INSTITUT NATIONAL DES SCIENCES APPLIQUÉES ROUEN NORMANDIE

TRAVAUX PRATIQUES DE CHIMIE ANALYTIQUE
CFI3 Semestre 6
EC TP ANALYSE

Année 2024-25

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 il est conseillé d'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, suivi actif pendant les exposés
- 3 points : exposé par binome (1 par séance de TP) selon le planning de rotation des TP, descriptif sur moodle, déposer l'exposé sur moodle avant la présentation
- 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 (à négotier avec l'enseignant responsable de l'EC).

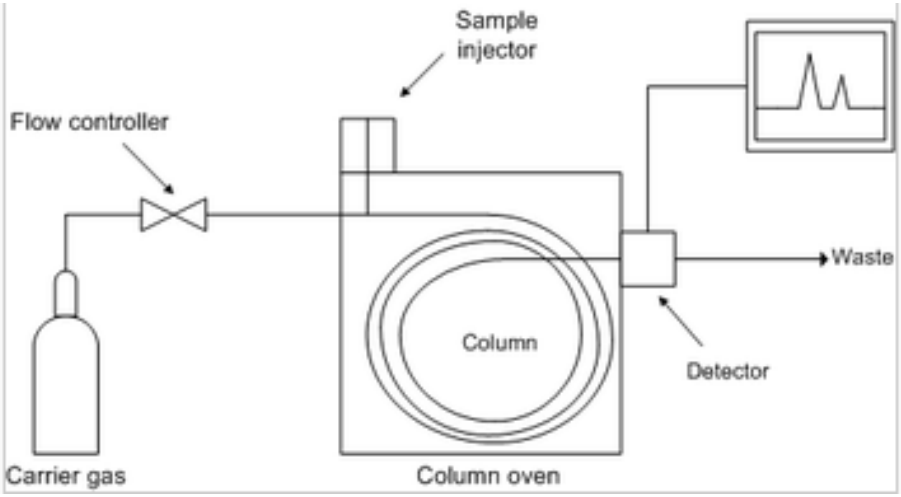
Les comptes rendus doivent comporter **au minimum** les éléments suivants :

- Vos noms et prénoms ainsi que le nom de votre encadrant
- 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 d'acide benzoïque/kg de shampoing. Il faudra aussi calculer l'incertitude sur ces résultats. Idéalement, chaque détermination de solution inconnue doit être tripliquée (trois analyses). Garder en tête que si vous ne détectez pas une molécule dans un échantillon, cela peut être dû à la sensibilité de votre méthode analytique, tenir compte des LD et LQ.
- 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.

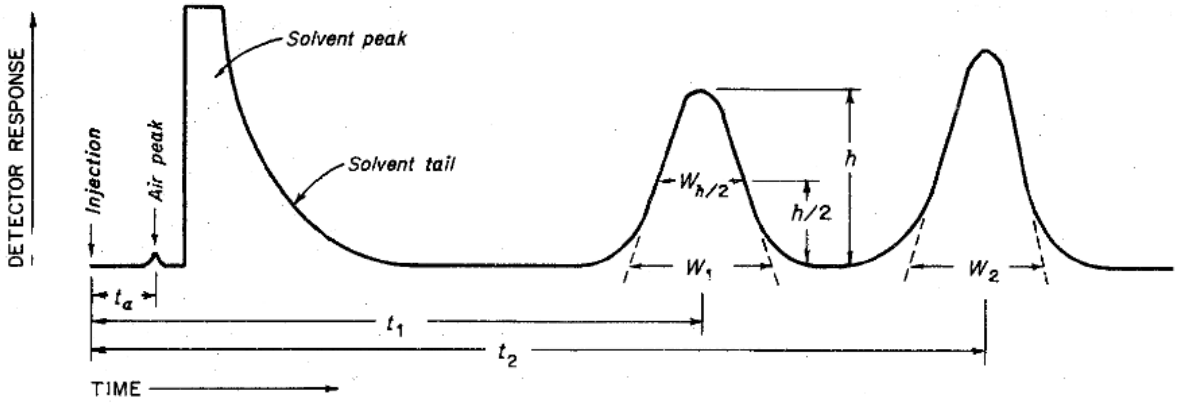
I. Principle of gas chromatography



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 injector is heated above the boiling point of the mixture. The liquid mixture is injected (using a micro syringe) in the insert of the injector. 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 times). 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 of carbon injected for a family of organic compounds.



Definitions:

- 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 $w_{1/2}$

Other parameters can characterize peaks (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} : $N_{eff}= 5.545(t_r'/w_{½})^2$ characterizes the efficiency of the separation. Increasing N_{eff} gives narrower peaks.
- The effective **H**eight **E**quivalent to a **T**heoretical **P**late H_{eff}: H_{eff}= column length/Neff. Low values of HETP will give better separation.
- - Reduced retention volume V_r': $V_r'=t_r'.d$ (d : column flow)
- Other parameters can characterize the 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: $R=1,177\ (t_{r2}-t_{r1})/(w_{½1}+w_{½2})$.

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

II. Optimization of the chromatographic method

Two parameters are modified to optimize the chromatographic separation:

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

During the development of a chromatographic method, other 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

A chromatograph Scion 436 (supplier: Scion, software: Compass) with an FID detector (See manual in the lab) is used. The column presents a stationary phase ZB-624 (6 %-cyanopropylphenyl-94 %-dimethylpolysiloxane) diameter 0.32 mm, length 30 m, film thickness 1.8 µm. Tmax = 260°C.
The temperature of the injector and detector are set to 200°C.
The liner used has the following dimensions: 6,3 x 78,5 mm – ID 4mm.

A solution with 20% by volume of toluene and 20% of methylcyclohexane in cyclohexane is injected. Predict the exit order of the 3 molecules and check with experimental results.

For the optimization part, the volume injected is **1µL**, the split ratio is 1/50, and the attenuation of the detector is set to 11.

For all chromatograms, you you need to extract the information from the results table.
An Excel sheet will enable you to calculate chromatographic parameters such as dead time t_0, effective plate number N_{eff}, retention factor k, selectivity α, and 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:

Note: The chromatograms in this section have already been prepared. Measure retention times and half-widths. Fill the Excel sheet and then clean the plastic sheets with ethanol.
The dead time will be calculated from the flow rate.
At a fixed oven temperature (T=150°C), The influence of the carrier gas flow on the column efficiency is studied by plotting HETP=f(flow) for flow=0.2 / 0.4 / 0.8 / 0.9 / 1 / 1.2 / 1.5 / 2 / 2.2 ml/min.

Exhaustively interpret the results on the different graphs with your knowledge from the course.

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 the temperature to have a minimum of 5 values (from 120°C to 200 °C). Inject the sample at different temperatures, plot chromatograms, and measure half-widths. Fill out the Excel sheet.
Exhaustively 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

The 2 molecules studied are present in complex mixtures such as kerosene that also contains heavier molecules like naphthalene. Would you use isothermal conditions or a gradient in this situation?
To answer this question, use the Restek software ProEZGC to try isothermal conditions (150°C) and different gradients ($\Delta T<50°C/min$) with an initial temperature of 150°C and conclude.

Adresse mail: hje89593@nezid.com Mot de passe: Laboana_01

3. Injection and detection

3.1 Expansion volume

For a flow rate of 1 mL/min (read the inlet pressure on the instrument) and some hypothesis, calculate the expansion volume of the liquid injected in the liner and check it with the calculator online (https://www.restek.com/fr/outils-et-calculateurs/outils/calculateur-d-expansion-de-solvant/).
What would be the problem if the expansion volume exceeded the volume of the liner?

3.2 Split ratio

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

3.3 Detector range

Change the range and observe the consequences.

A complete report is expected for this work including relevant screenshots, an interpretation of the results linked to the chromatography course and a conclusion to the study.

TP N°2: 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 a 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 hydrodistillation, 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%), and ketones like camphor but it also contains sesquiterpenes and esters.

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

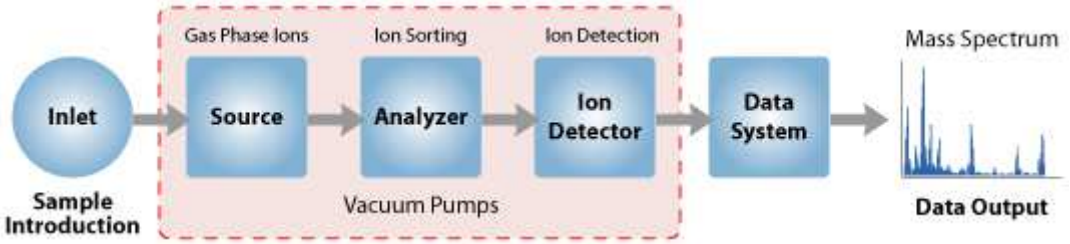
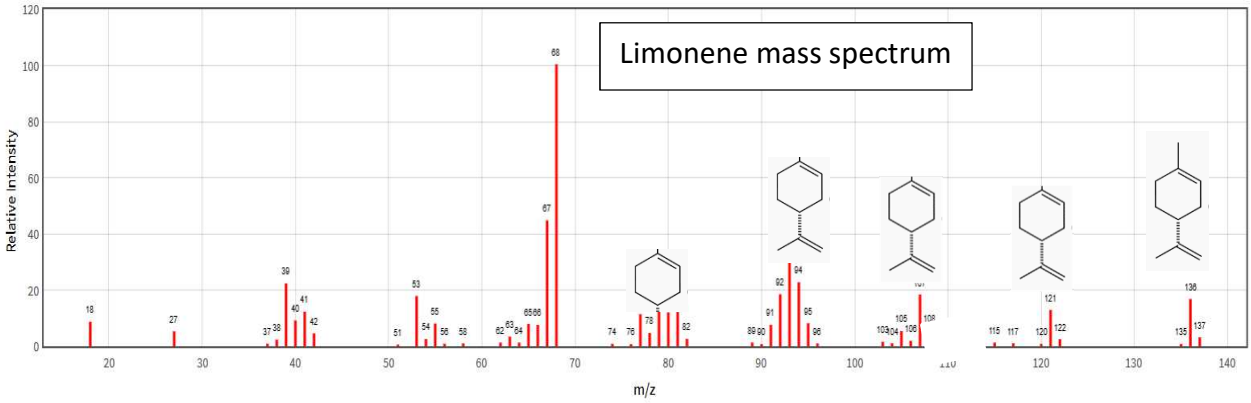


Figure 1: Principle of the spectrometer

In the case of soft ionization in the detector, the addition or 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 analyzer, and counted in the ion detector to give the mass spectrum. This spectrum is represented on the x-axis by the ion mass (m/z) and on the y-axis by the abundance of each ion.

Example: Limonene



Two modes are available in mass spectrometry:

- a SCAN mode if the detector analyses all the fragments between 40 and 200 m/z (for example
- a SIM (selected ion mode) mode if the detector analyzes only a few defined ions, characteristic of targeted compounds. The target ion for quantification is usually the highest and 2 other ions, named reference ions and specific to the molecule, are chosen on the spectrum (for limonene, the target ion will be 68 and reference ions could be 93 and 136).

With all the ions detected, the software draws a chromatogram TIC (total ion chromatogram). In SCAN mode, the NIST database (National Institute of Standards and Technology) identifies the compounds by comparison between the mass spectrum of the compound 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
- Limonene; eucalyptol; linalool: analytical standards

1. Operating conditions

Shimadzu GC 2030 Chromatograph fitted with mass spectrometer QP2020NX is used. The ionization method is electronic impact and the analyzer is a quadripole.
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. Preparation of solutions

Weigh 50 mg of the essential oil in a 50 mL volumetric flask. Complete to the mark with ethanol, and shake vigorously the solution.
Dilute the solution: Put 1 mL in a 10 mL volumetric flask and complete it with ethanol.
Prepare, in 10 mL volumetric flasks, 4 calibration solutions containing linalool ranging from 20 to 80 mg/L in ethanol.

3. Identification of compounds in essential oil

Inject the diluted solution of essential oil using the SCAN mode method named “TP linalool SCAN”. Identify peaks detected using the library. Look for the retention time of linalool.

4. Quantification of Linalool in SCAN mode

Inject the standard at 80 mg/L to choose the target and 2 reference ions of linalol for the SIM mode.
You can give an estimation of the concentration of linalool (the precise quantification will only be performed in SIM mode).

5. Quantification of Linalool in SIM mode

Inject the standards and the sample of essential oil with the SIM detection method named “TP linalool SIM”.
Draw the calibration curve Area = f(content of target ion in mg/L) in Excel.
Quantify the content of linalool in the oil.

6. NMR part

Pulsar NMR 60 MHz spectrometer from Oxford Instruments is used.
NMR analysis will be performed on linalool, eucalyptol, and limonene contained in the essential oil.
Under the hood, prepare 3 NMR tubes: 5 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 shifts on the spectrum.
Compare 60 MHz and 400 MHz spectra.

III. *Main points of discussion*

- Identify the more concentrated compounds in oil using mass spectra and the NIST database. Explain the formation of 3 major ions produced for 4 compounds including linalool.
- Quantify linalool in oil with SCAN and SIM mode. In SIM mode perform a complete statistic study. Consider the advantages and drawbacks of the 2 detection methods.
- Interpret completely the 400 MHz NMR spectra of linalool, eucalyptol, and limonene (assignment of protons, multiplicity and coupling constants). What are the limits of 60MHz spectra?

TP n° 3: 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: ≈50% but read the batch number and supplier reference on the bottle then consult the supplier's certificate of analysis
- capacity: to be determined (theoretically 5 meq*/g of dry resin)

** meq/g = mmol/g*

2. Separation by elution.

The work will consist of 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 to calculate some characteristic constants.

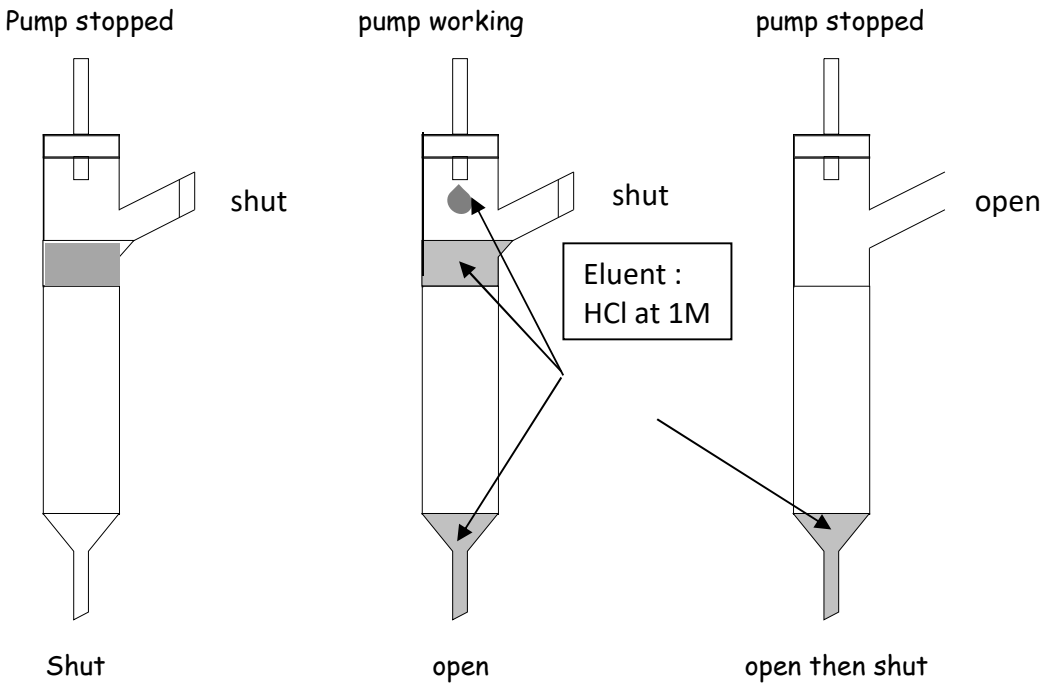
II. EXPERIMENTAL WORK.

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 rely 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 peristaltic pump after the HCl tank (first one) and after the elution column (second one). Open the valve under the elution column. Then start the two pumps at the selected flow.

- Step 2: The level of eluent must remain constant (20 to 25 mm). If this is not the case, increase or decrease the flow control valve under the HCl tank. Let the pre-elution occur for 10 minutes. Measure the eluent flow (during 4 or 5 minutes) and check it is between 1,5 and 2 mL/min.

- Step 3: Stop the first pump of the HCl tank and allow the eluent to flow until the level is aligned with that of the resin. Close the valve under the elution column and stop the second pump. 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.

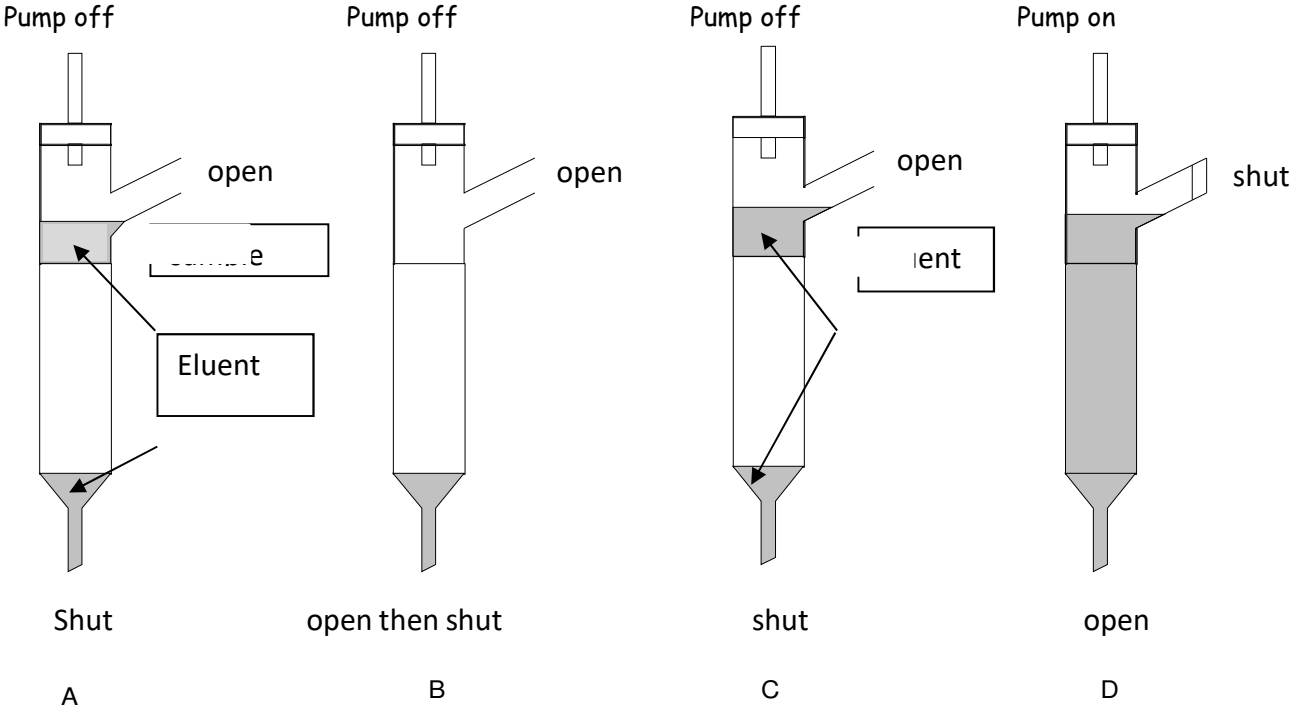
- Step A: Introduce slowly 0,4 mL of sample (0,005 $mol.L^{-1}$ in NaCl et 0,01 $mol.L^{-1}$ in KCl) using a pipetman*. You will demonstrate afterward that the cations of this sample are fixed on a small number of "theoretical plates", this condition being essential to a good separation.

  ** Test the pipetman with water and a precision balance before use and* ***write the results of the repeatability check.***

- Step B: To fix the sample on the resin, open the lower valve until the solution level is the same as the resin one then close the valve.

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

- Step D: To elute the sample, shut the side entry, and put under the second pump test tubes which will receive the different fractions during the elution process. Simultaneously, start the pumps, then open the lower valve, and activate the chronometer. Collect your fractions (30 tubes ¾ full) and record the time.



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 realize first the calibration of Na^+. For that, you have standards of Na^+: between 0,5 and 3.10^{-4} mol/L.

Afterward, measure the Na emission in each test tube (make sure to keep enough solution in each test tube for the second analysis). Then, realize 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 colored 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.
For that, H^+ ions contained in the resin will be released by an excess of NaCl and then titrated by NaOH.

Test procedure:
Weigh accurately approximately 0,25 g of wet resin, pour it into a 50 ml beaker using about 20 mL of ultrapur water, and 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 0,1 mol/L using methyl red as an indicator.
Express the capacity in meq / g of dry resin. Make 2 precise determinations

7. Determination of the moisture content of the resin.

2 methods will be compared: Karl-Fisher and thermobalance.

- the **Karl Fisher method** 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 water which is determined with this method.
(The reaction is carried out in the **aqualine™ solvent** which contains (non-aqueous) methanol in the presence of a base (imidazole $C_3H_4N_2$ noted RN)) and sulfur dioxide SO_2. The following reaction occurs: $CH_3OH + SO_2 + RN = [CH_3OSO_2^-, RNH^+]$

The titrant is **aqualine™ titrant 5**. It contains iodine in methanol.

The sample is added to the solvent and it is titrated by iodine, the following titration reaction occurs:
$[CH_3OSO_2^-, RNH^+] + I_2 + \mathbf{H_2O} + 2RN = [CH_3OSO_3^-, RNH^+] + 2RNHI$

See the instructions in the manual in the lab room, make 3 determinations, and write **all masses and stoichiometric volumes** (calibration + 3 determinations on resin) to calculate yourself the concentration of the titrant and the humidity (compare with the result of concentration of the titrant and humidity given by the software).

A determination can be repeated in the same solvent, up to 3 times.

An ion-exchange resin is sold in "wet" form, and the manufacturer indicates the moisture rate, that is to say, the % by mass of water present in the supplied product.

- The thermobalance records the mass of a sample that is heated to evaporate its water. Make 3 determinations at 150 °C (takes about 10 min each) knowing that a good precision requires at least 100 mg of mass loss.

  Consider the advantages and disadvantages of the 2 methods for any sample.

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 chromatograms C = f (V_{HCl}) and C=g(t).

2. Exploitation of results.

Calculation of the efficiency of the resin.

Check if 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 the resolution of the separation

Calculate the resolution between Na^+ and K^+. How can it be improved?

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 $K_{Na/K}$.
Définitions:

- 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:
$V = V_0 (1 + k)$ which becomes here $V_A = V_0 + m_r . D_A$

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°4: ANION ANALYSIS OF A MINERAL WATER BY ION CHROMATOGRAPHY

In this lab, use the skills you acquired in the analysis lab to develop your protocol.
Chemicals available: KCl (solid), $NaNO_3$ (solid), Na_2SO_4 (solid), Eluent. Do not forget to note the purity of the product.

I. Objective of the work involved:

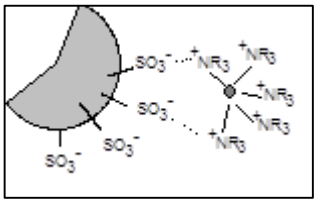
Implementation of a method for qualitative and quantitative analysis of anions in mineral water by ion chromatography.

Ion Chromatography (IC) is a high-performance liquid chromatography method performed on ion exchange resin columns with high specificity and high performance. We will use it in the installation configuration "ANION", under the conditions described below.

II. Chromatographic parameters:

System : Thermo Aquion
Software: Chromeleon
Column: IonPac AS22-fast
Capacity: 126 µeq
Dimensions: 150x4 mm ID
pH of the eluent: 0 < pH < 14
Guard column: AG22-fast
Suppressor: self-regenerating suppression mode SRS - ANION
Eluent: CO_3^{2-} / HCO_3^- (4.5/1.4 mM)
Flow rate: 1.2 ml/min
Detector: Sampling frequency : 5 Hz
Background conductivity: 15 to 20 µS

The column is filled with silica sulfonated particles (5 µm diameter) on the surface of which latex particles bearing quaternary ammonium groups are chemically grafted, in a thickness of about 0.1 µm.



III. Detection:

The detector is a conductimeter that measures conductance in µS. As the cell constant is k = 1 cm, the value corresponds to conductivity in $\mu S.cm^{-1}$.

This detector presents a linear range from 1 to 10 mg/L for each anion.

A suppressor is necessary to suppress the conductivity of the eluent. Without it, it would be impossible to distinguish the conductivity due to the anions analyzed (extremely low concentration) and the background conductivity of the eluent.
The self-regenerating suppressor (DIONEX SRS) is based on the principle of electrodialysis.

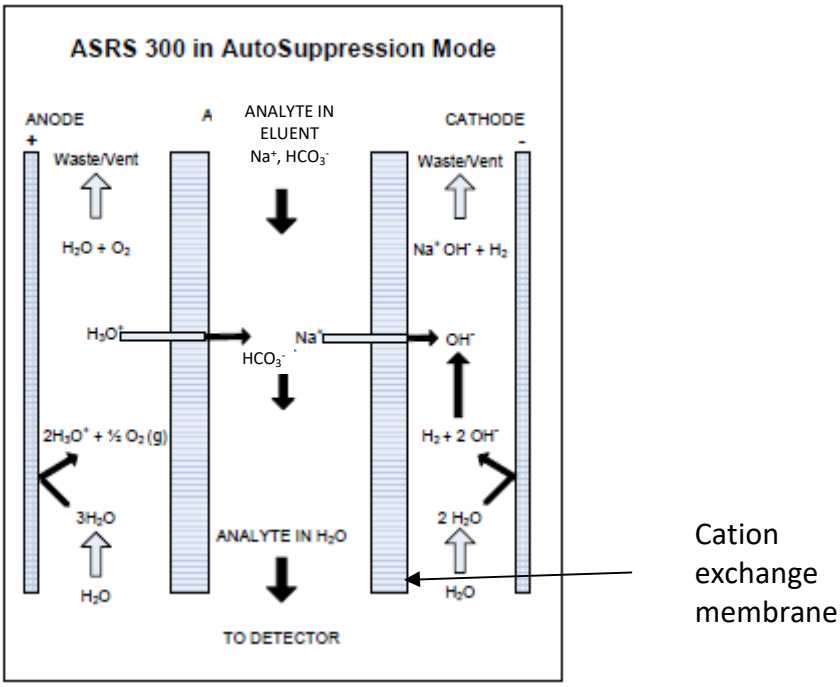


Figure 1: Principle of a self-generating suppressor with $NaHCO_3$ Eluent.

On the cathode, water is reduced to H_2 and OH^-. All cations present in the eluent (Na + and cations from the sample) are moved by the electric field towards the cathode - (migration) through the cation exchange membrane. They are evacuated in the cathode compartment.

At the anode, water is oxidized to O_2 and H^+. The H^+ formed also migrate to the cathode through the cation exchange membrane, then they encounter the weak anion HCO_3^- or CO_3^{2} and form a neutral solution of very weak acid (carbonic acid) CO_2, H_2O poorly conductive. It only remains to measure the conductivity due to the anions in solution in a very poorly conductive medium.

IV. WORK TO PERFORM

Prepare 4 standard solutions in HQ water.
After loading the chromatographic method, inject the blank, the calibration solutions, and the mineral water (Triplicate the preparation) to determine its anion contents (in Cl^-, NO_3^-, and SO_4^{2-}).
The software can do the quantification easily and automatically. You will test this function.
Find the concentrations of CO_3^{2-} and HCO_3^- experimentally using the AT1000 automatic titrator (titrating solution: HCl $\approx 10^{-2}$ mol/L, titration of 10,0 mL of eluent + 40 mL H_2O). Deduce the pH of the eluent.

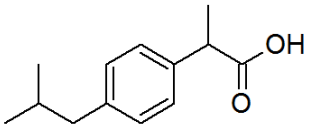
Main Points to discuss

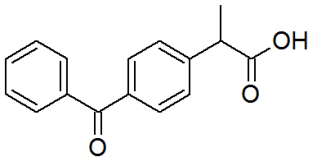
- Explain how selective retention of anions occurs, and justify the relative retention time of anions.
- Use the results of the titration to check the composition of the eluent and use the concentrations obtained to calculate its pH.
- Give your protocol for the preparation of standards and sample dilution
- Check for good peak separation and peak shape (parameters given by the software)
- Draw the calibration curve and perform statistical calculations to ensure linearity
- Determine the content and confidence interval for each ion
- Compare values to commercial and regulatory values for potable water

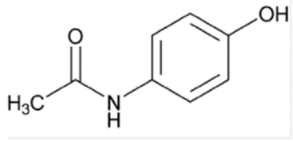
Don't print the full report, but add the relevant screenshots to report on your work.

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

Ibuprofen, Acetaminophen, and Ketoprofen are well-known 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 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 x 15 cm, 5µm particles, max pressure = 250 bar
(5) Detector diode array (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 for 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. Ibuprofen will be quantified at λ=226 nm (BW=4 nm) and Acetaminophen and Ketoprofen at λ=250 nm (BW=4 nm)
Injected volume: 20µL
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 (inject only 2).

I. Calibration

Prepare, in 10 mL volumetric flasks, 4 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: 8 tablets each containing 1g of acetaminophen were crushed. The overall mass obtained is written on the flask.

-Ibuprofen Sample: 8 tablets of ibuprofen each containing 400 mg of ibuprofen were crushed. The overall mass obtained is written on the flask.

-Ketoprofen Sample: 6 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 a 0.45 µm nylon filter before 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 calculations to ensure linearity
- Calculate the medicine content and calculate the confidence interval
- Considering the 3 samples studied as CRM (certified reference materials*), check the absolute and relative bias of the chromatographic method used.

  ***** A certified reference material is necessary to assess the accuracy of an analytical method. As it is very expensive (for example 215 euros for 100 mg of ketoprofen) we cannot afford it in lab sessions. Thus, we consider that the content on the box of medicine is given with a high degree of accuracy.

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.

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

Equipment :

- THERMO HPLC VANQUISH CORE, quaternary pump, UV-visible absorption detector (the wavelength will be the characteristic absorption one for aromatics).
- Autosampler (V=20 µL)
- Luna 2 column, C18, 0.46 cm x 15 cm, 5µm particles, max pressure = 250 bar, plus a guard column (length 0.5 cm; same material)
- Chromeleon 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 4 standard solutions ranging from 10 mg/L to 50 mg/L in 50:50 A: B (v:v).

I. SEPARATION OPTIMISATION

The optimization of the separation is performed using the most concentrated standards.
A chromatogram obtained with the eluent set at 85/15 A/B and a runtime of 20 min is already available on the desktop. Set the pump at 60/40 A/B. After stabilization of the pressure, inject with a run time of 8 min.
Using Osiris software (see on the computer and specific manual), optimize theoretically the separation to have a resolution of at least 1,9 (safety margin).
Set the pump at this composition and wait for at least 10 min before injection to allow the column to equilibrate with this composition of the eluent.

Inject and 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. Use software for calibration.

III. Extraction of cosmetics

Weigh **precisely** around 0,5 g of the cosmetic in a 100 mL volumetric flask. Add around 50 mL of ACN/water (50/50), and vigorously shake the solution. Complete to the mark. Filter an aliquot using a 0.45 µm nylon filter before 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	Risques (préciser sa catégorie et sa signification)	Sécurité : proposer la meilleure solution pour se protéger pour chaque risque (prévention ou protection) en plus de *	Matières incompatibles (donner 1 à 2 exemples)
Exemple : *Acide acétique concentré*	*1-Liquide et vapeur inflammable (catégorie 3)* *2- Brûlures de la peau et graves lésions des yeux (catégorie 1A)*	*1--Tenir à l'écart de toute source de chaleur (flamme,…)* *2-Travailler sous sorbonne pour se protéger des vapeurs corrosives* *2-Porter des gants adaptés*	*Les matières oxydantes (ex : permanganate de potassium)*
		!!!!Select the most important information for your use!!!	

RISQUES LIÉS AUX MACHINES (compléter en séance)

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