce the sample response to approximately 20% lower than the highest concentration. Concentrations are listed as sodium chlorate equivalents in water. Alternate solutions may be prepared as needed. All prepared calibration standard solutions are expected to be stable for extended periods when stored at room temperature.

10.0 ng/mL: Transfer 0.50 mL of the 1.00 µg/mL intermediate standard solution to a 50-mL volumetric flask. Bring to volume with water and mix well.

5.00 ng/mL: Transfer 0.25 mL of the 1.00 µg/mL intermediate standard solution to a 50-mL volumetric flask. Bring to volume with water and mix well.

2.00 ng/mL: Transfer 0.10 mL of the 1.00 µg/mL intermediate standard solution to a 50-mL volumetric flask. Bring to volume with water and mix well.

1.00 ng/mL: Transfer 0.50 mL of the 0.100 µg/mL intermediate standard solution to a 50-mL volumetric flask. Bring to volume with water and mix well.

0.500 ng/mL: Transfer 0.25 mL of the 0.100 µg/mL intermediate standard solution to a 50-mL volumetric flask. Bring to volume with water and mix well.

0.300 ng/mL: Transfer 0.15 mL of the 0.100 µg/mL intermediate standard solution to a 50-mL volumetric flask. Bring to volume with water and mix well.

7 SAMPLE WEIGHING/FORTIFICATION

1. Weigh 5.00 ± 0.01 g of homogenized sample into a 50-mL polypropylene centrifuge tube.
2. Fortify applicable samples with the appropriate amount of standard solution.
3. Proceed with Section 8, Step 1.

8 SAMPLE EXTRACTION

1. Add 25 mL of water and two ¼ inch steel bearings to the sample.
2. Securely cap the sample bottle and process in a Geno/Grinder® for 3 minutes at 1200 strokes per minute.
3. Centrifuge the sample at ~3500 rpm for ~3 minutes.
4. Decant the supernatant into a clean 50-mL polypropylene centrifuge tube.
5. Re-extract the solid sample pellet with 25 mL of water. Repeat Steps 2 and 3.
6. Combine the supernatant in the same 50-mL polypropylene centrifuge tube.
7. Dilute the combined sample extract to 50 mL with water.
8. Filter approximately 1.5 mL of the extract using a 0.45 µm PTFE syringe filter into a clean 2-mL glass autosampler vial. Final matrix concentration in undiluted samples is 0.10 g/mL.
9. Subsequent dilutions may be prepared using water, as required.
10. Quantify the samples via LC-MS/MS.

9 LIQUID CHROMATOGRAPHIC ANALYSIS

The column and conditions stated below have been satisfactory for the matrices being analyzed. The specific column packing, mobile phase, column temperature, and flow rate listed are typical conditions for this analysis. Alternate columns may be used depending on the need to resolve analyte and/or interfering responses. Specific conditions used should be noted with each chromatographic run.

9.1 Operating Conditions

Typical LC-MS/MS conditions used for this analysis were as follows:

System:	Applied Biosystems/Sciex API 5000 LC-MS/MS; Waters Acquity Column Manager UPM, Sample Manager UPA, Binary Solvent Manager UPB, Sample Organizer			
Data Acquisition Software:	Applied BioSystems Sciex Analyst, Version 1.6.2			
Column:	Imtakt Scherzo SM-C18 MF, 50 mm × 2 mm, 3-µm particle size			
Column Temperature:	40 °C			
Mobile Phase:	Mobile Phase A: 0.15% formic acid (aq) Mobile Phase B: 20 mM ammonium formate (aq)			
Autosampler Temperature:	10 °C			
Flow Rate:	0.500 mL/min (No Split)			
Gradient:	Time (min.)	% A	% B	
	0.00	95	5	
	3.00	5	95	
	4.00	5	95	
	4.01	95	5	
	5.00	Stop		
Interface:	TIS (turbo ion spray)			
Polarity	Negative (-)			
Scan Type	MRM			
Resolution:	Q1 – unit, Q3 – unit (Note: Unit equivalent to medium)			
Source Temperature:	600 °C			
Curtain Gas:	Nitrogen @ setting of "40"			
Collision Gas:	Nitrogen @ setting of "12"			
Transitions Monitored:	Analyte	Q1	Q3	CE,*v*
	Chlorate	83 83	67 51	-32 (quantitation) -42 (confirmation)
Injection Volume:	10 µL			
Retention Time:	Chlorate		~2.2 minutes	

9.2 Sample Analysis

Prepare a standard curve by injecting constant volumes of standard solutions (at least five concentrations). Use constant volume injections for sample extracts as well. Sample responses found greater than those produced by the highest concentration of standard in the standard curve require dilution and reinjection. Dilutions are made using water. Calibration standards should be injected intermixed with test samples before and after every 1-4 samples in each analytical set.

9.3 Assay Time

A typical analytical run would consist of at least five calibration standard concentrations encompassing the expected range of sample concentrations, a procedural control (non-fortified sample), a minimum of two fortified procedural controls (one of which should be at the LOQ), and associated samples. This typical analytical run requires approximately 5 hours to extract and prepare a set of 24 samples for LC-MS/MS analysis, followed by approximately 3 hours of instrumental analysis. Acceptable stopping points to allow for the continuation of a set the following day were not assessed and should be assessed as needed.

10 CALCULATIONS

Calculations for instrumental analysis are conducted using a validated software application (e.g., Applied BioSystems Sciex Analyst, version 1.6.2) to create a standard curve based on linear regression. The regression functions are used to calculate a best-fit line (from a set of standard concentrations in ng/mL versus peak area response) and to determine concentrations of the analyte found during sample analysis from the calculated best-fit line. For each analytical set, calibration standards are injected over the linear range of the instrument (typically 0.300 to 10.0 ng/mL). All standards injected and their corresponding peak responses are entered into the program to create the standard curve. Weighting (1/x) is typically used.

The equation used for the least squares fit is:

$$Y = \text{slope} \times X + \text{intercept}$$

Y = detector response (peak area) for each analyte

X = analyte concentration in the sample in ng/mL

$$X = \frac{Y - \text{intercept}}{\text{slope}} = \text{ng/mL found}$$

The standard (calibration) curve generated for each analytical set is used for the quantitation of chlorate (expressed as sodium chlorate equivalents) in the samples from the set. Correlation coefficients (r) for each calibration curve should be greater than 0.990 (r^2 equal to or greater than 0.98).

For the determination of chlorate in soil (expressed as ppb sodium chlorate equivalents), the following equation is used:

$$Found\ (ppb) = \frac{ng/mL\ found \times Extract\ Vol.\ (mL) \times DF}{Sample\ Weight\ (g)}$$

where:

Extract Vol.	=	500 mL
DF	=	Dilution factor
Sample Weight	=	5.00 g (nominal; actual sample weight used in calculations)

Procedural recovery data from fortified samples are calculated via the following equation:

$$Percent\ Recovery = \frac{ppb\ found - ppb\ found\ in\ control}{ppb\ added} \times 100$$

Example Calculations:

1. Sample Description: S18-05007-01, Control soil

   (Analysis ID: 87636-MV01-1, Set MV01-1):

$$0\ peak\ response\ (area) \rightarrow 0\ ng/mL$$

$$ppb = \frac{0\ ng/mL \times 50\ mL \times 1\ DF}{5.00\ g}$$

$$ppb = 0.00000\ ppb$$

$$Reported\ ppb = None\ detected$$

2. Sample Description: S18-05007-01 + 10.00 ppb, Fortified Control soil

   (Analysis ID: 87636-MV03-1, Set MV01-1):

$$15138\ peak\ response\ (area) \rightarrow 1.08361\ ng/mL$$

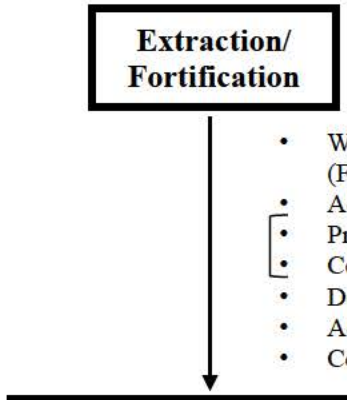
$$ppb = \frac{1.08361\ ng/mL \times 50\ mL \times 1\ DF}{5.00\ g}$$

$$ppb = 10.83610\ ppb$$

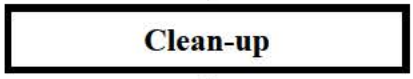
$$Percent\ Recovery = \frac{10.83610\ ppb - 0.00000\ ppb}{10.0\ ppb} \times 100$$

$$Percent\ Recovery = 108\%$$

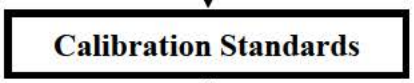
APPENDIX I Analysis Flowchart



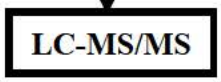
- Weigh 5.00 g of sample into a 50-mL polypropylene centrifuge tube. (Fortify as needed.)
- Add 25 mL of water and two ¼ inch steel bearings.
- Process in a Geno/Grinder® for 3 minutes at 1200 strokes per minute.
- Centrifuge for ~3 minutes at ~3500 rpm.
- Decant the supernatant into a clean 50-mL polypropylene centrifuge tube.
- Add 25 mL of water and repeat bracketed steps.
- Combine the supernatants into the same 50-mL tube and dilute to 50 mL with water.



- Filter approximately 1.5-mL of extract using a 0.45 µm PTFE syringe filter into a clean 2-mL glass autosampler vial. Final matrix concentration is 0.10 g/mL.
- Dilute sample to fit within the standard curve with water as necessary.



- Solvent standard injections are interspersed with the samples throughout the run.



- Analyze by negative-ion LC-MS/MS.