GAS CHROMATOGRAPHY

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Basic principles

Gas chromatography is a type of chromatography used for separating and analyzing gases, liquids and solid compounds that can be vaporized at temperatures lower than approx. 400 °C. Compounds are separated as they are distributed between two phases: a **stationary phase** and a **mobile phase** (or „moving phase"). The mobile phase is formed by a **carrier gas** and the stationary phase is placed in a chromatographic column.

There are two main types of columns for gas chromatography: packed columns and capillary columns. In packed columns, stationary phase is a solid material (e.g., activated charcoal, silica, aluminum oxide, polymeric sorbents) or a microscopic layer of high-boiling liquid on an inert solid support. In capillary columns, a thin film of stationary phase coats the inner wall of a fussed-silica capillary.

The separation of analytes is based on the following principle: The sample is vaporized in a heated chamber **(injector)** and further introduced into the head of the separation column together with a continuous flow of carrier gas. When passing through the column, the gaseous analytes interact with the stationary phase ("like-dissolves-like" rule). The strength of the interaction for each compound is expressed in the form of a **distribution constant** $K_D = c_s/c_m$, where c_s and c_m are the equilibrium concentrations of a component in/on the stationary phase and in the mobile phase, respectively. The higher distribution constant means the stronger interaction with the stationary phase and longer time it takes for the compound to migrate through the column (longer retention time). The analytes then enter the detector that measures the immediate concentration of the separated analytes in the carrier gas. Detector signal is registered and data is presented as a graph of the detector response (y-axis) against retention time (x-axis), such dependence is called a **chromatogram**.

Many compounds can be separated in one chromatographic run they elute in the form of chromatographic **peaks**. Retention time can be used to identify analytes if the method conditions are constant. The peak height and peak area are proportional to the amount of the analyte present in thesample. The ideal chromatographic peak is a symmetrical Gaussian profile defined by three main parameters: retention time t_R, peak height h and peak width at baseline w_d or at half maximum w_h (Fig. 1).

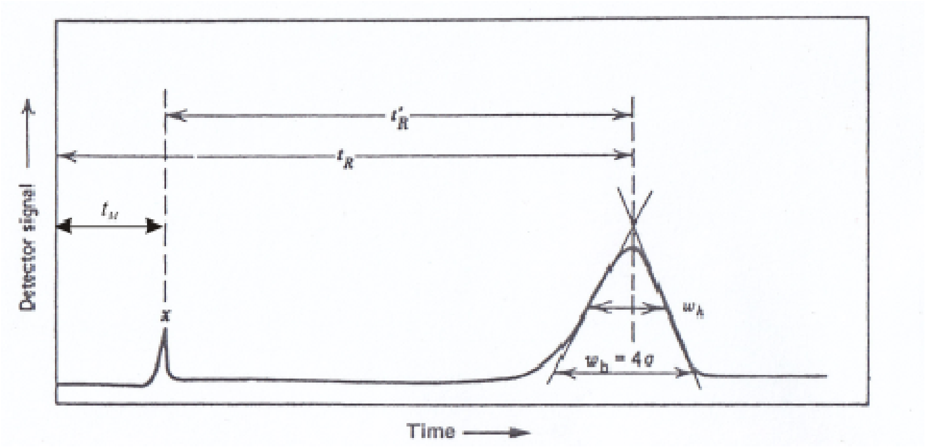

Fig.1. Retention parameters of a chromatographic peak

Retention time t_R [min] is a measure of the time needed for the compound to pass through a column, i.e. since injection to a peak maximum in a detector.

Retention volume V_R [ml] is the volume of the carrier gas that passed through the column while eluting a component with retention time t_R and with the carrier gas flow of F_m [ml min^{-1}]:

$$V_R = F_m t_R \tag{1}$$

Dead retention parameters are the retention parameters of a compound that is not retained under given conditions, i.e. its distribution constant $K_D = 0$. Those parameters are:

t_M - dead retention time (also known as *void time* or *holdup time*)
V_M - dead retention volume

Methane is commonly used as a non-retained compound in gas chromatography.
The calculation is identical to retention parameters of any other compound.

Adjusted retention parameters (also *net retention parameters*) t'_R, V'_R, represent the time for which a particular analyte is retained on the stationary phase, and are expressed as the difference between the retention time and the dead time:

$$t'_R = t_R - t_M \tag{2}$$
$$V'_R = V_R - V_M \tag{3}$$

Column efficiency

Column efficiency is a measure of the peak dispersion. Narrow peaks take up less space in the chromatogram, which means more peaks can be separated. Efficiency is usually explained using the concept of theoretical plates:

Number of theoretical plates n is commonly calculated using equation:

$$n = 5.545\ (t_R/w_h)^2 \tag{4}$$

where t_R [min] is retention time, w_h [min] is peak width at half height.

Height equivalent to a theoretical plate (*plate height*) H [cm] can be calculated using column length L [cm]:

$$H = \frac{L}{n} \tag{5}$$

Qualitative analysis

In gas chromatography, an unknown compound can be identified by comparing its t_R (or V_R) to a standard, but in such case, all chromatographic runs have to be performed under identical conditions on the same GC system. Otherwise, a correct comparison is not possible. For this reason, universal characteristics have been established. These can be used regardless of the used conditions.

Retention index I is used to convert retention times into system-dependent constants allowing for comparing values measured by different laboratories under varying conditions. Contrary to retention time or retention volume, the retention index I is dimensionless, and can be used to identify peaks by comparing obtained values with the tabulated data. The identification method interpolates peaks between bracketing standards: the *n*-alkanes are used for this purpose. The index I of n-alkane is equal to its carbon number times 100 (i.e., for *n*-alkane with z carbon atoms $I_z = 100z$). The index calculation is based on the application of logarithms of adjusted retention times because there is a linear relationship between the logarithms of the retention times and the carbon number of the components within a homologous series. The retention index I is given by the equation:

$$I_x = 100z + 100\frac{\log t'_x - \log t'_z}{\log t'_{z+1} - \log t'_z} \tag{6}$$

where t'_x, t'_z, t'_{z+1} stand for the adjusted retention times of an unknown compound x; z is the number of carbon atoms of the n-alkane eluting before, and z+1 is the number of atoms of the n-alkane eluting after the peak of interest. Ideally $t'_z < t'_x < t'_{z+1}$, with as close retention times as possible. If the index is calculated for an unknown sample, it can be compared to the tabulated values in order to identify the compound. However, if the retention indices of two unknown compounds yield in similar values, the compounds cannot be identified unequivocally.

Unambiguous identification or verification of the identity can be carried out by coupling gas chromatography with a suitable spectroscopic detector. The best results can be obtained by mass spectrometer as a detector (MSD). For each peak in the chromatogram, mass spectrometer provides a **mass spectrum**. If highly energetic electrons are used for ionization (electron ionization, EI), the analyzed compounds are extensively fragmented. The spectrum of the fragments, together with the molecular ion ($M^{+\cdot}$), can be compared to the spectral libraries and the individual compounds can be usually reliably identified.

Quantitative analysis

The amount of the separated analyte is measured by a detector. The most commonly used detectors measure the immediate concentration of the analyte eluting from a column. A peak is obtained, and the area under the peak is proportional to the amount of the analyte present. Such relationship is linear within a certain concentration range. The peak area is calculated by its integration – a feature commonly performed by a chromatographic software.

Several methods can be used for quantification: e.g., standard addition method, external standard method, or internal standard method. The **internal standard method** is based on the addition of a known amount of a carefully selected substance – **internal standard (IS)** – to the sample. The internal standard should behave similarly as the analytes but provide a signal that can be clearly distinguished from that of the analytes. In the ideal case, factors that affect the analyte will affect the internal standard to the same degree. One very practical benefit of the application of IS for quantification stems in the fact that there is no need for injecting precise volumes of the sample because any imprecision in sample volume injection will be compensated for. This is because for the quantitative calculations are based on ratios of peak areas and not on their absolute values (see below).

The relationship between peak area and the amount (weight, concentration) of an analyte can be described as follows:

$$A_x = k_x * m_x \tag{7}$$

where A_x is the peak area of the analyte x, k_x is a constant of proportionality (slope of the linear function) for the compound x, and m_x is the amount of the compound x.

Similarly, for the internal standard:

$$A_S = k_S * m_S \tag{8}$$

where A_S is the peak area of the IS, k_S is a constant of proportionality (slope of the linear function) for the IS, and m_S is the amount of the IS.

The peak area ratio equals to:

$$A_S/A_X = k_S * m_S/k_X * m_X = f * (m_S/m_x) \tag{9}$$

In practice, a calibration standard sample with known amounts of both the analyte and the internal standards is analyzed, and this allows for the k_S and k_x ratio (k_S/k_x) to be calculated. This value is known as the **response factor *f***. Then a separate solution with an unknown amount of analyte and a known amount of IS is injected. The response factor is applied to the data from the second solution, and by knowing the amount of IS in the sample, the unknown amount of the analyte can be found. The result can be expressed as mass percentage of the analyte (*wt%*).

Gas chromatograph

Fig. 2 shows a diagram of a gas chromatograph. The carrier gas flows from a gas cylinder or a gas generator (1) through a proportionating valve. Flow and pressure are controlled by regulators (2 and 3) and the gas enters an injector (4), where the sample is injected by an injection syringe. The injector is heated at a chosen temperature, which allows to evaporate the injected sample without its thermal decomposition. The temperatures of 150-350 °C are commonly used. The evaporated sample is transported by the carrier gas to a thermostatted column (6 and 7). Here, the analytes are separated based on the principle described above, and then enter the detector (5). The signal is collected (8) and registered by a data processing unit (9 and 10).

Compared to the packed columns, the capacity of the capillary columns in capillary gas chromatography is reduced. To avoid column overloading, **a split injector** is used for the sample injection. In the split injector, the vapor is split into a small portion that is transferred to the column and a large portion that is vented from the system via the split opening. The flow ratio between the two portions is called the **split ratio** and is chosen based on the capacity of the column. In this way, even small volumes of sample can be precisely measured and injected.

The **flame ionization detector (FID)** is one of the most widely used detectors in gas chromatography. It is suitable for a wide range of organic compounds and a wide range of concentrations. The operation of the FID is based on the detection of ions formed during combustion in a hydrogen flame. The generation of these ions is proportional to the concentration of organic species in the gas stream.

The flame ionization detector consists of a flame chamber at the end of a chromatographic column, air inlet, an inert „make-up“ gas (nitrogen) and a fuel gas (hydrogen). These two gases are mixed and enter the flame chamber by a nozzle, the air is then added as an oxidant, and the resulting flame serves to ionize any gas molecule that is not carrier gas. The charged particles of the organic sample produce a measurable current flow in the gap between two electrodes in the detector, while the current of the pure carrier gas or fuel gas is negligible. This current differential provides information about the sample. The resulting signal depends on the composition of the separated sample.

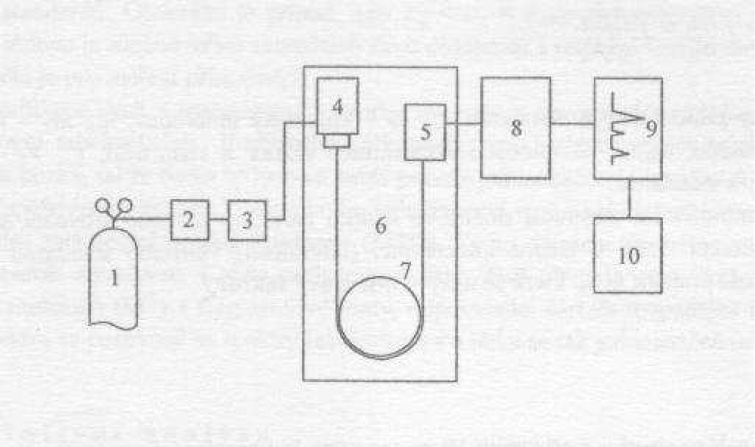

Fig. 2. Diagram of a gas chromatograph

1 – gas cylinder or gas generator as a source of the carrier gas; 2, 3 – flow and pressure regulators; 4 - injector; 5 - detector; 6 - thermostat; 7 - column; 8 – signal attenuator; 9+10 – data processing unit with a display.

Working instructions:

Gas chromatography analysis of a sample mixture

Tasks

1. In sample A, evaluate the retention indices *I* and identify the compounds in the mixture.
2. In sample B, calculate the mass percentage (*wt%*) of *p*-xylene in a mixture of aromatic hydrocarbons.
3. Calculate the number of theoretical plates *n* and the plate height *H* for *n*-nonane.
4. Evaluate the results.

Instrumentation

Gas chromatographs Scion 436-GC with flame ionization detector and capillary columns are used in the laboratory. The data processing unit operates with SW Compass CDS, which processes the signal from the chromatograph and allows to save and store the acquisition methods used during the measurements.

Column

The chromatographs are equipped with fused-silica capillary columns Scion - 1 MS, with a non-polar stationary phase, a column length is of 15 m, an inner diameter of 250 μm and a 0.25 μm stationary phase thickness.

Gas chromatograph and its use:

Instrument description

The diagram of the gas chromatograph Scion 436-GC is shown in Fig. 3. The power switch (1) is placed in the lower left corner of the front side of the instrument. Carrier gas flow and pressure are fully regulated by electronic regulators, and values can be set on the computer during method setup. The injector and the detector are both found in the upper part of the instrument. The instrument settings can be controlled by a touch display (3) or a computer (4). The computer is equipped by SW Compass CDS, which enables to both control the instrument and process the acquired data.

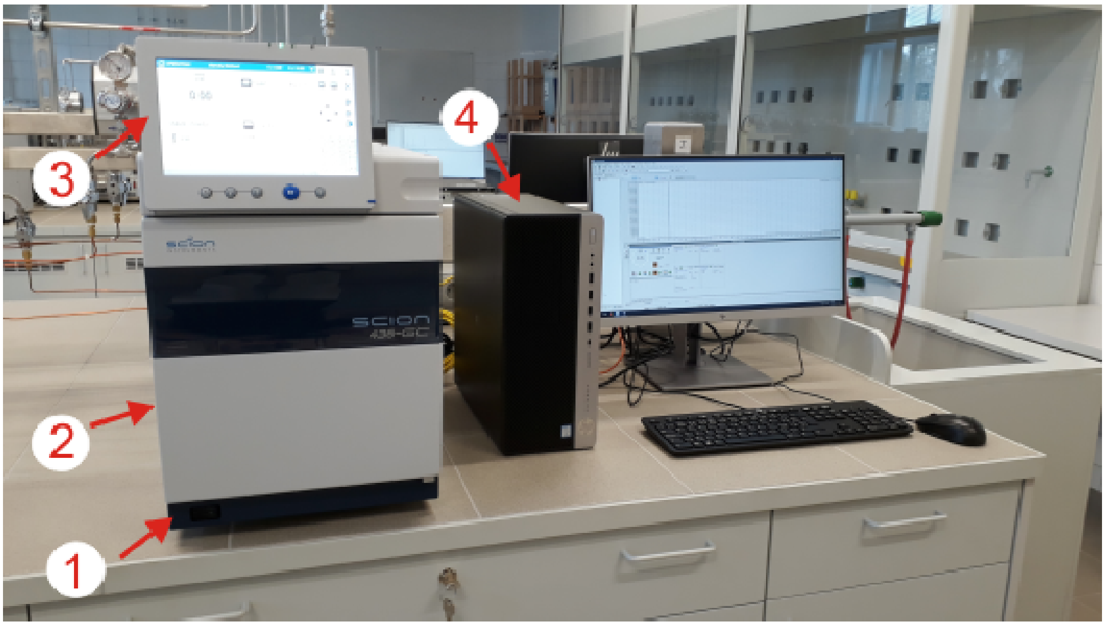

Fig. 3. Gas chromatograph Scion 436-GC

1 – Main power switch; 2 -Gas chromatograph; 3 – Touch display; 4 - PC

Setting up the instrument

Before the analysis, the instrument needs to be equilibrated. As this is a time-consuming process, the instrument setup will be performed by the laboratory assistant beforehand. Thus, gas temperature and flow will be set to appropriate values. The **Startup-FID** method will be running, the detector flame will be ignited and the temperature of the injector, column, and the detector will be equilibrated.

Injection of a liquid sample – a general procedure

Liquid samples (1 µL) are injected by a Hamilton syringe (with a volume of 10 µL). First, wash the syringe thoroughly by the injected liquid. Next, use the syringe to draw 1 µl of the injected liquid. Do not clean or wipe the end of the needle because the drawn liquid could be absorbed. Carefully (but quickly) insert the needle in the silicone septum of the injector, push the needle as far inside as possible, inject the liquid from the syringe and remove the syringe from the septum.

Work tasks:

1. Task 1 – Evaluation of retention indices *I* and identification of compounds in sample A

a) Review of the method parameters: **GC_Method_1**

All required instrument settings are saved in the acquisition method (GC_Method_1): It contains injector temperature, injector split ratio, column temperature, temperature gradient, detector settings, etc.

In the upper left corner of the menu bar, select *File* - *Open* - *Open Method* and select a method called **GC_Method_1.meth**. A number of menu items will appear in the lower left section, select the first - *control*. A GC instrument diagram will appear on the main screen. To display different parts of the method (acquisition parameters, integration parameters, etc.), click on the corresponding item in the diagram. The following parameters have to be set before the analysis:

On the injector section, check *Heater (On)*, set the *Set point* temperature of **200 ºC**, on the *Split state* check **ON**, and set the *Split ratio* to **100**. This indicates the sample will be split between the column and the waste in the ratio 1:100.

Next, select *Oven,* check the *Heater (On)*, and set the following parameters of the temperature gradient: set the initial column temperature to **40 ºC**, in the *Time* column insert **5**, in the *Rate* section (second row) insert **7**, in the *Temperature* column insert **80**, and in the *Time* column insert **0**. These parameters indicate that the initial column temperature (in the time of the injection) will be 40 ºC, this temperature will keep constant for 5 min, then it will be gradually raised by 7 °C per min, and when it reaches 80 °C, it will drop back to the initial temperature of 40 °C.

Next, select *Column pneumatic*, make sure *Enabled (ON)* is checked, *Constant flow* is selected, and the flow rate is set at **1 mL min^{-1}**. In addition, make sure *Pressure pulse* is unchecked. This indicates that the pressure and gas flow will be automatically regulated, and the gas flow will be 1 mL min^{-1} regardless of the column pressure. Pressure pulse during sample injection will be turned off.

Next, open the *Detectors* section and make sure the *Heater (On)* is checked, *Setpoint* is set at **200 ºC**, the *Electronics (On)* is checked and the *Time constant* is set as ***Slow***. Furthermore, in the *Range* column of the table (first row) insert number **12** and for the *Autozero* set **Yes**. The number 12 indicates a maximum sensitivity of the detector. The adjustment of the baseline (autozero) will be performed during sample injection.

When all parameters are set up, save the method by clicking on *File – Save - Save Method.*

In the upper section of the screen click on *Acquisition* and select *Quick Start*. A list of methods will pop up. Select *GC_Method_1.meth*. In the new screen, type in the name of the chromatogram (*Run Name)*.

Chromatogram names are generated according to the following pattern:
date _ student's initials _ sample name _ run number

Foer **sample names** use the following abbreviations:

Solution of *n*-alkanes (hexane, heptane, octane, nonane in pentane) => **STD**

For the sample A for qualitative analysis
AX (where X is the number of sample A) => **AX**

The mixture of *n*-alkanes and the sample AX => **STDAX**

Examples of chromatogram names:

Example 1
On **10.2.**2020, student **J**on **S**now injected a **standard** of *n*-alkanes. It was the **first** run of the standard.
The chromatogram will be named (following the above description):
1002_JS_STD_1

Example 2
On **11.2.**2020, student **M**ary **P**oppins re-injected the **sample A2**. Thus, it was the **second** injection of the sample.
The chromatogram will be named:
1102_MP_A2_2

Important: Do not forget to include the run number at the end of the chromatogram name. In some cases, the runs have to be repeated, and correct numbering will prevent confusion.

b) Sample injection

The samples can be injected once the LED light on the GC instrument display turns green.

Perform the following runs:

i. Analyze 1 μL of *n*-alkane mixture (1% mixture of hexane, heptane, octane, and nonane in pentane).
ii. Analyze 1 μL of sample A, which contains 4 unknown compounds dissolved in pentane.
iii. Analyze 1 μL of a mixture of *n*-alkanes and sample A. The mixture is prepared right in a Hamilton syringe by drawing 0.5 μL of *n*-alkane mixture and 0.5 μL of sample A, and it is injected directly from the syringe.

The analysis of *n*-alkane mixture (i.) and sample A (ii.) can be performed only **once**, the analysis of a mixture of *n*-alkanes and sample A (iii.) is performed at least **twice**.

2. Evaluation of results of Task 1

First, evaluate the retention times of all peaks, including *n*-alkanes, sample peaks, and even a solvent peak. This can be done following these steps: In the lower left section click on

Data. Then click on *File* - *Open* - *Open Chromatogram* and from the list of acquired data, select the corresponding chromatogram. In the upper left section under the chromatogram name, click on *results*. A table with retention times, peak areas (needed for quantification in Task 2), and peak widths at half height (needed for the calculation of theoretical plates) will appear. Note down the required values and include them in the final report.

Compare the chromatograms of the *n*-alkane solution (i.) with the sole sample A (ii.) and finally with the mixture of *n*-alkanes + sample A (iii.). Decide, which peaks in the sample iii. correspond to an *n*-alkane (hexane, heptane, octane, nonane) and which peaks are unknown compounds of the sample A. Then, calculate the retention indices I. The dead retention time t_M is the time of the first (smaller) peak preceding the largest (i.e. solvent) peak in the chromatogram. Compare the calculated retention indices to the tabulated values (available in the laboratory) and identify the compounds. Confirm your estimate by comparing the mass spectra of your compounds to a MS spectral library (available in the laboratory).

3. Task 2 – Determination of the mass percentage (*wt%*) of *p*-xylene in a mixture of aromatic hydrocarbons (sample B)

For this task, the above-mentioned acquisition method needs to be slightly adjusted: Open the method **GC_Method_1**, in the section *Oven* set the initial temperature of **45 °C**, in the *Time* column insert **2**, in the *Rate* section (second row) insert **7**, and in the *Time* column insert **0**. **Save the adjusted method by clicking on *File* - *Save* - *Save Method*.** In this setting, the column temperature will hold constant for 2 min after the injection, then it will rise by 7° C per min, and when it reaches 80 °C, it will drop back to the initial temperature of 45 °C.

The calculation will be performed by the internal standard (IS) method, with toluene as the IS. Peak areas will be used for the calculation.

a) Preparation of a calibration solution (iv.)

First, weigh a dry vial, then use a dedidated syringe to add approx. 20 µL of p-xylene, and weigh the vial again. In the same way, add approx. 20 µL of toluene (IS) (by a dedicated syringe) and weigh the vial one more time. Use a pipette to add 1 mL of hexane to the mixture, close the vial and mix the solution by shaking. The resulting calibration solution contains the m_{x1} g of p-xylene and m_{S1} g of toluene in 1 mL volume. This solution can be used to calculate the response factor f.

b) Preparation of a sample solution B with added IS (v.)

First, weigh a dry vial with a label B1-8, then use a dedicated syringe to add approx. 20 µL of a concentrated mixture of aromatic hydrocarbons and weigh the vial again. In the same way, add approx. 20 µL of toluene (IS) and weigh the vial one more time. Use a pipette to add 1 mL of hexane to the mixture, close the vial and mix the solution by shaking. The resulting sample solution contains m_{vz} g of sample and m_{S2} g of toluene in 1 mL volume.

Perform the following runs:

iv. Analyze 1 μL of the calibration solution
v. Analyze 1 μL of the sample solution B

Chromatogram names are formed in the same manner as in Task 1, using the following abbreviations:

Calibration solution	=>	**KALX**
Sample B used for quantification	=>	**BX**

Perform the analysis of both calibrator (iv.) and the sample B (v.) at least **twice**. Use the first run to correctly identify the peaks of *p*-xylene and toluene (IS). Note there will be more than two peaks in the sample B (as the sample contains several compounds nit only *p*-xylene and IS).

After the runs are finished, clean the column. For this purpose, use 1 μL of hexane and a pre-defined method named **GC_Method_2_cleaning.meth**. This method includes a temperature gradient of 50-200 °C with rate of 20 °C min^{-1}. First, select the section *Acquisition* and in the *Method name,* select *GC_Method_2_cleaning.meth* and click OK. Once the LED light on the GC display turns green, inject 1 μL of hexane. The resulting chromatogram is not evaluated and the instrumental part is thus finished. **Do not turn the instrument off, it will be switched off by a laboratory assistant or a technician.**

4. Evaluation of results of Task 2

In the chromatogram of the calibration solution (iv.), evaluate the peak areas of toluene (A_{S1}) and *p*-xylene (A_{x1}). Using the known amounts (weights) of toluene and *p*-xylene, calculate the response factor *f* for each individual run, and then calculate the average.

When the peaks of toluene and *p*-xylene in sample B (v.) are correctly identified, evaluate their corresponding peak areas (A_{S2} and A_{x2}). By using the response factor *f* and the weight of the IS added to the sample (m_{S2}), calculate the weight of the *p*-xylene (m_{x2}) in the sample. Then by using the weight of the sample added to the solution (m_{vz}), calculate the weight percentage (*wt%*) of p-xylene in the sample. Calculate the value for each individual run and present the result as an average from the (at least) two runs.

5. Evaluation of column efficiency, expressed as plate count and plate height

Use the chromatogram of the *n*-alkanes (i.) obtained in Task 1. Find the retention time t_R and the peak width at half height w_h (in the software found as *width 50%*) for *n*-nonane. Calculate the number of theoretical plates *n* using the equation (4) and plate height *H* using the equation (5). Calculate average values obtained from at least two measurements and present them in the final report.

Self-assessment questions

1. What are the stationary and mobile phases in gas chromatography?
2. Name the main parts of a gas chromatograph.
3. Describe the operating principle of a flame ionization detector (FID).
4. Which compounds can be detected by FID?
5. What is a chromatogram?
6. Which parameters define a chromatographic peak?
7. Define retention time and retention volume.
8. What is the relationship between retention time and retention volume?
9. Define reduced retention parameters.
10. What is the dead retention time and which compound is used for its calculation?
11. Which parameters define the column efficiency?
12. Which parameter found directly in the chromatogram can be used for compound identification?
13. Which compounds are used as standards for the calculation of retention indices *I*?
14. How do you quantify a compound in a sample?