Guide to Instructors

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Introduction

The materials comprising the Lake Nakuru project are designed in a modular fashion, allowing them to be used in different classes in a variety of ways. The vignettes by instructors who have used these materials show many approaches to using these materials in a variety of classroom settings. For example, one use for these materials could be as a capstone assignment in an Instrumental Analysis course. Alternatively, the modular nature of the materials could allow use of only the sampling unit in a Quantitative Analysis or an Environmental Chemistry course.

Instructor Vignettes

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The remainder of this Instructor's Guide addresses each unit providing a brief overview of how the content might be used, supplemental lecture content that might be helpful to the students before starting the module, and answers to the questions posed within each section.

Identifying the Problem

This module briefly lays out the scientific problem – what in Lake Nakuru is killing the flamingos? Like most real scientific questions, this one is complex. To our knowledge the "real" reason is not known and there may in fact be a variety of contributing factors. Even if only one or two of the modules will be used in your course, it would probably be useful to start with the Identifying the Problem unit to provide a context for the other sections.

Identifying Possible Analysis Methods

It is useful to have students explore the possible analytical methods that might be used to measure the chemicals involved in each of the three hypotheses for flamingo death.

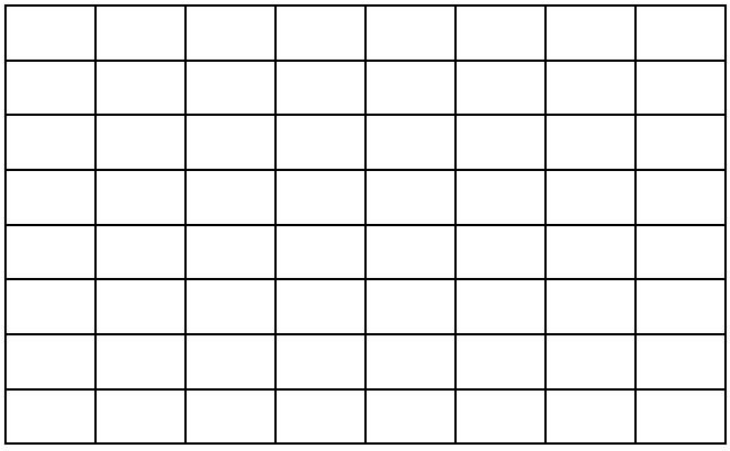
If this exercise is used toward the end of an analytical chemistry course, students may be asked to go back through each method that was covered in the course and explain whether or not it might work for the analysis of the species in question.

An alternative is to ask students to go to the scientific literature and find possible methods for the analysis. In this format, it is probably best to divide the class into groups and give each group one of the three hypotheses. After completion of the assignment, each group can report their findings to the rest of the class. This can lead to a useful discussion of the strengths and weaknesses of the various methods they identify. This discussion may turn up methods that are not covered or emphasized in the course and lead to the introduction of other analysis methods that are usually not discussed.

Sampling

After discussing the ***Identifying the Problem*** module and the first page of the ***Sampling*** section, students can work in groups on the questions that are provided. The answers to the questions are provided below.

Q1. Pick eight random samples from the grid laid out above. How do you ensure you sampling is random?



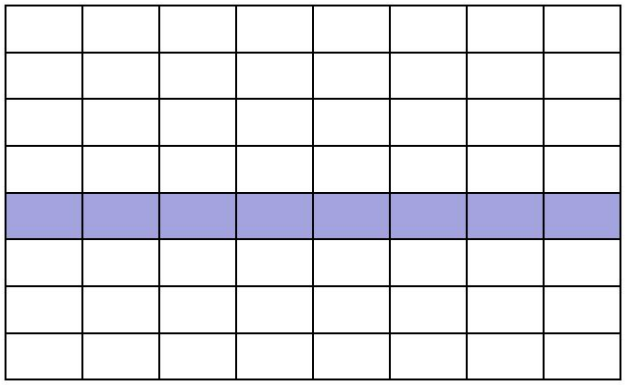
One way to get random samples is to use Excel. To get 8 random grids label the boxes 1-8, row 1, 9-16, row 2, etc. for 64 boxes. Then use Excel to generate 8 random numbers between 1 and 64; for example: 63, 35, 25, 46, 7, 53, 43, 5.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32
33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48
49	50	51	52	53	54	55	56
57	58	59	60	61	62	63	64

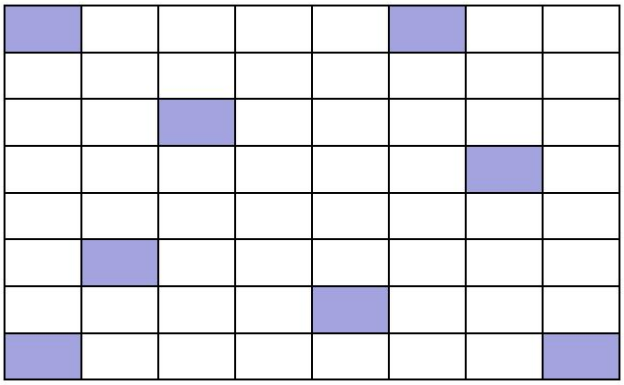
You might ask the students to discuss whether they think a random approach represents the best way to sample. They may realize that the answer depends in part on what you may already know about the system you are sampling. If there is a specific or point source of the chemical, then random sampling might not be the best option.

Now take a look at the following grids with the analyte of interest identified (colored squares).

A



B



Q2. Would you consider the samples above to be heterogeneous or homogeneous?

The analyte is heterogeneous because it is not evenly distributed throughout the entire grid.

Q3. Did your random sampling affect the potential accuracy or precision of your measurement of the analyte for the samples in grid A or grid B? If so how?

The random sampling would have been better for grid B because the analyte is more spread out than in grid A where the analyte is confined or stratified.

Q4. Each of the previous grids is an example of one of these cases. Can you identify which sample is which?

Sample B exhibits constitutional heterogeneity while Sample A exhibits distributional heterogeneity.

Q5. How does *distribution heterogeneity* affect accuracy and precision?

The answer to Q5 is included with the answer to Q6 below.

Q6. How does *constitutional heterogeneity* affect accuracy and precision?

When you overlay the sampling scheme with Sample A and Sample B neither sampling scheme is effective, as shown below. The analyte in sample "A" was sampled once (35) as it was in Sample "B" (53). With either sample, unless the sampling scheme could take this heterogeneity into account the accuracy and precision of the measurement would be compromised.

A. Distributional Heterogeneity

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32
33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48
49	50	51	52	53	54	55	56
57	58	59	60	61	62	63	64

B. Constitutional Heterogeneity

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32
33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48
49	50	51	52	53	54	55	56
57	58	59	60	61	62	63	64

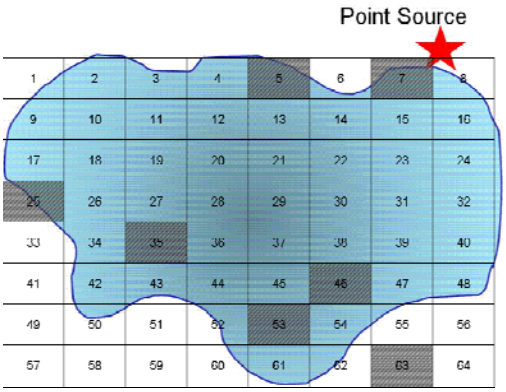
Q7. Do you see a scenario where distribution heterogeneity could be magnified by mixing and/or sampling?

Sampling is often by weight or by "grab". In this case settling may alter the sample composition.

Q8. What is the advantage of implementing judgmental sampling over random sampling if one knows the point source for the discharge an analyte into a system?

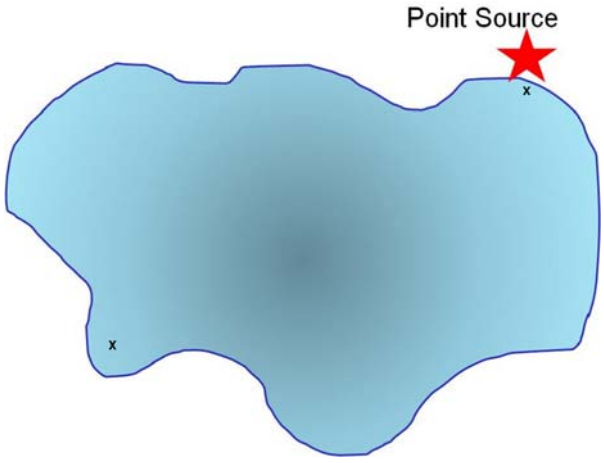
The advantage is that you can get larger number of relevant samples which should decrease the standard deviation of the average value measured for that sample. The cost should also decrease.

Q9. Assume you have a chosen a selective sampling plan to evaluate pollution from a point source into a lake. Use the diagram below and words to describe your sampling plan.



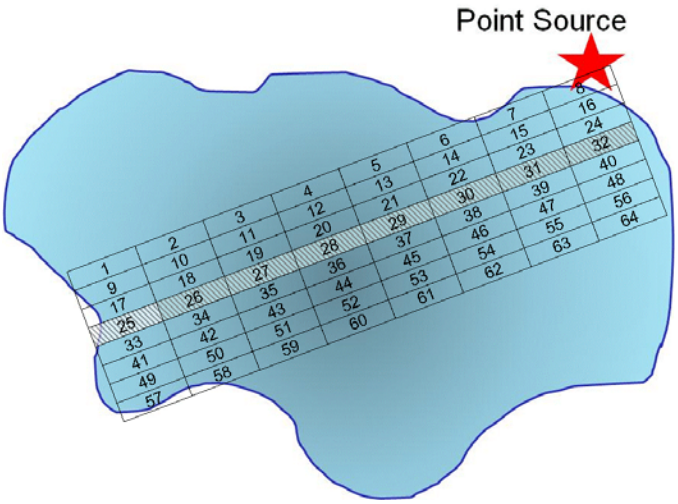
If we use a purely random grid over the lake we will be unable to tell what the effect of the point source is because we would have sampled only once at the source.

A selective method might be the following:



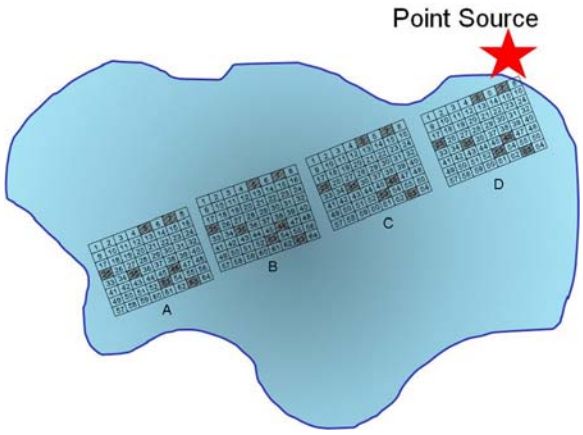
A sample is taken at the point source and for comparison a sample is taken at a distance from the point source.

Q10. Use a grid design (as we have previously done) to show how you would conduct systematic sampling (regular intervals in space and time) of the pollutant. Is there an advantage to what you might learn using this sampling method? What are the disadvantage(s)?



Here we have set up a grid along regular intervals. Because we collect only 8 samples, we may or may not collect a representative sample from within each grid point as we are only collecting one sample.

Q11. Describe how stratified sampling (random sampling within sub populations) might be applied to evaluate the pollutant in the lake? In general, what is the advantage of stratified sampling over cluster sampling?



In this example the trajectory along the longest distance from the point source is sampled a total of four times, with random grabs to be co-joined into a single sample.

Q12. What is a general rule with regard to sampling times or locations to increase the likelihood that samples will be representative?

The samples will be more representative if they are taken a larger number of times and at more locations.

Q13. What is the main disadvantage of grab and composite samples?

You cannot use them continuously for real time *in situ* monitoring.

Q14. Can you think of any control studies you might want to include when compositing samples?

You might want to retain portions of the grabs and analyze them separately.

Q15. Does the EPA Method 525.2 suggest a particular sampling method?

The EPA Method does not describe a particular sampling method because each sampling plan must be designed for the specific analytical question being addressed.

Q16. What sample size does EPA Method 525.2 suggest for analysis of pesticides in water? Why?

The EPA method suggests 1 L. The method does not say why but presumably the concentrations of the organochlorine pesticides are low and using 1 L helps create a "composite" sample of sufficient quantity that the target analytes can be extracted and measured at levels above the instrumental limit of detection.

Q17. An analytical method (not necessarily the Lake Nakura project) has a percent relative sampling variance of 0.10% and a percent relative method variance of 0.20%. The cost of collecting a sample is $20 and the cost of analyzing a sample is $50. Propose a sampling strategy that provides a maximum percent relative error of ±0.50% ($\alpha = 0.05$) and a maximum cost of $700.

$$error = Value_{Experimental} - Value_{Known} = \overline{X} - \mu$$

$$relative \quad error = \frac{\overline{X} - \mu}{\mu} = \frac{\overline{X}}{\mu} - 1$$

$$\%\, relative \quad error = 100\left[\frac{\overline{X} - \mu}{\mu}\right] = 100\left[\frac{\overline{X}}{\mu} - 1\right]$$

If there is no bias then the error is equal to the standard deviation, s and

$$\%\ relative \qquad error \ = 100\left|\frac{s}{\mu}\right|$$

To answer this question requires reading Harvey, specifically eq. 7.8

$$\overline{X} - \mu = error = t\sqrt{\frac{s_{samp}^2}{n_{samp}} + \frac{s_{meth}^2}{n_{samp} n_{rep}}}$$

In this equation μ is the true value and t is the value for the t test which depends on the number of samples and the confidence level. The value of α is 0.05. Since we are going to be limited by the cost, a rough guess of the sample size is:

Begin the iterations assuming t(10,0.05)=2.228 (from t table).

n=10. You have 10 samples you can work with to get the maximum percent relative error of 0.5%. The percent relative error is

The total variance associated with a measurement is

where …..σ_x is the variance from any other contributing factor. So we can write

$$\sigma^2_{total} = 0.5 = \sigma^2_{sampling} + \sigma^2_{method} = \sigma^2_{sampling} + 0.2$$

This sets the allowed variance associated with sampling at 0.3.

Q18. The auto sampler on the GC-MS you will be using for pesticide analysis has 200 vial locations. How will you choose your representative samples? Here is a picture of Lake Nakuru. Design your sampling plan. Think about random, systematic, clustering, etc. sample strategies. Will you take grab samples or pool samples together?

One way to use this question is to have the students discuss these questions in groups and put together a final plan. The plans could either be presented to the class or turned in as a graded written assignment.

3. Sample Preparation

Students who have already carried out project-based labs involving extensive sample preparation will likely be familiar with many of the concepts in this module. If this is not the case for your students, this section could be good preparation for a laboratory project or serve as a surrogate for actually conducting such a study. Although the focus of this section is necessarily on the preparation of aqueous samples for GC-MS analysis, many of the concepts are relevant to analysis of trace organic compounds in general. Students will likely need some guidance in answering questions 1-3 and may need to refer to both the EPA method and the **Identifying the Problem** module, especially to answer Q3. One way to handle these questions is to discuss the issues in class, without necessarily arriving at the correct answers. The remainder of the module is fairly self-contained and students should be able to work through the rest of the questions in small groups or as a homework assignment. Once they have completed this **Sample Preparation** module, they should be better able to answer questions 1-3.

Q1. Is the sample in the wrong physical state for the analysis method?

Yes. A sample for GC-MS analysis must be in the liquid or gaseous state. The sample is in the liquid state, but the aqueous solvent is incompatible with the column used in the gas chromatograph.

Q2. Does the sample have interfering matrix components that may give either a false positive or negative reading in the measurement?

This is difficult to answer definitively without knowing more about the sample and its constituents. For a matrix component to be a problem it would have to be relatively hydrophobic so that it would be retained by solid phase extraction and concentrated in the eluent along with the pesticides of interest. The use of a separation prior to detection also helps to reduce interferences from matrix compounds. However, even when a high-resolution separation method like GC is used, it is possible that a matrix component from the water might coelute with one of the pesticides of interest and interfere with its detection. The use of mass spectrometry as a detector can help identify matrix interferences and in many cases the use of selective ion monitoring can allow quantitation of the analytes even in this presence of the interferent. This is discussed in greater detail in the section on MS analysis.

Q3. Does the sample have too low an analyte concentration to be detected?

Again, it is difficult to know the answer to this question with certainty without knowing more about the sample. In most cases, however, organochlorine pesticides in lake water samples would require a preconcentration step to bring them into an appropriate concentration range for quantification.

Q4. Can you think of a procedure to remove the organochlorine pesticides from water?

Students will likely first think of a liquid-liquid extraction, since they are familiar with this from organic chemistry. Some may think of using a solid-phase extraction agent, perhaps because they are familiar with using filters on tap water purification or softening systems. If they do not think of using solid-phase extraction, you will eventually need to lead them to this idea or present it to the class.

Q5. Why is this procedure done over two hours instead of 10 minutes?

If the sample is passed through the solid phase too quickly, compounds may not be retained by the solid phase sorbent and the recovery will be low.

Q6. Why is octadecyl chosen as an extraction stationary phase for the study of pesticides? What would happen if bare silica was used as an extraction phase?

Unless the students have been previously exposed to normal and reverse phase liquid chromatography, you will likely need to explain what is meant by an octadecyl phase and bare silica before having them work on this question. Then they can probably determine that the solid phase must be matched in polarity to the compounds that you are adsorbing. The organochlorine pesticides are rather non-polar and so the solid phase must be non-polar as

well. Octadecyl phases are non-polar. Bare silica has a polar surface so it is doubtful that the organochlorine pesticides will adsorb to this material.

Q7. Discuss the following aspects of the solvent you would choose to elute the organochlorine pesticides.

- Polarity

The polarity of the eluting solvent must be such that it readily dissolves the adsorbed pesticides. In this case it ought to be relatively non-polar.

- Boiling point

If the eluted sample will be directly injected into the gas chromatograph, then the solvent needs a low boiling point so that it elutes from the chromatographic column prior to the pesticides. Also, the volume of organic solvent used for the elution usually dilutes the analyte too much so that it needs to be concentrated before injection into the gas chromatograph. This concentration step is facilitated by having a solvent with a low boiling point.

- Volume

The volume of eluting solvent needs to be large enough to remove all of the compound from the solid phase. But if the volume is too large, that will lengthen the time needed to concentrate the sample for injection into the gas chromatograph-mass spectrometer.

Q8. How would you remove any residual water in the organic phase?

Add a suitable drying agent such as sodium sulfate, similar to what students have likely done in the past in organic chemistry.

Q9. How would you reduce the volume of the organic extract that contains your pesticide?

Students will likely think of evaporation with heating as a means of reducing the solvent. A common procedure is to blow an inert gas such as nitrogen over the sample to reduce the volume.

Q10. What concerns might you have in the solvent reduction step?

One is that there is some loss of the analyte because some of it has evaporated as well. A second is that some of the compound decomposes. Evaporation under a nitrogen stream avoids the use of higher temperatures and is often used to reduce loss and decomposition of the analyte relative to an evaporation procedure involving heating.

Q11. What would be the final extraction concentration be if you plan to use EPA Method 525.2? Can you detect this level in the lake?

The LOD is given as 0.083 μg/L (ppb) for the sample injected into the GC-MS. The lake water is 150 ng/L or 150 ppt. The extraction step calls for a liter of water that is eventually reduced into a volume of 1 mL of organic solvent. This represents a 1,000-fold increase in the

concentration compared to the original sample of the lake (extract concentration is 150 ppb). This concentration is well above the detection limit. For quantitative purposes, it is usually recommended that the sample concentration be ten times the LOD or 0.83 μg/L (ppb). The sample is well above the value needed to complete a quantitative analysis of DDT in the lake.

Q12. What does it mean to determine the precision of the pesticide analysis? What does it mean to determine the percent recovery of the pesticide?

Precision is a measure of the reproducibility of the extraction process from run to run.

The percent recovery refers to the amount (typically a mass) of the compound that is removed from the solid phase sorbent compared to the amount that was introduced onto the solid phase in the sample. Percent recoveries are evaluated for a standard for which you know exactly how much you introduced to the solid phase.

Q13. Suppose a solid-phase extraction procedure provided a low percent recovery. What could you change in an attempt to raise this value?

There are three possibilities for why the percent recovery is low.

The first possibility could be that the sample was run through the solid phase sorbent at too high a flow rate such that the compounds did not have sufficient time and contact to adsorb to the surface. Correcting this would involve slowing the flow of the sample through the solid phase.

A second possibility is that the surface of the solid does not sufficiently match the polarity of the compounds you are trying to adsorb and the compounds are not being retained. In this case you would need to use a different solid phase. Also, many solid phase materials require a pre-treatment of the surface before using them for adsorption. If the pre-treatment was not performed, the adsorption might be compromised.

A third possibility is that the eluting solvent does not sufficiently match the polarity of the compounds being eluted. In this case, you would need to switch the solvent.

Another possibility that students might suggest would be that the sample could overload the capacity of the solid phase sorbent, however, this is very unlikely with environmental samples as contaminants are typically present in trace amounts.

Q14. Is the solid used in the cartridge effective for removing the pesticides from water?

Yes. All of the % recoveries in Table 8 are in the range of 98-118 percent, so the solid did remove the pesticides.

Q15. How is it possible to get a percent recovery of over 100?

Every step in the process is subject to error. To determine a percent recovery, you must introduce a known concentration onto the cartridge. There is error in the volume you introduce such that the volume is larger than expected.

Q16. From the chemistry described in 8.2 and 8.3 what might be the cause of pesticide degradation before extraction?

It would appear that the water can have microbes in it that can decompose the pesticide. One common chemical reaction that occurs with many compounds is hydrolysis.

Q17. What is a surrogate compound?

A surrogate is a compound that is not expected to be in your sample and has properties similar to the compounds that you are analyzing.

Q18. What assumptions must be made when choosing a surrogate?

That it behaves in an identical manner to the pesticides that are being analyzed.

Q19. When is the surrogate added during the sample preparation (i.e. before extraction or after). Why is it added then?

The surrogate should be added before the sample extraction so that it goes through the same set of workup steps as the analytes.

Q20. What could be responsible for the different percentages of true concentration for the surrogates in the two tables?

There are several things that could contribute to the different percents. One is inherent random error that occurs with any process. There are also differences in the two methods. One uses a disk extraction whereas the other uses a cartridge extraction. One uses a quadrapole mass spectrometer whereas the other uses an ion trap mass spectrometer.

Q21. What is an internal standard? Why is it used? At what point during the sample preparation are internal standards added?

An internal standard is similar in chemical composition to the analytes of interest and is added in a known concentration directly to the sample. The response of the internal standard is used as a normalization factor to which the signals for the analyte compounds are ratioed.

Q22. Why spike samples? Do you spike them before or after the sample preparation?

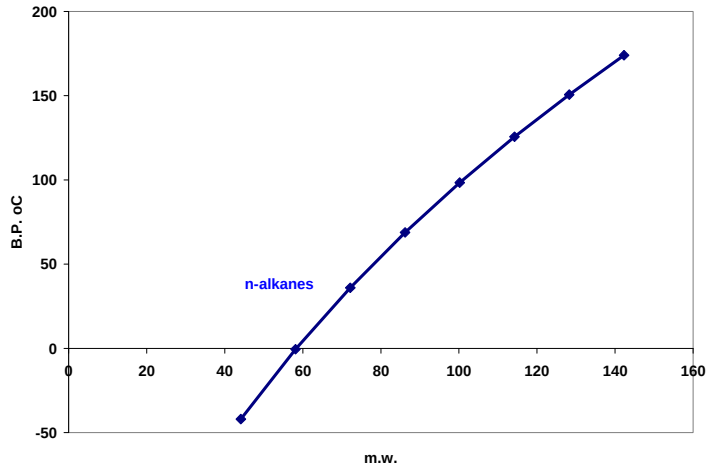
Spiking a sample involves adding a known amount of one of the actual analytes of interest. It is akin to a one-point standard addition process. The purpose of the spike is to get information about whether there is a matrix effect taking place. Spiking is usually done after the extraction step and before injection of the sample into the instrument.

4. Gas Chromatography

The focus of this unit is on practical chromatography. Before working through this unit, students will likely benefit from an introduction to chromatography theory, including the van Deemter equation, resolution, capacity, and selectivity. While some instructors may prefer to handle

these topics in a traditional lecture format, one could also consider the companion active learning module on separations as a way to teach these concepts.

Q1. Create a graph of the boiling points of a series of linear alkanes as a function of mass. Can you separate these compounds by boiling point? Describe the trend you observe in the graph?

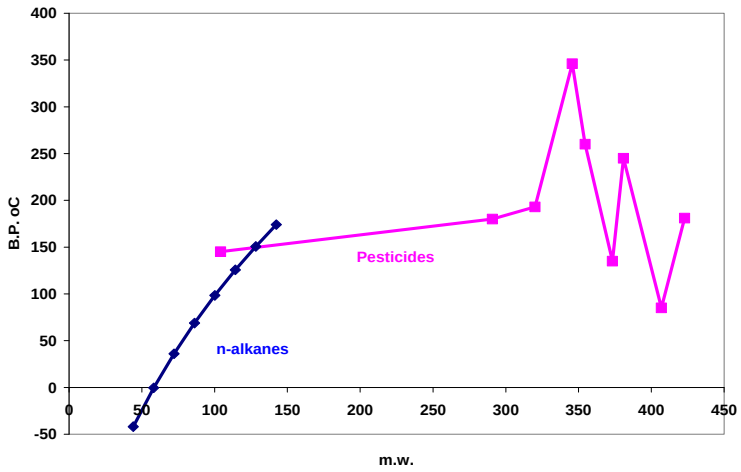


The graph is almost a linear curve in which the boiling point increases regularly with molecular weight.

Q2. Figure 1 contains a portion of chromatogram showing the elution of a sample of *n*-alkanes from a column held at a constant temperature of 87°C. Estimate the approximate time at which C16 will appear?

An examination of the chromatogram shows that each subsequent n-alkane in the homologous series has approximately double the retention time of the previous one. Since C15 takes about 12 minutes, C16 should take about 24 minutes.

Q3. Using the data in Table 2, create a graph of the boiling points as a function of molecular weight. Is the trend in this graph the same as you observed for the series of *n*-alkanes? If not, what do you think makes it different?



No. The graph is quite different and there is no overall correlation between molecular weight and boiling point.

Q4. Examine the molecular structures of the compounds in Table 2. These compounds can be divided into three general types. Which ones would you group together into the three different types? Within a compound group, is there a relationship between structure, molecular weight and boiling point? Do you think that the compounds in Table 2 can be separated based on their boiling points alone?

Group 1 – Compounds 1-4. The compounds in group 1 are all isomers with the same boiling point. These definitely cannot be separated on the basis of boiling points.

Group 2 – Compounds 5-8. There is no agreement between weights and boiling points. Compound 8 is the heaviest of the group but has the lowest boiling point.

Group 3 – Compounds 9-17. Several of these compounds do not have data because the compounds decompose before the boiling point. For those that do, there is no correlation between the boiling point and the molecular weight.

Final conclusion is that these compounds cannot be separated on the basis of boiling point alone. It will be necessary to select a stationary phase that has different interactions with the compounds to produce different partition coefficients.

Q5. Use the information in Table 3 above to find the column recommended for a boiling point-based separation of linear alkanes. What is the chemical composition of the columns suggested for the separation of alkanes?

Methyl polysiloxane. This phase only has methyl groups on a siloxane polymer.

Q6. From Table 3 and Figure 2 what are the compositions of the DB-5 and DB-1 stationary phases? Why is the DB-5 phase a better choice for separation of the organochlorine compounds listed in Table 2?

The DB-5 phase has 5% of the methyl groups replaced with phenyl groups. The DB-1 phase has only methyl groups attached to the polysiloxane. The addition of the phenyl group to the DB-5 provides an aromatic ring that can interact with the organochlorine pesticides to provide another factor in the partition coefficients besides just the effect of boiling point.

Q7. Of the column types listed in Table 3 what might you suggest as alternatives? What figures of merit would you use to determine whether a column provides a "better" separation?

For the methyl 5% phenyl polysiloxane, there are several equivalent columns that are provided by other commercial vendors. If that column did not complete the separation, you may want to try a column with a higher percentage of phenyl groups. The description of the 50% trifluoropropyl 50% methyl polysiloxane phase suggests that it is "widely used as a confirmatory phase for chlorinated pesticides" so this might be a good one to try.

The goal is to separate the compounds in the minimal amount of time. So one figure of merit is the resolution of the compounds you want to separate. The other is the time it takes to complete the analysis.

Q8. What is column bleed? From the structures in Figure 2, why do you think that the DB-5.MS columns have reduced column bleed than those using the standard DB-5 stationary phase?

Column bleed is the loss of stationary phase material. It is particularly problematic at higher temperatures and with mass spectrometry detection because it increases the background signal. The DB-5.MS stationary phase is less prone to column bleed because the phenyl and methyl siloxane components are part of the same polymer, and therefore less likely to degrade and elute at higher temperatures than the DB-5 co-polymer

Q9. The chromatogram in Figure 3 shows a separation of a standard containing 20 organochlorine pesticides plus an internal standard. How would you determine which peak correlates to each organochlorine compound? Be specific.

To conclusively identify each compound in the standard, you would need to individually inject each compound and verify that the retention times and mass spectra match. This would be a long process for the 21 compounds in this sample. EPA methods usually provide an example chromatogram for the mixture of standards on the recommended column, which then tells you the expected elution order. Knowing the elution order, it is possible to see if the chromatogram you obtain for the standards provides the same peaks at the same relative retention times. If the mass spectrum of each peak matches what you expect, it is usually not necessary to inject all 21 compounds individually.

Q10. Why is temperature programming commonly used with gas chromatography?

Running the chromatogram at a single, fixed temperature (an isothermal separation) often leads to two problems. First, those compounds with low boiling points may elute too rapidly from the column and overlap at the beginning of the chromatogram. Second, those compounds with high boiling points may elute too slowly from the column, leading to broad peaks with very long retention times. The temperature program starts with a low temperature and gradually raises it

during the chromatogram. Compounds with low boiling points stay on the column longer and are better separated. Compounds with high boiling points move through the column faster as the temperature is raised and have shorter retention times and more narrow peaks than they would in an isothermal run.

Q11. Look at the maximum temperature at which the stationary phases in Table 3 are stable. What is the maximum operating temperature for the DB-5 column?

325^{o}C.

Q12. What would an ideal chromatographic peak look like? What would a chromatographic peak look like if you overloaded the column? Why is it undesirable to overload a column?

An ideal peak exhibits a symmetrical Gaussian shape. If you overload the column, you will either get peaks that exhibit fronting or tailing, both of which are a form of asymmetry of the peak. The specifics of whether you observe fronting or tailing are rather complex and depend on particular parameters of the system. Whichever one you observe, peaks that show tailing or fronting will lead to a loss of column efficiency and a reduction in the resolution between adjacent chromatographic peaks. It can also be more difficult to achieve accurate quantitation with asymmetrical peaks.

Q13. A 1 L water sample containing 0.1 μg/L of DDT was concentrated to ~1 mL by the sample preparation procedure and 1 μL injected splitless onto a 30 m X 0.25 mm id fused silica capillary column coated with a 0.25 μm bonded film of DB-5.MS. Calculate the ng of material injected. Is this value greater or less than the capacity of the column?

The original concentrated sample (1 mL) contains 0.1 μg or 100 ng.

1 μL (1/1000) of the 1 mL sample is injected into the GC in a splitless mode.

(100 ng) x (1/1000) = 0.1 ng was injected into the instrument.

This is well under the column capacity of 50-100 ng.

Q14. Evaluate the GC data below for benz[a]anthracene and chrysene and determine if the GC column requires maintenance.

Height of benz[a]anthracene peak = 12 mm

Height of chrysene peak = 31 mm

Average height of the two peaks = 21.5 mm

Height of valley between peaks = 5 mm

(5 mm/21.5 mm) x (100) = 23.26%

This is less than 25% so the column does not require maintenance.

5. Mass Spectrometry

Much like the chromatography section, the focus of this unit is on the practical aspects of mass spectrometry measurements and data interpretation. The module introduces the basic components and there is a short discussion of electron ionization, however, the module does not explain how the various mass analyzers work or how the ions are detected. Students will likely benefit from a lecture on these topics before completing the module, although this knowledge is not required to answer the questions. The discussion of both quadrupole and ion trap mass analyzers in EPA method 525-2 opens the possibility of dissecting both mass analyzers in lecture and discussing their relative merits.

Q1. What important information would you need to unambiguously identify a pesticide like DDT in the presence of other similar compounds?

You would need to know its retention time in the separation and have a match of the mass spectrum of the unknown peak to a standard of DDT.

Q2. Why do you need ions instead of neutral species in mass spectrometry?

Ions can be removed from a gaseous mixture using an electric field whereas neutral species cannot. Ions also will interact with a magnetic or electric fields used in the mass analysis portion of the instrument.

Q3. Mass spectrometers operate under high vacuum conditions? Why?

To insure that the ion has a long enough lifetime to be analyzed by the mass analyzer. If the ion collides with another molecule, it is likely to pick up an electron, be fragmented or be deflected from the ion beam.

Q4. What is an advantage of having a large number of fragment ions present in the mass spectrum? Are there any disadvantages?

Large numbers of fragment ions allow for a more definitive match of the mass spectrum of an unknown compound to the known compound. The disadvantage of having a molecule form too many fragments is that the most intense peak (called the base peak) in the mass spectrum is used for quantitative analysis. A large amount of fragmentation may reduce the intensity of the most intense peak.

Q5. Compare the experimentally determined mass spectrum for DDT in Figure 3 to that from the NIST database in Figure 4. Do you think this is a good match? Why?

Yes. The most intense clusters of peaks do match up with each other. Many of the smaller clusters match as well.

Q6. Table 2 of the ***EPA method 525.2*** lists the quantitation ions for DDT as 235 and 165. Why do you think these ions were selected to determine the amount of DDT in our sample?

Because they are the two most intense peaks in the mass spectrum.

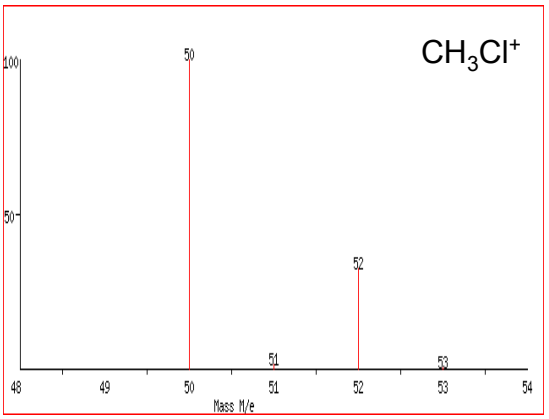
Q7. Using this program, calculate the isotopic abundances of the molecular ion peak for CH_3Cl^+. Are the calculated relative intensities of the ions at 50 and 52 what you expected? In addition to the expected peaks at 50 and 52 Da, the program lists low intensity peaks at 51 and 53. What is the source of these peaks?

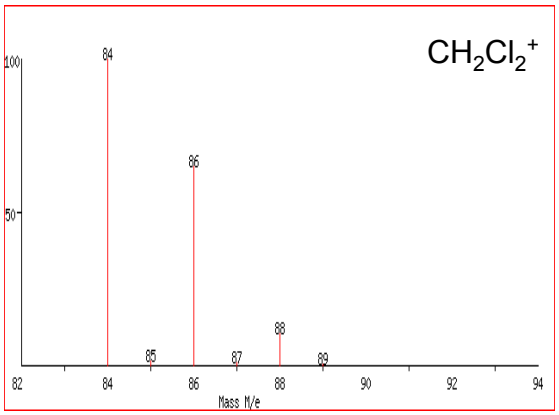
The peaks may not be what the students expect. Rather than having the 50 Da peak be 75.78 and the 52 peak 24.22, which are numbers equal to the percentage of each isotope, mass spectra are typically normalized to give the most intense peak a value of 100% so that the 50 Da peak has an intensity of 100 and the 52 peak is 32.4. These two relative intensities are those expected for a fragment with one chlorine atom.

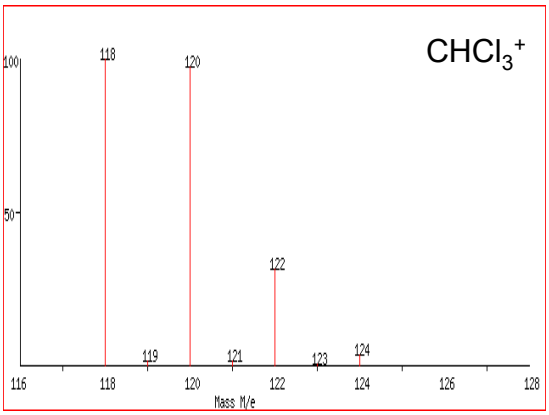
The smaller peaks at 51 and 53 occur because of the ^{13}C or ^{2}H. The natural abundance of ^{13}C is 1.1%, while that of ^{2}H is 0.012%.

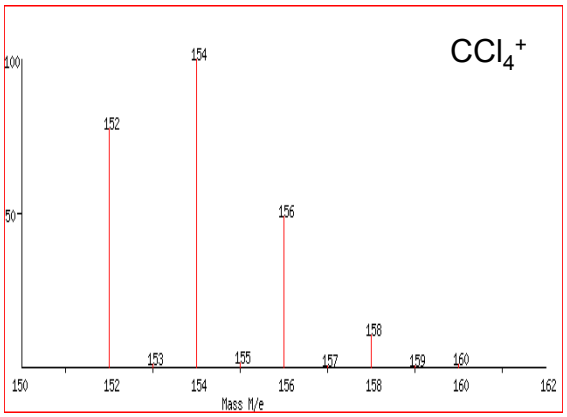
Q8. Now use this program to compare the isotopic distribution patterns of CH_3Cl^+ to $CH_2Cl_2^+$, $CHCl_3^+$ and CCl_4^+. What trends do you observe as the number of chlorine atoms increases?

The isotopic distribution patterns calculated for each species are pasted below. Several trends can be observed. First, as the number of chlorine atoms increases so does the mass of the molecular ion, M^+. The number of isotopic peaks observed also increases with increased numbers of chlorine atoms, and the pattern of intensity changes. For CCl_4, the most intense peak (and therefore the most probable configuration of the chlorine isotopes) is such that the M^++2 peak is the most abundant ion.









Q9. Now calculate the isotopic distribution expected for the molecular ion (M^+) of DDT. How many ^{35}Cl atoms are in the molecule that comprises the most intense peak?

Entering $C_{14}H_9Cl_5$ as the molecular ion, the chlorine isotopic distribution pattern shows peaks at 352, 354, 356, 358, 360, and 362 that correspond to chlorine fragments. The peak at 352 would have five ^{35}Cl atoms. The most intense peak is the 354 peak, so this corresponds to a fragment with four ^{35}Cl atoms and one ^{37}Cl atom.

Q10. In a complex environmental sample that has pesticide contaminants at low levels, the GC-MS spectrum extracted for the DDT molecular ion or base peak may have contributions from other compounds, naturally occurring or anthropogenic, that might be confused with DDT. How could the chlorine signature in the mass spectrum be used to help with compound identification?

While many molecules or fragments of molecules might have masses identical to the mass of some of the DDT fragments, the likelihood that these contaminants have the exact same number of chlorine atoms and the exact same pattern for the chlorine isotope distribution is much less likely.

Q11. Propose a structure for the 235 fragment ion of DDT. Use the isotopic distribution calculator to calculate the relative abundances of the ions expected for the structure you propose. How does the predicted spectrum match the intensities in Figure 2?

Usually the easiest way to determine the formula for a fragment is to examine the loss from the molecular ion. Using 352 as the molecular ion for DDT with only ^{35}Cl isotopes, the loss to 235 involves a mass of 117. One way to get 117 is to lose a CCl_3 group (12 + (3 x 35) = 117). The DDT molecule has a CCl_3 group in it so this loss does make sense. The formula for a fragment with a mass of 235 would then be $C_{13}H_9Cl_2$. The predicted masses and intensities for the chlorine isotope pattern are 235 (100), 237 (65.7) and 239 (11.1). This agrees well with the pattern shown in the mass spectrum.

Q12. Why was chrysene-d_{12} selected as an internal standard rather than chrysene with a natural isotopic abundance of hydrogen nuclei? Would you expect that the chrysene and chrysene-d_{12} to be resolved in the GC chromatogram?

While it is unlikely that chrysene would be found in a sample of the water, chrysene is found in some samples in nature and could be a contaminant in the lake water. The isotopically-substituted chrysene-d_{12} has a weight 12 units higher than normal chrysene and there is no expectation of finding this compound in samples of natural systems.

Sometimes it is possible to resolve molecules that differ only by isotope distribution in chromatographic separations, but the system has usually been designed specifically to facilitate such a separation. The column and conditions used to separate the pesticides in EPA Method 525.2 would not separate chrysene and chrysene-d_{12}.

Q13. Section 13.1.2 of EPA 525.2 describes the calculation of the method detection limit (MDL) for each analyte. Use the data in Tables 1 and 2 to calculate the MDL for DDT. How does this value compare with those found in Tables 3-6 of EPA Method 525.2?

According to the EPA Method, *"the MDL is defined as the statistically calculated minimum amount that can be measured with 99% confidence that the reported value is greater than zero*."

To calculate the MDL, students will use the variance in the calculated concentrations for the fortified blanks (Table 1) as shown below. Additional information about the calculation of the MDL for EPA methods can be found at http://water.usgs.gov/owq/OFR_99-193/detection.html .

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

For each of the fortified blanks, the first step is to normalize the intensity of the DDT peak relative to that of the internal standard for each measurement, as shown in the Table below. The data in Table 2 is then used to construct a calibration plot, from which the concentration values for each replicate can be calculated. (Note: some attention will need to be made to the fact that concentration values for calibration curve are in mg/L, while MDL should be calculated in values of μg/L. A subtle point is that the internal standard was added to the sample before the pre-concentration at 5.00 μg/L, and since we have normalized to this value, the calculated

concentrations for the fortified blanks from the calibration plot can be considered to be in ug/L.) The mean value for concentration for the 7 fortified blank solutions is 0.49_1 μg/L, with s = 0.03_9. From the table of Student's t values with 7-1 degrees of freedom, at the 99% confidence level, t = 3.143.

Table 1. Laboratory fortified blank data (0.50 μg/L DDT and 5.00 μg/L chrysene-d_{12})

Sample #	Intensity of m/z = 240 for chrysene-d_{12}	Intensity of m/z = 235 for 4,4'-DDT	Relative intensity 4,4'-DDT
1	2494373	143803.1	0.057651
2	2800996	148889.7	0.053156
3	2985785	182870.4	0.061247
4	2855369	180016.7	0.063045
5	2762538	154296.0	0.055853
6	2602473	152374.8	0.058550
7	2289537	125819.2	0.054954

$$MDL = 0.03_9 \times 3.143 = 0.12_2\ \mu g/L$$

The MDL listed in Table 3 of the EPA method is 0.083 μg/L.

Q14. What is the average percent recovery for DDT indicated for the given data?

% Recovery = [(Mean Calculated Concentration) / (Actual Concentration)] x 100

The mean calculated concentration for the seven fortified blank solutions from Table 1 was 0.49_1 μg/L.

All of the blanks had been fortified at an actual concentration of 0.50_o μg/L.

This gives a % recovery of $[(0.49_1)/(0.50_0)] \times 100 = 98\%$.

Q15. What is the concentration of DDT (μg/L) for each of the five samples you selected?

Ask students to choose 5 samples from the grid in Figure 8 and provide them with the chrysene-d12 and 4,4'-DDT peak intensities from the data table below. They can then use this data with their calibration plot to calculate the concentrations to achieve the values in the right hand column of the table below.

DDT sampling data.

Sample Area	Intensity of m/z = 240 for chrysene-d_{12} (5 mg/L)	Intensity of m/z = 235 for 4,4'-DDT	Calculated 4,4'-DDT Concentrations
1	2194322	1828941	9.19 μg/L
2	2990505	1392968	5.07

3	2871304	183603	0.56
4	2090152	1612462	8.50
5	2633190	1318854	5.46
6	2272639	378235	1.71
7	2307414	54199	Below MDL
8	2474594	1662106	7.38
9	2024419	619017	3.28
10	2848970	253889	0.85
11	2273543	37052	Below MDL
12	2119662	425187	2.10
13	2736101	229072	0.79
14	2951912	56069	Below MDL
15	3071910	41778	Below MDL
16	2240233	30467	Below MDL
17	2021236	123795	0.53
18	2378539	147817	0.54
19	2546930	57535	Below MDL
20	2214506	34099	Below MDL
21	2564591	37184	Below MDL

Q16. Are the levels of DDT that you observed in your samples from Lake Nakuru potentially toxic to flamingos there?

Once students arrive at the DDT concentrations for their samples they will probably not know what to do next without some guidance. You might suggest that they look up the LD50 for DDT. DDT is only moderately toxic; the acute oral LD_{50} value for rats is around 118 mg/kg. However, the flamingos are not likely exposed to the DDT by consuming the lake water. Instead, it is more likely that the DDT is bioconcentrated by the algae which are then consumed by the flamingos. Detecting high concentrations of DDT in the lake water is an important first step in addressing the hypothesis that organochlorine pesticides may contribute to the flamingo die-off events at Lake Nakuru, but it is not a smoking gun.

Q17. If you were not limited to water samples, what other types of samples would you collect for analysis and why?

It would be important, as a next step to collect algae samples from the lake and analyze these for DDT and other organochlorine pesticides. If the algae bioconcentrate the pesticides, as suspected, then the flamingos could be exposed to much larger concentrations than are in the water samples. Because DDT may absorb to particles and deposit in the sediment, it could also be interesting to collect sediment samples. Sediment samples might also provide a history of DDT levels in the lake.

6. Validation of the Method

The students will need a copy of EPA Method 525.2, especially the sections on *Quality Control* (Section 9.0) and *Method Performance* (Section 10.0).

Q1. Define the terms accuracy and precision. What types of error are associated with each?

Accuracy refers to how close the measurement is to the true value. Accuracy may be impaired if systematic bias exists. Precision refers to the range of error associated with various points in the analytical process, it is associated with random error.

Q2. What is the general definition of a blank?

A blank ideally contains all of the components of the sample matrix except for the analyte.

Q3. Explain the difference between a field reagent blank and a lab reagent blank.

Field reagent blank is known water that is placed in the sampling container and treated similar to the field samples. If the containers leach phthalates on acidification because of a temporary high temperature in shipment, then the field reagent blank would tell you this. The lab reagent blank is the solvent and reagents used in the preparation process. What one wants to find out from this measurement is if, for example, the nitric acid used to digest your sample already contained lead in it that would contribute substantially to your measurement of lead in samples.

Q4. What are fortified solutions? What is the purpose of a fortified solution?

Fortified solutions are the same as above, but to which a known amount of the target compound have been added. The purpose is to determine if the lab is capable of making an accurate measurement.

Q5. The purpose of internal standards and surrogate analytes were covered in the section on sample preparation. Review these materials and describe how internal standards and surrogate analytes differ?

A surrogate could possibly be a target analyte, although one attempts to find a surrogate that is not in the sample. The surrogate(s) should have chemistry similar to the targets so that any loss of the target molecules caused by the extraction procedure can be evaluated. An internal standard is a compound that is added immediately prior to the instrumental measurement at a concentration that will be constant across all samples. This allows the response of the target analyte to be normalized to the response of the internal standard.

Q6. Why is decafluorotriphenylphosphine (DFTPP) used in the method?

DFTPP is an internal standard that is used to calibrate the MS instrument.

Q7. Table 3.1 gives the results of DFTPP performance tests. What are the three parts to the performance test?

Low mass resolution, mid-mass resolution, and high-mass resolution.