Experiment GC : Analysis of BTEX by GC-FID

Learning Goals:

- Familiarity with gas chromatography
- Gain experience in temperature programming and method development
- Correctly use an internal standard, and compare results with and without
- Learn proper injection technique

Reading:

Before coming to the lab, please do some reading on gas chromatography from a reliable analytical source, like Skoog et al, Instrumental Analysis; Harris, D., Quantitative Analysis, 6th ed.; and/or Harvey, Modern Analytical Chemistry.

Prelab:

1. What are the boiling points of benzene, toluene, ethylbenzene, o-xylene and m-xylene? Based on your answers, decide on suitable temperatures for the injector and detector of the GC, bearing the following in mind: the injector temperature should be at least 20°C-40°C higher than the highest boiling point in the mixture, and the detector should be at least 220°C, not higher than 280°C, and 20°C-40°C higher than the injector.

2. The Varian 3400 GC is equipped with the choice of split or splitless injection. Given the conditions of this experiment, which mode is more appropriate? Why?

3. The Varian 3400 GC has an FID detector. What is an FID detector? What are the advantages and disadvantages of this detector? Would you say it is a good choice for the analysis of BTEX? Why or why not?

Introduction:

Gas Chromatography (GC):

Chromatography involves separating a mixture of analytes according to their partitioning between a stationary phase and a mobile phase. In gas chromatography, the mobile phase is an inert carrier gas, and the stationary phase is found in the column. The instrument being used in this lab is the Varian 3400 Gas Chromatograph. It is equipped with a capillary (or open tubular) column, which is 30 m in length. These long narrow columns provide much better resolution than the alternative, packed columns, which now find their best use in preparative GC applications.

The volatile liquid or gaseous analyte mixture is injected onto the GC through a septum into a heated injection port. The temperature of the injector is selected so as to vapourize the sample upon injection. The sample vapour is then carried through the column by the carrier gas. As
they interact with the stationary phase to varying degrees, they are separated. With nonpolar stationary phases, the principal determining factor as to relative retention times on the column is the volatility of the analytes. Therefore one would expect to see an elution order that followed, or nearly followed, the order of the boiling points of the analytes. The detector temperature is chosen to be at least 20°C higher than the highest boiling point, in order to ensure all analytes are detected as gases.

To get a better and more efficient separation of analytes, temperature programming is often employed (see Figure 1). The temperature of the column is raised during the course of the analysis. If there are analyte peaks at the beginning of a chromatogram that are overlapping, you might choose to start the analysis at a lower temperature, and raise the temperature back up after a few minutes. As well, there could be some late eluting peaks that can be forced to elute faster if the temperature is raised during the analysis. Raising column temperature decreases retention time for analytes. Since analyte peaks broaden as time on the column increases, so decreasing retention time will improve resolution.

**BTEX:**

BTEX are an important class of volatile environmental contaminants, and are frequently analyzed in environmental and drinking waters. Current CRD regulations, for example, require that all waste entering the municipal sewer system contain no more than 1 mg/L BTEX; the other organic contaminants which must be monitored are chlorinated phenols, polycyclic aromatic hydrocarbons (PAH), phenols and petroleum hydrocarbons. In this experiment, % levels of BTEX are quantified using the internal standard technique as described below. Pentane is used as an internal standard, as it fulfills the necessary requirements for an internal standard. Hexane is used as a solvent.

Sources of BTEX include:
- Benzene: solvents in printing, paints, dry cleaning solutions, etc.
- Ethyl benzene: solvent for coatings; used to make styrene, and is a component in rubber and plastic wrap.
- Toluene: used to make benzene and urethane
- Xylenes: gasoline, solvents; used to make plasticizers, polyester film, film.

**Procedure:**

**Week 1:**

*Varian 3400 GC:*

The Varian 3400 GC is equipped with an FID detector. From your prelab, you will know a hydrogen flame is used in detection. As most people are aware, hydrogen is an explosive gas, and must be handled properly.

1. You will need to turn on all of the necessary gases at the cylinders. Helium is the inert carrier gas, medical air is used as the oxidant, and hydrogen for the fuel. Open all of these cylinders.
The regulators should be set at 80 psi for each already, so there is no need to adjust these. Next, open the helium tap on the benchtop. Make sure this is the tap that is connected to the 3400 GC. Leave the other taps closed for now.

2. Next you will have to build a method. Compare your selected temperatures for the injector and detector with your partner. Decide together on temperatures that you agree upon, and check that your selected injector and detector temperatures are ok with your TA. Select a column temperature. Remember the column temperature is now bound between room temperature, and at least 20°C below your injector temperature. You can run your first injection isothermally if you like, or you can try a program. Take careful notes of your conditions, so that you can track your changes as you run trials.

3. To build the method, press BUILD/MODIFY, then press METHOD 1. Always use method 1 for this lab. Next you will be asked for an initial column temperature. Enter the temperature you have chosen, and press enter. The screen will keep leading you through a series of prompts:

   - initial column hold time: enter the amount of time (in minutes) you want the column to remain fixed at the initial temperature.
   - add column program? – enter yes or no. Yes means you want to add a temperature program, so that the temperature will change over the course of the run.

If you select yes, you will be prompted to enter a final column temperature, a hold time for the final temperature, and a rate of temperature increase, in °C/minute.

![Figure 1. Schematic of a possible GC temperature program](image)

In the example shown in Figure 1, a column is held at an initial column temperature of 50°C for 5.00 minutes, then the temperature is ramped at 10°C/min to a final temperature of 150°C. The final temperature is then held for an additional 6 minutes.

4. After entering this information, you will be asked if you want to add an additional column program. This is used if you want to ramp the temperature at two different rates, at different
times in the run, or if you want to hold the column at a particular temperature partway through, then ramp again afterwards. For our purposes, this probably won’t be necessary, but is an option.

5. Next, you will be asked to enter an injector temperature. Enter the approved temperature, then press enter.

6. The last part you will have to build is the detector program. You will be prompted to enter a detector temperature, attenuation and range. The attenuation should be 8, and the range should be 9. You can adjust these later if needed.

7. There will be further prompts, but at this point, you can just press ACTIVATE then press METHOD 1. A red “NOT READY” light will remain on until the temperatures you have set have been reached. To see where the temperatures are at any moment, push status.

8. When the detector has reached about 200˚C, you can light the detector. Please get your TA to supervise this.

Turn on the medical air on the benchtop tap. Do the same for the hydrogen. Then, on the front of the GC, push IGNITE and hold for about 4 seconds. You might hear a faint ‘pop’ as the flame lights. After releasing the button, check for ignition by holding the glass slide over the exhaust. If it fogs up immediately, the flame is lit. If not, try pressing ignite for another 4 seconds. If there is still no luck, turn off the hydrogen and air taps, and try again in a couple of minutes. If there is still no success, get further assistance from the SLI.

Once the detector has been lit, you are ready to proceed with the analysis.

9. Injection technique is important for this experiment. You should inject one or two microlitres. Choose an injection volume in this range, note it, and stick with it. You can adjust the attenuation if your peaks are too small or too large. You and your partner can both take turns injecting.

10. You can use the QC for the method development portion of the experiment. This part is done in pairs, so either of you (or both) can use your highest standards. This is not a quantitative exercise. You are looking for a good separation of the analyte peaks, as well as the sharpest possible peaks, and fastest possible method. You should only budget one hour for this part of the experiment, so get the best possible method you can in this time frame. If you need any guidance, ask your TA.

11. After you have a suitable method, you and your partner can start your standard preparation. Only one set of standards needs to be prepared between the two of you. You will want to calibrate from 0-10% each of benzene, toluene, ethylbenzene, o-xylene, and m-xylene. The easiest way to do this is to prepare several (at least 5) multistandards, that contain a mixture of the analytes, in varying concentrations. As well, in this experiment we will use an internal standard, pentane. Each prepared standard solution should therefore contain a constant amount (5%) of pentane. The solvent for the preparation is hexanes.
Week 2:

12. Follow the same procedure to start the instrument. Re-enter your method parameters, as many other people have been using different programs, and yours is probably no longer on the instrument. You and your partner will inject all of your standards, a QC, and one sample each. As many times as you feel necessary.

13. After all of the data has been collected, activate method 4, turn off the hydrogen at the cylinder, then on the benchtop. Then turn off the medical air at the cylinder and benchtop. After you have finished cleaning up your things and are ready to leave, turn off the helium at the benchtop, then at the cylinder.

Report

A full report is required for the experiment. Answers to the points below are to be included in the discussion.

1. Calculate the ratio of the peak area component/peak area of pentane internal standard for each of the 5 components of the BTEX standards and unknown. Plot analytical calibration curves for all 5 BTEX components. From these curves, determine and report the concentration of each component of BTEX in the unknown sample as %; remember that the unknown contains 5 % pentane internal standard.

NOTE: the integrator is linear over several orders of magnitude; it’s a good idea to examine the areas of off-scale peaks, and decide if they also represent accurate/adequate data.

2. Hand in your labeled chromatograms as per formatting guidelines.

3. Calculate the mean retention time (RT) ± standard deviation for all components.

4. Calculate the relative retention time (RRT) for each component. The RRT is calculated by dividing the retention time of the compound of interest by the retention time of the pentane internal standard. Calculate the mean RRT ± standard deviation. Compare with the results from 3 above and discuss.

5. Discuss results for two components (you can pick any two) obtained from both internal standard and external standard calibration, with respect to this experiment. This means you must prepare two calibration curves for each of the two components that you have chosen, one using the internal standard results, and one using the external standard results. Then calculate the concentration of unknown, QC and spike samples from each of the cal. curves. Compare accuracy and precision between internal/external calculations.