

Chromatography Lab – Assignment I Chem 543/443

1. Introduction

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 correct, the knowledge on the operation of injectors and detectors **is necessary** as well. Therefore, the aim of this lab assignment is to gain experience in the setup of standard GC instrumentation and in the optimization of basic operating parameters.

2. Instrumentation

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

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 should suffice for 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 methylmyristate ~ 3 mg/mL will be used. For optimization of temperature programs and splitless injection two solutions 100 $\mu\text{g/mL}$ benzene and solution consisting of benzene, phenanthrene, tricosane ~ 100 $\mu\text{g/mL}$ of each, will be used. Solvents, such as methylene chloride will be provided.

Each student will also receive one liner for the GC, glass wool, one pair of gloves, 2 vials (2 mL), and 10 μL syringe for the sample injection. At the end of the lab assignment, students are responsible for returning stock solutions and cleaned syringes. 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. 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.

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

Within this lab assignment you will learn to:

- 1) Install the liner
- 2) Optimize average linear velocity.
- 3) Perform splitless injection.

- 4) Optimize temperature program and evaluate reproducibility of the injection.

4.1. Installation of the liner

Before starting, open the helium line and see whether the inlet is pressurized, then close the helium. The liner obtained from the instructor is precleaned with a series of solvents. Any contact with parts inside the injector should be performed in gloves. Before installing the liner, insert a small piece ($\sim 0.3 \text{ cm}^3$) of precleaned glass wool ca. 4 cm deep into the liner. This has to be performed with a solvent-cleaned (dried) forceps and a new Pasteur pipette. The video of the liner installation is available via Agilent website (links are provided in the computer lab AH 343).

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 **GC operation manual** and provide the results in the report including the GC#. The first gas introduced to the GC should be a carrier (e.g., helium). Report in MS Excel spread sheet all flow rates measured including the number of GC you have worked on. If significant deviation from the default values is observed let know your instructor. Follow the guideline to turn on the GC, detector gases, and light the detector **AFTER IT REACHES REQUIRED TEMPERATURE!**

Set temperatures of 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 detailed protocol). **Every time you inject, make sure the purge is ON.** Before initializing any work (on each day), inject 1 μL of methylene chloride and set the temperature on the oven to 250 °C; leave it for about 20 min or until the signal is stable.

4.3. Optimization of average linear velocity

You will optimize the average linear velocity (u) based on the minimum height of theoretical plate (H).

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

Perform isocratic analysis (200 °C) at four different column head pressures ranging from 4 – 20 psi. When increasing the column head pressure, make sure you still have sufficient flow rate on the split vent $\sim 30 \text{ mL/min}$. As mentioned above the injector should be operated in split mode (make sure (observe) the purge valve is on at the beginning of the analysis).

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

The average linear velocity can be obtained from the equation $u = L/t_m$ where L is length of the column (cm) and t_m (s) is hold-up time. For its the determination, of hold-up time, assume that the solvent peak represents unretained analyte.

The plate height is inversely proportional to a number of theoretical plates $H = L/N$.

The number of theoretical plates is determined using equation $N = 16(\text{tr}/w)^2 = 5.55(\text{tr}/w_{1/2})^2$. For calculation you can simply measure " t_r " and " w " distance by a ruler. Report all the calculation within MS Excel spreadsheet, calculate all data using excel formulas. Report optimal average linear velocity and column head pressure and **employ those for the next task**. Provide electronic copy of your chromatograms in MS PowerPoint file (use export function in the Clarity software)

Calculate inlet and outlet flow rate for the optimum average linear velocity (see instruction file on these calculations).

4.4. Application of splitless injection

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

Employ *optimal head pressure* determined in the previous assignment. Setup the following temperature program. Note, methylene chloride is a very tolerant solvent thus you can use start temperature program at 35 °C/ hold for 2 min, followed by 35 °C/min to 80 °C for 2 min, and followed by 8 °C/min gradient to 250 °C with hold for 5 min.

Perform three injections with different splitless times 0.1 min, 0.2 min and 0.4 min (to open the splitless valve, see the manual for setup) of methylene chloride (as a blank), benzene, and mixture of three analytes. 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. The expected elution order for your compounds is benzene, phenanthrene, and tricosane. Compare injection of blank (solvent alone) versus benzene solution to determine its retention time determine retention times of other two species as well and evaluate the benefits of different splitless times, with respect to peaks' shape, area and retention time.

4.5. Optimization of temperature program and reproducibility

The aim of temperature program optimization is a fast analysis with all peaks resolved.

Thus, modify the program used in the section 4.4. to achieve fast elution and separation of all compounds. Inject the mixture of analytes three time and report average, standard deviation (SD), and relative standard deviation (RSD) values for retention time and areas. Repeat the injections until your get RSD of ~ 5%.

5. Report

You will be evaluated on the basis of your report (in the electronic form), capability to deal with assigned problems, and attached chromatographic data. The files should be named XXGClab.docx/xlsx/pptx replacing the XX with the initials.

The chromatograms should include the label with description of analyte and analysis conditions.

The report should include a title page with the date the experiments were performed and name of the person performing experiments. The report should consist of sections addressing each task (4.1-4.5) briefly describing what was performed, tabulated data obtained, and discussion of parameters affecting the results. The calculations and charts should be enclosed as the MS Excel spreadsheet in a comprehensive form (all column should be labeled) and formulas of calculation embedded within MS Excel.

The last section of report should address the following questions.

- 1) Why do you use glass wool in liner?
- 2) Why do you inject methylene chloride before the start of work?
- 3) At what temperature did you light FID and why?
- 4) What is the base for selection of operation temperature of FID?
- 5) Which analyte would be more suitable for determination of hold-up time and why?
- 6) When the pressure is constant, how and why does change of temperature affect the velocity? (You can use flow calculator on Agilent website (also installed in computer labs) to evaluate this.)
- 7) Explain and calculate the difference between the inlet and outlet flow.
- 8) Explain the mechanism and why do we use solvent trapping in splitless injection?

- 9) What defines elution order of the analytes? How does it correspond to your measurements?
- 10) What was the most critical parameter in the temperature program optimization?