université PARIS-SACLAY
FACULTÉ DE PHARMACIE

FACULTÉ DE PHARMACIE DE CHÂTENAY-MALABRY

Identification number :

Last NAME :

First Name :

MASTER 1: Development of Drugs and Health Products

TU09 Analytical sciences

Exam (no documents allowed)
February 4, 2021 from 13:30 to 15:30

INSTRUCTIONS ON HOW TO WRITE ANSWERS

- FILL IN VERY CAREFULLY THE HEADINGS ON THIS FIRST PAGE WHICH ALLOWS YOUR COPY TO REMAIN ANONYMOUS.

- NO MENTION OR SIGN OF RECOGNITION MUST BE INDICATED ON THE FOLLOWING PAGES, UNDER PENALTY OF NULLITY

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

Attention: Notation of multiple choices questions: For each question: No difference with the correct response=100% of the note; one difference=50%; two differences= 20%; three or more or no response=0%.

Electrophoresis

1) ***What are the mode(s) of electrophoresis that allow(s) the separation of proteins by their sizes?***

☐ A. Native PAGE
☐ B. SDS-PAGE
☐ C. Isoelectric focusing (IEF)
☐ D. 2D Electrophoresis

2) ***What are the mode(s) of electrophoresis that allow(s) the separation of proteins by their charges?***

☐ A. Native PAGE
☐ B. SDS-PAGE
☐ C. Isoelectric focusing (IEF)
☐ D. 2D Electrophoresis

3) ***We use a zone capillary electrophoresis (CZE) setup with untreated fused silica capillaries. What are the parameters that influence the electroosmotic flow (EOF)?***

☐ A. The charge of the capillary's internal surface
☐ B. The pH of the buffer (background electrolyte)
☐ C. The ionic strength of the buffer
☐ D. The viscosity of the buffer

Protein analysis and analysis of drugs and macromolecules

4) Critical aspects for protein analysis using size exclusion chromatography are:

☐ A. Define the permeation range of the column under defined conditions
☐ B. Avoid interactions protein - stationary phase
☐ C. Form aggregates during the analysis
☐ D. Insure that interactions protein – excipients occurred during the analysis

5) ***Which chromatography mode can be used for detecting charged variants induced by degradations (deamidation, oxidation) or glycosylation?***

☐ A. Size exclusion chromatography
☐ B. Reverse phase chromatography
☐ C. Ion exchange chromatography
☐ D. Hydrophobic interaction chromatography
☐ E. Hydrophilic interaction chromatography

6*) Different approaches for chiral separations:*

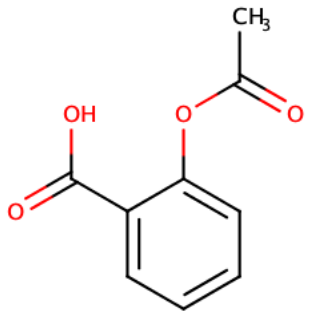
☐ A. Chromatographic enantiomer separations can be carried out directly using chiral selectors incorporated either in the stationary phase or mobile phase to form diastereomeric derivatives.
☐ B. Chromatographic enantiomer separations can be carried out indirectly by using chiral derivatization reagents to form diastereomeric derivatives
☐ C. Indirect and direct ways are possible in CE
☐ D. Only indirect way is possible in CE

7) ***Enantiomer separation using cyclodextrins (CDs) in CE***

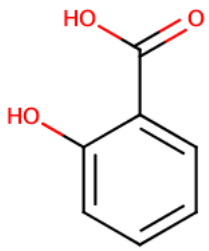
☐ A. The chiral recognition mechanism is based on inclusion of a hydrophobic group of the analyte into the cavity of the CD
☐ B. The chiral recognition is based on the possibility to form hydrogen bonds between the hydroxyl groups at the mouth of the CD and polar substituents close to the chiral center of the analyte
☐ C. Only neutral substituted CDs can be used
☐ D. γCD-derivatives are the most frequently used for molecules bearing aliphatic or substituted aromatic group.

Chromatography exercise

A mixture of salicylic acid and acetylsalicylic acid (standard solution **(b)**) was analyzed by Reversed Phase LC. In the same conditions a standard solution containing only salicylic acid (standard solution **(a)**) was analyzed.



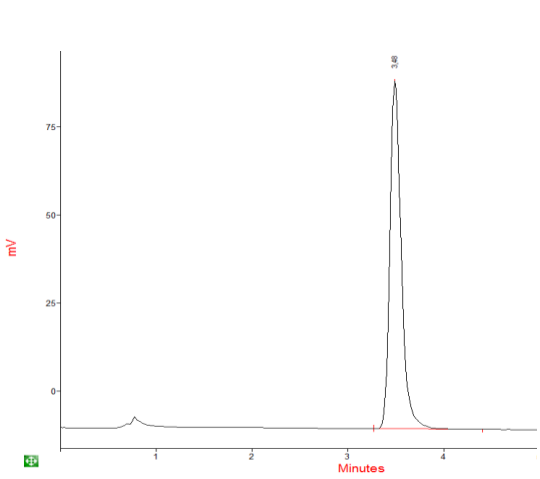
Acetylsalicylic acid
logP=1,24; pKa=3,41



Salicylic acid
logP=1,98; pKa=2,79

Figure 1 : Compounds structure

The operating conditions are: *Column: Kromasil C18 ; L=12,5 cm ; i.d.=4,0 mm ; dp = 5 µm;* Mobile phase: *methanol / 0,1% phosphoric acid (50/50 v/v); Flow rate: 1,2 mL/min; Detection: 237 nm.*
The obtained chromatograms are shown on figure 2 and the corresponding data on table 1.



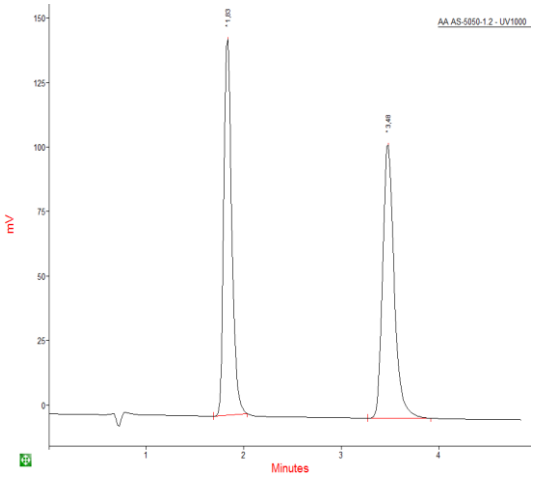


Figure 2: Standard solution **(a)** Standard solution (**b**)

	Standard (**a**)			Standard (**b**)		
	t_R (min)	**$\omega_{0,5}$** (min)	**Area**	**t_R** (min)	**$\omega_{0,5}$** (min)	**Area**
Acetylsalicylic acid	-	-	-	1,83	0,09	831,02
Salicylic acid	3,48	0,12	807,73	3,48	0,12	871,18

Table 1

t_R is the retention time and $\omega_{0,5}$ is the width of the peak at half of its maximum height and Area the peak area.

Dead time, determined by injection of a non-retained compound, is t_M=0,75min.

1. Calculate, for standard (**b**) chromatogram, the retention factors (k) for the 2 compounds and as well as the selectivity (α) and resolution (R) between them.

2. Concentration of salicylic acid in standard solution (**a)** is 0,01g/L. Calculate salicylic acid concentration in standard solution **(b)**.
3. We increased the methanol percentage to 60%. How would this affect dead time and retention time?
4. Would a gradient mode be useful for this chromatographic separation or an isocratic mode is sufficient? Explain.

List of equations:

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

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

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

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

$$logk = logk_w - S\varphi \quad \text{(S=3 for methanol)}$$

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

True or false (5 points):

1. Near infrared spectroscopy is based on light diffusion while mid infrared spectroscopy is based on absorption.
   True ☐ false ☐
2. Only the vibrations inducing changes in the dipolar moment can be detected in infrared
   True ☐ false ☐
3. The light absorption by water molecules limits the application of near infrared spectroscopy on aqueous solutions
   True ☐ false ☐
4. The energy necessary to pass an electronic level is 1/10 lower than that necessary for a vibrational transition.
   True ☐ false ☐
5. Beer's law can be applied in UV -visible, mid-infrared and near-infrared absorption spectroscopies
   True ☐ false ☐
6. In UV-Visible spectroscopy, the transmittance is directly proportional to the concentration.
   True ☐ false ☐
7. The X-rays are made of energy quanta called photons
   True ☐ false ☐
8. The wavelength of X rays is between 10^{-8} to 10^{-11} m
   True ☐ false ☐
9. The energy of X-rays is much lower than infrared
   True ☐ false ☐
10. X-rays diffraction arises from constructive interferences between the radiation diffracted by the periodically arranged atoms of molecules composing matter
    True ☐ false ☐
11. X-ray Diffraction is mainly devoted to determination of crystalline structures at micrometric scale
    True ☐ false ☐

12. STM technique is based on tunneling effect that allows the passage of electrons between the tip and the surface, creating an electric current.
    True ☐ false ☐

13. In mass spectrometry, the Maldi ionization is considered as a soft technique
    True ☐ false ☐

14. In mass spectrometry, different types of analyzers can be used, for example: Quadrupole, Time-of-Flight or ion trap
    True ☐ false ☐

15. Electron ionization and chemical ionization can be used when the mass spectrometer is coupled to Gaz chromatography
    True ☐ false ☐

NMR Spectroscopy (2.5 points)

All answers have to be properly justified

Exercice 1

1) In the **Table 1** are gathered several isotopes of chemical elements identified following the official nomenclature by the symbol ${}^{A}_{Z}X$, where A is the mass number of the chemical element X, and Z its number of protons. In the **Table 2**, are indicated different nuclear spin values.

Associate a nuclear spin value found in **Table 2** to each isotope of **Table 1**.

${}^{28}_{14}Si$	${}^{29}_{14}Si$	${}^{6}_{3}Li$	${}^{31}_{15}P$

***Table 1** : Several isotopes of chemical elements identified following the official nomenclature by the symbol (${}^{A}_{Z}X$, where A is the mass number of the chemical element* X*, and Z its number of protons*

0	1/2	1

***Table 2** : Different nuclear spin values*

2) Boron possess different isotopes, among which one of them has a nuclear spin number equal to 3/2.

Indicate for this isotope in the two following cases (denoted **a** and **b**), the number of nuclear energy levels and their relative position in term of energy:

a) absence of any homogeneous and static magnetic field

b) in the presence of an homogeneous and static magnetic field denoted $\boldsymbol{B}_0$.

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

DSC : High density polyethylene (HDPE) is analyzed by Differential Scanning Calorimetry (DSC).

A glass transition at -125°C and a melting peak at 135°C are highlighted.

1) Draw the thermogram corresponding to the first temperature rise program.
2) Are these transitions of the first or second order?
3) Are they reversible or irreversible?

TGA : 1) What is the main information given by TGA? Briefly recall the principle.

2) In order to identify the volatile compounds emitted during a TGA experiment, which couplings with other analytical techniques can be set up ?