

Standard Operating Procedure

**for the USGS Reston Biogeochemical Processes in Groundwater Laboratory
(RBPGL)**

Determination of Low Molecular Weight Organic Acid (LMWOA) in Water Samples

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Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$^{\circ}\text{F} = (1.8 \times ^{\circ}\text{C}) + 32$$

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

$$^{\circ}\text{C} = (^{\circ}\text{F} - 32) / 1.8$$

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L).

Acronyms and Abbreviations

Ag	Silver
ASTM	American Society for Testing and Materials
Ba	Barium
CV	Coefficient Variation
g	Gram
IC	Ion chromatography
ID	Identification
KOH	Potassium hydroxide
LIMS-LMWOA	Laboratory Information Management System for Low-molecular Weight Organic Acid
LMWOA	Low-molecular Weight Organic Acid
mg/L	Milligram per liter
MDL	Method detection limit
mL	Milliliter
mL/min	Milliliter Per Minute
n	Number
OSHA	Occupational Safety and Health Administration
P/N	Part number
%RSD	Percent Relative Standard Deviation
RBPGL	Reston Biogeochemical Processes in Groundwater Laboratory
RSD	Relative Standard Deviation
R ²	Coefficient of Determination
SD	Standard Deviation
SDS	Safety Data Sheet
Std	Standard
SOP	Standard Operating Procedure
TM	Trademark
USGS	United States Geological Survey
USEPA	United States Environmental Protection Agency
VFW	Volatile Free Water
VOA	Volatile Organic Analysis
18.2 Ω	18.2 Ohm Milli-Q

Symbols

Ω	Ohm
\leq	Less than or equal to
%	Percent
$^{\circ}\text{C}$	Degree Celsius

Determination of Low-molecular Weight Organic Acid (LMWOA) in Water Samples

By Tracey A. Spencer, Jeanne B. Jaeschke, and Isabelle M. Cozzarelli

Summary of Procedure

This document describes the Standard Operating Procedure (SOP) for the determination of low molecular weight organic acids (LMWOA) of the U.S. Geological Survey Reston Biogeochemical Processes in Groundwater Laboratory (RBPGL), Reston Virginia;

<http://water.usgs.gov/nrp/biogeochemical-processes-in-groundwater/>. Water samples are analyzed for LMWOA by ion chromatography using a Thermo Scientific™ Dionex™ Ion Chromatograph with a Dionex™ ICS Series 2100 AS DV auto sampler, herein referred to as IC, and Chromeleon® software. Prior to analysis, frozen raw water samples are thawed. Each sample is analyzed initially at a 1:5 and 1:20 dilution for quantitative results. Once the analyst has determined the approximate concentration of the samples, the dilution factor is adjusted so that the sample is analyzed as close to a 1:1 dilution as possible. If there are matrix interferences, the sample will be filtered using a Dionex™ OnGuard Ag, OnGuard AgII, or OnGuard Ba cartridge prior to analysis. A Thermo Scientific™ Dionex™ IonPac AS₁₁-HC Anion-Exchange Column is used for identifying each analyte. A detailed description of the sampling methodology can be found at the USGS RBPGL web site

<http://water.usgs.gov/nrp/biogeochemical-processes-in-groundwater/>.

Operating Range

The typical analytical linear range of this method is 0.1 to 10 mg/L. Samples with higher concentrations must be diluted. Some samples with matrix interferences must be filtered using a Thermo Scientific™ Dionex™ OnGuard Ag, OnGuard AgII, or OnGuard Ba cartridge prior to analysis. Data are reported with two significant figures.

Reference Materials and Documentation

Reference Materials Used, Storage Requirements, and Stability

The RBPGL uses two standards, a 100 mg/L mixture of Lactate, Acetate, Propionate, Formate, Butyrate, and Benzoate from Inorganic Ventures, Christiansburg, VA. The second is a 100 mg/L concentration standard of Pyruvate that is mixed from a dry chemical (0.1009 g into a 1000 mL volumetric flask to yield a 100 mg/L solution) from Acros Organics, Geel, Belgium.

Documentation

All calibration results are stored in the Reston Laboratory Information Management System for Low-molecular Weight Organic Acid Samples (LIMS-LMWOA) associated with field id number, sample submission date, and project identifier. The requisite Microsoft Excel® data spreadsheet is created by the RBPGL analyst. Blanks and calibration standards are run with every batch of samples.

Sample Collection, Preparation, Analysis, Retention Time and Disposal

Field Sampling Containers, Collection Procedures and Handling Requirements

Water samples are collected in 20-mL clear borosilicate glass precleaned USEPA certified Volatile Organic Analysis vials (VOA) with open-top, septa-lined caps (septa are made of Teflon-lined silicone). This is a recommended size, however 40-mL borosilicate glass precleaned EPA certified (Level II) VOA vials are acceptable. 20-mL or 40-mL vials with septa can be provided by RBPGL at a cost of \$3.00 each plus shipping to the sample submitter on request through e-mail or can be purchased at Scientific Specialties Service (www.scispec.com). The 20-mL P/N 276720-clear, P/N 276820-amber glass. The 40-mL P/N 276740-clear, 276840-amber. Other scientific products retailer may also provide this product. For smaller sample volumes the submitter must first contact RBPGL (RBPGL@usgs.gov). The vials must be labelled clearly with a waterproof marker. All labels must list field id, date and time of sample collection, analyses to be done. If duplicate samples are collected at one site they should be labelled "1 of 2" and "2 of 2". Documentation accompanying the samples must indicate whether or not any samples contain a known or suspected hazard. If a hazard is noted, please provide a detailed description. If there is no known or suspected hazard, the documentation must include the word "None." Samples to be analyzed by this method can be collected following sampling procedures outlined in the "USGS National field manual, variously dated" available at <http://water.usgs.gov/owq/FieldManual/>. Samples are collected raw, unfiltered and the vial is filled 2/3- rds leaving enough headspace for sample expansion when frozen. A minimum of 15-mL of sample in a 20-mL vial or a minimum of 30-mL of sample in the 40-mL vial is required for analysis unless special arrangements are made in advance. Screw the cap onto the vial firmly, but do not overtighten. If the septum appears to be creased or if it is off-center, the vial may leak.

Place each vial into a foam sleeve, then into a Ziploc-type bag. Freeze with dry ice if available in the field before shipping, if not, the sample will be frozen when received at the lab. Keep all vials from the same site together. Secure the vials with additional packing materials and tape so they will not break during transport. Sample vials should be shipped, if available, on dry ice overnight, notifying RBPGL by e-mail that the samples having been shipped. The coolers must be sent Monday-Thursday by overnight delivery. If samples cannot be shipped on the day of collection, they should be frozen if possible. Label the inside lid of the cooler with return address and telephone number using a waterproof marker. Coolers will be returned by RBPGL to the sample submitter at the submitter's expense. Shipper must include either a Federal Express shipping label identifying the USGS internal cost code or Federal Express account number, or a return mailing label and metered with correct postage for return of the cooler by parcel post. RBPGL is not open on Saturday, Sunday or Federal holidays, therefore, coolers should not be sent the day before a holiday. Any cooler or container not accompanied by a prepaid shipping return label will not be returned automatically. For answers to additional questions sample submitters should email *RBPGL@usgs.gov*.

Sample Preparation

Sample preparation at the RBPGL includes logging samples into LIMS-LMWOA in batches of 1-50 samples per batch, printing labels for each sample and a summary project report. Each sample label contains the field id (the identifier by which the sample submitter knows each sample) and the unique RBPGL lab id assigned by the LIMS-LMWOA log in procedure. This information is e-mailed to the submitter of the samples for identification of any errors. The processing of samples involves including the field id and dates correspond to the list provided by the submitter.

Holding Time, Sample Analysis, and Time Requirements

The maximum time that raw, frozen samples can be held prior to analysis is 45 days. A sample template for as many as 15 samples per day is created in LIMS-LMWOA. The analyst sets up blanks, duplicate or triplicate samples, and calibration standards in the template. Each sample is run in duplicate at a minimum. The standard error for duplicate runs of the same sample should be $\leq 3\%$. An average of the duplicates is the reported value. Average, median, standard deviation (SD), and percent relative standard deviation (%RSD) for duplicate or triplicate samples are reported. The LIMS-LMWOA will not release preliminary data to submitters until the statistics are acceptable. The time requirement for performing the LMWOA analyses is a minimum of twenty days for every 15 samples. If a submitter requires a shorter turnaround time for the analyses, contact RBPGL directly at RBPGL@usgs.gov.

Problematic Samples

Problematic samples can include surface water and groundwater impacted by landfill leachate, brines, industrial and domestic wastewater, hydraulic fracturing and oil exploration sites. Such samples may require additional preparation in order to improve analytical results and/or remove interferences. Insufficient dilution or filtration of samples may lead to instrument interferences that can preclude obtaining acceptable results from the RBPGL.

Sample Retention Time and Disposal

Water samples are retained by the RBPGL for 30 days after data are reported. Samples are then discarded unless the submitter requests that the samples be returned. Return shipping costs are paid for by the sample submitter. Sample-analysis raw-data files generated from the Chromeleon® software are

exported in Microsoft Excel® format and copies are kept for 6 months on two different servers, one on the instrument computer and one on a shared network.

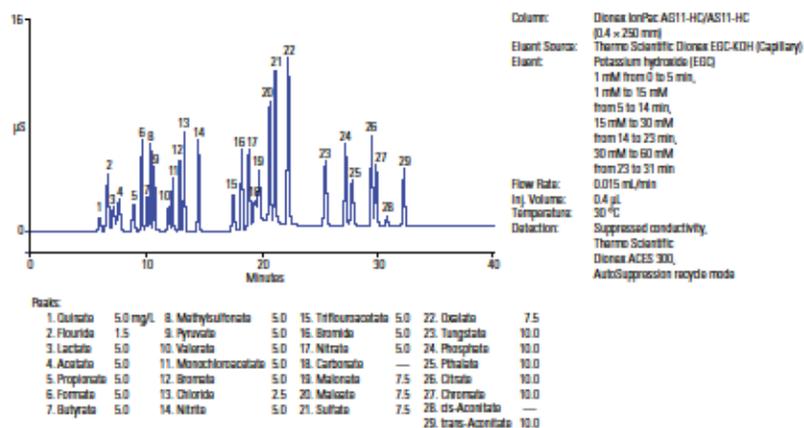
The Analytical System and Measurement of LMWOA, Thermo Scientific™ Dionex™ Ion Chromatograph, Thermo Scientific™ Dionex™ ICS 2100 Series AS DV Auto Sampler

Equipment

The LMWOA are analyzed on the Thermo Scientific™ Dionex™ Ion Chromatograph and Thermo Scientific™ Dionex™ ICS Series 2100 AS DV (referred to herein as IC) using a Thermo Scientific™ Dionex™ IonPac AS₁₁-HC Anion-Exchange Column (http://www.dionex.com/en-us/webdocs/4157-PS-Dionex-IonPac-AS11-AS11-HC-28Mar2012-PS70024_E-R2.pdf). This column uses a potassium hydroxide gradient eluent to provide fast profiling of organic acids. It is designed for use in complex matrices with a large number of organic acid constituents.

Separate a wide variety of inorganic anions and organic acids using the Dionex IonPac™ AS11 and AS11-HC Anion-Exchange Columns with potassium hydroxide gradients.

Thermo Scientific Dionex IonPac AS11 and AS11-HC Anion-Exchange Column



The Dionex IonPac AS11 and AS11-HC anion-exchange columns are designed for the separation of organic acids and inorganic anions in various matrices. Simpler matrices can quickly be profiled using the AS11 while the AS11-HC is best for more complex, higher ionic strength matrices.

The Dionex IonPac AS11 anion-exchange column provides fast profiling of inorganic anions and organic acid anions using sodium hydroxide or potassium hydroxide gradient elution. The AS11 column is available in 2 mm and 4 mm formats. The Dionex IonPac AS11-HC is a high-capacity anion-exchange column with selectivity similar to the AS11 column.

This high-capacity column is designed to resolve a large number of organic acids and inorganic anions in complex matrices, using potassium hydroxide gradient elution. The Dionex IonPac AS11-HC is ideal for the determination of trace components and for profiling organic acids in uncharacterized samples, and is available in 4 mm, 2 mm, and 0.4 mm formats.

Figure 1. Thermo Scientific™ Dionex™ IonPac AS11-HC Anion-Exchange Column Chromatogram

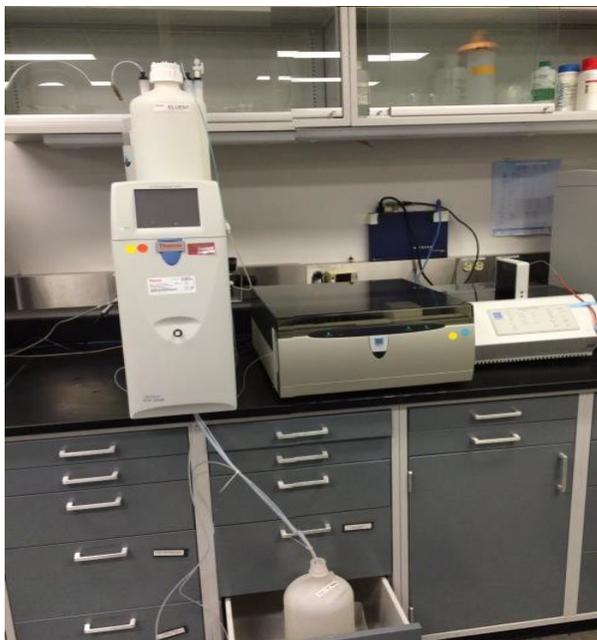


Figure 2. Pictorial Representation of the Thermo Scientific™ Dionex™ ICS 2100 for LMWOA in Water Samples.

The IC and Chromeleon® Software are the major components of the analytical system. The average analytical run time is approximately 45 minutes per sample. Replicate analyses of each sample are measured to meet the system-specified range of a SD of $\leq 3\%$. The sample tray holds a maximum of (6) 6-mL vials. The auto sampler can hold 50 samples. The carrier gas is high purity helium. For instrument operating instructions and parameters, refer to Appendix A.

Reagents, Blanks, Standards, Quality Assurance and Quality Control

Distilled 18.2 Ω water is used in preparation of reagents, blanks, standards, reference material and dilution of samples. Class A volumetric flasks and pipettes are used to prepare all solutions. All glassware is thoroughly cleaned by soaking for several hours in Alconox®, (White Plains, NY) and then

rinsed one time with tap water and multiple times with 18.2 Ω water. Foil is used to wrap the glassware, and non-volumetric items are baked in a muffle furnace for 4 hours at 425 °C. Pasteur Pipettes are rinsed three times with 18.2 Ω water, and then air-dried. After air-drying over night, they are baked in a muffle furnace for 4 hours at 225 °C, allowed to cool, and then wrapped in foil. Volumetric glassware is dried for 4 hours at 225 °C. Glassware is cooled to room temperature before use. 18.2 Ω water is used as blanks for each run. A minimum of two blanks are run at the beginning of each batch and at the end of each batch. The blanks are run at the end of the batch to confirm a stable baseline was attained. A median value of the blanks is determined to be the absolute value of the y-intercept from peaks integrated with Chromeleon® Software.

Table 1. Batch data with peak areas, concentrations, and calculations for LMWOA analyses of blanks. [Standard deviation, SD; Percent relative standard deviation, %RSD]

Blank sample numbers	Blank area counts	Blank concentrations in mg/L	Calculations	Data values
		Analysis		
1	0.015	0.040		
2	0.017	0.041		
3	0.014	0.039		
4	0.018	0.042		
5	0.011	0.036		
6	0.013	0.038		
7	0.019	0.043	Median 0.04	
8	0.010	0.035	Average 0.04	
9	0.014	0.039	SD 0.003	
10	0.013	0.038	%RSD 6.43	

Standard curves are visually inspected for the presence of outliers or non-linear values. When noted, these are excluded from the calibration curve. A 100 mg/L mixture of Lactate, Acetate, Propionate, Formate, Butyrate, and Benzoate are used to make the calibration standard (Inorganic Ventures, Christiansburg, VA); a 100 mg/L concentration standard of Pyruvate (Across Organics, Geel, Belgium) is purchased separately and combined with the above to make a 100 mg/L stock solution.

Concentrations for blanks and standards are calculated from the R^2 equation derived from the standard calibration curve, fig. 2. Equation 2.

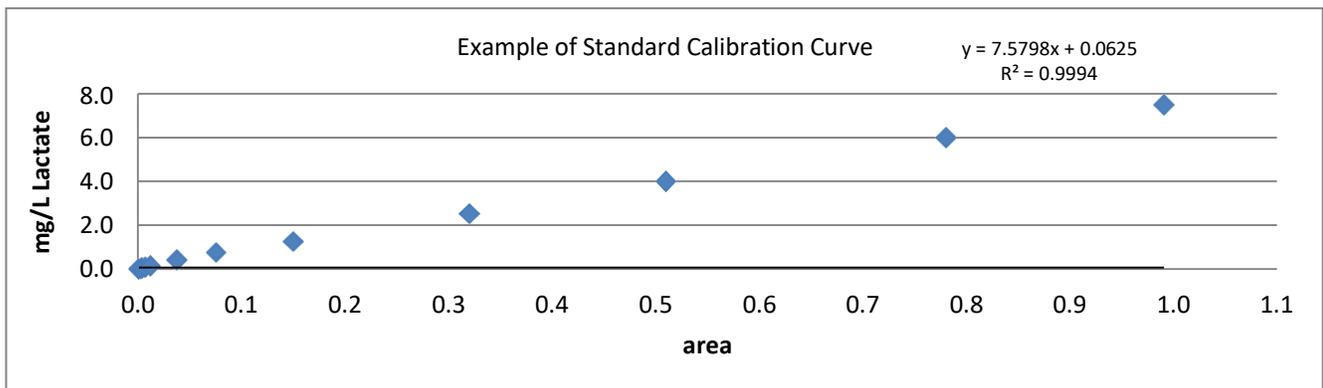


Figure 3. Typical calibration curve for the determination of lactate in water samples.

The LMWOA sample concentration is calculated using the following equation: $y = mx + b$

Where:

$$y = \text{LMWOA mg/L}$$

m = the slope of the regression line x = the area count

b = the y-intercept

Preparation of Reagents

Add 20 mL of 100 mg/L Organic Acid Standard each (mixture thawed and the pyruvate combined) to 200-mL volumetric flask containing approximately 150 mL 18.2 Ω water and fill flask with additional 18.2 Ω water to the fill line. A final concentration accuracy of $\pm 2\%$ is acceptable.

Preparation of Standards

Pyruvate standard:

0.1009 g Pyruvate into a 1000-mL volumetric flask = 100 mg/L solution Stock Standard

Preparation Calculations:

160 mL 18.2 Ω water + 20 mL standard mixture + 20 mL of pyruvate standard

Calibration Standards:

Calibrations standards are made from a multi-analyte mixture and pyruvate standard. Appendix I. contains exact standard concentrations.

Quality Assurance and Quality Control

Raw field samples are run in duplicate or triplicate with blanks and calibration standards in each batch. The blanks are run to establish a stable baseline and monitor any carryover contamination during analysis of samples. Conditioning blanks are run before and after the calibration curve. They help in decreasing the instrument blank concentrations by reducing carryover and help restore baseline conditions. Calibration standards are used to calibrate the instrument.

Detection Limits

The method detection limit (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. This is determined from analysis of a standard in a given matrix containing the analyte. This procedure is described in 40 CFR part 136, Appendix B, rev. EPA Method 415.1, 1.11 (http://www.caslab.com/EPA-Method-415_1/). The statistical method and data for nine replicate standards being analyzed are listed in table 2. The MDL is calculated using the following equation:

$$MDL = t_{(n-1, 1-\alpha=0.99)} (S)$$

Where: $t_{(n-1, 1-\alpha=0.99)}$ = the student's t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. (The t value for 10 replicates = 2.821), (S) = standard deviation of the replicate analyses (%) (<http://dnr.wi.gov/regulations/labcert/documents/guidance/-lodguide.pdf>).

Table 2. Batch Data With Peak Areas, Concentrations, and Calculations for LMWOA

Analyses of lactate

n of stds analyzed	Lactate area	Lactate concentrations in mg/L	Calculations	Data values
		Analysis		
1	0.023	0.100	Median (mg/L)	0.101
2	0.027	0.101	Average (mg/L)	0.101
3	0.021	0.099	SD (mg/L)	0.036
4	0.029	0.114	%RSD (mg/L)	34.35
5	0.033	0.127		
6	0.019	0.091		
7	0.026	0.101	MDL (mg/L)	0.090
8	0.034	0.128		
9	0.038	0.128		
10	0.022	0.100		

Potential Interferences

Certain matrices can cause interferences during sample analysis, yielding a high bias. If necessary, lab personnel will use IC OnGuard Ag, OnGuard II Ag, or OnGuard II Ba filters to filter samples to remove particles in the water samples. The IC OnGuard Ag and II Ag cartridges contain a silver form, high capacity, sulfonated, cation-exchange resin (Dionex.com) and may be used to remove chloride, bromide, and iodide from concentrated matrices. The IC OnGuard II Ba cartridge is a styrene-based, sulfonic acid resin in the form of barium (Dionex.com) and may be used to remove high concentrations of sulfate from sample matrices.

Sample Run

A batch of 50 samples including duplicates or triplicates is run with blanks and a calibration curve. An analyst examines the raw data and calculates the LMWOA concentrations. Average, median, SD and % RSD are calculated. If the % RSD exceeds 3% for any sample, that sample is re-examined and is added back on the next available day's analytical template.

Data Management and Records

Reported results are sent to submitters. Raw data runs are filed by project and date. Schedule calendars are updated as new submission and sample analyses requests are received.

Health, Safety, and Waste-Disposal Information

Applicable Health and Safety Issues

Personal Protection

Safety glasses, protective gloves, enclosed shoes and lab coats are required whenever samples area analyzed for LMWOA, especially when the samples are of biological origin. For other precautions and safety procedures, consult Safety Data Sheets (SDSs), (<http://www.ilpi.com/msds/index.html>).

Chemical Hazards

Site specific hazards will be documented and handled accordingly to OSHA regulations.

Specific Waste Disposal Requirements

Sample containers may be disposed of in the regular trash receptacle unless hazards are noted.

References Cited and Recommended Readings

American Society for Testing and Material, 1991, Standard Specifications for Reagent Water. D1193-77 (Reapproved 1983). Annual Book of ASTM Standards, Vole 11.01. ASTM: Philadelphia, PA.

U.S. Environmental Protection Agency Method Detection Limit (MDL) procedure found in Title 40 Code of Federal Regulations Part 136 (40 CFR 136, Appendix B, revision 1.11).

U.S. Environmental Protection Agency Report EPA 2000, 2000-National water quality inventory—1998 report 841-F-00-006, 45 p.

U.S. Environmental Protection Agency Report 40 CFR 136, Guidelines for Establishing Test Procedures for the Analysis of Pollutants, 1984 pp5-335

U.S. Geological Survey, 2002, USGS Handbook 445-3-H, Safety and Environmental Health Handbook, 435 p

U.S. Geological Survey, variously dated, National field manual For the collection of water-quality data: U.S. Geological Survey Techniques of Water-Resources Investigations, <http://water.usgs.gov/owq/Fieldprocedures.html>, chapter 4, collection of water samples

Book9, chaps. A1-A9. (Also available at <http://pubs.er.usgs.gov/publication/twri09>)

http://www.caslab.com/EPA-Method-415_1/ access date 4_23_2015

Dionex.com access date 04_29_2015

http://www.dionex.com/en-us/webdocs/4157-PS-Dionex-IonPac-AS11-AS11-HC-28Mar2012-PS70024_E-R2.pdf access date 4_23_2015

<http://dnr.wi.gov/regulations/labcert/documents/guidance/-lodguide.pdf> access date 4_23-2015

<http://www.ilpi.com/msds/index.html> access date 02_19_2014 *water.usgs.gov/nrp/biogeochemical-processes-in-groundwater* (USGS, 2015)