université PARIS-SACLAY
FACULTÉ DE PHARMACIE

FACULTÉ DE PHARMACIE
Henri MOISSAN, ORSAY

Identification number :

Last NAME :

First Name :

MASTER 1: Development of Drugs and Health Products

TU09 Analytical sciences

Module A

Exam (no documents allowed)
January 27, 2025 from 10:00 to 12:00

INSTRUCTIONS ON HOW TO WRITE ANSWERS

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A. SEPARATION TECHNIQUES (1 hour: 10 points/20)

Electrophoresis (D. Mai)

Multiple choices questions

1) Which of the following statements regarding electrophoresis are correct?

- ■ A. Electrophoretic separation performance is influenced by the temperature.
- ☐ B. Capillary electrophoresis cannot be used for separations of anionic and cationic in a single run.
- ☐ C. Electrophoretic separation of weak acids is independent of the pH of the background electrolyte.
- ☐ D. Electrophoresis allows separation of inorganic ions but not for proteins nor for amino acids.

2) What are the detection mode(s) that can be used for detection of proteins after their separation by electrophoresis?

- ■ A. UV
- ■ B. LIF
- ■ C. Immunoassays
- ■ D. MS.

3) In capillary electrophoresis, the electrophoretic mobility of the analyte depends on:

- ■ A. The charge of the analyte
- ■ B. The size of the analyte
- ■ C. The length of the capillary
- ■ D. The voltage applied on the capillary.

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Gas chromatography and Pharmaceutical drug analysis (A. G. Ismail)

1) What property makes Gas Chromatography unsuitable for certain analytes?

☐ A. Thermolability
☐ B. Low volatility
☐ C. High molecular weight
■ D. All of the above.

2) What does robustness testing assess in an analytical method?

■ A. Consistency of results under deliberate variations in parameters
☐ B. Precision across multiple labs
☐ C. Minimum quantifiable analyte amount
☐ D. Speed of the method.

3) What does the parameter 'specificity' refer to in method validation?

■ A. The ability of a method to measure the analyte in the presence of impurities
☐ B. The consistency of results across different analysts
☐ C. The ability to measure low concentrations of an analyte
☐ D. The accuracy of the instrument calibration.

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Analysis of chiral substances (T. Tran)

Multiple choices questions

1) Which technique(s) can be used to differentiate two enantiomers*?*

- ■ A. Capillary electrophoresis
- ■ B. High performance liquid chromatography
- ☐ C. UV spectroscopy
- ■ D. Gaz chromatography
- ■ E Polarimetry
- ■ F. Calorimetry.

2) What is the principle of a chiral separation? Choose the right item(s).

- ☐ A. Formation of enantiomers
- ■ B. Formation of diastereoisomers
- ■ C. Use of a chiral selector
- ☐ D. Use of a racemic selector.

3) The development of a chiral separation method depends on:

- ☐ A. The sample purity
- ■ B. The acido-basic properties of the analyte
- ■ C. The hydrophobicity of the solute
- ■ D. The charge of the enantiomers.

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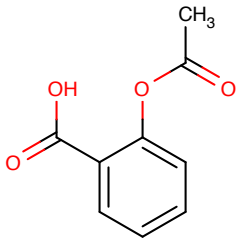
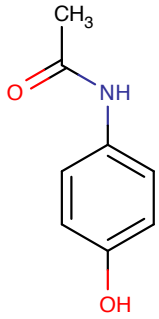
Chromatography exercise (A. Kasselouri)

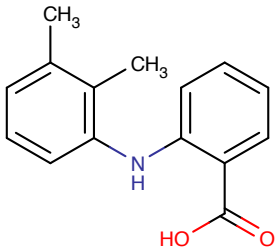
A recent article describes the separation of four active ingredients: acetylsalicylic acid, paracetamol, mefenamic acid and cetirizine (Figure 1). The chromatographic conditions used for this separation are: Column: NUCLEODUR® Gravity C18, L=125mm, i.d.=4,6 mm, packed with 5µm particles; Mobile phase: acetonitrile:buffer (20 mM sodium phosphate, pH=6,5) 60:40 (v/v); Flow rate: 1mL/min.



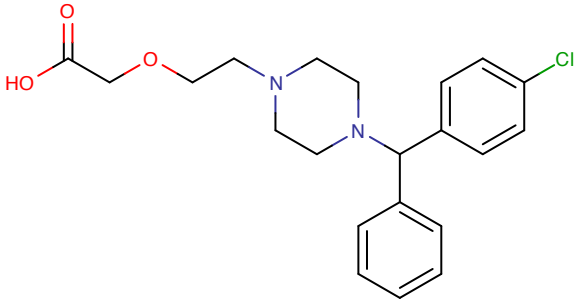
Acetylsalicylic acid



Paracetamol



Mefenamic acid



Cetirizine

Figure 1 : *Compound structures*

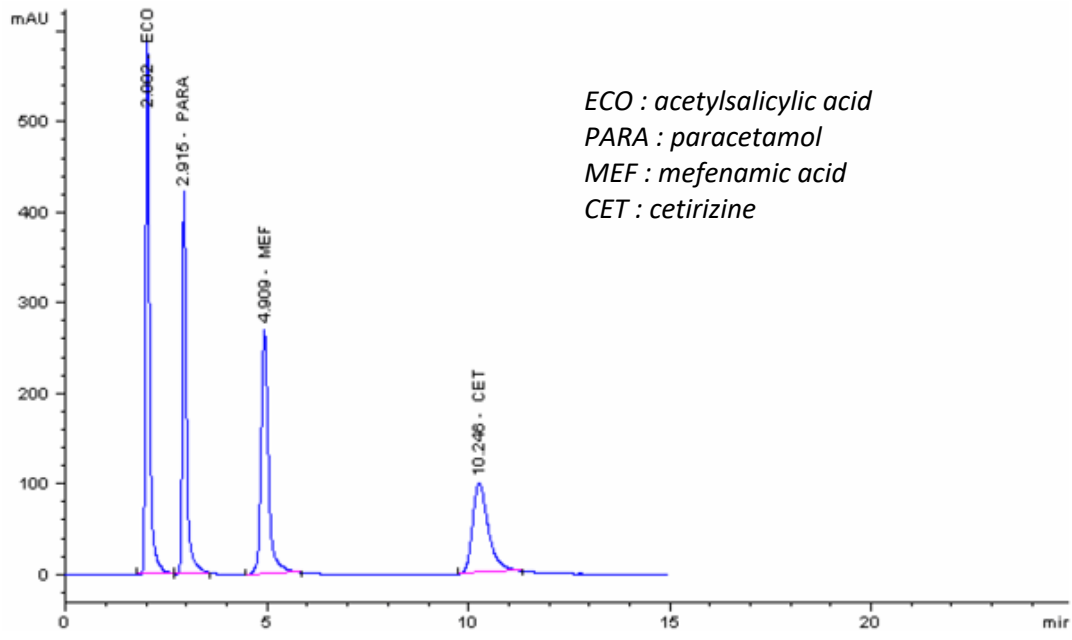


Figure 2: *Chromatogram*

The obtained chromatogram is shown on figure 2 and the data for the chromatogram on table 1.

Compound	t_R (min)	$\omega_{0,5}$(min)
Acetylsalicylic acid	2,01	0,138
Paracetamol	2,92	0,112
Mefenamic acid	4,91	0,195
Cetirizine	10,2	0,417

Table 1: Data of Figure 2

t_R is the retention time and $\omega_{0,5}$ is the width of the peak at half of its maximum height. Dead time, evaluated in a previous analysis using uracil, is **t_M=1,50 min**. The back pressure (ΔP) is 140 bar.

1. Calculate the retention factors (k) of Acetylsalicylic acid and Cetirizine.

$$k = \frac{t_R - t_M}{t_M}$$

$$k_{Acetylsalicylic\ acid} = \frac{2{,}01min - 1{,}5min}{1{,}5min} = 0{,}34$$

$$k_{Cetirizine} = \frac{10{,}2min - 1{,}5min}{1{,}5min} = 5{,}8$$

2. Calculate the resolution (R) between Paracetamol and Acetylsalicylic acid. Are the two compounds well separated?

$$R_S = 1{,}18\frac{\left(t_{R(2)} - t_{R(1)}\right)}{\left(\omega_{0,5(1)} + \omega_{0,5(2)}\right)}$$

$$R_S = 1{,}18\frac{2{,}92min - 2{,}01min}{0{,}138min + 0{,}112min} = 4{,}3$$

Resolution is superior to 1,5 (R=4,3>1,5), so the compounds are well separated. Furthermore, we can see at Figure 2 that there is no overlap between the peaks.

3. Calculate the column efficiency (N) and evaluate the plate height (H) using the peak of Mefenamic acid.

$$N = 5{,}54\left(\frac{t_R}{\omega_{0,5}}\right)^2 \Rightarrow N = 5{,}54\left(\frac{4{,}91\ min}{0{,}195\ min}\right)^2 = 3512$$

$$H = \frac{L}{N} \Rightarrow H = \frac{125\ mm}{3512} = \frac{125000\ \mu m}{3512} = 35{,}6\ \mu m$$

4. We can use another column with L=50mm, keeping all the other operating conditions similar to the conditions in Figure 2. How this will affect retention factors (k), column efficiency (N), and resolution (R)? Explain.

Decrease of column length decreases dead time and retention time (both proportional to L). Retention factors remain unchanged: calculated as the ratio $k=(t_R-t_M)/t_M$.

We consider that column composition and manufacturing did not change so the plate height (H) is the same for the new column. We also know that N=L/H. So N is proportional to L and will decrease for the new column: $\frac{N_{new}}{N} = \frac{L_{new}}{L} = \frac{50\ mm}{125mm} = 0{,}4$

Resolution decreases as a consequence of efficiency (N) decrease.

5. Propose a mobile phase modification to increase retention factors (k). Justify.

To change the retention factor (for the same column and the same solutes) we need to modify mobile phase composition.
The column used here is a non-polar C18 column. The type of chromatography is Reversed Phase liquid chromatography (RP-HPLC). In order to increase the retention, we have to choose a more POLAR mobile phase. The mobile phase can become more polar by increasing the percentage of the aqueous buffer (and so decrease the percentage of the organic solvent). We can propose for example to use a mobile phase acetonitrile:buffer 50:50 (v/v), instead of 60:40 (v/v).

A second approach (not required in this answer) is to decrease the buffer pH: at pH=6,5 the studied acid solutes are ionized; but at lower pH they will be un-ionized, so less polar and more retained on the non-polar stationary phase. This approach is less used than the first one as method development is more complex.

List of equations:

$$k = \frac{\mathrm{t_R} - \mathrm{t_M}}{\mathrm{t_M}}$$

$$N = 16\left(\frac{t_R}{\omega}\right)^2 \quad or \quad N = 5{,}54\left(\frac{t_R}{\omega_{0,5}}\right)^2$$

$$R_S = 2\frac{\left(t_{R(2)} - t_{R(1)}\right)}{\left(\omega_{(1)} + \omega_{(2)}\right)} \quad or \; R_S = 1{,}18\frac{\left(t_{R(2)} - t_{R(1)}\right)}{\left(\omega_{0,5(1)} + \omega_{0,5(2)}\right)}$$

$$\Delta P = \frac{\eta\, u\, \Phi_r L}{d_p^2}$$

$logk = logk_w - S\varphi$ (S=3 for methanol and acetonitrile)

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Modules B and C

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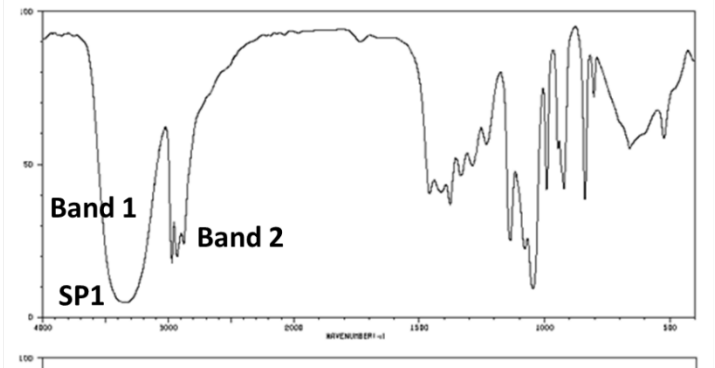
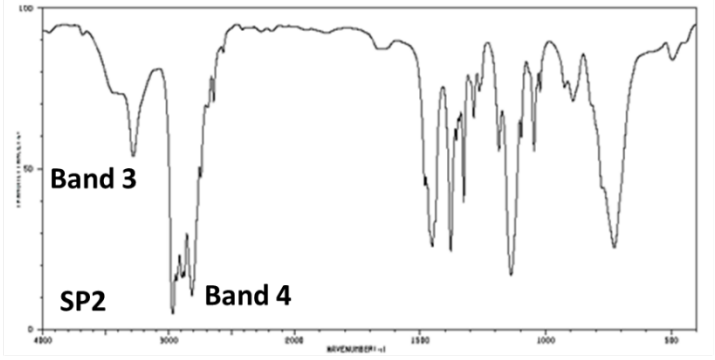
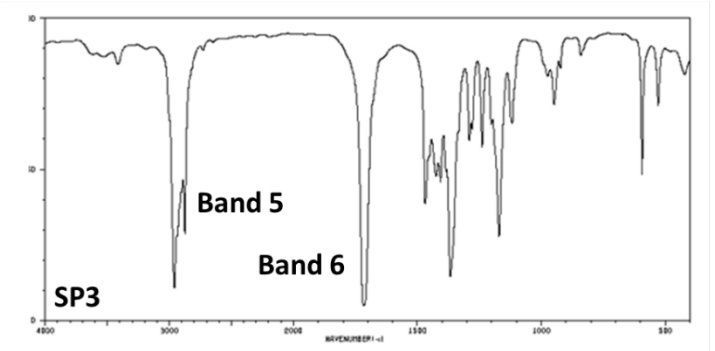
B. SPECTRAL METHODS (45 min: 7.5 points /20)

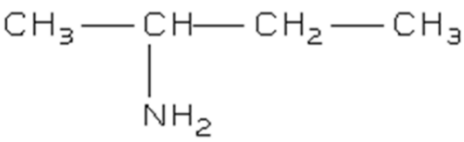
Mid infrared spectroscopy exercise (A. Tfayli)



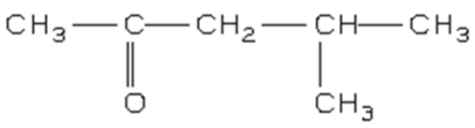




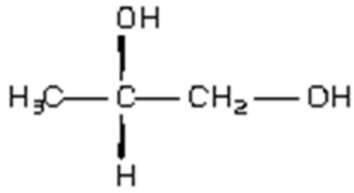
Mol A



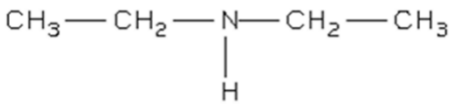
Mol B



Mol C



Mol D



1- Link the right spectrum to the right molecule:

- Sp 1: Mol C
- Sp 2: Mol D
- Sp 3: Mol B

2-Give the right band assignment among the following list:

a: OH stretching; **b:** OH bending; **c:** NH_2 stretching; **d:** C=O stretching; **e:** CH_2 and/or CH_3 stretching; **f:** CH_2 scissoring; **g:** NH stretching, **h:** C≡C stretching,

Band 1 a	Band 4 e
Band 2 e	Band 5 e
Band 3 g	Band 6 d

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NMR spectroscopy test

We study the nucleoside **Uridine** by ^{1}H NMR spectroscopy; which molecular structure is presented on **Figure 1**. Its 600.13MHz NMR ^{1}H spectrum obtained when dissolved in deuterated water is presented on the **Figure 2**. Note that within the sampling conditions used, NMR signals of amide and alcohols are not observed on the spectrum. The experimental spectral error is ±0.1Hz. **The study here is focused only on H2, H3, H4, H5, H10 and H11**. Protons H14 are fully disregarded on purpose from this study.

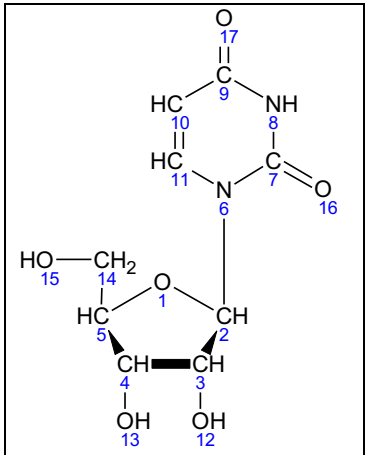

Figure 1 : *Molecular structure of Uridine and atom numbering*

Q1. The spin number of the isotope ^{2}H is 1. Justify that the spin number of the ^{2}H isotope is an integer. Precise the number and relative position of ^{2}H energy levels : i) in absence of any static magnetic field; ii) in presence of a static magnetic field denoted $\overrightarrow{B_0}$.

Q2. According to the **Figure 1**, justify that the nuclei denoted H_2, H_3, H_4 and H_5 are not chemically equivalent.

Q3. According to the **Figure 1**, indicate the spectral multiplicity expected for nuclei denoted H10, H11 and H2. Justify your answer.

Q4. The signal **(a)** observed around 7.82 ppm correspond to one of both ethylenic protons (H10 or H11). Determine the value of their mutual *J*-coupling, denoted $J_{10\text{-}11}$, in Hertz.

Q5. The signal **(a)** observed around 7.82 ppm correspond to one of both ethylenic proton (H10 or H11), and the signal **(b)** observed around 5.85 ppm correspond to the superposition of the signals of two protons : one is H2, the other one is the ethylenic proton which is not resonating around 7.82ppm. Determine with the best precision the *J*-coupling value between H2 and H3.

Q6. Complete the **Table 1** according to the ^{1}H NMR spectrum analysis.

Table 1 *: NMR data of the compound* ***Uridine*** *dissolved in deuterated water measured on a 600.13MHz NMR spectrometer.*

Signals	**H10**	**H11**	**H2**	**H3**	**H4**
Frequencies ν_i (Hertz)					
Chemical shifts δ_i (ppm)					
Integrals					
Apparent spectral multiplicity					
Numbers of *J*-coupling partners					
Experimental *J*-coupling values (Hertz)					
Actual spectral multiplicity					

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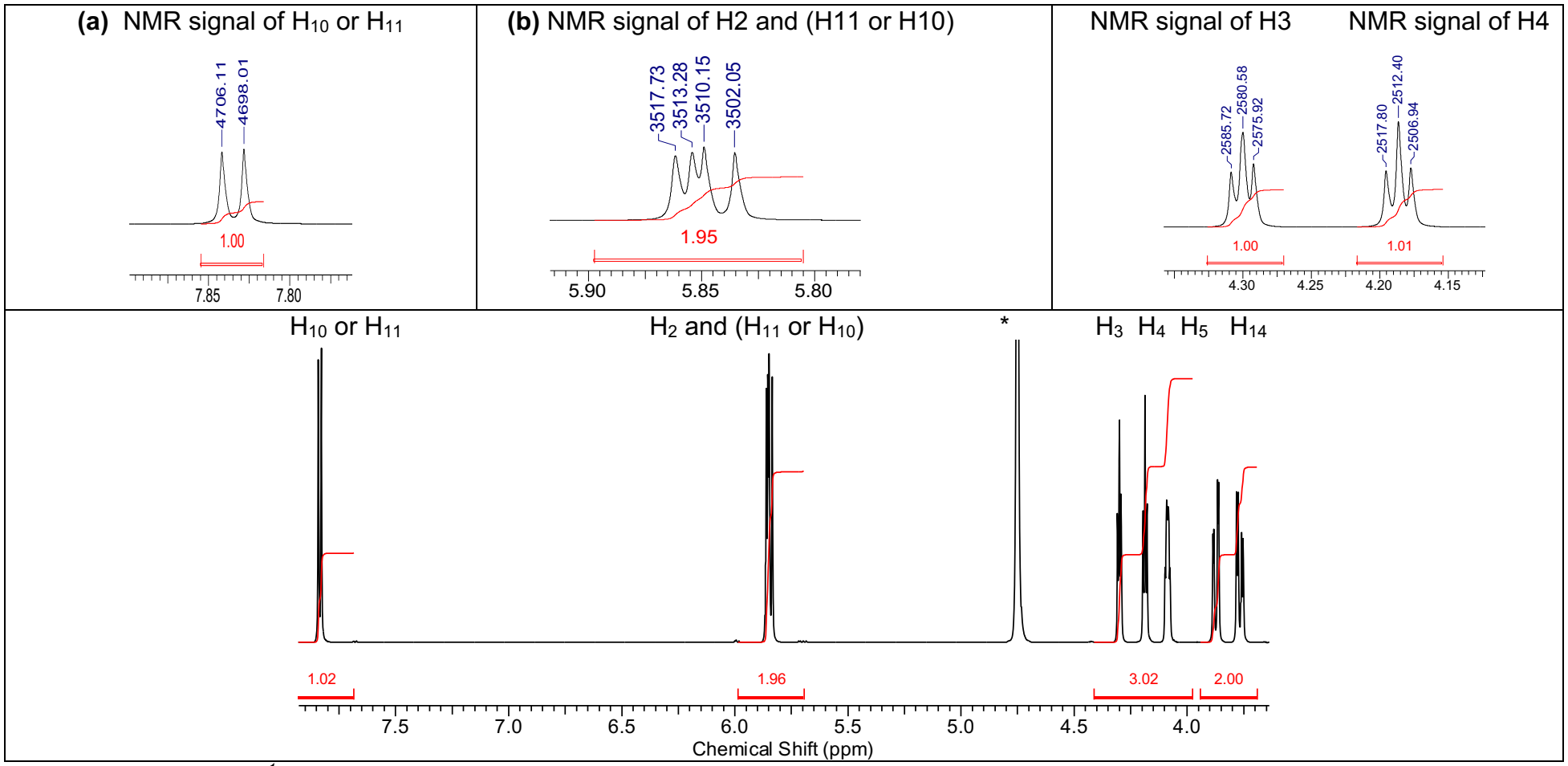

***Figure 2** : 600.13MHz ^{1}H NMR spectrum of the compound **Uridine**, and zooms for the regions of interest. The solvent of the NMR sample is deuterated water (signal not integrated). The values of integrals are the data reported below the signals. The horizontal scale is done in ppm in the full spectrum and The peak picking is done in Hertz in the zooms.*

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NMR spectroscopy test (C. AROULANDA

Question 1 (Q1)

Question 2 (Q2)

Question 3 (Q3)

Identification number: .....

Question 4 (Q4)

Question 5 (Q5)

Question 6 (Q6) : complete the table 1

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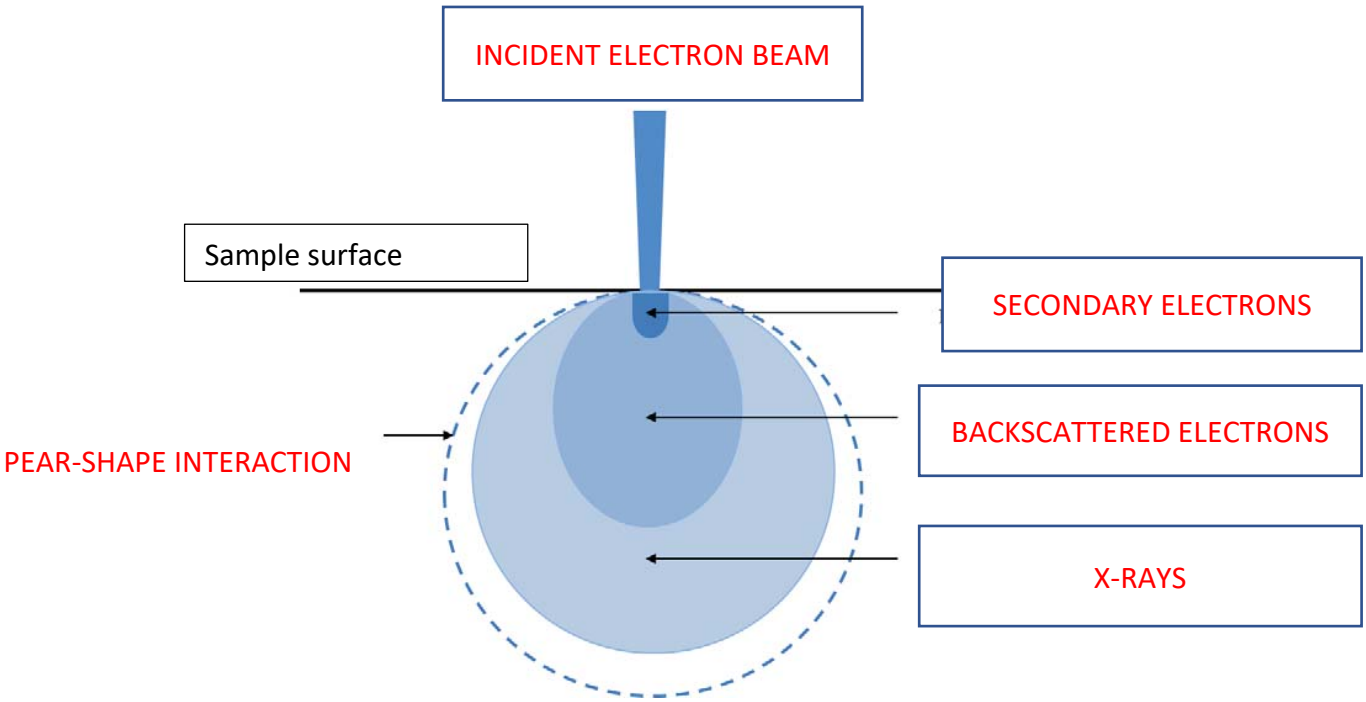
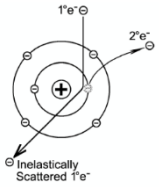
Scanning Electron Microscopy (SEM) - 10 min (C. AYMES CHODUR)



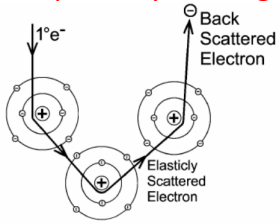
1- Feel in the blanks and give a name to the volume defined by the dashed line.

2- Choose one interaction between the incident electron beam and the sample and describe it.

Secondary electrons : ineslastic events : the energy is transferred from the incident electrons to the sample producing secondary electrons. Those are weekly bonded electrons. Electrons are deflected by the electric field of an atom causing the electrons to deviate from its previous path onto a new trajectory.



Backscattered electrons : elastic events : no energy is transferred from the electrons to the sample, only a change in direction of the electron beam (deflection back out of the specimen).



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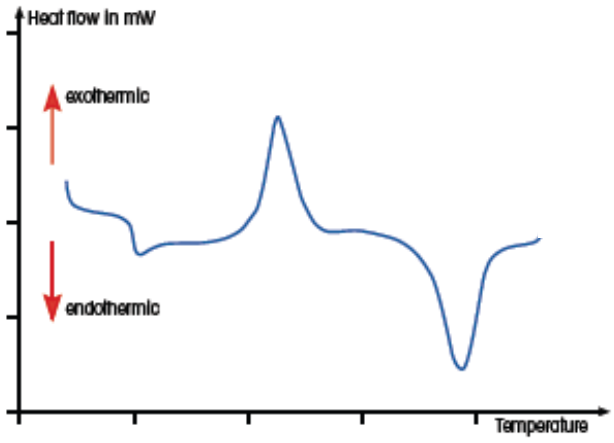
C. THERMAL ANALYSIS (15 min, 2.5 points/20) (C. AYMES-CHODUR)

A) DSC

Plot the DSC thermograms of a PET sample, extracted from a plastic bottle:

- A) As received at 10°C/min (First heating scanning curve)
- B) After the first heating/cooling program at 10°C/min (Second heating scanning curve)

A) As received at 10°C/min (First heating scanning curve)
Tg,/ Cold crystallization /melting peak



B) After the first heating/cooling program at 10°C/min (Second heating scanning curve)
No more Cold crystallization : Tg/ melting peak more important in surface.

You will give a brief explanation for each curve.

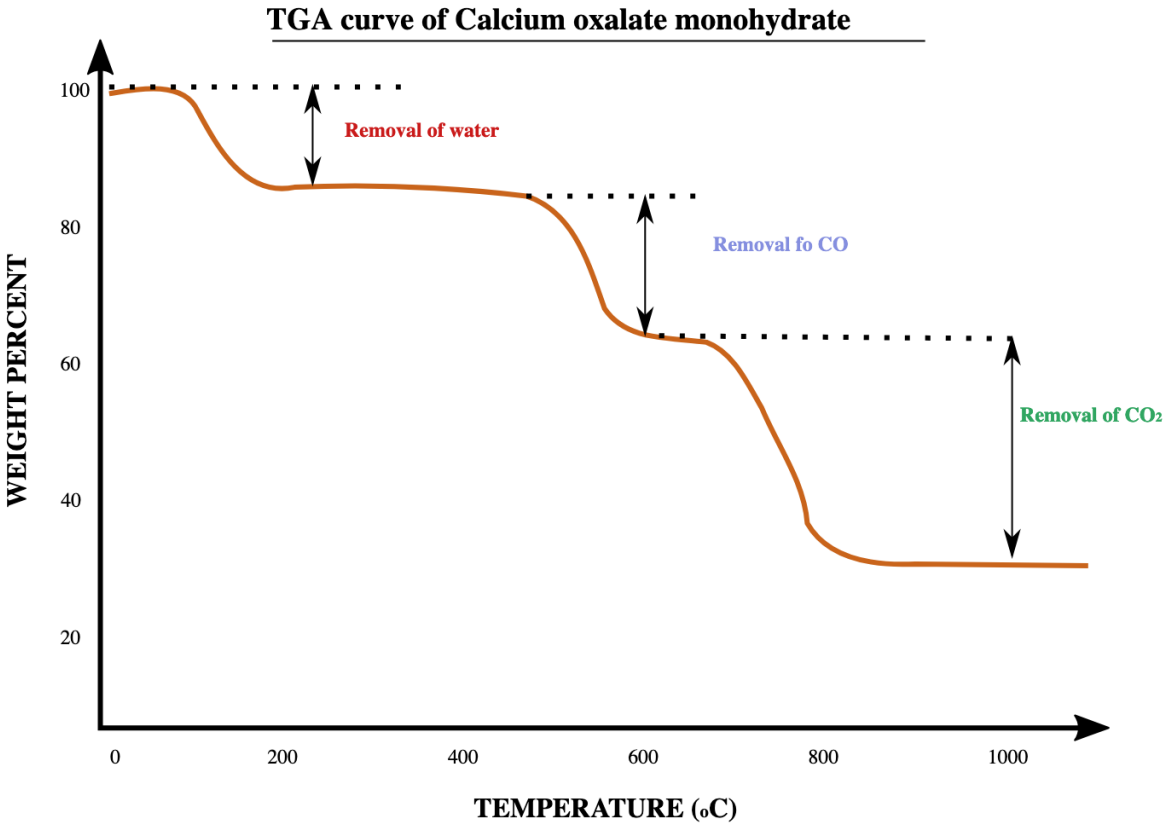
Define the sign of the transition (exo/endothermic), define the transitions (Tg, cold crystallization, melting)

After heating/cooling cycle at low scanning rate, the cold crystallization disappears because the system had enough time to melt and recrystallize slowly before the 2nd heating treatment.

B) TGA

Here is the TGA curve of CaC_2O_4 at 10°C/min.

Comment the curve and explain how to get the identification of the 3 volatile components.



3 decomposition steps of volatile compounds

1) at around 100°C corresponding to adsorbed water (around 18 % in weight). After that step, CaC_2O_4 is no longer hydrated;

2) at around 500°C, removal of CO and formation of $CaCO_3$ (around 22 % in weight).

3) at around 700°C, removal of CO_2 and formation of CaCO (around 28 % in weight).

At 1000°C the weight % is not back to 0 which means all the compound is not degraded (30%)