Gas Chromatography User Training Chem 543

1. Motivation

Capillary gas chromatography (GC) is one of the most popular analytical techniques used in today's research. Its popularity is mainly due to efficient separation of complex mixtures of various analytes. Although GC analysis is very common, a large number of users are not aware of all the options chromatographic equipment offers. Consequently, the GC instrumentation is often used incorrectly leading to wrong conclusions about sensitivity and selectivity of methods.

Most researchers think of a GC method development as a simple modification of temperature program. Although this is indeed the main aspect of optimization, the knowledge on the operation of injectors and detectors **is essential**. Therefore, the aim of this lab assignment is to gain experience in the setup of standard GC instrumentation and in the optimization of fundamental GC parameters.

2. Instrumentation

The experiments will be performed on the departmental HP5890 Series II GC system equipped with split/splitless injector and a flame ionization detector (FID). For separation, a 15 m long DB-5 (5% phenyldimethylpolysiloxane) capillary column with 0.25 mm internal diameter and 0.25 μm film thickness will be used.

3. Materials

The stock solutions of standards dissolved in methylene chloride will be made available by the instructor and each student is responsible for asking for them in advance and returning them after the lab. The stock solutions are available and shared by all students. Thus, pay attention to how you use it. Inappropriate use will result in an additional assignment consisting of a preparation of new stock solutions.

For optimization of linear velocity, methyl myristate (~3 mg/mL in methylene chloride) will be used.

For optimization of temperature programs and splitless injection, **an evaluation mixture** consisting of benzene (b.p. 80.1 °C), tridecene (233 °C), tetradecene (251 °C), pentadecene (268 °C), hexadecene (274 °C) and hexadecane (287 °C) ~100 μg/mL of each (also in methylene chloride) will be used.

Solvents, such as methylene chloride, will be provided.

Each student will also receive one GC liner, glass wool, Pasteur pipette, one pair of gloves, 2 tubes (7 mL), a 10 μL syringe for the sample injection, a liner wrench, and an adapter for flow rate measurements. At the end of the lab assignment, students are responsible for returning all tools, stock solutions, and cleaned syringe. All disposable materials must be disposed of, and glassware washed with water, and acetone. The samples should be disposed into the properly labeled waste bottle.

4. Instructions

The lab assignment is performed on an individual basis or in pairs (Chem 443). Students have to sign up to ensure availability of the instrument. Students need to provide record of all their measurements in order to complete the training. Prior to the 1st lab session students must submit a handwritten home to take test, unsatisfactory, the lack of this test submission or lack of pertinent knowledge (oral) will require rescheduling of the lab session based on availability and loss of points.

NOTE! Before proceeding to the individual tasks, make sure that you follow the particular guidelines in the **GC operation manual**.

Within this lab assignment, you will learn to:

1) Install the liner
2) Verify the gas' flow rates and start the GC
3) Optimize the average linear velocity.
4) Evaluate splitless conditions.
5) Optimize the temperature program and
6) Evaluate the repeatability of injection.

4.1. Installation of the liner

Before starting, open the nitrogen line and see whether the inlet is pressurized, then close the nitrogen valve prior to the installation of the liner. The liner obtained from the instructor is pre-cleaned with a series of solvents. Any contact with parts inside the injector should be performed while wearing gloves. Before installing the liner, insert a small piece (~0.3 cm^3) of pre-cleaned glass wool, ca. 4 cm deep, into the liner. This has to be performed with solvent-cleaned (dried) forceps and a new Pasteur pipette. The video of the liner installation is available via the Agilent website.

4.2. Verification of flow rates and starting GC

Record the pressures in the gas cylinders at the beginning and the end of your lab.

Measure all flow rates as described in the **GC operation manual** and provide the results in the report including the GC# as part of experimental section (note that only one sentence with measured values is sufficient). The first gas introduced to the GC should be a carrier (e.g., nitrogen). This gas should be measured at the outlet of the column (detector) to provide the flow rate, then measure a septum purge, and split flow rates. Finally, determine the flow rates for hydrogen, air, and auxiliary gas on the detector. This protocol is employed any time you are starting to work on an instrument which was not used for some time. Report in a spreadsheet all flow rates measured, including the number of the specific GC instrument you have worked on. If a significant deviation from the default values is observed, let your instructor know. **Check with the instructor or TA whether you may continue in the assignment**, then follow the guideline to

turn on the GC, detector gases, and light the detector – but only AFTER IT REACHES THE REQUIRED TEMPERATURE!

For Task 4.3, set the temperatures of the injector and detector to 250 °C and 300 °C, respectively. The injector should be operated in the split mode: set the **purge to "ON"** (see the manual for a detailed protocol). **Every time you inject, make sure the purge is ON.** Before initializing any work (on each day), inject 1.0 µL of methylene chloride and set the oven temperature to 300 °C; leave it for about 20 min or until the signal is stable. The labeling of run files should always start with the run order number (i.e., 01 for the first run and 02 for the second run).

4.3. Optimization of average linear velocity

You will optimize the average linear velocity ($\bar{u}$) based on the minimum height of theoretical plate (H).

For the optimization use the provided solution of methyl myristate (**3 mg/mL**).

Perform isocratic analysis (200 °C) at four different column head pressures ranging from 2 – 15 psi (you do not have to test the full range of pressures as long as you determine the optimum). As mentioned above, the injector should be operated in split mode [make sure (observe) that the purge valve is on at the beginning of the analysis]. You should see two peaks: a solvent peak and methyl myristate peak. Typically, you should not stop the analysis before the run is completed, but in this lab you may have to reduce the waiting time.

For each analysis determine the average linear velocity and the height of a theoretical plate.

The average linear velocity can be obtained from equation 1,

$$\bar{u} = L/t_m \qquad \text{Eq. 1,}$$

where L is the column length (cm) and t_m (s) is hold-up time. For the determination of hold-up time, assume that the solvent peak represents the unretained analyte. The theoretical plate height (H) is inversely proportional to a number of theoretical plates (N) (Eq. 2).

$$H = L/N \qquad \text{Eq. 2}$$

The number of theoretical plates is determined using equation 3.

$$N = 5.54(t_r/w_{1/2})^2 \qquad \text{Eq. 3,}$$

where t_r is retention time of the analyte peak and $w_{1/2}$ is the peak width at half of the height (sometimes also reported as $w_{0.5}$).

Report all the calculations within the MS Excel spreadsheet Table, calculate all data using formulas (do not insert values). Report the optimal average linear velocity (cm/s) and column head pressure and calculate inlet and outlet flow rates (mL/min) for the optimum average linear velocity (see an instruction file for these calculations). Employ the optimum average linear velocity in **the next task**. Provide an electronic copy of your chromatograms as Figure 1 in MS PowerPoint (use the export function in the Clarity software).

4.4. Evaluation of splitless injection

Splitless injection is the technique used for a trace analysis. Thus, only low concentrations of analytes are used.

Employ the **optimal head pressure** determined in the previous assignment. Set up the following temperature program as described in the manual. Note that the methylene chloride boiling point is 37 °C, however this is the case only at atmospheric pressure. Thus you can use the start temperature program at 35 °C with hold for 1 min, followed by 35 °C/min to 60 °C for 3 min, and followed by 25 °C/min gradient to 300 °C with hold for 5 min.

Perform injections with different splitless times 0.2 min, 0.5 min and 1 min (to open the splitless valve, see the manual for the setup) of an evaluation mixture. To determine the identity of different peaks, inject methylene chloride (as a blank), and (if needed) also benzene solution. The elution of later eluting analytes (alkenes) may be determined based on their boiling points. Make sure that at the beginning of analysis **the splitless valve is closed** (PURGE = OFF), watch the purge valve during the beginning of analysis whether it really opens at the preset time.

Report tabulated results in MS Excel and evaluate the benefits of different splitless times, with respect to **peaks' shape, area, peak width and retention time**. Provide obtained chromatograms in MS PowerPoint file as Figure 2.

4.5. Optimization of temperature program

The aim of temperature program optimization is enabling a fast high-sensitivity analysis with all peaks resolved. Thus, modify the program used in Section 4.4. to achieve faster elution and separation of all compounds, also ensuring high response. **You need to propose and analyze your sample using only two additional programs.**

Summarize all three temperature program conditions evaluated in the form of a table. In your MS PowerPoint file, show the resulting chromatograms. Highlight (explain) the contribution of varied parameters to the separation and propose how you would further optimize the program to improve separation. Evaluate the resolution of hexadecene and hexadecane.

4.6. Evaluation of repeatability

Inject the mixture of analytes at least three times and report the average, standard deviation (SD), and relative standard deviation (RSD) values for the retention time and peak areas. Repeat the injections until you obtain a RSD of ~ 10% for the majority of peaks. Ensure the correct integration of all peaks. Discuss your results.

5. Report

For the class, the two reports (intermediate and final) will be submitted within PowerPoint and Excel files. The intermediate report needs to show all measure flow rates and optimization of linear velocity based on sections 4.2 and 4.3.

The final report must be comprehensive, addressing all experimental sections (4.3-4.6) and providing interpretive and conclusive statements for each section. Students must ensure clear

slide titles and labeling to data to ensure clarity of the report. The file name should start with the initials of the student.

6. Lab Check Out

At the end of this lab assignment, each student will be responsible for returning the cleaned syringes and any materials to the instructor. All disposable materials must be discarded, and glassware must be washed with water and ethanol. Each student needs to demonstrate proper use of the laboratory notebook to the instructor.

7. Grading

The grading will be based on
(1) Prelab test (5 pt) – see section 8
(2) Intermediate report submitted as Excel and PowerPoint files (10 pt)
(3) Final Report (60 points), consisting of the PowerPoint presentation and supported by MS Excel file sheet.
(4) Lab check out and demonstration of effective use of the lab notebook (5 pt)

8. Test Prior to the Lab

The answers must be provided prior to the lab; however, the student should have the knowledge of the content without looking at notes. Lack of preparation will result in the cancelation of the lab and its delay to a further date. The uploaded answers must be handwritten.

1. Sketch major components of gas chromatograph.
2. Sketch split/splitless injector, explain the operation principle, and explain difference between split and splitless injection.
3. What is the purpose of the septum purge?
4. What is a backflash in gas chromatography? How can you limit it?
5. When is a split injection on a gas chromatograph normally preferred?
6. Explain the principle of solvent/cold trapping. How is it performed?
7. Would be the easiest approach to optimize the separation?
8. Why do you optimize linear velocity? Do you have to do it before each analysis? Why yes or no?
9. Apart from getting into big trouble, what will happen if you leave the column heated without the flow?
10. Which chromatographic parameters do you affect when you are changing the splitless time?
11. Explain the principle of FID operation.
12. How high would you set the temperature on FID for the ignition and for the operation?
13. Which part of the instrument defines the volume of the injector?
14. Which physical parameter (property of the analyte) controls elution and separation on a nonpolar stationary phase.
15. What are the main assignments you have to complete within the lab?