

## **Chromatography/Mass Spectrometric Lab – Assignment II room 318 Chem 543**

### **1. Introduction**

GC/MS is a technique used for the identification and quantification of numerous compounds. The qualitative character of analysis is given by the retention time and the mass spectra. Quantitative analysis in a total ion current mode (TIC) can be as in a traditional GC analysis based on the total peak area, or if more accuracy is required on the peak area of a specific ion. Depending which type of a mass analyzer is used (e.g., quadrupole, ion trap), the selected ion monitoring (SIM) can enhance the sensitivity. In addition to the instrumentation, quantification is also dependent on the type of the calibration used. Various calibration methods such as external standard calibration, internal standard calibration, and method of standard addition may be performed. Today the most common in the gas chromatography is the internal standard calibration.

The aim of this lab assignment is to gain experience in the internal standard calibration, including the setup of GC/MS for TIC and SIM analysis, quantification using a series of standards, obtaining calibration curves, determination of the limit of quantification (LOQ), and the limit of detection (LOD).

### **2. Instrumentation**

The experiments will be performed on the HP5890 Series II system equipped with split/splitless injector and a mass spectrometric detector (HP 5972) with electron ionization source, quadrupole analyzer, and HP 6890 autosampler. The analyses will be performed on a 60 m long ZB-5 (5% phenyldimethylpolysiloxane) capillary column with 0.25 mm internal diameter and 0.25  $\mu\text{m}$  film thickness with a 10 m build in guard column.

### **3. Materials**

The concentrated mixture of benzene, phenanthrene, and tricosane (500  $\mu\text{g}/\text{mL}$  each) prepared within the chromatography lab I, and methyl myristate 3  $\text{mg}/\text{mL}$ , used as an internal standard (IS).

Each student will also receive 9 vials (2 mL), methylene chloride, syringes (100  $\mu\text{L}$  and 1 mL) for the solution preparation, and pasteur pipettes to ensure sufficient amount of solvent wash in the autosampler. At the end of the lab assignment, each student is responsible for returning stock solutions and cleaned syringes. All disposable materials must be discarded, and glassware washed with acetone and methylene chloride. The samples should be disposed of in the properly labeled waste bottle.

### **4. Instructions**

**The lab assignment is performed on the individual basis. Students have to sign up to ensure availability of the instrument.**

NOTE! Students can perform this assignment using their own analytes, but need to get approval from the instructor.

For this lab assignment you will:

- 1) Prepare solutions for internal standard calibration.

- 2) Modify GC method for TIC analysis and perform the analysis.
- 3) Based on the retention times and mass spectra, generate SIM method, and analyze the standards and the sample.
- 4) Develop calibration curves using selected ions in TIC and SIM analysis.
- 5) Determine LOD, LOQ and content of analytes in the unknown sample.

#### **4.1. Preparation of solutions**

**Calibration solutions.** The easiest way of the internal standard calibration method is to prepare standards by a serial dilution, then to add IS to each solution ( using the same volume ratios), and add IS to the sample (in the same ratio).

If your solution is 500 µg/mL stock you can prepare five solutions of 5x fold dilutions (e.g., 200 µL of mixture + 800 µL of solvent). Thus you obtain 5 calibration standards of 800 µL (from the lowest concentration, you have to remove 200 µL). To each of those standards add 4 µL of IS.

**Other solutions.** Take sample, prepare 800 µL of 10x diluted sample in methylene chloride add 4 µL of IS. Perform the sample preparation in triplicate. Prepare one vial with methylene chloride only.

#### **4.2. TIC and SIM method setup and method review**

In the logbook (with GC/MS) record your name, date, the pressure in the He gas cylinder, look at the last autotune report, and to determine if there was a leak. If GC/MS software is running (if not turn it on by clicking the instrument ion “MS Top 1”) click the “method/load CH543.mth” to upload the method.

Edit the method by clicking “method/edit entire method,” confirm each window by “OK.” Use atune.u as a tune file, scan: **TIC**, make sure you have a correct mass range for your analysis (MS scan parameters – mass range of low and high m/z) to detect all your compounds.

Record temperatures of injector and detector, temperature program, the pressure on the injector (B), split/splitless program, and how much sample is being injected. Save the new method using the same file name and your initials (max 8 characters).

You can prepare **method SIM** method after you have finished analysis in TIC mode, and know the retention times and mass spectra of your compounds. To modify the method (edit method):

- Select SIM.
- In the group ID write in the name of a compound you want to monitor.
- Keep dwell time at 100 ms.
- Set the start time to the time before the retention time of the compound.
- Type in two m/z ions you want to monitor.
- Continue with next group ID, tr, and m/z values
- Save the method using original file name, your initials, and “S”.

#### **4.3. Setup of sequence & RUN**

To run samples using autosampler you need to prepare a sequence. Click “*Sequence/Sample log table*” to insert samples you will be analyzing.

Start with blank and continue with standards from lowest to highest concentration. To prevent cross contamination, inject blank again, and then add your samples. You need to specify sample name, file name (your filename should always start with your initials), name of the method, and vial position on the carousel. You can remove rows in the table by “cut” and add rows by “repeat.” Save “*Sequence/save*,” name your sequence by YY-MMDD.

Before running the sequence, **make sure the vials with wash solvents on the autosampler are filled with methylene chloride.**

To run sequence, go to “*sequence/run*”, in the data file directory specify C:\HPCHEM\1\DATA\CH543\, click off overwrite existing datafiles, and hit run data sequence. The autosampler should start injecting. Make sure whether sample is injected and after the first run look at the analysis.

#### **4.4. Data Processing**

On the desktop click the icon “MS data analysis 1.” You can view already acquired data during the sequence. To view the data file, go to “File/load data file”, find directory to which you have saved the data, and open the file. If you want to view the current “run” go to “File/Take snapshot.”

##### **Commands to review the data**

**Go back to original view** = left double click

**Extract ions** = “Chromatogram/extract ion chromatogram” type in m/z

**Integrate** = “Chromatogram/Integrate or autointegrate”

Integration Parameters = “Chromatogram/Integrate Parameters”

**Integrate manually** = “options/manual integration”, then you can integrate by right key on mouse (click and drag)

**Integrate smaller peaks** = “Chromatogram/Integration Parameters” change threshold to lower number ENTER “Chromatogram/Integrate”

**Integration report** = “Chromatogram/Integration results”

**Magnify** = Left click, drag and view

**Mass spectra of the peak** = Right double click (if you cannot get it go to Tools/options and make sure the manual integration is off)

**Search spectra in the library** = Right double click on the spectra

**Select library** = “Spectrum/select library” type in “?” select NIST98.L

**Subtract spectra** = right double click on the peak, right double click on the background “Spectrum/subtract”

**View extracted ions overlay (on/off)** = “options/overlay graph”

##### **Processing analysis data**

For the data processing use commands listed above.

To generate a calibration curve, you need to get the area of each peak. Higher accuracy and sensitivity is achieved when processing m/z ions instead of the total area. Thus, select the m/z ions you want to quantify for each compound, and extract them. You can integrate your peaks manually or automatically. For automatic integration (if you don’t get correct integration) you need to set integration parameters – mainly threshold. For manual integration, you need to switch

to manual integration mode. For integration results, click Integration report, and note the areas and retention times in your notebook, so you can later process it.

Using the areas and concentrations, generate calibration curve for SIM and TIC analysis.

Determine LOD and LOQ (see lecture notes) for SIM and TIC analysis.

Calculate concentration of analytes in your sample and determine reproducibility of your analysis for SIM and TIC analysis.

## 5. Report

You will be evaluated on the basis (1) of capability to deal with assigned problems (10 points); (2) of your report (in electronic form) (40 points), consisting of written and spreadsheet sections (20 points each).

The report should include the date the experiments were performed, and name of the person performing experiments. The report should include

- 1) Description of the method conditions used for the analysis, including SIM and TIC settings. This should be written in the manner of experimental section in Journal of Chromatography.
- 2) Description of approach used for determination of LODs and LOQs.
- 3) Report calibration curves and LODs, LOQs for SIM and TIC analyses of individual compounds, include Excel spreadsheets showing how this was calculated.
- 4) Be sure to address the following topics:
  - a) Compare the mass spectra of different compounds. Is there difference in fragmentation? What is the base peak?
  - b) Why do we use the IS method, and did it affect your measurements? If you left your samples for 1 month and reanalyzed them, would there be any advantage for the use of the IS method.
  - c) Explain reasoning for the selection of ions for quantification in TIC and SIM?
  - d) Explain the difference between TIC and SIM with respect to LOD and LOQ.
  - e) Include graphical version of calibration curves.
  - f) What is the purpose of the wash solvent in the vials (4 mL) on the autosampler.