1. Introduction

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 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 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 was 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 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 methyl myristate ~3 mg/mL will be used.

For optimization of temperature programs and splitless injection a two solutions 100 μg/mL benzene (100 μg/mL) in methylene chloride and an evaluation mixture consisting of benzene (b.p. 80.1 °C), tridecane (233 °C), tetradecene (251 °C), pentadecene (268 °C), hexadecane (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 liner for the GC, glass wool, Pasteur pipette, one pair of gloves, 2 tubes (7 mL), 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. At the beginning of the 1st lab session students must submit handwritten home to take test, unsatisfactory, lack of this test or lack of pertinent knowledge (oral) will require rescheduling of the lab session based on availability.

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

4.1. Installation of the liner

Before starting, open the nitrogen line and see whether the inlet is pressurized, then close the nitrogen. 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 (~0.3 cm³) 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.

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# as part of experimental section (note 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 flow rate, as auxiliary (makeup) gas for the detector, septum purge, and split flow. Then determine flow rates for hydrogen and air on the detector. This protocol is employed anytime you are starting to work on an instrument, which was not used for some time. Report in a 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. 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 AFTER IT REACHES REQUIRED TEMPERATURE!
For the Task 4.3, 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 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. 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). 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 to reduce 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 the equation 1,

$$\bar{u} = \frac{L}{t_m} \quad \text{Eq. 1}$$

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 theoretical plate height ($H$) is inversely proportional to a number of theoretical plates ($N$) (Eq. 2).

$$H = \frac{L}{N} \quad \text{Eq. 2}$$

The number of theoretical plates is determined using equation 3.

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

Where $t_r$ is retention time of the analyte peak and $w_{1/2}$ is a peak width at half of the height (sometimes also reported as $w_{0.5}$). Report all the calculation within MS Excel spreadsheet Table, calculate all data using formulas (do not insert values). Report optimal average linear velocity and column head pressure and calculate inlet and outlet flow rate for the optimum average linear velocity (see an instruction file on these calculations). Employ the optimum average linear velocity in the next task. Provide electronic copy of your chromatograms as Figure 1 in MS PowerPoint or MS Word file (use 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 necessary.

Employ optimal head pressure determined in the previous assignment. Setup the following temperature program as described in the manual. Note, methylene chloride boiling point is
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37 °C, however this is at atmospheric pressure. Thus you can use 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 identity of different peaks inject methylene chloride (as a blank), and if needed also benzene solution. The elution of later eluting analytes (alkanes) 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. Evaluate the benefits of different splitless times, with respect to peaks’ shape, area, peak width and retention time.

4.5. Optimization of temperature program

The aim of temperature program optimization is a fast high sensitivity analysis with all peaks resolved. Thus, modify the program used in the section 4.4. to achieve faster elution and separation of all compounds ensuring also high response. In experimental section report all the temperature programs evaluated. In result section discuss contribution of varied parameter to the separation.

4.6. Evaluation of repeatability

Inject the mixture of analytes at least three times 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 ~ 10% for majority of peaks. Ensure correct integration of all peaks. Discuss your results.

5. Report

For the class, the report should be prepared as a manuscript following the J. Chromatography Authors' guideline. For the training purposes (outside of the class) it is sufficient to report all data in a form of Excel spreadsheets and a text reporting conclusions and also a series of questions listed at the end of the section 5.1.

It is suggested to use one of the printed articles as a reference (http://www.elsevier.com/wps/find/journaldescription.cws_home/502688/authorinstruct) However, the following exceptions must be applied. The document has to be submitted written in MS Word. The Tables, Figures, should be provided in the MS Excel or Power Point files, respectively. Both Tables and Figures should be properly referenced in the text and have captions. The Excel file must show all optimization results including the calculations. The text may not be longer than 3000 words excluding the references and abstract. The references must be based on ACS style and other references besides this lab report guideline should be used. The example of file labeling is "AK_102511_GClab_a" indicating your initials (AK), date when the lab was performed (102511), and"MSlab_a or b" indicating 1st and 2nd submission, respectively. Similar labeling needs to be used for the MS Excel file.
5.1. Grading

Timely delivery of the report by required deadline is required (5 pt); every day of delay will result in the deduction of 1 point. After receiving their grade, student will have exactly 1-week for resubmission of corrected report.

The proofreading and language expressions will be for 4 points. The distribution of the rest of points (40) is listed within the report requirements below. Students must prepare reports individually including the mass spectra information!!

Grading will be based on the following items

a) Lab preparation, performance, notebook (4 pt).

b) Proofreading and language expressions (4 pt).

c) Following the J. Chromatography guideline with respect to the nomenclature and organization of the manuscript (4 pt).

d) Content of individual sections required in the guideline for J. Chromatography and exceptions specified above (30 pt).

The main sections should include:

- Abstract (5 pt) including description of results
- Introduction (3 pt) = paragraph explaining importance of optimization of GC optimization particularly with regard to splitless injection
- Experimental (3 pt) = see any J. Chromatogr. paper as an example, the description of work performed in section 4.1 and 4.2 should be included in this section only as brief statement (1 sentence).
- Results and Discussion (12 pt) should address section 4.3. 4.4, 4.5 and 4.6, note the results needs to evaluated in this section.
- References in ACS format (2 pt) = should be used in the introduction
- Tables and/or Figures (5 pt) = should be provided In MS Excel and Power Point files. They should follow guideline of J. Chromatography with clear labeling, using proper units and captions.

e) Provide answers to questions below ( 3 pt)

The last section of report should address the following questions.

1) Why do you use a 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 tan methyl chloride and why?
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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?

11) When performing routine work on real samples, why the analysis should not be stopped prematurely even though all analytes eluted.

12) Why it may be useful to know in which order were the analyses performed?