

STANDARD OPERATING PROCEDURE Procedure for Measuring Organic Compounds using High Performance Liquid Chromatography (HPLC)

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STANDARD OPERATING PROCEDURE

Procedure for Measuring Organic Compounds using High Performance Liquid Chromatography (HPLC)

BACKGROUND

The [Great Ships Initiative](#) (GSI) is a collaborative effort to end the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System through independent research and demonstration of environmental technology, financial incentives and consistent basin-wide harbor monitoring. To that end, GSI has established research capabilities at three scales—bench, land-based, and shipboard. Each scale is dedicated to addressing specific evaluation objectives, with protocols as consistent with IMO and federal requirements as practicable. Developers of ballast treatment systems apply for GSI research services [online](#), and awards are offered based on an objective review process. GSI incubation/testing will allow meritorious ballast treatment systems to progress as rapidly as possible to an approval-ready and market-ready condition.

GSI bench-scale tests take place year-round at the University of Wisconsin-Superior's Lake Superior Research Institute (LSRI) in Superior, Wisconsin. The LSRI is amply equipped with staff expertise and resources to conduct the tests, and has a long history of successfully undertaking similar tests.

The overarching goals of GSI bench-scale testing are to explore dose-effectiveness, chemical degradation, residual toxicity, and sensitivity to challenge conditions of a proposed ballast treatment method about which little is known. To that end, the tests are “range-finding” missions, to determine the optimal treatment dose/intensity that would maximize effectiveness and minimize residual toxicity. Findings help treatment developers better design an effective system and/or to move to the next stage of treatment evaluation. The tests are also a form of trouble-shooting to encounter possible problems with the proposed treatment in advance of more extensive and larger scale tests.

INTRODUCTION

This GSI Standard Operating Procedure (SOP) describes the method used to measure organic compounds using High Performance Liquid Chromatography (HPLC). HPLC is an analytical technique in which a liquid mobile phase transports a sample through a column containing a stationary phase. The interaction of the sample with the stationary phase selectively retains individual compounds and permits separation of sample components. Detection of separated organic compounds is achieved mainly through the use of UV absorbance or fluorescence detectors. In addition to separating the components of a sample, HPLC can be used to determine the concentration of the components.

The instrument should only be operated by persons who are trained in its use. Any problems or unusual conditions encountered should be addressed by experienced personnel.

This SOP is strictly for guidance purposes and is not intended as an instruction manual. The HPLC instrument manual should be consulted for further information regarding use and care of the instrument components.

DEFINITIONS

Brackish Water (BW): Synthetic water created from laboratory water (LW) with the addition of commercially prepared salts, such as Instant Ocean, to obtain a salinity of 16 parts per thousand (as measured by a refractometer).

Duluth-Superior Harbor Water (HW): Natural surface water collected from the Duluth-Superior Harbor of Lake Superior at a depth of approximately 3 m.

High Organic Content Laboratory Water (HOC-LW): Synthetic water created from laboratory water that is used as a surrogate in place of Duluth-Superior Harbor water.

Laboratory Water (LW): City of Superior, Wisconsin municipal water that has been dechlorinated by passage through an activated carbon filter. Note: Based on data from previous testing, background levels of chlorine from below the limit of detection (i.e., $<3 \mu\text{g/L}$) to $10 \mu\text{g/L}$ are expected in dechlorinated laboratory water, depending on where the dechlorinated water is taken from.

Prospective Ballast Treatment System (BTS): A system containing an active substance and/or component that mechanically, physically, chemically, or biologically serves to remove, render harmless, or avoid the uptake or discharge of potentially invasive organisms within ballast water (IMO, 2005).

Salt Water (SW): Synthetic water created from laboratory water (LW) with the addition of commercially prepared salts, such as Instant Ocean, to obtain a salinity of 32 parts per thousand (as measured by a refractometer).

EQUIPMENT LIST

- Solvent Reservoirs (Thick, Glass Vacuum Safe Bottles).
- High Pressure Pump (i.e., Shimadzu Model LC-10AT).
- Solvent Degassing System (i.e., Shimadzu Model DGU-20A).
- Solvent Mixing Unit (i.e., Shimadzu Model FCV-10AL).
- HPLC Autoinjector (i.e., Gilson Model 234).
- Variable Wavelength UV Detector (i.e., HP Series 1050).
- Integrator or Chromatography Data System.
- Analytical Column (i.e., LiChrospher 100 RP-18, 5μ Packing).
- Waste Container.
- Solvents Appropriate For the Mobile Phase.
- Autoinjector Vials and Caps.
- Deionized Water.

- Pasteur Pipets.
- Ultrasonic Bath.
- Vacuum Pump.
- Material Safety Data Sheets.
- Lab Coat.
- Safety Glasses/Goggles.
- Gloves.
- Instrument Maintenance Log Book.

PROCEDURE

Labware Cleaning

1. Use glass or Teflon sample containers whenever possible because these are the least likely materials to bind organic compounds.
2. Do not pre-clean new Pasteur pipets, test tubes, or autoinjector vials if it can be reasonably assumed that they are free of the analyte. The use of a blank sample during analysis will verify that no contamination or interference is present on new labware.
3. Clean all other labware used in analyzing organic compounds using the following procedure:
 - a. Use a laboratory approved detergent (e.g., LiquiNox) and wash in warm water.
 - b. Rinse labware thoroughly in hot water to remove all traces of detergent, followed by a rinse with deionized water.
 - c. Dry the items or remove excess water with an organic solvent (i.e., acetone, methanol, acetonitrile). Use only high-grade organic solvents so as not to interfere with the analysis. The solvent used as the mobile phase organic component would be a good choice.

Method Development

1. Develop an appropriate HPLC method prior to sample analysis. The following parameters/conditions must be defined for each chemical and matrix to be analyzed by consulting the literature, through trial and error testing, or a combination of both:
 - a. Sample preparation method (if required)
 - b. Type of chromatography column
 - c. Solvent(s) appropriate for the mobile phase
 - d. Various instrumental conditions and settings used to analyze a particular compound or group of compounds in a specific matrix
2. Complete the Operating Conditions Summary Sheet (Appendix 1) once a method has been established.

Instrument Preparation

1. Degas all HPLC solvents prior to use by applying a vacuum to the solvent reservoir while sonicating the bottle until the vigorous bubbling stops.
2. Ensure that the solvent that is in the system lines and column is compatible (i.e., miscible) with the mobile phase prior to starting the high pressure pump. If not compatible, pump a solvent that is miscible in both the mobile phase and the solvent in the system lines into the system for several minutes to avoid contact between the immiscible solvents.
3. If buffers are used in the mobile phase, use an intermediate solvent of the same organic solvent composition, but with water in place of the buffer, whenever switching between the mobile phase and a solution of higher organic solvent content. This prevents buffer salts from precipitating in the system.

HPLC Pump

1. Turn the HPLC pump on (Shimadzu LC-AT10).
2. Set the eluant to the percentages determined in the method development. To do this press the "FUNC" key until the "BCONC" screen appears. Enter the value for the percentage of eluant B required for the method. Press the "ENTER" key followed by the "CE" key. The percentage for eluant A will be the difference between 100 % and the value entered for eluant B.
3. Open the "DRAIN" valve about one half turn. Press the "PURGE" key. Allow to purge until ~20 mL of eluant has collected in the syringe. Stop purge by pressing the "PURGE" key. Close the "DRAIN" valve. Empty the syringe into the labeled HPLC waste bottle.
4. To avoid pressure shock to the analytical column the eluant pumping rate should be increased gradually. Set the pumping rate to 0.5 mL/min by pressing "0.5" on the keyboard followed by "ENTER". After about 30 seconds, increase the eluant flow to 1.0 mL/min by entering "1.0" on the keypad followed by "ENTER". If the desired pumping rate is greater than 1.0 mL/min, increase the pump rate to the desired value after pumping at 1.0 mL/min for about one minute.
5. Allow the eluant to flow for about 30 minutes before starting the analysis. This gives the system time to equilibrate to the mobile phase being used. The column must be completely equilibrated with the mobile phase for consistency of peak shape and retention time.

HPLC Detector

1. Turn the HP Series 1050 detector on.

2. The detector goes through a brief self-check. When the detector display indicates "HP 1050 UV", press the "LAMP ON/OFF" key followed by the "ENTER" key. The detector lamp will warm up and display the wavelength that the detector is set at. If the wavelength displayed is not the wavelength to be utilized for the analysis, change to the desired wavelength. To change the wavelength, press the " λ " key followed by the desired wavelength value and then "ENTER". The detector should then display the desired wavelength.
3. If other special detector conditions were determined in the method development, check to see that they are properly set. If needed, the HP 1050 detector manual should be used to help with making these changes.

HPLC Autoinjector

1. Turn on the Gilson Model 234 Autoinjector. After a brief warm-up, the "READY TO RUN" screen is displayed.
2. Select the autoinjector file that was created during the method development procedure. This is done by entering the desired "File #" followed by the "ENTER" and "RUN" keys.
3. Place the autoinjector vials containing the standards and samples into the sample tray. You will need to indicate the position of the first sample to be analyzed and the total number of samples to be analyzed. Press the "RUN" key. Indicate the position on the first sample in the sample tray and press "ENTER". Press the "NEXT" key and indicate the total number of standards/samples (vials) to be analyzed. Press "ENTER".
4. When ready to start analysis, press the "START" key.

HPLC Data System

1. Turn on computer, monitor, and printer.
2. After the computer boots, enter the User name ("LSRI") and the password ("HPLC").
3. Double click the "PEAK SIMPLE 3.29" icon on the desktop. When the software has finished loading, select "OPEN CONTROL FILE" under the "FILE" menu. Double click the icon for the control file that was created during the method development to open that file.
4. Before beginning analysis, press the "BALANCE" key on the HP detector. This zeroes the detector response. Also click the "AUTOZERO" icon located on the left side of the computer screen between the plus and minus magnifying lenses. This zeroes the data system input. This can be confirmed by checking the "STANDBY" mV reading in the upper right corner of the computer screen. It should read approximately 0.0 mV.

Analysis of Samples

1. The HPLC system is connected to a timer that automatically shuts the system down at a designated time. Be sure to check that the timer will not shut the system down before the analysis has been completed.
2. After having prepared the various HPLC components for sample analysis as described in the previous sections, press the "START" key on the autoinjector.
3. Observe the first few injections to see that autoinjector draws up sample from the appropriate sample vials and that the response on the computer screen (i.e., retention time, peak shape, integration, and baseline noise) is what is expected. If problems are encountered, press the "STOP" key on the autoinjector and determine where the problem is originating. Make any needed changes and restart the autoinjector. Again, watch to see that the analysis is proceeding properly.
4. When the analysis has been completed, label each chromatogram with the ID of the sample, date, and operator's initials. Before disposing of the autoinjector vials, double check the sample IDs on the vials against the sample IDs on the chromatograms. The vials can then be disposed of in the waste vial bucket.

HPLC System Shutdown

1. When analysis is completed, change the eluant to 100% strong solvent (i.e., acetonitrile). On the HPLC pump press "FUNC" until the "B CONC" screen appears. Change "B CONC" to 0.0 by entering 0.0 on the keypad and pressing "ENTER" followed by the "CE" key. Allow 100 % strong solvent to flow through the system for ~20 minutes. This is done to remove any tightly bound impurities that may have accumulated on the column.
2. Change the pump rate to 0.5 mL/min by entering "0.5" on the keypad followed by "ENTER". The pump can then be turned off by pressing the power button.
3. The detector, autoinjector, and data system can be shut down as soon as the analysis has been completed.
4. On the detector, press the "LAMP ON/OFF" key followed by "ENTER". Turn the power to the detector off by pressing the power button.
5. Turn the power to the Gilson autoinjector off.
6. Shut down the computer and turn the monitor and printer off.
7. Fill in the requested information in the HPLC logbook or on the data recording form. This includes the date of analysis, project, number of injections, number of hours system

was operating and operator's initials. Any problems or maintenance performed should also be recorded.

8. Waste solvent from the analysis should be collected and stored for later disposal as hazardous waste. Records of the volume and contents of the waste container should be kept.

Data Reduction

1. Use the data from the standards analyzed to generate a calibration curve. The slope and y-intercept of the calibration line are used to determine concentrations of analyte in the samples.
2. The raw data and analysis results should be summarized in a spreadsheet that is maintained in a 3-ring binder for the project. The chromatograms should also be stored in the 3-ring binder. The binder should be labeled with the project ID code, location where binder is kept and the name of the scientist responsible for the binder.

QUALITY ASSURANCE/QUALITY CONTROL

1. Conduct all quality assurance/quality control procedures according to the GSI/QAPP/1 - Quality Assurance Project Plan (QAPP) for Great Ships Initiative Bench-Scale and Land-Based Biological Tests (2009). Analyze data to ensure that all applicable data quality criteria are met.
2. Collect and analyze in duplicate at least 10 % of the samples to document sampling and analytical variability. At least 10% of samples should be spiked to determine spike recoveries.
3. Follow all procedures outlined in this SOP. Any deviations known ahead of time must be approved by the GSI Lead Investigator for Bench-Scale Studies. Any deviations made during the experiment must be recorded and also approved by the GSI Lead Investigator for Bench-Scale Studies as soon as practicable.
4. Record data on data collection forms or in specific laboratory notebooks. All instrument data output and data forms must be stored in a project-specific three-ring binder. Ensure hard copies of instrument data output and data collection forms are scanned and stored electronically.

DATA STORAGE AND ARCHIVING

1. Store and archive data according to GSI/QAPP/1 - Quality Assurance Project Plan (QAPP) for Great Ships Initiative Bench-Scale and Land-Based Biological Tests (2009).
2. Archive all hard- and electronic-copies of data and records generated for a period of five

years.

REFERENCES AND RELATED DOCUMENTS

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Gilson Medical Electronics. Gilson Model 234 Users Guide. Middleton, Wisconsin.

Hewlett Packard Company Waldbronn Analytical Division. Using Your Variable Wavelength Detector. HP 1050 Series of HPLC Modules, Federal Republic of Germany.

International Maritime Organization (IMO) (2005). Guidelines for Approval of Ballast Water Management Systems (G8) Adopted by Resolution MEPC.125 (53). London, England.

Shimadzu Corporation Chromatographic Instruments Division. Shimadzu High Performance Liquid Chromatograph Instruction Manual for Solvent Delivery Module LC-10AT. Kyoto, Japan.

SRI Instruments. PeakSimple 2000 Chromatography Integration Software Operation Manual. Torrance, California.

APPENDIX 1

HPLC OPERATING CONDITIONS SUMMARY SHEET

HPLC SYSTEM OPERATING CONDITIONS

EQUIPMENT: Shimadzu LC-10AT Pump	
Eluent Ratio:	
Solvent:	
Brand:	
Grade:	
Lot Number:	
Flow Rate:	
Operating Pressure:	
Maximum Pressure:	
Minimum Pressure:	
Analytical Column:	
Brand:	
Dimensions:	
Packing:	

EQUIPMENT: HP Series 1050 Detector	
Wavelength:	
Response Time:	
Recording Device:	
Output Range:	

EQUIPMENT: Gilson Model 234 Autoinjector	
File Number:	Rack Number:
Injection Method:	Loop Filling Method:
Injection Volume:	Number of Injections/Sample:
Chromatography Time:	Syringe Volume:
Transfer Tubing Volume:	Injection Loop Volume:
Injection Flush Volume:	GSIOC Number:
Inside Needle Rinsing Volume:	Outside Needle Rinsing Volume:
Injection Valve Rinse Volume:	Air Gap Volume:
Air Gap Asp. Flow Rate:	Vail Asp./Disp. Flowrate:
Priming Flow Rate:	Mixing Flow Rate:
Diluent Asp. Flow Rate:	

EQUIPMENT:		Peak Simple Data System		
CHANNEL DETAILS				
Description:				
End Time:				
Sample Rate:				
Remote Start:				
Default Display Limits				
Max mV:				
Min mV:				
Time Base:				
EVENTS				
Time:				Event:
Time:				Event:
Time:				Event:
Time:				Event:
Time:				Event:
Time:				Event:
Time:				Event:
Time:				Event:
INTEGRATION				
Peak (%):				
Baseline (%):				
Area Reject:				
Spike Channel:				
Standard Weight:				
Sample Weight:				
COMPONENTS				
Peak	Name	Start	End	Calibration
POSTRUN				
<input type="checkbox"/> Save file as:		<input type="checkbox"/> Save results (check if yes)		
<input type="checkbox"/> Autoincrement (check if yes)		<input type="checkbox"/> Print results (check if yes, see "Print" section)		
<input type="checkbox"/> Add to results log (check if yes)				
OVERALL CONTROLS				
Unknown Peaks are Labeled:				
<input type="checkbox"/> Show Retention Windows (check if yes)				

Label Peaks on Screen	
By: <input type="checkbox"/> Number <input type="checkbox"/> Name <input type="checkbox"/> Abbreviated Name <input type="checkbox"/> Retention Time	
Default Display Period	
Start:	
End:	
<input type="checkbox"/> Draw Label Vertically (check if yes) <input type="checkbox"/> Postrun File Overwrite Protection (check if yes)	
Graph Labels	
Scale Time By/Decimal Places:	
Scale mV By/Decimal Places:	
Method Detection Limit:	
Report Concentrations Below Detection Limit As:	
Data Automatically Saved Every (min):	
<input type="checkbox"/> Reset Relays at End of Run (check if yes) <input type="checkbox"/> Save Thumbnail Images (check if yes)	
PRINT	
<input type="checkbox"/> Print Header (check if yes) <input type="checkbox"/> Print Chromatogram (check if yes)	
<input type="checkbox"/> Print Report (check if yes) <input type="checkbox"/> Use Channel 1 Format for all Channels (check if yes)	