INSTRUCTOR'S MANUAL FOR LABORATORY PROJECTS USING CHROMATOGRAPHY

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Learning Objectives

After completing a laboratory project, a student will be able to:

1. Complete a literature search using Scifinder Scholar
2. Using literature as a guide, devise a procedure for collecting samples
3. Collect samples
4. Identify a set of criteria to use to evaluate possible sample workup methods
5. Use those criteria to compare, contrast, and critique different sample workup methods from the literature
6. Apply those methods to the workup of the samples
7. Modify sample workup methods if necessary
8. Using literature as a guide, design a procedure to prepare standards
9. Prepare a set of standards
10. Validate an analysis method using appropriate procedures
11. Operate the instrument necessary to complete the project
12. Collect and present data in graphical and other appropriate forms
13. Determine whether repetitive analyses are reproducible
14. Interpret data and make judgments based on this interpretation
15. Calculate the concentration of the analyte in the samples
16. Explain the methods used to carry out the project
17. Summarize the findings of the project
18. Defend the conclusions of the project
19. Communicate with group members in the execution of the project
20. Negotiate with group members when making decisions about the project
21. Collaborate with group members in writing a project proposal
22. Develop respect in their ability to complete an independent project
23. Develop respect for the skills of their group members
24. Practice leadership skills
25. Employ ethical practices in the utilization and interpretation of data
26. Prepare and give an oral presentation using Powerpoint
27. Write a final report that takes the form of a journal article
28. Recommend future work for continuing the project

Introduction

The approach I use in the laboratory with my separation science course is to have students work in groups of 2-3 on a single, semester-long project. The students in the course are usually in the second semester of their sophomore or junior year, although some first-year students and seniors are also enrolled, and this is usually their first analytical chemistry course. Because most of the students have no background in analytical chemistry, I identify for them the projects and the instrumental method that will be used to complete the analysis. The following is a list of the most common analysis projects that I have done over the years I have taught the course.

- Caffeine, theobromine and theophylline in chocolate – HPLC-UV
- Catechins (polyphenols) in green tea, wine and chocolate – HPLC-UV
- Amino acid analysis – HPLC-Fluorescence
- Volatiles in coffee – GC-MS
- Trihalomethanes in drinking water – GC-MS
- Methylbenzenes from car exhaust in air – GC-MS
- Polycyclic aromatic hydrocarbons in charred meats or creosote – GC-MS
- Nitrate and nitrite in hot dogs/cured meats – Ion Chromatography
- Chloride content of frozen foods – Ion Chromatography
- DNA restriction fragment analysis – Capillary Electrophoresis
- Additives in soft drinks – Capillary Electrophoresis

I assign the groups based on information I have collected the first day of class. I usually try to form groups that are mixed gender and mixed in terms of student experience. In the first laboratory period, we pair up groups with projects from a list I have selected for that year. My only constraint is that, if a group member has extensive experience with a particular instrument (e.g., she or he used GC-MS extensively during a summer job or research position), the group undertakes a project that uses a different instrument. Every group does a different project.

With a topic and instrumental method, each group must then write a proposal that describes how they intend to complete the project. **Appendix 1** is the handout I provide to the students that describes the Project Proposal, and we go over this document in the first laboratory period. We have twelve week semesters and the proposal is due at the end of the fifth week. On the same day that the proposal is due, students also must submit an individual peer- and self-evaluation on the preparation of the proposal. **Appendix 2** is the evaluation that is used.

The first step in constructing the proposal is for the students to search the scientific literature using Scifinder Scholar to find articles relevant to their analysis. The final activity I do in the first lab period is to provide instruction on the use of Scifinder Scholar. Groups are then expected to begin their literature search and to collect articles for their project.

In the second week of the semester I schedule a one-hour meeting with the groups that are using a particular instrumental method (e.g., HPLC, GC-MS, ion chromatograph). At this

meeting I provide background relevant to their projects. This will include a brief discussion of the instrumental method. I introduce terms that they are likely to encounter in their literature articles. I identify items that they will need to decide on to complete the project (e.g., sample workup, mobile phase, preparation of standards, etc.) and will need to examine carefully in the articles. See **Appendix 5** for lists of the items I cover for each project.

In each of the following weeks until the proposal is due, I meet individually with each group to review what they are finding in their literature search. At these meetings, which typically run about 30-45 minutes each, I end up explaining terms and concepts that they are uncertain about from their articles. We identify strategies for comparing methods (e.g., cost, time, ease of use, etc.). This gives me a chance to further identify steps that will be needed to complete the project and decisions that they must make and explain in the proposal. Also, I am often aware of a sample workup method that they may not have found yet. I point this out to them and that they will need to cast a broader net in their literature search. Even if I tell them generally what they need to be looking for, the specific details of now the implement the method requires that they find the actual article. During these weeks each group is also shown how to operate the instrument. Group members are then encouraged to come in at an off hour either individually or collectively and run the instrument on their own to become more familiar with its operation. As they begin to decide on particular procedures, they also begin to collect glassware and chemicals they will need to carry them out. If we identify items that they will need to order to complete the project, they must include these in the proposal with their cost and the supplier, but we order them in advance to have them in hand when they begin the laboratory portion of the project.

Because of these meetings, I usually have a good idea what to expect in each proposal. I read them carefully and approve the steps that have been put forward by the group. Often I will raise additional questions that still need to be addressed. Once these are resolved, the students begin experimental work in earnest. The policy is to have an open lab where students are allowed to come in at off-hours to undertake preapproved tasks. Students keep an annotated log in the lab notebook of all of the hours they spend on their project (all hours count except those that go into preparing the final Powerpoint presentation and writing their final individual laboratory reports). Students must put in a minimum of 30 hours on their project, and it is common to have a third to half the class often log in more than 60 hours. I encourage them to coordinate their activities so that they avoid situations where one student is undertaking an activity (e.g., preparing standards) while the others are watching. I stress the importance of having each student become proficient on all of the facets of the project so that they do not develop specific roles (e.g., one student prepares samples, another runs the instrument, and the third cleans up).

During the experimental phase of the work, my association with the students is comparable to the way I interact with students conducting independent research projects in my lab. I am available to consult with them as needed. I make a point to circulate through the lab whenever I see students working on the projects to inquire about how things are going.

On the last week of lab each group gives a 20-30 minute oral Powerpoint presentation on their project. Every student must give a section of the presentation, and in a three-person group they usually have one introduce the project, another describe the experimental procedures, and the third present the results and conclusions.

Each student must also submit an individual final report that takes the form of a published article. **Appendix 3** provides the instructions for writing the final report. I have them write individual reports so that I can better distinguish the extent to which each student grasps the project and is able to present the project in written form. They also must submit a peer- and self-evaluation with the final report, which is included as **Appendix 4**.

Appendix 5 contains specific information about each of these projects that includes the topics I go over with each group in an introductory session, key items they need to find through a literature search, key things they find from the literature, and common problems or issues they often face in the lab when carrying out the project.

Appendix 1: Separation Science – Laboratory Project Proposal

Introduction

The proposal must have a section that thoroughly describes the significance of the analysis project that is being undertaken. It should include information about why the compounds being measured are important and why we care about them. If the compounds are important in the environment or living systems, explain their significance and the effects they have that warrant their analysis.

Comparison of Prior Methods/Procedures

The proposal must have a thorough discussion of prior primary literature reports that describe the analysis of the compounds being measured. This section should summarize, compare and contrast prior methods and findings. If more recent literature describes improvements in methodology that are reportedly better than prior procedures, this progression of methodology should be noted.

Selection of Methods/Procedures

A discussion of the rationale for the particular methods and procedures that have been selected for implementation of the project must be provided. Factors such as time, money, ease of implementation, and availability of equipment for certain procedures are all appropriate criteria to utilize and incorporate into the rationale.

Experimental Protocols

A thorough description of the experimental procedures and protocols that will be used in the implementation of the project must be provided. This includes specific protocols and procedures for sampling (e.g., sampling procedure, number of samples, etc.), sample workup, preparation of standards, and sample measurement (e.g., chromatographic conditions). Any specialized equipment that will be used in the project for sampling, sample workup, standard preparation, or measurement must be described. An itemized list with cost of any equipment, chemicals or supplies that need to be purchased must be included.

Appendix 2: Peer and Self-Assessment of Laboratory Proposal

The information requested is **CONFIDENTIAL** and will not be shared with other members of the class. It will be used to assess how well student assessment of the work on the proposal portion of the laboratory projects agrees with my own. These evaluations must be turned in at the same time that your proposal is due.

Please evaluate everyone's contribution to the proposal, including your own, for each of the following criteria. Provide a mark out of 10 for each category.

Evaluation Scale:

10-9 - Outstanding contribution

8-7 - Very good contribution

6-5 - Good contribution

4-3 - Fairly satisfactory contribution

2-1 - Unsatisfactory contribution

0 - No or virtually no contribution

Criteria:

A – Gathering literature

B – Reading and evaluating the literature

C – Helping to develop and write the proposal

D – Attendance at meetings to develop and write the proposal

E – Undertaking a fair share of the work

F – Ability to arrive at consensus/overcome difficulties

G – Ability to facilitate the group's efforts

Your name _____

Name of person you are evaluating _____

Category	Score	Comments
A		
B		
C		
D		
E		
F		
G		

What percent of the total effort that went into writing the proposal did this individual contribute (The total for all of your group members must be 100%)?

Any additional comments you wish to make about this individual's contribution to the project:

Appendix 3: Final Laboratory Report

Each person in the course is required to submit an **individual report** on the laboratory project.

I do realize that you will be discussing your results with the other members of your group. That is not only expected, but encouraged. The report, though, is to be your own individual document reflecting how you choose to describe the work you have undertaken over the semester. As such, I expect that each member of a particular group with submit a unique document that might have considerable differences in style and the manner in which data is presented and discussed. Although I can also imagine reports from members of a group in which plots or tables of data are the same. The report is to be patterned after scientific journal articles published in *Analytical Chemistry*. It should be comprehensible to other students who have taken Separation Science at Bates. There is no length restriction; however, the report should be concise, yet complete. The report is to be a typed, double-spaced, size-12 font.

The report should contain the following sections:

Title

Abstract: The abstract consists of a short paragraph containing a brief description of the focus of your experiment, a short statement describing in general terms the results obtained, and any major conclusions of your study. The abstract should only contain statements about what you did, how you did it, and the results obtained. It should be in the range of 100-200 words.

Introduction: The introduction should include a section justifying the work that has been done and explaining why the work was important to perform (why it is important to analyze for the particular species that you measured). The introduction also ought to describe in general terms (specific details will be provided in the Experimental Section) the methods that were used to perform the measurement. New techniques, instruments, or methods that would not be familiar to someone in the Separation Science course should be described. Relevant background literature that is important to the substance(s) you were analyzing or that helped you in designing particular aspects of your project are included in this section.

Experimental: This section should include a thorough description of all procedures that were followed in carrying out experiments and collecting samples and data. It should contain complete descriptions of equipment or apparatus including brand name and model numbers that were used, parameters that were set on instrumentation (e.g., flow rate, etc.), detailed procedures for preparing solutions, thorough descriptions of how samples were obtained, and procedures for workup and analysis of data. A common mistake that is made in this section is the omission of important details that another person would need to replicate the experiment. Another common error is for the writer to start reporting results or conclusions in this section, or to start explaining why a particular experimental protocol was used. These types of discussions belong in the Results and Discussions section. The Experimental section is essentially a cookbook (although it is not written as an itemized list of procedures, but written

in a textual form) that describes to anyone else how to perform an identical experiment to what you have done. This is an important section in a scientific paper because it is critical to anyone else who wants to repeat the work.

Results and Discussion: This section involves a presentation and discussion of the data. The presentation and discussion are integrated together. In other words, there is not a separation section that merely presents all of the data and then a following section that discusses its significance. The results of your work are to be communicated in an organized manner, and the significance of your results are to be discussed. The following items are usually included:

- Data: Relevant primary data should be included. This may best be done in the form of a table or figures with appropriate reference and discussion in the text.

- Tables: Tables should generally be integrated into the text. Tables are given titles and are numbered consecutively. Tables must be referred to and discussed in the text.

- Figures: Figures should generally be integrated into the text and are numbered consecutively. Each figure is described by a caption that appropriate describes the figure. Each figure must be referred to and discussed in the text. A common error for many first-time writers is to provide figure captions that tell too little about the figure.

Any conclusions that can be drawn from the data must be stated and supported by a discussion of the data. If the data appears flawed in some way, thereby preventing definitive conclusions from being drawn, this should be discussed. Data that you measured ought to be compared to prior results reported in the literature. A common error in these reports is for the writer to overstate the degree to which a conclusion is valid. This section should also include discussions of your experimental protocols; why you chose to perform the experiment the way you did and what you would retain and change if you were to continue the work.

This section should also contain a description of any future work that would be done should you continue this project. What would be done to obtain better data with a higher degree of accuracy and reliability? What experiments would you have performed if you had the time? Is there equipment that would have helped in the execution of your project?

References: Any information that is used in the report but obtained from another source (text, journal article, etc.) should be referenced.

Appendix 4 - Peer and Self-Assessment of Laboratory Project

The information requested is **CONFIDENTIAL** and will not be shared with other members of the class. It will be used to assess how well student assessment of the work on the laboratory projects agrees with my own. These evaluations must be turned in with your laboratory report.

Please evaluate everyone's contribution to the laboratory project, including your own, for each of the following criteria. Provide a mark out of 10 for each category.

Evaluation Scale:

10-9 - Outstanding contribution

8-7 - Very good contribution

6-6 - Good contribution

4-3 - Fairly satisfactory contribution

2-2 - Unsatisfactory contribution

0 - No or virtually no contribution

Criteria:

A – Gathering preliminary literature

B – Helping to develop and write the project plan

C – Attendance

D – Undertaking a fair share of the work

E – Ability to generate good ideas/solve problems

F – Ability to arrive at consensus/overcome difficulties

G – Ability to facilitate the group's efforts

Your name _____

Name of person you are evaluating _____

Category	Score	Comments
A		
B		
C		
D		
E		
F		
G		

Any additional comments you wish to make about this person's contribution to the project:

Appendix 5: Comments about the Projects

Analysis of caffeine, theobromine and theophylline in chocolate using HPLC with UV detection.

Introductory session:

- Explain to them the chemical nature of a C-18 bonded phase column and how this allows them to use an aqueous-based mobile phase.
- Explain why fats are not compatible with C-18 bonded columns.
- Point out the origin of the term "reversed-phase", since they will likely encounter it in the literature.
- Discuss the difference between isocratic and gradient elution, and how an isocratic method without any buffer is preferable to methods with a buffer and/or a gradient.
- Discuss why ultraviolet absorption is a suitable method for the detection of these compounds.
- Indicate that at a minimum they need to analyze one sample of dark, milk and white chocolate

Key items to look for in the literature:

- Procedure for defatting chocolate
- Mobile phase
- Detection wavelengths
- Concentration of standards (reminding them of the dilution of the analytes that will occur in the sample defatting and workup procedures). Whether to use internal or external standards.

Key findings from the literature:

- There are two methods that they should find for defatting the chocolate. One involves the use of hexane or petroleum ether to extract out the fats. The other is to use a C-18 cartridge to adsorb out the fats. I push students to find both and encourage them to try both to compare the samples.
- After defatting the chocolate with the hexane extraction, the compounds are extracted using water.
- The compounds can be separated using isocratic phases with methanol or acetonitrile as the organic modifier. No buffer is needed in the mobile phase.

Key problems that come up when performing the experiment:

- Getting a clear enough sample to be able to inject it into the LC. Dark chocolate is the easiest one to get clear enough for injection. Milk and white chocolate are more difficult. Because of suspended particles, we have had considerable difficulty getting a clear enough sample using only filtering, even with 0.2 um pore filter media. We have usually resorted to high-speed centrifuging as a necessary step prior to a final filtration to get samples suitable for injection.

Analysis of catechins (polyphenols) in green tea, wine and chocolate using HPLC with UV detection.

Introductory session:

- Explain to them the chemical nature of a C-18 bonded phase column and how this allows them to use an aqueous-based mobile phase.
- Explain why fats are not compatible with C-18 bonded columns.
- Point out the origin of the term "reversed-phase".
- Discuss the difference between isocratic and gradient elution, and how an isocratic method without any buffer is preferable to methods with a buffer and/or a gradient.
- Discuss why ultraviolet absorption is a suitable method for the detection.

Key items to look for in the literature:

- Which of the many possible catechins should we analyze for?
- Procedure for defatting chocolate
- Mobile phase
- Detection wavelengths
- Concentration of standards (reminding them of the dilution of the analytes that will occur in the chocolate defatting and workup procedures). Whether to use internal or external standards.

Key findings from the literature:

- There are two methods that they should find for defatting the chocolate. One involves the use of hexane or petroleum ether to extract out the fats. The other is to use a C-18 cartridge to adsorb out the fats. I push students to find both and encourage them to try both to compare the samples.
- After defatting the chocolate with hexane, the compounds are extracted using water.
- Samples of tea and wine can usually be analyzed directly after suitable filtration.
- They will find a range of mobile phases, most of which involve gradient elution.
- Catechin, epicatechin, resveratrol, gallocatechin, and epigallocatechin gallate are good compounds to analyze for

Key problems that come up when performing the experiment:

- We have had some difficulty getting reproducible chromatograms. Even though they may find isocratic mobile phases reported in the literature, we have had troubles with them and find gradient methods to be more reproducible. Some compounds seem to chromatograph more reproducibly than others. Because of this, I usually encourage them to start with green tea and wine samples instead of trying chocolate, which requires a substantial amount of sample workup. Once the tea and wine samples show reproducible results, they can then move on to chocolate samples.
- When analyzing chocolate, there can be problems getting a clear enough sample to be able to inject it into the LC. Dark chocolate is the easiest one to get clear enough. Milk and white chocolate are more difficult. Because of suspended particles, we have had considerable difficulty getting a clear enough sample using only filtering, even with 0.2 um pore filter media. We have usually resorted to high-speed centrifuging as a necessary step prior to a final filtration to get samples suitable for injection.

Analysis of amino acids using HPLC with fluorescence detection.

Introductory session:

- Explain to them the chemical nature of a C-18 bonded phase column and how this allows them to use an aqueous-based mobile phase.
- Explain why fats are not compatible with C-18 bonded columns, and if the sample they will analyze has fats, how they will need to find defatting procedures (we have analyzed amino acids in skimmed milk, beer, coffee and popcorn).
- Point out the origin of the term "reversed-phase", since they will likely encounter it in the literature.
- Discuss the difference between isocratic and gradient elution, and how an isocratic method without any buffer is preferable to methods with a buffer and methods that use a gradient.
- Explain the use of *o*-phthaldehyde as a fluorescent derivatizing agent for amino acids
- Discuss the nature of fluorescence and explain why it is a suitable method for the detection of these compounds.

Key items to look for in the literature:

- Procedure for isolating and hydrolyzing proteins
- Procedure for defatting samples if necessary
- Procedure for preparing the *o*-phthaldehyde derivatives
- Mobile phase
- Detection wavelengths
- Concentration of standards (reminding them of the dilution of the analytes that will occur in the sample workup procedures). Whether to use internal or external standards.

Key findings from the literature:

- The separation of up to 20 amino acids is a challenging one that will require the use of gradient elution and a buffered mobile phase.

Key problems that come up when performing the experiment:

- This is an ambitious project with many steps to finally get data. Just working out all of the *o*-phthaldehyde derivatization and separation procedures on a complex amino acid standard is a gratifying accomplishment. Because of the complexity of the sample, each chromatographic run takes about an hour to complete.
- Precipitation and isolation of the proteins from skimmed milk is relatively straightforward and a good sample to analyze after getting reproducible chromatograms of the standards.

Analysis of volatiles in coffee or trihalomethanes in drinking water using GC-MS.

Introductory session:

- Explain how water is incompatible with the GC columns we will use for the analysis and that a suitable sample workup procedure will be necessary.
- Explain the possibility of using an organic extraction (undesirable), purge and trap procedure (I have a GC-MS that has an injection system specifically designed for desorption of sorbent traps, but this is likely unusual in an undergraduate curriculum), or headspace analysis.
- Explain how headspace analysis could be performed using a gas-tight syringe or solid phase microextraction system (SPME).
- If using purge and trap, explain breakthrough, backflushing and thermal focusing.
- If using headspace or SPME, explain how that injection system works.
- Explain the design of a fused silica capillary column.
- Explain the concept of a temperature program.
- Briefly explain how mass spectrometric detection works.

Key items to look for in the literature:

- Procedures for purge and trap analysis
- Procedures for head space and SPME analysis
- What GC column to use
- What temperature program to use
- Mass spectrometer settings
- Procedures for preparing standards and whether to use internal or external standards.
- Procedures for preparing, collecting and/or storing samples
- What compounds to analyze for – especially when analyzing volatiles from coffee, there are many possibilities from which to choose.

Key findings from the literature:

- That the most likely procedure for doing the analysis (if purge and trap is not an option) is SPME analysis.
- That there are many conflicting reports about what represents the best SPME system to use for the analysis and many conflicting reports about the best set of conditions to use for SPME analysis.
- That the preparation of the trace level standards is not a simple process of dissolving the compounds in water. The compounds will usually need to be dissolved at a higher concentration in an organic solvent such as methanol that is miscible with water.

Key problems that come up when performing the experiment:

- Each analysis takes a long time to perform (about an hour) so it is not possible to generate a lot of data quickly.
- Finding the best conditions for the SPME analysis is likely to involve a trial-and-error process informed in part by procedures in the literature.
- Reproducibility can be a challenge, especially in the early stages of the project.
- Preparation of reproducible standards is a challenge because of the compound's high vapor pressure.

Analysis of polycyclic aromatic hydrocarbons (PAHs) in charred meats or creosote using GC-MS.

Introductory session:

- Explain that we will need to find a procedure for the extraction of the PAHs from the creosote and meat samples that avoids high molecular weight fats that would decompose in the injection port of the GC-MS.
- Explain the injection system for liquid samples.
- Explain the design of a fused silica capillary column.
- Explain the concept of a temperature program.
- Explain how mass spectrometric detection works.

Key items to look for in the literature:

- Procedures for sample workup.
- What GC column to use.
- What temperature program to use.
- Mass spectrometer settings.
- Procedures for preparing standards and whether it is possible to use an internal standard.
- Procedures for preparing, collecting and/or storing samples.
- What compounds to analyze for as there are many possible PAHs.

Key findings from the literature:

- The first step in the analysis of charred meats is a digestion step with KOH.
- Sample cleanup and extraction of the PAHs is likely to involve the use of one or more cartridge systems.

Key problems that come up when performing the experiment:

- The digestion step for charred meats is prone to frothing and bumping in the round-bottomed flask/reflux condenser system.
- Sample cleanup is an involved process prone to loss of sample and compromising of reproducibility.

Analysis of methylbenzenes from car exhaust in air using GC-MS

Introductory session:

- Explain how the concentration of these compounds in an air sample is too dilute to just inject a small volume of air into the GC-MS.
- Describe the use of sorbent traps to concentrate the organic compounds from a large volume of air. Conducting this project needs a GC-MS that has an injection system specifically designed for desorption of sorbent traps. We also have a device specifically designed to draw air through a sorbent trap at a set rate.
- Explain the concepts of breakthrough, backflushing and thermal focusing.
- Explain the design of a fused silica capillary column.
- Explain the concept of a temperature program.
- Briefly explain how mass spectrometric detection works.

Key items to look for in the literature:

- Procedures for obtaining air samples, including whether it is possible to store samples
- What GC column to use
- What temperature program to use
- Mass spectrometer settings
- Procedures for preparing standards
- What compounds to analyze for

Key findings from the literature:

- Procedures for obtaining air samples.
- That the preparation of the trace level standards is not a simple process.

Key problems that come up when performing the experiment:

- Each analysis takes a long time to perform (about an hour) so it is not possible to generate a lot of data quickly.
- Preparation of standards is a significant problem. What we have used as a fall-back is to prepare a trace level standard via serial dilution in methanol. We then inject a small volume onto a Tenax trap, evaporate the methanol under a flow of helium, and then analyze the trapped organic compounds.
- Reproducibility is a problem because they usually can only collect one to two samples in a short period of time and levels can change significantly with the time of day, weather conditions, etc.

Analysis of nitrate and nitrite in hot dogs/cured meats using ion chromatography

Introductory session:

- Explain how an ion exchange resin is designed and can be used to separate ions
- Explain why the mobile phase needs an eluent ion
- Explain why conductivity is suitable for detection of these ions provided the conductivity of the eluent ion is suppressed
- Explain why injection of fats may not be compatible with the column
- Explain how the samples will have very high chloride levels that might interfere with the nitrate and nitrite analysis

Key items to look for in the literature:

- Possible mobile phases
- Methods to prepare standards
- Work up procedures to remove fats and extract ions
- Procedures to remove chloride interference

Key findings from the literature:

- Many articles are "silent" on the exclusion of fats from the extracts
- Most extraction steps involve blending the meat with water followed by filtration
- There are two possible procedures for the removal of chloride. One is to add a solution of silver sulfate to precipitate it. The other is to use a cation exchange cartridge in the silver form to precipitate out chloride. These cartridges are commercially available.

Key problems that come up when performing the experiment:

- Obtaining clear enough samples to inject into the IC can be a problem. Using appropriate cartridges to remove fats may be necessary. High speed centrifuging may lead to three layers for the blended extract sample – meat particles on the bottom, aqueous extract in the middle, fats on the top.
- We have tried the silver cartridge procedure once and it did not work as well removing the chloride as adding a solution of silver sulfate. The sulfate peak coming latest in the chromatogram does not interfere with the other ions.
- Nitrite levels are exceptionally small and students have to zoom in on that part of the chromatogram to see the peak.

Analysis of chloride content of frozen foods using ion chromatography

Introductory session:

- Explain how an ion exchange resin is designed and can be used to separate ions
- Explain why the mobile phase needs an eluent ion
- Explain why conductivity is suitable for detection of chloride ion provided the conductivity of the eluent ion is suppressed
- Explain why injection of fats may not be compatible with the column
- Will need to decide what foods to analyze

Key items to look for in the literature:

- Possible mobile phases
- Methods to prepare standards
- Work up procedures to remove fats and extract chloride ion

Key findings from the literature:

- Many articles are "silent" on the exclusion of fats from the extracts
- Most extraction steps involve blending the sample with water followed by filtration

Key problems that come up when performing the experiment:

- Depending on the food being analyzed, obtaining clear enough samples to inject into the IC can be a problem. Using appropriate cartridges to remove fats may be necessary. High speed centrifuging may lead to three layers for the blended extract sample – food particles on the bottom, aqueous extract in the middle, fats on the top.
- Chloride levels are usually quite high requiring appropriate dilution of the samples.

DNA restriction fragment analysis using capillary electrophoresis

Introductory session:

- Background on CE and how it works, including possible injection techniques
- Background on DNA restriction fragment analysis

Key items to look for in the literature:

- CE column and mobile phase to use for the separation
- Procedure for the restriction fragmentation of DNA

Key findings from the literature:

- Best to use a commercially available kit as a test process before moving on to actual samples

Key problems that come up when performing the experiment:

- This is an ambitious experiment and we have had problems with getting good separations and reproducibility

Analysis of additives in soft drinks using capillary electrophoresis

Introductory session:

- Background on CE and how it works, including possible injection techniques
- Background on the types of additives expected in soft drinks that they can analyze

Key items to look for in the literature:

- What to analyze for
- CE column and mobile phase to use for the separation
- Procedures for the preparation of standards
- Whether any sample workup is needed

Key findings from the literature:

- In theory, this should be a rather straightforward analysis to perform

Key problems that come up when performing the experiment:

- Even though this would seem to be a straightforward experiment to carry out, we have had problems with getting good separations and reproducibility