Application of Ion Chromatography to the Investigation of Real-World Samples

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Lab Documentation

Student Handout:

Chemistry 134

Experiment #4: Ion Chromatography

Handout prepared by David Rakestraw, Rebecca Whelan, Theresa Hannon, and Cheol Keun Chung

I. Summary of Laboratory

In this laboratory we will use ion chromatography to analyze a variety of small anions and cations. Ion chromatography is a specific implementation of a more general technique, high performance liquid chromatography. Ion chromatography is used extensively in the modern chemical analysis laboratory. The development of new detection methods and advances in separation materials continues to expand the application of ion chromatography. This experiment will allow us to develop an understanding of the general principles of chromatography and explore some of the some of the specific applications of ion chromatography. This experiment will be conducted over six laboratory periods and will have three main components.

Each lab group will complete:

(1) either the "Anion Analysis" or the "Transition Metal Analysis"

(2) the "Contributions to Band Broadening" study using the same chromatography system used in step 1

(3) "Experimental Design and Analysis of 'Real' Unknown"

Material safety data sheets or Dionex product sheets will be provided for all chemicals used in the first two sections of this laboratory. The 4-(2-pyridylazo) resorcinol solution and some of the transition metal ion solutions discussed below are especially toxic. Keep in mind, also, that the transition metal solution containers and MSDS sheets may say they contain "copper in HNO_3" or something similar; remember that these metals react with the acids to form ionic compounds that may be significantly more hazardous than the solid metals themselves. You must review this information and write appropriate sample handling strategies in your lab notebook **before beginning any of these investigations**. Each team will be responsible for obtaining appropriate safety information for the chemicals employed in the self-designed experiment.

Anion Analysis

An unknown sample that contains as many as seven different anions (F^-, Cl^-, Br^-, SO_4^{2-}, NO_2^-, NO_3^-, PO_4^{3-}) will be analyzed. The identity of the anions will be determined by their retention times. The concentration of the anions will be determined by preparing a calibration curve from a standard solution containing known concentrations of all the anions of interest. The detection of the anions will be achieved by measuring the conductivity of the separated anions as they elute from the separation column. Detection limits for the seven anions will be determined.

Transition Metal Analysis

The analysis of transition metal ions will be similar to the anion analysis. An unknown sample containing up to six transition metal ions (Pb^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+}) will be analyzed for composition and concentration. In this case, the eluted species will be detected using absorption spectroscopy. This detection makes use of a strong chromophore that is created by complexing the transition metals with 4-(2-pyridylazo) resorcinol (PAR) following the separation. Detection limits for the transition metals will be determined.

Measuring Contributions to Band Broadening

There are a number of contributions to separation efficiencies obtained in high performance liquid chromatography. Many of the processes leading to band broadening are a function of flow rate. In this component of the laboratory we will measure the plate height, H, under different flow velocities. This will allow identification of the main contributions to band broadening in ion chromatography.

Experimental Design and Analysis of "Real" Unknown

This component of the laboratory will challenge our capabilities as analytical chemists. Each team of two to three students will define an analysis strategy that uses one of the ion chromatography instruments. This will require several important steps: 1) define target problem and analyte(s); 2) develop a sampling strategy; 3) determine method of sample preparation; 4) develop a calibration strategy; 5) run the sample mixture on the ion chromatograph; 6) evaluate the data. Some examples of "real" unknowns that were evaluated in previous years include different brands of soda beverages, mouthwashes, tea, zinc tablets, and swamp water. Almost anything that is completely soluble in water can be analyzed for anions, cations, and transition metals by ion chromatography.

II. Theory of Chromatography

Basic Concepts of Liquid Chromatography

The basic theory of liquid chromatography will be covered in lecture and can be found in Harris's textbook entitled *Quantitative Chemical Analysis*.

Introduction to Ion Chromatography

Ion Chromatography by Hamish Small, compliments of Dionex Corporation, can be found in the library. This material will be summarized in lecture.

III. Laboratory Schedule and Procedure

Each laboratory section (TTh and WF) should divide into teams of two to three students. The teams will be assigned by the TA. **This final laboratory report will be written in teams.** The schedule for the six laboratory periods is given below

Laboratory Schedule

	Group A	Group B	Group C	Group D
Day 1	--Preparation of Transition Metal Standards --Transition Metal Analysis	--Planning for Real World Sample	--Preparation of Anion Standards --Anion Analysis	--Planning for Real World Sample
Day 2	--Planning for Real World Sample	--Preparation of Transition Metal Standards --Transition Metal Analysis	--Planning for Real World Sample	--Preparation of Anion Standards --Anion Analysis
Day 3	--Band Broadening Experiment Using Transition Metals --Data Analysis	--Confirm Plan for Real Sample Analysis --Begin Sample Preparation	--Band Broadening Experiment Using Anions --Data Analysis	--Confirm Plan for Real Sample Analysis --Begin Sample Preparation
Day 4	--Confirm Plan for Real Sample Analysis --Begin Sample Preparation	--Band Broadening Experiment Using Transition Metals --Data Analysis	--Confirm Plan for Real Sample Analysis --Begin Sample Preparation	--Band Broadening Experiment Using Anions --Data Analysis
Day 5	--Sample Preparation --Initial Screening	--Sample Preparation --Initial Screening	--Sample Preparation --Initial Screening	--Sample Preparation --Initial Screening
Day 6	--Analysis of Real Sample	--Analysis of Real Sample	--Analysis of Real Sample	--Analysis of Real Sample

Two ion chromatography instruments are available for this experiment. One is configured for anion analysis and the other for transition metal detection.

1) The anion analysis system can measure a wide range of anions using an anion exchange column (Dionex, AS12) and conductivity detection. This system can also be configured for cation detection (e.g., Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}) by replacing the column (Dionex, CS12A) and changing the mobile phase (eluent).

2) The transition metal ion chromatography system uses a cation exchange column (Dionex, CS5A). This system detects the transition metals by complexing them with 4-(2-pyridylazo) resorcinol following elution from the column. The complexes have a maximum absorbance at 530 nm.

Detailed information on the columns, optimized operation parameters and sample applications can be found on the Dionex application notes disk. It is recommended that you examine this information to gain a better understanding of the system operation.

General Instructions

Concentrated stock solutions of known concentrations for each of the seven anions are available. Use these solutions sparingly. Using the stock solutions, you should prepare 100 mL of standard solution that contains all the target analytes (either anions or transition metals) at a 10-fold dilution of the stock solutions. This standard solution will contain the maximum concentration of each analyte. Use this standard solution to prepare a series of dilute solutions for obtaining calibration curves. The stock solutions, used to prepare your standard solutions, may contain slightly different concentrations of each analyte. This is a first-order attempt to correct for the different response functions of the detectors for the various analytes.

Each sample injection on the ion chromatograph will take about 3 mL of solution to assure complete rinsing of the syringe and the injection loop. Keep this in mind when making up solutions for analysis.

General instructions for instrument operation will be given in sections IV and V.

A. Anion Analysis (Br⁻, Cl⁻, F⁻, NO₃⁻, NO₂⁻, PO₄³⁻, SO₄²⁻)

The experiment will be composed of four steps.

a. ***Determination of Retention Times of the Anions***: One way to determine the retention time of each anion would be to obtain a separate chromatogram for each analyte independently. However, this approach is time-consuming. Instead you should devise a procedure that will allow you to identify the retention times of the anions with a small number of chromatographic runs that contain a combination of analytes at different concentrations. Consider an example of four analytes. First run a standard that contains all four analytes at known concentration. Then make a second run that contains only three of the analytes but at a concentration ratio that has been changed to be 1:0.5:0.25 of the standard solution concentrations. This should allow the determination of the retention times of all the analytes in two chromatographic runs. You should develop a plan and write it in your notebook prior to the laboratory period. The solutions for these experiments should be made by dilution of the stock solutions. (Note: these solutions will be different from the solutions used for your calibration curves.)

b. ***Identification of Anions in Unknown Sample:*** This will only require a single run of your unknown. Compare the retention times of your sample with that of the known retention times for the target anions. Note the approximate response of the unknown anions for the next step.

c. ***Calibration Curves and Determination of Unknown Concentrations:*** Based on the response of the analytes in your unknown sample, determine the appropriate concentration range over which to make a calibration curve. Using your standard solution, prepare dilutions that will cover approximately one order of magnitude and encompass the concentrations of the all the analytes in your unknown. Use four different concentrations for the calibration curves. Make two measurements at each concentration.

d. ***Determination of Detection Limits:*** Estimate the limit of detection for each of the anions by determining the concentration that would provide a signal-to-noise (S/N) ratio of approximately 3:1. Although it might be possible to estimate this from the lowest concentration used in your calibration curves, a more accurate determination could be made by making a chromatographic run at a concentration that gives approximately 10:1 S/N ratio. Prepare a dilute solution using your standard solution (containing all the analytes) that will give a response in this range for the analytes. Make two chromatographic runs if time allows.

B. Transition Metal Analysis (Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+})

The experiment will be composed of four steps. (a) Identify the retention time of the transition metal ions. (b) Identify the transition metal ions in your unknown sample. (c) Create a calibration curve and determine concentrations of the transition metal ions in your unknown sample. (d) Determine detection limits.

The steps for the transition metal analysis will be identical to those described for the anion analysis except the concentrations and number of analytes will be different. Note that the transition metal stock solutions have been prepared in dilute nitric acid to maintain a pH of ~4. Take appropriate precautions.

C. Measuring Contributions to Band Broadening

In this experiment, each team will perform a series of separations to determine the dependence of the separation efficiency on flow velocity. Measurements will be conducted on either transition metal ions or anions (see schedule). The measurements should be made with a standard solution containing multiple analytes. Choose a concentration that will give a signal-to-noise ratio of ~50:1. (Too high a concentration can lead to increased peak widths.) Collect one chromatogram at approximately 6 different flow velocities ranging from 1.0 to 1.6 mL/minute. From each chromatogram determine the plate height for each analyte by measuring the peak width and the retention time. Create a van Deemter plot for each of the analytes.

If time allows, the teams should consider conducting an experiment that examines other potential band broadening mechanisms. Some potential options are listed below.

- Test the effect of increasing the concentration of the analyte by a factor of 50.
- Increase or decrease the concentration of the eluent.

D. Experimental Design and Analysis of "Real" Unknown

Define Target Problem and Analyte

The ability to measure a wide range of anions, cations and transition metals at relatively low concentrations allows us to investigate many interesting chemical analysis problems. Dionex has compiled a number of application notes that are on a CD in the laboratory. These notes will give you some ideas about the types of problems ion chromatography can address. The scientific literature will provide an even greater database. Use these sources and your imagination to come up with a problem that is of interest to your team.

Your team should attempt to identify a problem involving anions or transition metal ions and discuss it with the TAs and the instructor by the end of the second laboratory period. This will allow time to acquire any additional chemicals that might be required for the analysis. It will also be necessary to develop a time schedule for using the two instruments during the last three lab periods. We will need to spread out the experiments over the two instruments. Early planning will assure you can do the experiments you desire.

** Your team must obtain material safety data sheets (MSDS) or other appropriate safety information for all chemical species you intend to use in your investigation. You must plan

appropriate sample handling procedures and discuss them with the TAs <u>prior to</u> the start of sample

collection.

Sampling Strategy

Each team will be responsible for gathering its samples outside of class. This might be simple or complex depending on the problem. For example, a study of the fluoride concentration in Bay Area water supplies might require collection of samples from numerous municipal water suppliers. It would be important to collect samples at several locations within the municipal supplier district and maybe even collect the samples on different days. A second example might be the analysis of aquarium water for nitrates and nitrites. This analysis might be an effective way to test filter systems or the dependence of the concentration on the time since the last aquarium cleaning.

The type and timing of sampling will be coupled to the scientific purpose of the experiment. Think about the stability of the samples. What is the best way to store the samples and maintain the integrity of the sample? What type of collection vessels should be used? Make sure to label samples carefully.

Sample Preparation

Sample preparation can be also be very simple or very complex. Clearly, water quality studies present a simpler sample preparation problem than identifying the transition metal content in battery acid. The preparation of solid samples will require some research to determine the best methods to dissolve the samples without changing the chemical nature of the analytes. There will often be protocols in the literature (try SciFinder Scholar if you need help in finding journal articles) describing sample preparation methods.

Calibration Curve and Sample Chromatograph

Once a prepared sample is available, obtain a sample chromatogram to determine the complexity of the sample and the approximate concentration of the analyte. Run a standard solution containing the analyte(s) of interest to determine the approximate concentration and retention time. Confirm that the analyte(s) of interest are in the sample by spiking the sample with a solution containing a known amount of the analyte. The concentrations of the analytes can be determined using a standard calibration curve or by using the method of standard addition. Adjust number of replicate measurements to allow statistical analysis as time allows.

Data Analysis

As you collect your chromatographic data, attempt to make a rough evaluation of the data while in the laboratory to help identify any gross errors in the measurements. Ask yourself questions such as: Do the data for the sample match the concentration range used for the calibration curve? Do the standard addition data appear to fall on a straight line?   Collect repeated measurements if they are necessary.

IV. Description of Ion Chromatographic Instruments

The instruments are controlled via PeakNet, a software package developed by Dionex. This software will control the operation of the injection valve, the pumps, and the detectors. The TAs will give a brief introduction to the software at the beginning of the laboratory period. Detailed descriptions of the instrumentation and software are available in the Dionex manuals. There is a manual for each ion chromatography system and two manuals for the PeakNet software. In general the procedure will be as follows.

1) Start the PeakNet software.

2) Load a "method" with preset parameters for running the pumps and detectors.

3) Establish that the baseline is stable.

4) Fill injection loop with the sample to be analyzed using a syringe. Approximately 1 mL of sample should be flushed through the injection loop to assure effective rinsing.

5) Run the sample. This will require providing a filename.

6) A data report will be generated containing the information necessary for analysis (such as peak areas and retention times). Further data analysis can be conducted using the *Optimize* function of the PeakNet software or by analysis with Excel or Kaleidagraph.

More specifics:

The typical procedure for operating **anion chromatography** is:

- Turn on ED 40, GP 50, and computer.

- Launch PeakNet; click on run button; then click on "file"; find and click on "load method", then "browse"; go to c:\chem134\method folder and find the correct method to load.

- Click on "baseline" under "run"; wait until you get a stable baseline.

- Rinse the inject valve with DI water two or three times, each time 0.5-1.00ml DI water; then rinse it with analyte solution.

- Then, without removing the syringe from the injection port, click on "run." If you find that all ions come off the column, click on "end" to stop the run. The data will be stored automatically to the folder you have set up. If you click on "abort", the run will end and no data will be saved.

- Repeat this procedure until you complete your job.

- Click on "Load Method" and go to c:\chem134\method folder. Find "stop" method and load it. Now it is safe to turn off computer and chromatograph.

The typical procedure for **transition metal (T-metal) analysis** is almost the same as anion, except that it is necessary to warm up the lamp in advance, and the shutdown procedure is different. The shutdown procedure is as follows:

- Load "PAROFF" method to turn down PAR, our complexation ligand solution; wait for about 5 minutes to allow the eluent to rinse PAR out of our system.

- Load "T-Metals SHUTDOWN" method; then you are safe to turn it off.

A schematic diagram of the anion analysis system is shown below. The key components are: 1) injection valve and loop; 2) guard column; 3) separation column; 4) ion suppressor; 5) conductivity cell. The guard column is used to protect the separation column from the injection of particulates or high analyte concentrations that might damage the expensive separation column. The ion suppressor removes the high concentration of eluent ions before directing the mobile phase to the conductivity cell. (More detailed information is available in the text, *Ion Chromatography,* and will also be presented in lecture.)
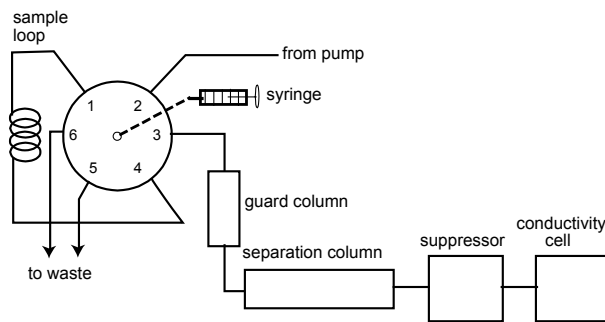
The transition metal ion chromatography system is similar, except that the ion suppressor and the conductivity detection cell is replaced by a postcolumn mixer and an absorption cell. A schematic diagram of the optical system used for the absorption measurements is presented below.
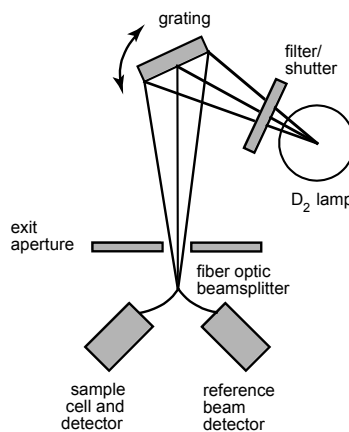
V. Description of the PeakNet Chromatography Software

In addition to running the instruments, the PeakNet software can be used to analyze your data using the "Optimize" function. This function is described in great detail in the Dionex manuals. One key piece of information that is provided by the software is the integrated area of each peak (peak area $\propto$ concentration). The software has been preset by the TAs to provide peak integrals. However, this integration will sometimes fail, and you may need to change the parameters for integrating the peaks.

Instructor Notes:

Background Information

Because this is the last laboratory experience in our Quantitative Analysis course, it is expected that the students have previously learned how to make a calibration curve and to use that calibration curve to determine unknown concentrations and associated errors. Therefore, they are not provided with detailed instructions regarding these tasks. Students learn about the chemical hazards (which have been discussed in the Lab Summary and in the Student Handout) by means of material safety data sheets (MSDS), Dionex product sheets, and/or labels on chemical containers. Material safety data sheets can be found on several websites. Information about the Dionex instrumentation, consumables, and reagents can be found on the Dionex Consumables/Instrumentation Manuals and Literature CD (Part Number 053891-14. Copyright 2001).

**Instructors and students should note that some material safety data sheets are more complete than others. Also, bottles and MSDS for atomic absorption standards often simply list the transition metal itself and not the ionic compound formed when the metal combines with nitric or hydrochloric acid, such as lead nitrate or cupric nitrate. It is important to realize that these standards likely contain the ionic forms of these metals, because species such as lead ion and cadmium ion may present much greater hazards than their solid metal counterparts.

Regarding assessment of student work, the instructor and TAs intentionally provide only minimal specific guidelines for the written report. The students have received extensive guidelines for the three previous laboratory reports they complete in the course, and it is expected that they have learned how to decide what is relevant enough to present and how most clearly to present it. Additionally, each team's report will have a different focus, specific to the self-designed experiment. In past years, each team has written an extensive report which encompasses all of the activities described in the above lab handout. This year, the students completed shorter structured exercises on the assigned activities and focused their lab reports entirely on their self-designed experiments. The resulting lab reports were found to be more cohesive and to more closely resemble real journal articles.

Experiment Details

The Dionex ion chromatography instruments we used have been described generally in the Lab Summary section. More detailed information about the instruments, reagents, and conditions can be found below. This information was modified from the Dionex Consumables/Instrumentation Manuals and Literature CD.

Separator columns are expensive; samples should always first be passed through the shorter and less expensive guard column to protect the separator column from potentially damaging sample material. In addition, samples should be prepared with the goal of making them innocuous to the columns. In the real-world sample component of this experiment, students passed all samples through filter paper and verified that sample pH was not extreme. Using dilute, filtered aqueous samples that are transparent to the eye, we have experienced no problems with column damage resulting from sample injection. Instructor discretion should be used to direct students away from real-world systems that might cause damage to the columns. When not in use, columns were stored according to instructions from Dionex. Under these conditions, we found that a single guard/separator column pair gave acceptable results for three years (being used for about one month each year), after which time sensitivity and resolution significantly degraded and the columns were replaced.

An ion suppression system was used with the conductivity detector. For anion detection, the suppressor was the ASRS-Ultra anion self-regenerating suppressor (4 mm); cation detection used the CSRS-Ultra cation self-regenerating suppressor (4 mm). Both suppressors are from Dionex and use the electrolysis of water for regeneration.

The transition metal ion samples were prepared from commercially available atomic absorption (AA) standards. AA standards with metal ion concentrations of approximately 1 mg/mL (1000 ppm) were given to the students, who prepared working solutions by dilution. Individual cation and anion solutions may be obtained from Dionex or prepared from salts of the ions. Anion standard solutions given to the students were 1-10 mM. Standard solutions containing either several transition metals or several anions are available from Dionex as well. For the transition metal cations, the following concentrations give reasonably sized peaks after complexation with PAR: 5 mg/L Pb^{2+}, 0.7 mg/L Cu^{2+}, 3.3 mg/L Cd^{2+}, 3.3 mg/L Cd^{2+}, 0.7 mg/L Co^{2+}, 1.3 mg/L Zn^{2+} (1mg/L = 1 ppm). Recommended concentrations of anions are: 3.0 mg/L F^-, 6.0 mg/L Cl^-, 10.0 mg/L NO_2^-, 20.0 mg/L Br^-, 20.0 mg/L

NO_3^-, 30.0 mg/L PO_4^{3-}, 20.0 mg/L SO_4^{2-}. Concentrations of cations to aim for include: 0.5 mg/L Li^+, 2.0 mg/L Na^+, 2.5 mg/L NH_4^+, 5.0 mg/L K^+, 2.5 mg/L Mg^{2+}, 5.0 mg/L Ca^{2+}. These are the concentration values suggested by Dionex for testing new columns. Higher concentrations of the ions may be needed as the columns age, and different instruments may have slightly different working concentration ranges.

Transition metals were detected after reaction with PAR. To detect transition metals present at concentrations greater than 0.5 ppm, Dionex recommends using a solution of 0.5 mM PAR in diluent. The diluent is 1.0 M 2-dimethylaminoethanol, 0.5 M ammonium hydroxide, 0.3 M sodium bicarbonate in water, pH 10.4. We prepared the working solution from 0.12 g of PAR in 1000 mL of diluent. Because PAR oxidizes in air, nitrogen or helium should be used instead of air to pressurize the PAR reservoir. In addition, PAR solution should be stored under nitrogen and should not be stored for more than two weeks. Dionex recommends adding PAR to the effluent stream at 0.6-0.8 mL/min. A 375-µL knitted reaction coil was used in our system for the mixing of effluent and PAR. After using PAR, eluent alone should be flowed for an additional minute, to remove residual PAR solution from the reaction coil and detector.

Determination of peak parameters (area, height, retention time) was done with Dionex software. Detection limits were not determined rigorously, but were estimated by serial dilution of a known concentration standard until the analyte peak was approximately three times the size of any background noise peaks.

We recommend that instructors and/or teaching assistants allot extra time beyond that expected for the completion of the experiment to allow flexibility in case of instrument break-down or high or low pressure warnings. It is also necessary for an instructor or teaching assistant to warm up the instruments (particularly the conductivity detector) an hour or more prior to the start of the lab each day.

Example Chromatograms

The following is an example chromatogram gathered by a student group using the transition metal IC set up to investigate an unknown solution prepared by the instructors. The solution was run at 1.2 mL/min. The students identified the numbered peaks as: 1: Pb^{2+}, 2: Cu^{2+}, 3: Cd^{2+}, 4: Co^{2+}, 5: Zn^{2+}, 6: Ni^{2+}. Resolution and time required to complete the separation were similar when the anion/cation system was used.
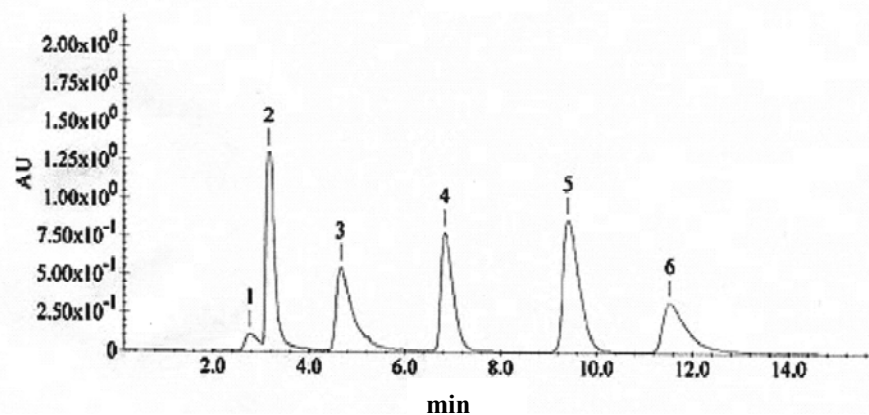
The following is a chromatogram gathered by the student team that investigated the concentration of anions in orange juice. The sample was syrup from Safeway brand canned mandarin oranges that was filtered and diluted with deionized water. The sample was run at 1.5 mL/min.
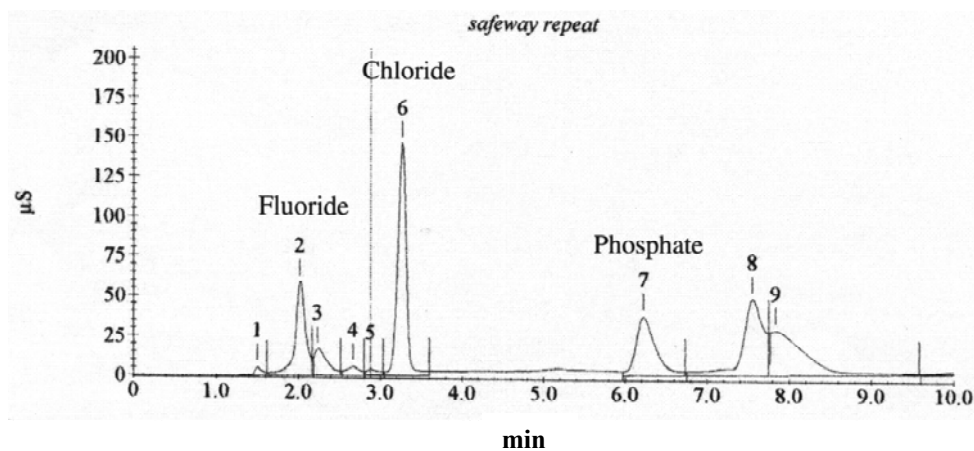
CAS Registry Numbers for Chemicals Used:

        Deionized water   7732-18-5

For anion solutions:

Sodium chloride	7647-14-5
Sodium fluoride	7681-49-4
Sodium nitrite	7632-00-0
Sodium nitrate	7631-99-4
Sodium sulfate	7757-82-6
Sodium phosphate	7632-05-5
Potassium bromide	7758-02-3

For transition metal solutions:

Atomic absorption standard solutions were used to make transition metal standards. The AA standard bottles typically say "Pb in HNO_3," for example. The predominating metal species in these solutions is the ion; therefore, we have included the CAS numbers for the metal nitrate or chloride compound as well.

Lead	7439-92-1	Lead nitrate	10099-74-8
Copper	7440-50-8	Copper nitrate	3251-23-8
Cadmium	7440-43-9	Cadmium nitrate	10022-68-1
Cobalt	7440-48-4	Cobalt nitrate	10141-05-6
Zinc	7440-66-6	Zinc chloride	7646-85-7
Nitric acid	7697-37-2		
Hydrochloric acid	7647-01-0		
Ferric Chloride	7705-08-0		

For cation solutions:

Lithium chloride	7447-41-8
Sodium chloride	7647-14-5
Ammonium chloride	12125-02-9
Potassium chloride	7447-40-7
Magnesium chloride	7786-30-3
Calcium chloride	10043-52-4

For transition metal post-column reagent:

4-(2-pyridylazo)resorcinol	1141-59-9

PAR diluent:

2-Dimethylaminoethanol	108-01-0
ammonium hydroxide	1336-21-6
Sodium bicarbonate	144-55-8

For eluents:

Sodium carbonate	497-19-8
Sodium bicarbonate	144-55-8

Dionex MetPac Oxalic Acid Eluent Concentrate:

Oxalic acid	144-62-7
Tetramethylammonium hydroxide	75-59-2
Potassium hydroxide	1310-58-3