INSTITUT NATIONAL
DES SCIENCES
APPLIQUÉES
ROUEN NORMANDIE

TRAVAUX PRATIQUES DE CHIMIE ANALYTIQUE
CFI3 Semestre 6
EC TP ANALYSE

Année 2021-22

Contenu

ORGANISATION DES TRAVAUX PRATIQUES

Les travaux pratiques ont une durée de 5h. Ils sont organisés sous forme tournante. Les élèves doivent consulter la fiche de rotation avant le début des séances pour savoir quel TP préparer La présence est obligatoire dès le début du TP. Les retards seront sanctionnés.

I. Sécurité

Avant chaque TP, les élèves doivent avoir pris connaissance des risques liés à l'utilisation des produits chimiques en consultant leur fiche FDS sur internet et en remplissant la fiche de sécurité donnée à la fin du poly. Il en est de même, pour les risques liés à l'utilisation des appareils. Pour ce faire, ils peuvent consulter la fiche de risque de l'appareil qui est apposée près de celui-ci.

Cette fiche de sécurité doit être remise avec le compte rendu (elle fera partie de l'évaluation).

Les règles de sécurité inhérentes à un laboratoire de chimie doivent être respectées (Blouse, lunette, gants…).

II. Feuilles de résultats

Afin d'éviter toute falsification de résultats, les feuilles de résultats sorties par les appareils **doivent être remises avec votre compte rendu**.

Toute absence de ces feuilles conduira à une diminution de la note d'évaluation.

III. Déroulement des séances

Les élèves doivent avoir pris connaissance du TP avant de venir. Au cours de la séance, ils doivent s'avancer dans le traitement des résultats et dans l'élaboration du compte rendu :

- Les droites d'étalonnage ou autres courbes doivent être tracées pendant la séance (il y a des ordinateurs mais vous pouvez apporter un portable). Cela permettra de corriger immédiatement toute erreur de calibration.
- Les calculs des concentrations finales des échantillons doivent être effectués.
- Il est conseillé de débuter la rédaction du compte rendu lors des phases d'attente.

IV. Evaluation

La note d'évaluation de la séance de travaux pratiques est décomposée de la manière suivante :

- 2 points : Comportement en séance (ponctualité, autonomie, efficacité, exploitation immédiate des résultats (tracé des courbes, calculs…), propreté de la paillasse, travail d'équipe en présentiel
- 3 points : **entretien oral individuel** avec l'enseignant (10' lors des séances 5 ou 6) sur la compréhension des TP réalisés lors des séances précédentes
- 15 points : compte rendu

V. Qualités d'eau utilisées

Le laboratoire de chimie analytique utilise 2 types d'eau : l'eau ultrapure et l'eau désionisée. L'eau ultrapure est la plus pure mais elle coute aussi plus cher à produire.

Bien suivre les consignes des TP pour utiliser l'eau de façon optimale.

VI. Compte rendu

1 compte-rendu sur feuille réponse (TP 6) sera rendu en fin de séance ou au plus tard le lendemain.
Les autres comptes-rendus seront remis **15 jours** après la séance hors période d'examen où un délai peut être obtenu. Cependant, pour les séances placées tôt dans l'année où une partie du cours n'aurait pas été réalisée, vous pouvez le rendre lorsque le cours est terminé.

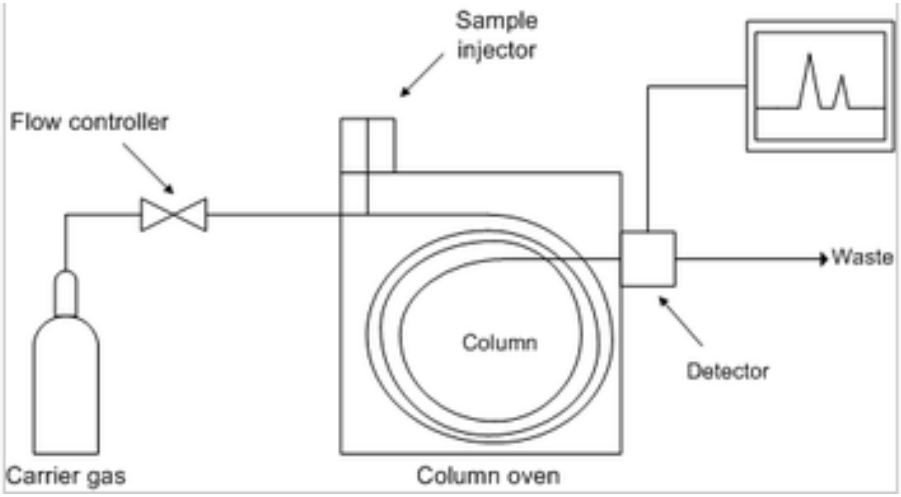
Les comptes rendus doivent comporter **au minimum** les éléments suivants :

- Une présentation succincte de la méthode et de l'appareil.
- La description de l'appareil et de ses réglages comme vous feriez en entreprise par exemple : Chromatographe Varian 3300 équipé d'une colonne capillaire de 30 m de long (phase stationnaire polyethyleneglycol), de diamètre interne 0.32 mm et d'épaisseur de film 0.25 µm. Injecteur en mode split (30 :1) à 250°C, détecteur FID à 250°C, débit de He à 1 mL/Min. Température de colonne…
- La réponse aux questions posées dans l'énoncé de TP
- Le tracé des droites d'étalonnage et leur évaluation d'un point de vue statistique (passage par l'origine, linéarité).
- Le cas échéant, l'étude de la répétabilité (assortie du calcul du coefficient de variation)
- Le calcul des concentrations dans les échantillons (pas seulement dans les solutions finales) par exemple : µg de mercure/kg de thon. Il faudra aussi calculer l'incertitude sur ces résultats. Idéalement, chaque détermination de solution inconnue doit être tripliquée (trois analyses).
- Une conclusion présentant l'intérêt du travail et ces résultats (éventuellement en comparant à des normes ou teneurs usuelles).

TP n°1: GAS CHROMATOGRAPHY - OPTIMIZATION OF A CHROMATOGRAPHIC SEPARATION

The goal of this work is to optimize the separation of methylcyclohexane and toluene by gas chromatography. We will see in this lab session the influence of flow and temperature in gas chromatographic separation. We will also see how to choose the injection volume, the split ratio and the attenuation of the detector.

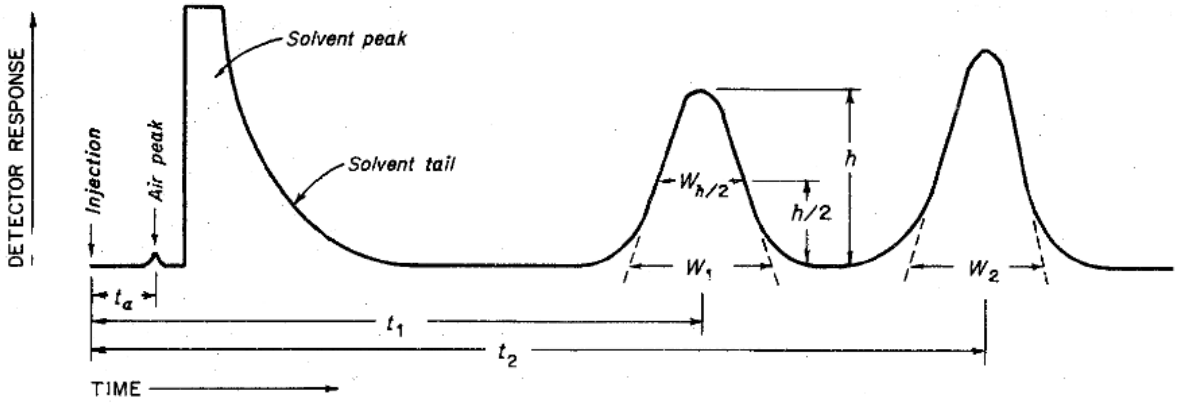
I. Principle of gas chromatography



Source Wikipedia

Gas phase chromatography is a separation method used in analytical chemistry. Volatile components of a mixture are separated by eluting them using a carrier gas (mobile phase) through a column (narrow tube coated with a stationary phase (polymer)). Compounds elute at different rates depending on their relative interaction with the stationary phase.

The liquid mixture is injected (using a micro syringe) in the injector's insert heated above the boiling point mixture. After fast volatilization, compounds are swept by the carrier gas (here Helium) through the column. Depending on their affinity with the stationary phase, they will have different rates of progression. Therefore, they will reach the end of the column at different times (retention time). A detector is used to monitor the outlet gas. The detector here is a flame ionization detector which gives a signal area proportional to the mass concentration of carbon for a family of organic compounds.



We will have to give first some definitions:

- The dead time t_0: it's the time a non-retained compound spends in the mobile phase from the injector to the detector.
- Retention time it's the time a retained compound takes to go from the injector to the detector.
- Half width $d_{1/2}$ (or $w_{1/2}$)

Other parameters can characterize peaks (you will see that in chromatography course) :

- Adjusted retention time t_r' : $t_r'=t_r-t_0$
- Retention factor $k=(t_r-t_0)/t_0$
- Effective plate number N_{eff} : $N_{eff}= 5.545(t_r'/w_{½})^2$
- This later parameter characterizes the efficiency of the separation. Increasing the plate number will give narrow peaks.
- The effective **H**eight **E**quivalent to a **T**heoretical **P**late H_{eff}: H_{eff}= column length/Neff. Low value of HETP will give better separation.
- - Effective retention volume V_r': $V_r'=t_r'.d$ (d : column flow)
- Other parameters can characterize separation between two peaks:
  - ✓ The selectivity α : $\alpha =k_2/k_1$

  A selectivity over one is a condition required to obtain a separation.
  - ✓ The resolution R: $R=1,177\ (t_{r2}-t_{r1})/(d_{½1}+d_{½2})$.

  We consider that peaks are separated if the resolution is above 1.5.

II. Optimization of the chromatographic method

Two parameters will be modified here to optimize the chromatographic separation:

- The carrier gas flow should be chosen to have the smallest HETP
- The oven temperature so that the analysis time is the smallest keeping resolution above 1.5.

During the development of a chromatographic method, others parameters must be checked:

- The expansion volume of the sample injected must be lower than the liner volume
- The split ratio must be chosen to avoid saturation of the column leading to a peak asymmetry
- The attenuation of the detector must be chosen to have a good signal to noise ratio and no flat top peaks.

III. Experimental part

We will use a chromatograph Clarus 580 with FID detector. (See manual in the lab). The column used presents a stationary phase ZB-624 (6 %-cyanopropylphenyl-94 %-dimethylpolysiloxane) diameter 0.32 mm, length 30 m, film thickness 1.8µm. Tmax=260°C.
The Temperature of the injector and detector are set to 200°C.

A solution with 20% of toluene, 20% of methylcyclohexane in cyclohexane is injected.

For the optimization part, the volume injected is **1µL**, the split ratio is 1/50 and attenuation of the detector is set to 6.

The liner used has the following dimensions : 6,2 x 92,1mm – ID 4mm.
Make some hypothesis to calculate the expansion volume of the liner and check it with the calculator on line (https://www.restek.com/fr/outils-et-calculateurs/outils/calculateur-d-expansion-de-solvant/).

For all chromatograms, you will need to determine the peak width at half height graphically, be as precise as possible.
An Excel sheet will calculate chromatographic parameters such as effective plate number N_{eff}, retention factor k, selectivity α, resolution R.

DON'T USE SPECIAL CHARACTERS FOR THE CREATION OF THE FOLDER OR THE NAME OF THE METHOD

1. Optimization of carrier gas flow:

At a fixed oven temperature (T=150°C), we will study influence of the carrier gas flow on the column efficiency by plotting HETP=f(flow) for flow=0.2/0.4/0.8/0.9/1/1.2/1.5/2/2.2ml/min.

The chromatograms have already been prepared. Measure half-widths. Fill the Excel sheet.

Interpret the results on the different graphs with your knowledge from the course. Does k and α depend on the volume entering the column? Suggest an explanation for the dispersion observed.

2. Optimization of oven temperature

2.a. Isothermal conditions

Using previous work, you have found optimal carrier gas flow. Set the flow to this value and modify temperature to have at minimum 5 values (from 120°C to 200 °C). Inject the sample at different temperatures, plot chromatograms and measure half-width. Fill the Excel sheet.
Interpret the results on the different graphs with your knowledge from the course.

Separation is optimal when peaks are well separated (R > 1.5) and analysis time is fast. Choose the best temperature.

2.b. Temperature gradient

Use the Restek software ProEZGC in order to try different gradients with an initial temperature of 150°C. Once you have found an optimal gradient, inject in order to compare the simulation and real chromatogram.
The 2 molecules we studied are present in complex mixtures such as kerosene that contain heavier molecules such as naphtalene. Would you use isothermal conditions or a gradient in this situation?

3. Injection and detection

What is the problem if the expansion volume is too high?

3.1 Split ratio

Change the split to 1/10. Calculate the mass of each solute injected, estimate* the asymmetry and conclude.

* The peaks may be saturated at 1/10

3.2 Attenuation

In the detector, the signal generated by the solute is amplified. If the signal is too high, the amplifier can no longer amplify it, resulting in a flat top peak.
Change attenuation of detector at “-2”. And conclude.

A complete report is expected for this work with an interpretation of the results linked to the chromatography course and a conclusion to the study will be made.

TP n° 2: STUDY OF A SEPARATION USING AN ION EXCHANGE RESIN - **SEPARATION BY ELUTION**

I. INTRODUCTION.

1. Characteristics of the resin

The stationary phase is a sulphonate cation exchanger resin: DOWEX 50W-X8 (copolymer styrene + divinylbenzene, on which $-SO_3H$ are grafted).

The exchangeable sites of the resin used are all initially occupied by H^+ ions.

Characteristics:

- granulometry: 50-100 mesh
- humidity: 54%
- capacity: to be determined (theoretically 5 meq / g of dry resin)

2. Separation by elution.

The work will consist in performing experimentally a separation by elution of Na^+ and K^+ cations. The column used for this purpose contains a known mass of resin, and HCl 1 mol L^{-1} elutes at a constant flow through the column.

Elution curves will be studied in order to calculate some characteristic constants.

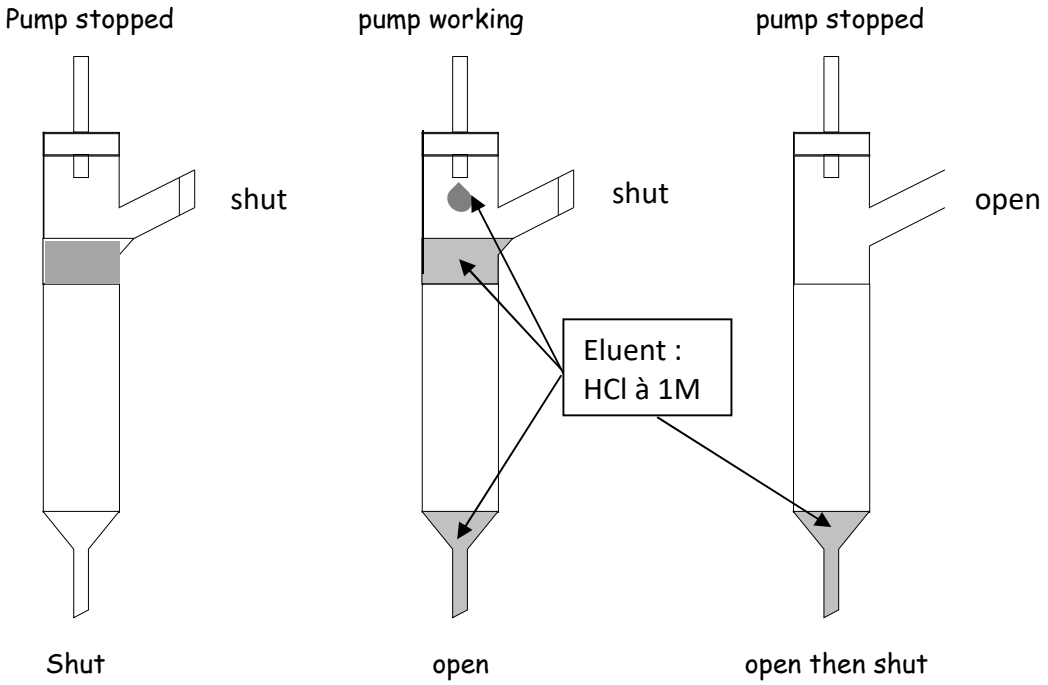
II. EXPERIMENTAL WORK.

1. Pre-elution.

We will make sure that the equipment is as tight as possible, so that the resin **will never be dry.** In these conditions, the value of the flow will relay on the peristaltic pump.

This pre-elution step is necessary to suppress Na^+ et K^+ ions in the column that may come from the previous elution. It is also useful to check the tightness of the equipment.

Different steps to start the separation device.



- Step 1: Insert the flexible pipe on the peristatic pump. Simultaneously, open the valve under the HCl tank (be careful: a little) and the valve under the elution column. Then start the pump (maximum flow)

- Step 2: The level of eluent must remain constant (20 to 25 mm). If this is not the case (non-constant level), increase or decrease the valve opening under the HCl tank. Let the pre-elution occur during 10 minutes. Measure the eluent flow (during 4 or 5 minutes).

- Step 3: Close the HCl tank valve and allow the eluent to flow until the level is aligned with that of the resin. Stop the pump and at the same time shut off the valve under the elution column before the resin is dry. Then, the column is ready to receive the sample.

2. Separation of Na^+ and K^+ ions.

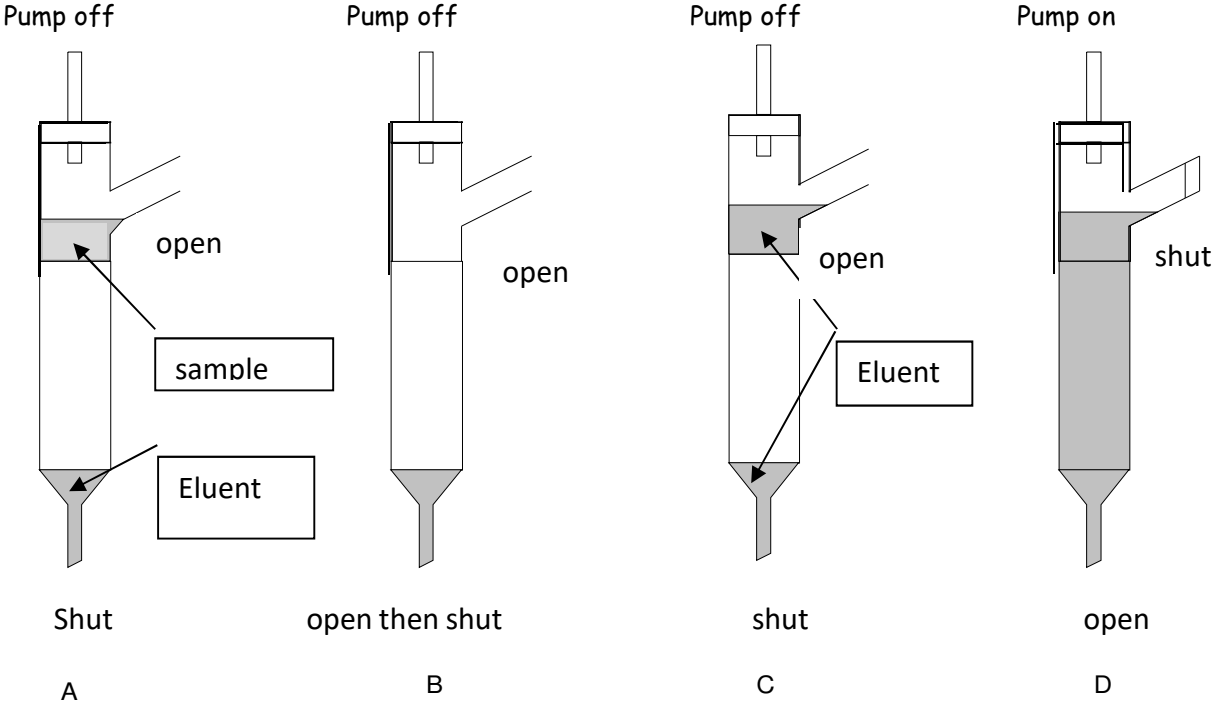
A to D figures show the different steps until the beginning of the elution itself.

- Step A: Introduction of 0,4 mL of sample (0,005 $mol.L^{-1}$ in NaCl et 0,01 $mol.L^{-1}$ in KCl). You will demonstrate afterwards that the cations of this sample are fixed on a small number of “theoretical plates”, this condition being essential to a good separation.

- Step B: Fixation of the sample on the resin (open the lower valve until the solution level is the same as the resin one then close the valve or use pump).

- Step C: Introduce afterwards with caution the eluent until its level is about 10 mm above the resin one.

- Step D: Elution: after shutting the side entry, put under the exit pump test tubes which will receive the different fractions (about 2,5 mL ie every 2 min 30 s) during the elution process. Simultaneously, start the pump, open the lower valve and activate the chronometer. Collect your fractions (40 tubes).



3. Preliminary check-up of the flame photometer.

Eluent analysis will be performed by flame spectroscopy.
It is thus necessary to assess that traces of Na^+ and $K^+$are absent from:

- the eluent
- the glassware used, which must be rinsed carefully.

For that, the flame photometer will be promptly ignited, the zero will be performed on "high purity" water, which will be used for dilutions. The maximum deviation will be adjusted on the highest concentrated solution (3.10^{-4} $mol.L^{-1}$)

The purity of the solvent will be checked in these amplification conditions.

4. Analysis of collected fractions

Note: Na^+ being eluted faster than K^+, it is advised to realise first the calibration of Na^+. For that, you have standard of Na+: between 0,5 and 3.10^{-4} $mol.L^{-1}$.
Afterwards, measure the Na emission in each test tube (make sure to keep enough solution in each test tube for the second analysis). Then, realise the calibration of potassium (standard of K^+: between 0,5 and 3.10^{-4} $mol.L^{-1}$) and measure its emission in each test tube.

5. Determination of the hold-up volume of our separating system.

The knowledge of this parameter is essential to interpret experimental results.
It is obtained by measuring the volume of eluent necessary for a solute easily detected and without interaction with the resin to go through all the separation device.
To determine the hold-up volume, 0.2 mL of an aqueous solution of a non ionic coloured polymer: DEXTRAN Blue (molar mass 2 000 000) will be introduced under the same conditions as for the sample.

6. Determination of the resin capacity.

The goal of this part is to determine the number of equivalents of cations that the resin may exchange per unit of mass.
It will implement a static method of moving H^+ ions contained in the resin by an excess of NaCl and then titrate by NaOH protons so liberated.

Test procedure:
Weigh accurately approximately 0,25 g of wet resin, pour it in a 50 ml beaker using about 20 mL of MilliQ water, add approximately 0.1 g of NaCl to help to move the equilibrium towards the right.

$$H^+_r \quad + \quad Na^+_s \rightarrow \quad H^+_s \quad + Na^+_r$$

Assay with NaOH using methyl red as indicator.
Express capacity in meq / g of dry resin.

7. Determination of the moisture content of the resin.

We use the Karl Fisher method. It is widely used for the determination of water. The titration reaction is based on the reaction described by RW Bunsen:

$$I_2 + SO_2 + 2\ H_2O = 2HI + H_2SO_4$$

This redox reaction requires absolutely water. Thus, water is determined with this method.
(The reaction is carried out in (non-aqueous) methanol in the presence of a base (imidazole $C_3H_4N_2$ noted RN)) and sulphur dioxide SO_2. The following reaction occurs:
$CH_3OH + SO_2 + RN = [CH_3OSO_2^-, RNH^+]$

The sample is added and it is titrated by iodine ($I_2 + I^-$), the following titration reaction occurs:
$[CH_3OSO_2^-, RNH^+] + I_2 + \mathbf{H_2O} + 2RN = [CH_3OSO_3^-, RNH^+] + 2RNHI$
See the instruction in the manual in lab room.

The determination can be repeated in the same solvent, up to 3 times.
An ion-exchange resin is sold in "wet" form, the manufacturer indicates the moisture rate, that is to say the % by mass of water present in the supplied product.

III. INTERPRETATION.

1. Plotting of the elution curve

Using the calibration curves and measures carried out on fractions (see § II-4), present the results as a curve C = f (V_{HCl}). Check the linearity of the calibration curves.

2. Exploitation of results.

Calculation of the efficiency of the resin.

Show that the elution curves obtained can be assimilated to Gaussians. In these conditions, calculate the height equivalent to a theoretical plate (HEPT) for this column.
We will focus on verifying the assumption made in the § II-2: step A.
Calculate, from the value of the resin capacity determined previously and quantities of Na^+ and K^+ introduced, the number of theoretical plates occupied for the fixation of the sample.
Conclusion?

Calculation of partition coefficients (or distribution).

Taking into account the hold-up (or interstitial) volume calculated in § II-5, calculate, under the operating conditions, the distribution coefficients D_{Na} and D_K and the Exchange constant $K_{Na/K}$.
Reminder of definitions:

Distribution coefficient of the X ion: $D_x = \frac{[X_r]}{[X_s]}$

Exchange constant between two X and Y ions:

$$X_r + Y_s \rightleftarrows X_s + Y_r$$

$$K_{X/Y} = \frac{[X_s][Y_r]}{[X_r][Y_s]}$$

The concentration of X in the resin is expressed in meq / g of dry resin
and the one of X in the solution in meq / mL.
Distribution coefficients will be obtained by application of the general relationship:
V = V0 (1 + k') which becomes here $V_A = V_0 + m_r \cdot D_A$

With:
- V_A = Retention volume of A (ml)
- V_0 = hold-up volume (ml)
- m_r = Mass of dry resin (g)
- D_A = Distribution coefficient of molecule A

IV. Main Points to discuss

For this labwork, you have to complete a report with an outline on moodle

TP N°3: IDENTIFICATION OF VOLATILE COMPOUNDS IN ESSENTIAL OIL BY GC-MS AND NMR

The goal of this lab session is to perform an identification of volatile compounds in an essential oil by GC coupled to mass spectrometer and realize some NMR spectra.

I. Introduction

The essential oil of lavender ASPIC is extracted from the flower and a part of the stem by hydro distillation, maceration in solvents and filtration. Lavender has antiseptic, soothing and healing properties.
Lavender essential oil is mainly composed of oxides like Cineol (25 to 38%), terpenic alcohol like Linalool (40 to 45%), ketones like camphor but it also contains sesquiterpenes and esters.

These volatile compounds are analysed by gas chromatography and can be identified and quantified using a mass spectrometer.
After separation in the chromatograph, isolated compounds enter in the ion source through gases and are ionised (Figure 1).

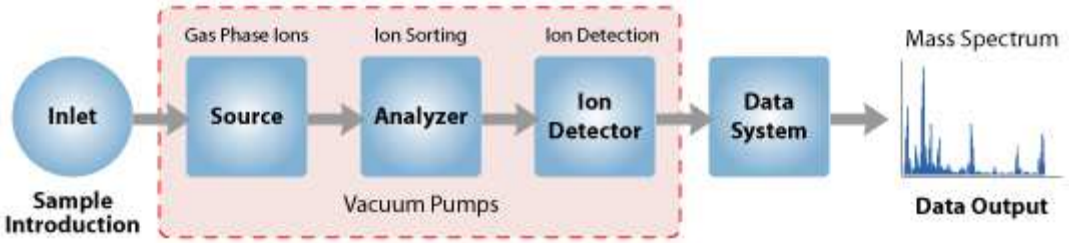
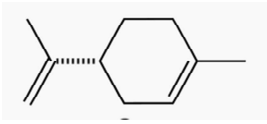
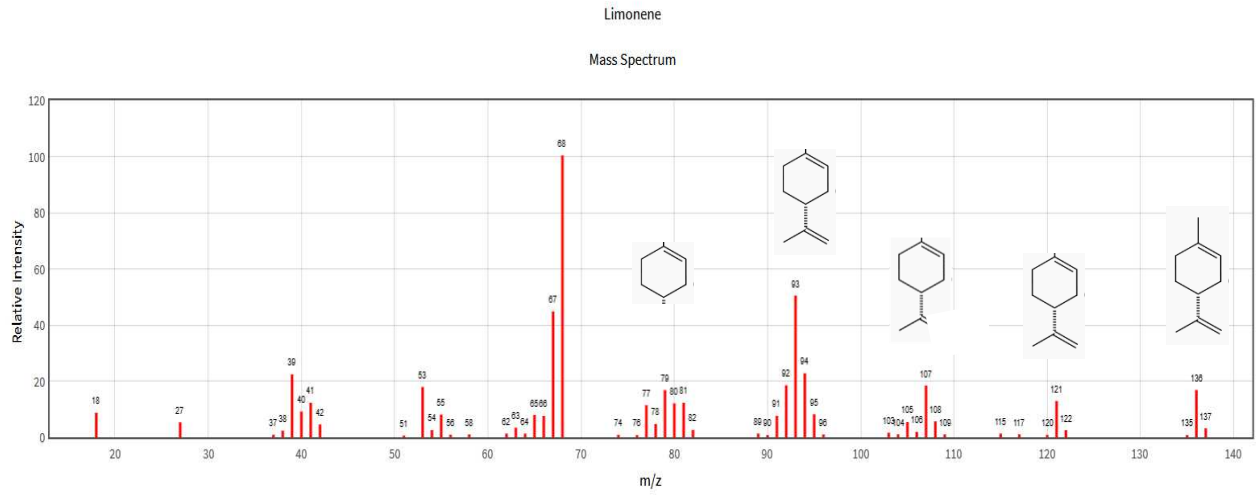


Figure 1 : Principle of spectrometer

In the case of soft ionization in the detector, the addition or the subtraction of an ion (such as H^+) or an electron in the source gives an ion called pseudo molecular ion or molecular ion that allows the molecular weight determination. In the case of hard ionization or in a collision cell, molecular ions are fragmented to lead to daughter ions. All the fragments formed are selected, ranged in the analyser and counted in the ion detector to give the mass spectrum. This spectrum is represented on x-axis by the ion mass (m/z) and on y-axis by the abundance of each ion.
Example: Limonene





Two modes are available in mass spectrometry: a SCAN mode if the detector analyses fragments between 40 and 200 m/z (for example); and a SIM (selected ion mode) mode if the detector looks only for a few defined ions, characteristic of targeted compounds. The target ion for quantification is usually the biggest and 2 other ions named reference ions are chosen on spectrum (for limonene, the target ion will be 68 and reference ions could be 93 and 136). With the spectrum of each detected peak, the software will draw a chromatogram TIC (total ion chromatogram). And the NIST data base (National Institute of standards and Technology) identifies the compounds by comparison between the compound mass spectrum and mass spectra in the library. Usually, identification becomes reliable when the probability is higher than 80 %.

II. Experimental work

You have:
- A solution containing linalool (CAS number: 78-70-6, purity: 97%) at 500 mg/L in ethanol
- Essential oil of lavender
- p-cymene; eucalyptol; linalool: analytical standards

1. Operating conditions

Shimadzu GC 2030 Chromatograph fitted with mass spectrometer QP2020NX is used.
See the equipment and software notice.

+

- **Stationary phase:** ZB5 MS Plus (5% diphenyl 95% polydimethylsiloxane), diameter 0,25 mm, length 30 m, Stationary phase thickness 0,25 µm. T_{max} = 325 °C
- **Injector temperature:** 250 °C split mode (30:1)
- **Carrier gas:** helium 1mL/min
- **Oven temperature programming:** 80 °C then 7 °C/min until 130 °C then 20 °C/min until 200 °C
- **Mass spectrometer:** Scan mass from 40 to 200 m/z, Ion source temperature: 200 °C and Interface temperature: 250 °C
- **Injected volume:** 1 µL

2. Identification of compounds in essential oil

Weight 50 mg of the essential oil in a 50 mL volumetric flask. Complete to the mark with ethanol, shake vigorously the solution.
Dilute the solution: Put 1 mL in a 10 mL volumetric flask and complete with ethanol.
Inject the solution using the method with SCAN detection named “TP linalool SCAN”. Identify peaks detected. Look for retention time of linalool.

3. Quantification of Linalool in scan mode

Prepare, in 10 mL volumetric flasks, 5 standard solutions containing linalool ranging from 20 to 80 mg/L in ethanol.
Inject standards with SCAN detection method and draw the calibration curve
Area = f(content of compound in mg/L)
Quantify the content of linalool in the oil.

4. Quantification of Linalool in SIM mode

Inject the same standards with the SIM detection method named “TP linalool SIM”. In this method, ions have already been chosen; check that these ions are present in the linalool spectrum.
Draw the calibration curve Area = f(content of target ion in mg/L).
Quantify the content of linalool in the oil.
Compare results in SCAN mode and SIM mode. Is the composition confirmed?

5. NMR part

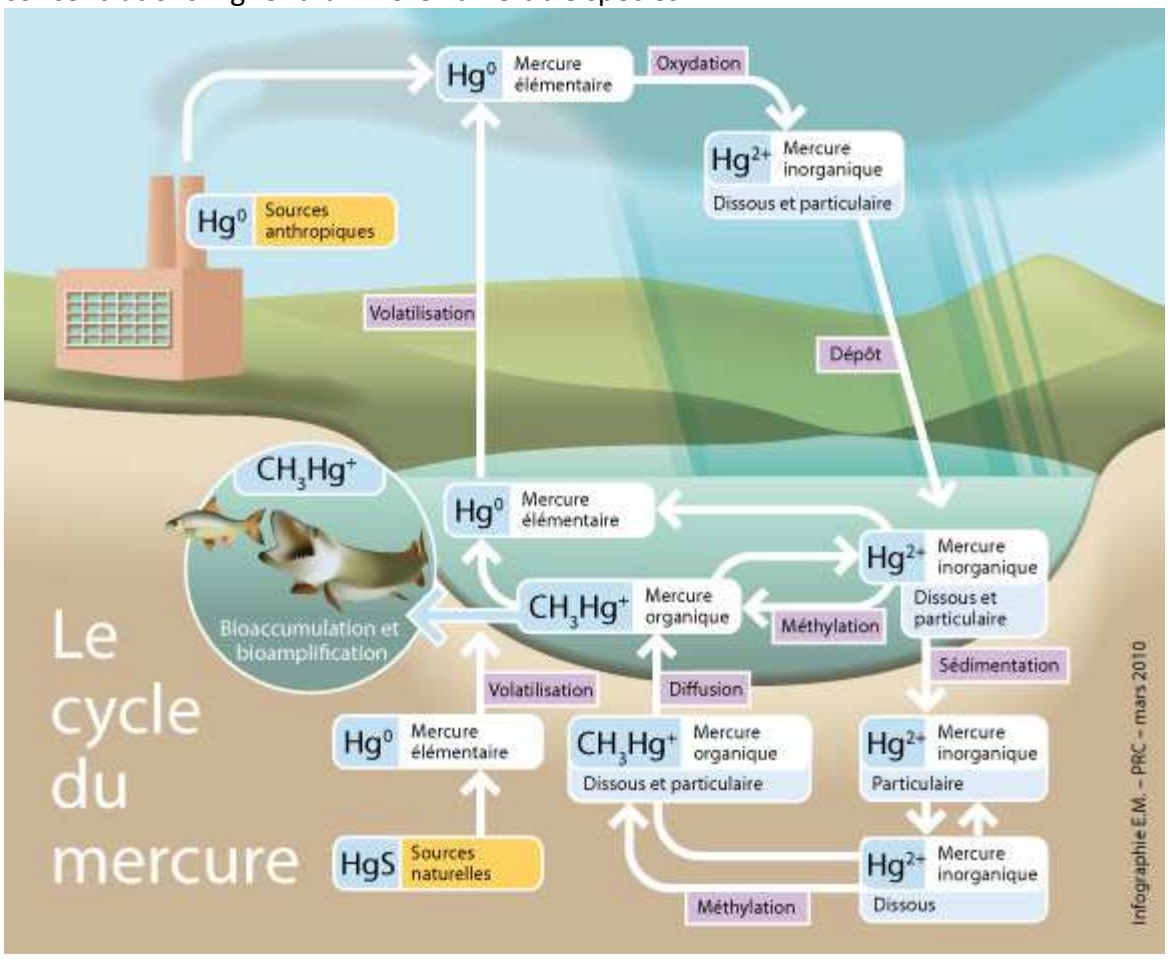
Pulsar NMR 60 MHz spectrometer from Oxford Instruments is used.
NMR analysis will be performed on linalool, eucalyptol and p-Cymene contained in the essential oil.
Under the hood, prepare 3 NMR tubes: 10 drops of the compound + 0.75 mL of $CDCl_3$. Cap and then shake the tube vigorously.
With the manual, perform the acquisition of the NMR spectra (Integration and Peak picking).
Assign chemical shift on the spectrum.
Compare 60 MHz; 300 MHz and 600 MHz spectra.

III. *Main points of discussion*

- Identify the more concentrated compounds in oil using mass spectra and NIST data base. Explain the formation of 3 major ions produced for 4 compounds including linalool.
- Quantify linalool in oil with SCAN mode and SIM including a statistic study. Compare the 2 detection methods.
- Interpret completely the NMR spectra of linalool, eucalyptol and p-Cymene (assignment of protons, multiplicity and coupling constants).
- Would the MS and RMN spectra of o-cymene and p-cymene be the same? Explain your reasoning

TP n°4: DETERMINATION OF THE CONTENT IN MERCURY, MAGNESIUM AND CALCIUM IN TUNA AND SHRIMPS

Fish has always been appreciated for its culinary and nutritional qualities and is among the healthiest food. We propose, in this regard, to determine here its magnesium and calcium content. However, in recent years, some species of see fish, specifically tuna, are the subject of special attention due to a mercury content regularly exceeding the limit value of 1.0 mg/kg **wet** weight (European decision EC 629/2008). In the aquatic environment, fish ranging at the end of the food chain, such as tuna, swordfish, sharks and other large fish accumulate mercury (in the form of methyl mercury) in their tissues for several years and thus contain concentrations higher than more vulnerable species.



Objectives of the work:

The aim of this work is to measure mercury, calcium and magnesium in tuna and shrimps sold in cans. The aim is, to prepare samples so that they can be analyzed by cold vapor atomic absorption spectrometry and inductively coupled plasma optical emission spectroscopy. Thus, a wet digestion will have to be conducted first.

Educational objectives:

- Familiarizing oneself with sample digestion.
- Use of a spectrometer with atomic absorption Thermo Solaar S2 cold vapour mode (thanks to the generator of hydrides VP100) for the mercury determination.

- Use of a inductively coupled plasma optical emission spectroscopy ICP-OES ICAP 6300 in simultaneous mode for Ca and Mg determination.

Organisation of working time:

- Launch the wet digestion simultaneously of one tuna, one shrimp samples and one blank.
- During 1 h of the wet digestion, create the atomic absorption method, ask the technician ' to open the gas on the ICP. Prepare solutions for external calibration (1 range for ICP, 1 range for mercury).
- Once the digestion is performed, prepare your samples, analyze them using ICP and then using the cold vapor atomic absorption.

I. Digestion under sonication.

The protocol was adapted from Niwat Manutsewee, Wanlapa Aeungmaitrepirom, Pakorn Varanusupakul, Apichat Imyim Determination of Cd, Cu, and Zn in fish and mussel by AAS after ultrasound-assisted acid leaching extraction Food Chemistry 101 (2007) 817–824

Wearing nitrile gloves (blue gloves) in addition to a lab coat and security glasses is essential in the preparation of the sample. In addition, stay next to the ultrasonic bath to check that the digestion is going on well. The preparation and the process of digestion will be under the control of a teacher or a technician. If problem, very quickly tell the teacher or the technician and evacuate the room.
1 digestion per sample leading to A_{tuna} and $A_{shrimps}$ solutions.

Sample preparation (done by the technician): Weigh then dry natural tuna at 100°C in an oven for 20h. Weigh dry tuna then ground it to small pieces (<300µm) dry tuna and keep them in a closed vessel in a desiccator.

A sample digestion: Weigh with accuracy around 0,2 g of dried tuna in a 50 mL plastic tube. Repeate this operation with dry shrimps. Add in these 2 plastic tubes and also in an empty plastic tube (method blank*) 2 mL of concentrated nitric acid (4M), 2 mL of hydrochloric acid (4M) and 0,25 mL of hydrogen peroxide to 35%. Put an unclosed cap. Place all 3 tubes in the beaker containing water then in the sonicated bath for 30 min. Then close the cap and centrifuge the tube at 4000 rpm for 10 min. Then carefully transfer the liquid phase in a 50 ml volumetric flask by filtration on filter paper. Then wash the solid residue with 5 mL of water and centrifuged again at 4000 rpm for 10 min. Add the liquid phase to the previous flask by filtration on filter paper and complete to the mark with Milli Q water. These flasks are called A_{tuna} and $A_{shrimps}$. Then dilute at 1/10 the previous solution to give respectively B_{tuna} and $B_{shrimps}$. You will dilute at 1/5 the first solution $A_{shrimps}$ to give respectively $C_{shrimps}$.

* A method blank (MB) is an analyte-free matrix that is processed exactly in the same manner as the samples. Its main function is to check there is no contamination resulting from the analytical process. It is different from the instrument zero (reference).

Analyses :

ICP - AES ICAP 6300	SOLAAR S2 atomic absorption spectrophotometer
MB (Method Blank)	MB (Method Blank)
A_{tuna}	B_{tuna}
$A_{shrimps}$	$C_{shrimps}$
$B_{shrimps}$	

II. Set up of SOLAAR S2 atomic absorption spectrophotometer

Mercury will be determined by the cold vapour hydrides method (VP100 coupled with Solaar S2).
An external calibration method needs to be created.
Refer to the manual, available near the apparatus, for the creation of methods as well as for the device ignition (turn on the cathode hollow lamp 15 minutes before the analysis). Don't light the flame.

III. Setting up of the ICP - AES ICAP 6300

Calcium and magnesium will be analyzed simultaneously on an ICP - OES (Inductively coupled plasma - optical emission spectroscopy). The analysis will be performed in the axial mode.

Element	Wavelength: λ (nm)	Mode
Ca	184,0	Axial
Mg	202,5	Axial

Refer to the manual, available near the instrument, for the creation of the method. The technician or teacher is with you and helps for the plasma ignition.

IV. Preparation of calibration solutions

1. Preparation of calcium and magnesium calibration

There are 2 solutions respectively at 1000 mg/L in magnesium and Calcium.
From these solutions, prepare the following range of solutions in 50 mL volumetric flask:
Intermediate solutions will be necessary (sampling below 1 mL volumes are not enough reliable).

Flask	1	2	3	4	5
Ca mg/L	0	1	2	5	10
Mg mg/L	0	1	2	5	10

2. Preparation of the calibration range for mercury

50 mL of a solution 100 mg/L in mercury (Hg^{2+}) is available. Dilute this solution using a **pipetman** (after testing the pipetman with water and a precision balance before use). 100 mL of a solution 50 µg/L in Hg. This will be the D solution.

Use the pipetman only for the preparation of the D solution.

Prepare these 5 solutions in 50mL volumetric flasks with the following concentrations of Hg.

Number of flasks	1	2	3	4	5
C (Hg) (µg/L)	2	5	10	15	20

V. Analysis and Results

1. Analysis in cold vapor mode, determination of mercury.

Using the manual, measure your solutions on the VP100 coupled with the spectrometer (ask the teacher or the technician to monitor operations).
Use the external calibration method with B_{tuna} and $C_{shrimps}$ samples.
Results will be quickly processed with Excel (you need to prepare your own calculation sheet).
The content of mercury in dried tuna will be expressed in mg/kg.

2. ICP analysis: determination of calcium, magnesium.

Ask the Professor or the technician to come with you for ICP analysis.

Prepare your method on the software by following the manual indications. Switch the plasma on and let it equilibrate.
Using the manual, pass your solutions on the spectrometer (ask the teacher or the technician to monitor operations).
Use the external calibration method with Blank sample, A_{tuna}, $A_{shrimps}$ and $B_{shrimps}$. Check for physical interferences (peak overlapping) on these samples with the help of your instructor.
Results will be quickly processed with Excel (you need to prepare your own calculation sheet).
The content of magnesium and calcium in tuna will be expressed in mg/kg.

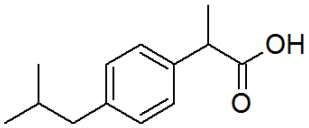
VI. Main Points to discuss

- In the introduction, give the main sources of mercury, explain the main effects Hg_0 and $CH3\text{-}Hg^+$ on health and indicate the content of the Minamata convention enforced In 2017.
- Explain the principle of ICP-OES and Cold vapor hydrides method
- Explain the digestion step
- Determine the content in Hg, Ca and Mg in Tuna and shrimps in mg/kg of dry and ***wet*** products
- Determine the confidence intervals and conclude.
- In annex, check the accuracy of the pipetman used to prepare the intermediate solution of Hg

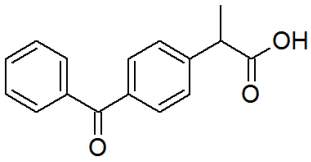
TP n°5: DETERMINATION of IBUPROFEN, ACETAMINOPHEN AND KETOPROFEN IN MEDECIN TABLETS BY HPLC

Ibuprofen, Acetaminophen and Ketoprofen are well none analgesic molecules. They can be used for the management of fever or for pain relief. The purpose of this work is to perform their separation and quantification in order to check their content in two different tablets.

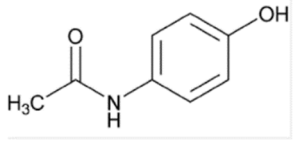
- Ibuprofen:



- Ketoprofen:



- Acetaminophen:



Chromatographic separation and calibration

Equipment:

A SCION LC6000 including:

(1) Quaternary pump for the mixture of four solvents under low pressure Flow = 1.5 mL/min
(2) Vacuum degasser.
(3) Autosampler
(4) Luna 2 column, C18, 0,46 cm, l=15 cm, 5µm particles, max pressure = 250 bar
(5) Detector : PDA **P**hoto **D**iode **A**rray (190 to 950 nm)

Getting started:

Actuate the HPLC as explained in the manual. Create the method.
Solvent A: 0.1% formic acid in water at pH=3 (adjusted with triethylamine)
Solvent B: ACN
Choose a flow of 1.5 mL/min and an analysis time of 10 minutes. The eluent will contain 90%/10% A/B during 2 min and then a gradient to 100% B at t=8 min and then holding at 100% B for two minutes. A reequilibration time of 6 min (Stabilisation time) will be chosen. The maximum pressure will be 250 bar.
The column temperature is 25 °C.
Injected volume: 20µL
The detector is set to register the signal at λ=226 nm (BW=4 nm) and at λ=250 nm (BW=4 nm) and a spectrum is recorded between 220 and 400 nm.

Set the report parameters as indicated in the manual.

Solutions provided:

You have:

-A stock solution containing Ketoprofen, ibuprofen and acetaminophen at 2500 mg/L in Ethanol/water (50:50, v: v)

-individual vials of Ketoprofen, ibuprofen and acetaminophen at 50 mg/L for peak assignment.

I. Calibration

Prepare, in 10 mL volumetric flasks, standard solutions containing Ketoprofen, acetaminophen and ibuprofen ranging from 10 mg/L to 200 mg/L in 50:50 ethanol: water (v: v).
Inject standards as soon as possible and then draw the calibration curve for each compound. Make sure that the criteria for a good separation are checked (resolution and peak asymmetry, use the HPLC software to calculate them).

II. Extraction of active compounds from tablets.

-Paracetamol sample: five tablets each containing 1g of acetaminophen were crushed. The overall mass obtained is written on the flask.
-Ibuprofen Sample: five tablets of ibuprofen each containing 400 mg of ibuprofen were crushed. The overall mass obtained is written on the flask.
-Ketoprofen Sample: five tablets of Ketoprofen each containing 25 mg of Ketoprofen were crushed. The overall mass obtained is written on the flask.

Determine the amount of acetaminophen, ibuprofen and ketoprofen in tablets (analysis won't be replicated). For this purpose, an adequate mass (*Mass between 50 and 100 mg) of the crushed tablets will be solubilized in 50 mL of 50/50 (ethanol/water) solvent in a volumetric flask. Put the flask in a sonicating bath for 10 min. Dilute the solution if needed and then filter an aliquot of the final solution using 0.45 µm nylon filter prior to injection.

III. Main Points to discuss

- Justify the choice of the wavelengths
- Check the resolution and peak asymmetry
- Draw the calibration curve and perform statistical calculation to ensure linearity
- Calculate the medicine content and calculate the confidence interval

TP n°6: OPTIMISATION OF A SEPARATION AND DETERMINATION OF BENZOIC ACID, BENZYL ALCOHOL AND PHENOXYETHANOL IN COSMETIC PRODUCTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Goal

Find optimum conditions (efficiency, resolution, run time) for the separation of a mixture of compounds using high-performance liquid chromatography and apply the conditions to the measuring of 3 preservative agents widely used in cosmetics: benzoic acid, benzyl alcohol and phenoxy ethanol.

We perform a « reversed-phase » separation: C-18 bonded silica column, using the following solvents:
A: formic acid 0.1% in water pH=3 (adjusted with NEt_3)
B: acetonitrile CAN

Equipment :

- Agilent 1100 HPLC, quaternary pump, UV-Visible absorption detector (the wavelength will be the characteristic absorption one for aromatics).
- Autosampler (V=20 µL)
- Pursuit, C18, 0,46 cm, l=15 cm, 5µm particules, max pressure = 250 bar, plus a guard column (length 1 cm; same material)
- HP Chem station software
- Tcol= 25°C, flow = 1 ml/min
- Diode array detector set at λ=262 nm (BW=8 nm). (Reference 350 nm (BW 50 nm)).

Standard preparation

Available solutions:

- A stock solution of benzoic acid, benzyl alcohol and phenoxy ethanol in 50:50 ACN:water (v:v) at 250 mg/L
- Individual solution of benzoic acid, benzyl alcohol and phenoxyethanol in 50:50 ACN:water (v:v) at 250 mg/L

Prepare 5 standards solution ranging from 10 mg/L to 50 mg/L in 50:50 A: B (v:v).

I. SEPARATION OPTIMISATION

The optimization of the separation will be performed using the most concentrated standards. An injection with the eluent set at 85/15 A/B with a runtime of 20 min has been performed. The chromatogram is available on the desktop (**do not inject**).
Perform a second injection with the eluent set at 70/30 A/B with a run time of 8 min. (Note that the column should be equilibrating for at least 10 min with the second eluent prior to the second injection).
Using Osiris software (see on computer and specific manual), optimize the separation in order to have a resolution of at least 1,9.

Check the good separation using the optimized conditions. Calculate the resolution and peak asymmetry using the HPLC software.

II. Calibration

Inject the remaining standards using the optimized conditions (put post-time at 0 min as no equilibration is necessary between runs). Assign all peaks with individual standards. Draw the calibration curve.

III. Extraction of cosmetics

Weight 0,5 g of the cosmetic in a 100 mL volumetric flask. Add around 50 mL of ACN/water (50/50), vigorously shake the solution. Complete to the mark. Filter an aliquot using 0.45 µm nylon filter prior to HPLC analysis.
The sample won't be duplicated.

For this labwork, you have to complete an answer sheet and give it back at the end of the session (or the next day).

FEUILLE DE SECURITE (à photocopier une par TP, à remplir avant et à rendre avec le compte rendu).

RISQUES et SÉCURITÉS LIÉS AUX PRODUITS

LA BLOUSE ET LES LUNETTES DE SECURITE SONT OBLIGATOIRES EN LABORATOIRE. Ne pas les indiquer dans la rubrique sécurité

Réactifs, Solvants et solution	Pictogramme (N° SGH)	Risques (préciser sa catégorie et sa signification)	Sécurité : proposer la meilleure solution pour se protéger pour chaque risque (prévention ou protection)	Mesure à prendre en cas de déversement accidentel	Matières incompatibles (donner 1 à 2 exemples)
Exemple : *Acide acétique concentré*	*SGH 02 et 05*	*Liquide et vapeur inflammable (catégorie 3)* *Brûlures de la peau et graves lésions des yeux (catégorie 1A)*	*Travailler sous sorbonne pour se protéger des vapeurs corrosives* *Tenir à l'écart de toute source de chaleur (flamme,)* *Porter des gants adaptés*	*Evacuer la salle avant d'aérer la pièce en se protégeant avec un masque adapté. Capturer l'épandage avec de la vermiculite avant de le récupérer le tout dans un fût bleu vide.*	*Les matières oxydantes (ex : permanganate de potassium)*

RISQUES LIÉS AUX MACHINES (compléter en séance)

Appareils	Risques (Il faut faire attention à quoi ?)	Sécurités (Comment s'en prémunir ?)