Villanova University
Villanova Urban Stormwater Partnership
Watersheds Laboratory

Standard Operating Procedure – VUSP – C

Chloride, Nitrate, Nitrite, Phosphate Determination
High Performance Liquid Chromatography (HPLC) Procedures

METHOD RETIRED SAVED FOR HISTORICAL PURPOSES
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Approved: ____________________________________________
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Distribution

Mary Ellen Dukart – Quality Assurance Officer
Laboratory Copy, maintained by Quality Assurance Officer

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The following laboratory staff have read and been trained on this Manual. A copy of this page will be maintained in the Laboratory. Training is good for one year.

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1.0 Scope and Application

The purpose of this standard operating procedure (SOP) is to demonstrate the proper setup and maintenance steps for the Water Model HPLC. This document serves as the basis for maintaining proper calibration and repeatability between samples as an addendum of the EPA Method 300.1 Determination of Inorganic Anions in Drinking Water by Ion Chromatography.

This SOP scope refers to the HPLC system, which consist of following components: a Waters Model 626 HPLC Pump with IonPac® ASII-HC Anion-Exchange Column, a Waters Model 431 Conductivity Detector, a Waters Model 600s Controller, a Waters Model 717plus Autosampler, a Dionex AMMS® III Eluent Suppressor, Galaxie Chromatography Data System Version 1.7.4.5, IonPac ATC-3 Trap Column 9x24mm, AG11-HC Guard Column, 4x50mm, and IonPac ASH11-HC Analytical Column, 4x250mm.

Blanks and calibration curves/checks are the quality control methods employed to validate or reject the resulting data, within the following detection limits:

<table>
<thead>
<tr>
<th>Conductivity uS/cm²</th>
<th>PO4 Detection Limits</th>
</tr>
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<tbody>
<tr>
<td>&lt; 80mg/L</td>
<td>0.2mg/L</td>
</tr>
<tr>
<td>150 mg/L</td>
<td>0.37mg/L</td>
</tr>
<tr>
<td>250mg/L</td>
<td>0.625 mg/L</td>
</tr>
<tr>
<td>350 mg/L</td>
<td>0.91mg/L</td>
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PO4 Detection Limits
August 10, 2005
Reference Research Study Form Number: HPLC2
## Chloride Concentration

<table>
<thead>
<tr>
<th>Conductivity (\text{uS/cm}^2)</th>
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<tr>
<td>&lt; 430uS/cm(^2)</td>
<td>0.05mg/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>710</td>
<td></td>
<td>0.0925mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1105</td>
<td></td>
<td></td>
<td>0.15625mg/L</td>
<td></td>
</tr>
<tr>
<td>1500</td>
<td></td>
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<td></td>
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August 10, 2005
Reference Research Study Form Number: HPLC2

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</tr>
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<td>1105</td>
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<tr>
<td>1500</td>
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</table>

#### 2.0. Method Summary

Concentrations of the following ions found in rainwater samples are determined via High Performance Liquid Chromatography (HPLC): chlorides (Cl⁻), nitrates (NO₃⁻), nitrites (NO₂⁻), and phosphates (PO₄⁻²). The HPLC is designed to separate the above noted components of the rainwater mixture via chemical reactions between the analyte and the chromatography column. In this process, the analyte is forced through a stationary phased column packed with microporous resin via pumping a hydroxide eluent of high pressure (mobile phase), which carries the analyte through the column. The velocity with which the analyte moves through the column is retarded via chemical interactions between the analyte and the column. The time at which the analyte elutes, or comes off the column, is noted as the retention time, and is used to determine the amount/identity of anionic components of the rainwater being tested.

#### 3.0. Definitions

- **Eluent**- sodium hydroxide; used as a solvent in separating materials.
- **Suppressor**- sulfuric acid; used to suppress conductivity detection
### 4.0 Health and Safety Warnings

*See Civil and Environmental Laboratory Safety Methods*

### 5.0. Interferences

Reference Product Manual IONPAC AG11-HC Guard Column IONPAC AS11-HC Analytical Column

Obtaining reliable, consistent, and accurate results requires that eluents be free of ionic impurities. Chemicals, solvents, and deionized water used to prepare eluents must be of the highest purity available. Reagent grade inorganic chemicals should always be used to prepare ionic eluents. Low trace impurities and low particle levels in eluents also protect the ion exchange columns and system components.

The deionized water used to prepare eluents should be Type I Reagent Grade Water with a specific resistance of 18.2 megohm-cm. The reagent water should be free of ionized impurities, organics, microorganisms, and particulate matter larger than 0.2 µm.

Carbon dioxide also readily dissolves in dilute basic solutions, forming carbonate. Carbonate contamination of eluents can affect the retention times of the anions being analyzed. Therefore, eluents must be maintained under an inert helium atmosphere to avoid carbonate interferences.

### 6.0. Handling and Preservation

All glassware and plastic ware used for collection, transportation, and laboratory analysis of the samples are acid-washed using the following procedure specified by laboratory protocol. The glassware and plastic ware is first cleaned with a laboratory detergent and rinsed with tap water. Next, the container is rinsed in 1:1 Hydrochloric Acid Solution. The container is then rinsed with deionized water three times and allowed to air dry.

The samples are analyzed immediately upon collection. All samples/standards are filtered through a 0.20 µm pore size, non-sterile, hydrophilic SFCA-membrane to remove any outstanding particulates and placed into 8x40 mm SEPCAP vials for analysis.
7.0. Equipment and Supplies

HPLC System
0.20 µm pore size, non-sterile, hydrophilic SFCA-membrane syringe filter
BD 3 mL Luer Lock syringes
8x40 mm SEPCAP vials
Reagents
Sulfuric Acid, ACS Grade
Sodium Hydroxide Solution, Certified 50% w/w
Deionized water, 18.2 megohm-cm

8.0. Quality Control

A system of standard solutions and blanks is used in every sample tray as a means of checking or validating the data.

Six standards are prepared from a stock solution that contains approximately 1000 mg/L Cl−, and 100 mg/L each of NO3-N, NO2-N, and PO4-P. The standards represent a linear range of concentrations from approximately 2-80 mg/L Cl−. A linear curve of peak area as a function of the concentration (mg/L) is generated and used to determine the unknown concentrations of each inorganic anion on the basis of its peak area. The regression must produce a correlation coefficient of at least 0.995 to be used. New standards are produced from the stock solution for each storm’s samples. A curve is generated for these fresh standards prior to their analysis, and again at the end of the sample tray. The second standard curve is not used to solve for unknown sample concentrations, but instead serves as a check for entire system. If the final curve agrees with the initial curve by at least 20% it may be assumed that all sample readings between the two curves are also valid. If the curves do not agree, the system is checked for malfunctions and the procedure rerun.

Finally, at least one set of duplicate samples are run for every batch analyzed. In this test, one sampling site is chosen at random in which two separate samples are taken and treated/analyzed separately. Both samples should render readings within 10% of each other to be considered valid. If they do not fall within this range, procedures are examined to be sure that all samples were treated in the same manner.
9.0 HPLC Procedures

The following instructions prepare the HPLC system for sampling.

9.1 Reagent Water Preparation
9.1.1 Reagent water: MQ 18.2 ohm cm
9.1.2 De-gas 1.5 L of reagent water for 30 minutes using the vacuum pump

9.2 Eluent Preparation
9.2.1 Prepare a solution of sodium hydroxide 50%w/w (NaOH 25 mN) by adding 2.6 mL of sodium hydroxide 50%w/w to reagent water and dilute to 2 L
9.2.2 De-gas the solution for 30 minutes using the vacuum pump

9.3 Sulfuric Acid Preparation
9.3.1 Prepare a solution of 25 mM sulfuric Acid (H₂SO₄ FW 98.06 CAS 7664-93-9) by adding 2.8 mL of sulfuric acid to reagent water and dilute to 2 L
9.3.2 De-gas the solution for 30 minutes using the vacuum pump

9.4 Master Standard Preparation
The standards solution (master solution) is used to identify the concentration of the inorganic anions by creating a calibration curve with the known concentrations vs. area under the peak identified by the chromatograph. The standards have a detection limit of 6 months.
9.4.1 Weigh 2.0 g of NaCl, 0.7000 g of NaNO₃, 0.700 g of NaNO₂, and 0.7000 g of KH₂PO₄ in separated aluminum cups
9.4.2 Place the cups in the oven at 105°C for 30 minute to evaporate any moisture lock in the compound.
9.4.3 Dissolve 1.6481 g NaCl, 0.6068 g NaNO₃, 0.4925 g NaNO₂, and 0.4393 g KH₂PO₄ in 1 L volumetric flask and top it with reagent water.
For Nitrate:

\[
0.1 \frac{g}{L} N \times \left( \frac{1 \text{ mol} N}{14.007 \text{ g} N} \right) \times \left( \frac{1 \text{ mol} NO_3}{1 \text{ mol} N} \right) \times \left( \frac{1 \text{ mol} NaNO_3}{1 \text{ mol} NO_3} \right) \times \left( \frac{84.996 \text{ g} NaNO_3}{1 \text{ mol} NaNO_3} \right) = 0.6068 \text{ g} NaNO_3
\]

For Nitrite:

\[
0.1 \frac{g}{L} N \times \left( \frac{1 \text{ mol} N}{14.007 \text{ g} N} \right) \times \left( \frac{1 \text{ mol} NO_2}{1 \text{ mol} N} \right) \times \left( \frac{1 \text{ mol} NaNO_2}{1 \text{ mol} NO_2} \right) \times \left( \frac{68.996 \text{ g} NaNO_2}{1 \text{ mol} NaNO_2} \right) = 0.4925 \text{ g} NO_2
\]

For Orthophosphate:

\[
0.1 \frac{g}{L} P \times \left( \frac{1 \text{ mol} P}{30.974 \text{ g} P} \right) \times \left( \frac{1 \text{ mol} KH_2PO_4}{1 \text{ mol} P} \right) \times \left( \frac{1 \text{ mol} KH_2PO_4}{1 \text{ mol} PO_4} \right) \times \left( \frac{136.084 \text{ g} KH_2PO_4}{1 \text{ mol} KH_2PO_4} \right) = 0.4393 \text{ g} KH_2PO_4
\]

For Chloride (E.g. 50mg./L):

\[
1.0 \frac{g}{L} Cl \times \left( \frac{1 \text{ mol} Cl}{35.453 \text{ g} Cl} \right) \times \left( \frac{1 \text{ mol} NaCl}{1 \text{ mol} Cl} \right) \times \left( \frac{58.442 \text{ g} NaCl}{1 \text{ mol} NaCl} \right) = 1.6484 \text{ g} NaCl
\]

This will give you an inorganic anions concentration of:

<table>
<thead>
<tr>
<th>Anions</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride (Cl-)</td>
<td>1000.0 mg/L</td>
</tr>
<tr>
<td>Nitrate (NO_3-N)</td>
<td>100.0 mg/L</td>
</tr>
<tr>
<td>Nitrite (NO_2-N)</td>
<td>100.0 mg/L</td>
</tr>
<tr>
<td>Orthophosphate (PO_4-P)</td>
<td>100.0 mg/L</td>
</tr>
</tbody>
</table>

9.4.4 Store the solution in the refrigerator
9.4.5 Use this solution to prepare your standards for a run storm sampling event
9.4.3.1 A typical standards curve look like the following:
These concentrations were prepared using “Dilution for Storm” spreadsheet.

### 9.5Initiate Flow of Instrument

To initiate the flow:

9.5.1 Make sure the conductivity detector is off
9.5.2 Turn on the controller and auto sampler equipment
9.5.3 Turn off the pump on the Direct Control Screen
9.5.4 Turn off the Helium pump in the HPLC System
9.5.5 Open the Eluent, MQ, and Supressor containers
9.5.6 Remove each respective cap and the pressure line. (Make sure the pressure lines do not touch the table)
9.5.7 Place all waste in waste container located in the enclosed cabinet below the HPLC, and rinse each container twice with reagent water
9.5.8 Remove the de-gassed new Eluent from the vacuum pump and add the new Eluent solutions to the Eluent HPLC container
9.5.9 Remove the de-gassed reagent water from the vacuum pump and add the new de-gassed reagent water solution to the Reagent Water HPLC container
9.5.10 Remove the de-gassed sulfuric acid from the vacuum pump and add the new sulfuric acid solution to the Sulfuric Acid HPLC container. Top it with reagent water
9.5.11 Place the cap/lines inside the container and close it.
9.5.12 Turn on the pump via the Direct Control Screen
9.5.13 Turn On the Helium pump to 40 psi
9.5.14 Rotate the inlet manifold valve handle located on the front of the pump to the Run Position
9.5.15 Place a beaker under the vent tube to collect the diverted eluent.
9.5.16 Turn the vent valve handle located on the injector panel to the Vent position to isolate the column from the flow path
9.5.17 Press the Direct key. At the Flow field, type a flow rate of 0.1 mL/min (the gradient proportioning valve is not actuated at 0 mL/min)

### 9.6 Drawing the Eluent

Once you have initiated flow, draw off the eluent as:

- **9.6.1** Move the cursor to the %A field for the MQ and type 100 in the New column. Press Enter.
- **9.6.2** Type 0 and press Enter for the remaining composition fields.
- **9.6.3** Attach the priming syringe to the Luer fitting on the inlet manifold valve.
- **9.6.4** Rotate the inlet manifold valve handle fully counterclockwise to the Draw position.
- **9.6.5** Draw off 5 mL of eluent then rotate the inlet manifold valve to the Run position.
- **9.6.6** Discard the liquid properly.
- **9.6.7** Repeat steps 6.1 through 6.4 for all remaining reservoirs you intend to use.

### 9.7 Priming

To prime the pump:

- **9.7.1** Move the cursor to the %A field for the MQ and type 100. Press Enter. Type 0 and Press Enter for the remaining % composition field.
- **9.7.2** Move the cursor to the Flow field and type 5.0. Press Enter. This sets the flow rate to 5.0 mL/min for reservoir A.

**Attention:** Confirm that the Vent valve is set to the Vent position when selecting high flow rates or when making rapid changes in flow rate. Sudden flow and backpressure variations or high flow rate may damage installed columns.

- **9.7.3** After one minute, or when the flow from the vent valve outlet is constant, press the Stop Flow screen key to stop the pump.
- **9.7.4** Repeat steps 7.1 through 7.3 above for all reservoirs in use.
- **9.7.5** Rotate the inlet manifold valve to the Run position.

### 9.8 Flushing the System

Flushing the system ensures that all eluent in the fluid system is replaced with new eluent before running samples.

Flushing involves:

- **9.8.1** Place the manifold valve to system
- **9.8.2** Press the Direct key to display the Direct Control screen
9.8.3 Move the cursor to the Flow field and type 1.0. Press Enter. This set the flow rate to 1.0 mL/min. Eluent now flows through the system at 1.0 mL/min. Be sure that %B (eluent reservoir) runs 100%.

9.8.4 Turn on the Conductivity Detector

Attention: Before starting to run a Method make sure that there are no air bubbles in the Sulfuric acid regeneration line to the suppressor and that the Conductivity value is low (< 0.021). Higher values could result in a loss of sensitivity at low concentrations, less than 0.1 mg/L

9.8.5 The system is ready for sampling
9.8.6 Fill out the Pre-HPLC Table page

For additional Information Refer to the Waters Autosampler Operator’s Manual.

9.9 Sampling Preparation

9.9.1 Conductivity
9.9.2 Open file “Dilution for Storm” Spreadsheet
9.9.3 Measure the conductivity of the samples
9.9.4 Copy the conductivity value to the spreadsheet
9.9.5 If the conductivity vs. Chloride concentration results in a value more than 75 mg/L of Cl-, then the samples need to be diluted or reacted with a solution of Ag₂SO₄ to precipitate chloride from the solution.
9.9.6 To prepare a Master Solution of Ag₂SO₄ add 2 grams of Ag₂SO₄ to a 1L volumetric flask topped with MQ water. The concentration will be 2000mg/L of Ag₂SO₄. Shake the solution until all Ag₂SO₄ is dissolved.
9.9.7 The Dilution for Storm Spreadsheet contains a matrix that will calculate the amount of solution required for dilution or reaction using a 50 mL volumetric flask. The reaction mass reaction between chloride and silver sulfate is 1:2
9.9.8 The reaction time for this process is less than 20 minutes per sample.
9.9.9 For reaction, add the calculated amount of Ag₂SO₄ from the matrix, to a 50 mL volumetric flask and top with the sample solution
9.9.10 Take about 3 mL of sample using a syringe.
9.9.11 Connect the 0.2 um filter to the syringe
9.9.12 Take out a few drop of the sample, and then fill the HPLC vial.
9.9.13 Close the vial.
9.9.14 Place the vial on the HPLC vial rack.
9.9.15 Repeat for all other samples
10.0 **Maintenance**

10.1 For the ATC-3 Column

Regenerate the ATC-3 using a fresh 2.0 M NaOH solution from a 50% w/w NaOH solution. Under normal operation conditions, the ATC-3 column should be generated every month to remove any contaminants that may be collected on it, including carbonate or when the conductivity baseline is ≤ 0.21. The daily regeneration of the ATC-3 column ensures that the IC system is systematically equilibrated for the most reproducible determinations of those anions being eluted by the weak eluent.

Amount of 50% w/w of NaOH Required:

Molarity = mol NaOH/L solution

Using a 200mL volumetric flask, calculate the amount required of a 50% w/w of NaOH solution to produce a solution of 2.0M NaOH.

\[
x \ M \ NaOH = \frac{50\% \times 1.53 \ g/mL}{40.01 \ g/mol} \times 1000 = 19.12 \ M \ NaOH
\]

, where 1.53 g/mL is the density of 50% w/w NaOH

Using dilution equation,

\[
(2.0M)(200mL) = (xmL)(19.12M)
\]

\[
xmL = \frac{(2.0)(200)}{19.12} = 20.92mL
\]

The amount required is 21 mL of 50% w/w of NaOH to produce a solution of 2.0 M NaOH.

**Procedure**

10.1.1 Prepare a fresh 2.0 M NaOH solution (21mL and dilute to 200mL using MQ water) from a 50% w/w NaOH solution. Degas for 30 min

10.1.2 Prepare a fresh 25 mM NaOH solution (eluent) as per HPLC Verification and Calibration SOP

10.1.3 Use the Analytical Pump to pump 100 mL of 2.0M NaOH solution through the 4-mm ATC-3 column or 50 mL for the 2-mm ATC-3 column. Direct the column effluent to a waste container

10.1.4 Install the ATC-3 column in the IC system and pump 20 mL of eluent (25 mM NaOH) through the 4-mm ATC-3 or 10 mL for the 2-mm ATC-3 column. Direct the effluent to a waste container
10.1.5 Turn on the IC system and allow it to equilibrate prior to starting your gradient

10.1.6 Fill out the cleaning schedule date

10.2 For the AG11-HC Columns

The Columns should be generated every month. Always ensure that the cleanup protocol used does not switch between eluent which may create high pressure eluent interface zones in the column. High pressure zones can disrupt the uniformity of the packing of the column bed and irreversibly damage the performance of the column. High pressure zones in the column can be created by pumping successive eluent through the column that are not miscible. Components in one eluent will precipitate out into the other eluent when using an acid eluent followed by a base eluent, thus creating neutralization pressure band.

The precipitation of the salts in solvents during column rinses can result in very high pressure zones. High viscosity mixing zones can be created between two eluent having solvents with a very high energy mixing. When in doubt, always include shorts column rinses steps to reduce content of the eluent to $\leq 5\%$ levels and the ionic strength of the eluent to $\leq 50\text{mM}$ levels to avoid creating high pressure zones in the column that may disrupt the uniformity of the column packing.

Choose the Appropriate Cleanup Solution.

A. **Concentrated hydroxide solutions** such as 10X concentrate of the most concentrated eluent used in the application is sufficient to remove hydrophilic contamination of low valence.

B. **Concentrated acid solution** such as 1 to 3 M HCl, remove high valency hydrophilic ions by ion suppression and elution by the chloride ion.

C. **Metal contamination** often results in asymmetric peak shapes, and/or variable analytes recoveries. For example, iron or aluminum contamination often results in tailing of sulfate and phosphate. Aluminum contamination can also result in low phosphate recoveries. Concentrated acid solutions such as 1 to 3 M HCl remove a variety of metals. If after acid treatment, the chromatography still suggests metal contamination, treatment with chelating acid such as 0.2 M oxalic acid is recommended.

D. **Organic solvents** can be used alone if the contamination is nonionic and hydrophobic.

E. **Concentrated acid solution such as 1 to 3 M HCl** can be used with compatible organic solvents to remove contamination that is ionic and hydrophobic. A frequent used cleanup solution is 200 mM HCl in 80% acetonitrile. This solution must be made immediately before use because the acetonitrile will decompose in the acid solution during long term storage.
Regardless the clean up solution chosen, use the following steps:

10.2.1 Prepare a 200 mL solution of the appropriate clean up solution
10.2.2 Disconnect the IonPac ATC-3 column and connect directly to the analytical column
10.2.3 Disconnect the IonPac AG11-HC guard column and analytical column, reverse the order of the guard and analytical column in the eluent flow path
10.2.4 Ensure that the guard column is placed after the analytical column in the eluent flow path
10.2.5 Set the pump flow rate to 1.0 ml/min. If backpressure occurs, set the flow rate to 0.5 ml/min and run it for two hours
10.2.6 Rinse the column for 10 min with reagent water
10.2.7 Pump the cleanup solution through the column for 60 min
10.2.8 Rinse the column for 10 min using reagent water
10.2.9 Return column to original positions
10.2.10 Equilibrate the column with eluent for at least 30 min.
10.2.11 Set your system as was originally configured
10.2.12 Fill out the cleaning schedule page

10.3 Waters 431 Flow Cell Cleanup

To clean the Water 431 Flow Cell with 20 mL of 6N Nitric Acid. Foreign materials in the flow cell may cause baseline drift, cycling, or noise. Even though this drift in the baseline does not create a problem in the identification of the anions, if it continues to drift, it will create a repeatability problem in the results. The drift should be < 0.21.

Amount Nitric Acid Required:

Using dilution equation,

\[(6N)(50mL) = (x mL)(15.8N)\]

\[x mL = \frac{(6)(50)}{15.8} = 18.98mL\]

The amount required is 19 mL of 15.8N of HNO₃ to produce a solution of 6N HNO₃.

Procedure

10.3.1 Prepare a 6N HNO₃ solution (19mL and dilute to 50mL using MQ water) from a 15.8N of HNO₃ solution
10.3.2 Disconnect the in and out lines
10.3.3 With a syringe, inject 20 mL of 6N Nitric Acid
10.3.4 Flush with Reagent MQ water
10.3.5 Equilibrate the HPLC system with eluent

For additional information refer to the Water 431 Conductivity Detector Operators Manual

10.4 Column Storage

To prevent organic growth inside the column during long period of non-use or drought, store each of column in the refrigerator at 4°C. Make sure each end is closed tightly.

11.0 **Galaxie Chromatography Data System**

The following program works directly with the HPLC system to plot the relationship between conductivity and time. The area under the curve is calculated by the program algorithm and it is used to calculate the concentration of the selected inorganic materials.

11.1 **START SOFTWARE**

11.1.1 Turn on the computer and load Window XP
11.1.2 Username: Administrator
11.1.3 Password: ceelab123
11.1.4 Load the Galaxie software
11.1.5 Username: mdukart or clay
11.1.6 Password: ceelab123

11.2 **Sequence**

The sequence allows the user to setup the order, method and any repetition for the samples. This is the main part of the software that controls the HPLC system.

11.2.1 Go to File
11.2.2 Select New Sequence
11.2.3 Select the number of samples
11.2.4 Select the method to be use, in this case is 2AMMSMETHOD10uL.METH
11.2.5 2AMMSMETHOD10uL have the following parameters:
11.2.5.1 Flow at 1.2mg/L
11.2.5.2 Acquisition length 20.00min
11.2.6 For the last sample select the following method, 2STOP.METH
11.2.7 2STOP.METH contain the following parameters:
   11.2.7.1 Flow at 0.5mg/L
   11.2.7.2 Acquisition length 30 min, then until equipment is manually turn off.
11.2.8 Copy the name of the sample on Run Name
11.2.9 Copy the number of injections
11.2.10 Copy vial id
11.2.11 Copy vial position number
11.2.12 Select the vial for which the sample is located
11.2.13 As soon as all the standard and samples names and location order are loaded in the software:
   11.2.13.1 Press Zero button in the Conductivity Detector
   11.2.13.2 Press the Play Icon in the Galaxy Software
   11.2.13.3 The equipment begins with the analysis

11.3 Load the Data

As soon as the sequence section finalize with its analysis, a COMPLETE sign will appear at the top of the software window. This tells the user that the program has concluded.

To load the data:

11.3.1 Close all the sequence windows
11.3.2 Go to File
11.3.3 Open Chromatograph
11.3.4 Select the standards chromatographs: The chromatographs will be open. Make sure the baseline remain constant throughout the test time and that the pick are well form
11.3.5 The following is an example of the graphical results for a solution containing 30.0066mg/L Cl-, 2.9994 mg/L NO3-N, 3.0415 mg/L NO2-N and 2.9983 mg/L PO4-P. Make sure the retention values are within that stated in the methods.
11.3.6 Copy in a separate paper the location of the know picks for all the standards.
11.3.7 Close chromatograph

**11.4 Load Reprocessing List**

The reprocessing list help the user to identify any know pick by selecting were the picks are going to be located at an average time frame.

11.4.1 Make sure all chromatograph are close
11.4.2 GO to File
11.4.3 Select New Reprocessing List
11.4.4 Set the number of chromatogram to be reprocess
11.4.5 Open the selected chromatograph
11.4.6 Select the “Result Method” to be used. * Make sure the standards peaks are writhing the values of the method.
11.4.7 Press Play
11.4.8 Save the reprocessing list.
11.4.9 Close the reprocessing list windows
11.4.10 Open the selected chromatograph and make sure that all known picks are identified

11.5 Summary Information

The summary information is to transfer all the chromatograph data to Microsoft Excel Program for further analyzes.

11.5.1 Close all chromatographs
11.5.2 Go to File
11.5.3 Select Open Summary Report
11.5.4 Select File “Storm Report”
11.5.5 Delete all the chromatograph
11.5.6 Select the desired chromatograph to be transferred
11.5.7 Change the file name on the selected areas
11.5.8 When finished, click on the Export Report Icon. All data regarding the chromatograph will be copied to your selected file location. All data is now ready for proper analyzes.

For additional information, read the Galaxie Chromatography Data System Version 1.7.4.5. Instruction Manual.
12.0. Waste Management

All eluent and suppressor waste is placed in a sealed container, kept in a cabinet under the HPLC instrument. When the container if full, the waste is neutralized and poured down the drain.

13.0. References


Waters Company “Waters 616/626 LC System Users Guide,” Milford, MA.
