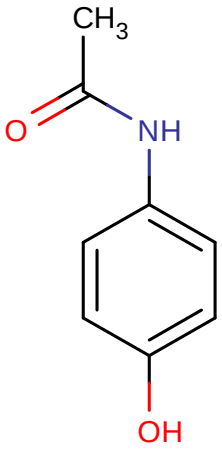
TU09: ANALYTICAL SCIENCES 1

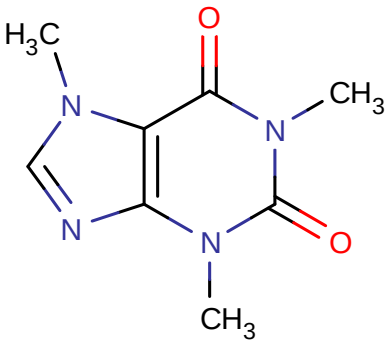
Tutorial 1 : Exercises

Liquid chromatography

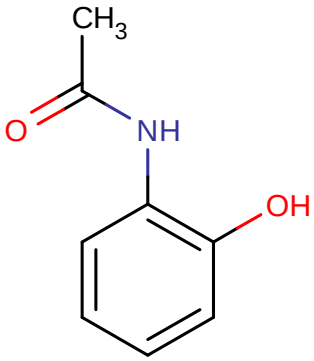
A mixture of analgesics (Figure 1) is analysed by liquid chromatography. The chromatographic conditions are: Column: NUCLEODUR Gravity C8 ; L=12,5 cm ; i.d.=4,0 mm ; dp = 5 µm; Eluent: methanol / 0,1% phosphoric acid (40/60 v/v); Flow rate : 1mL/min ; Detection UV : 240 nm ; Temperature 25°C. Pressure 80,4 bar.



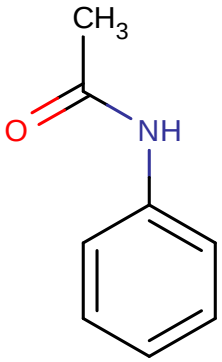
1. Paracetamol
logP=0,91; pKa=9,5



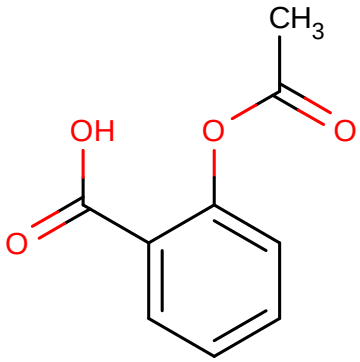
2. Caffeine
logP=-0,55



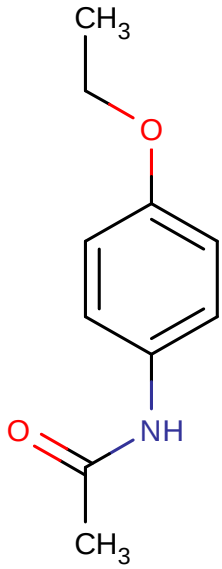
3. 2-acetamidophenol
logP=0,91; pKa=8,8



4. Acetanilide
logP=1,21



5. Acetylsalicylic acid
logP=1,24; pKa=3,4



6. Phenacetin
logP=1,41

Figure 1 : Analgesics structures; logP and pKa are calculated using MarvinSketch.

The chromatograms obtained after analysis of a) Thomapyrin® tablets and b) a solution of standards are shown in figure 2.

a) Thomapyrin® tablet; b) standard

Thomapyrin® is a trademark of Boehringer Ingelheim Pharma KG

Peaks:

1. Paracetamol
2. Caffeine
3. 2-Acetamidophen
4. Acetanilide
5. Acetylsalicylic acid
6. Phenactin

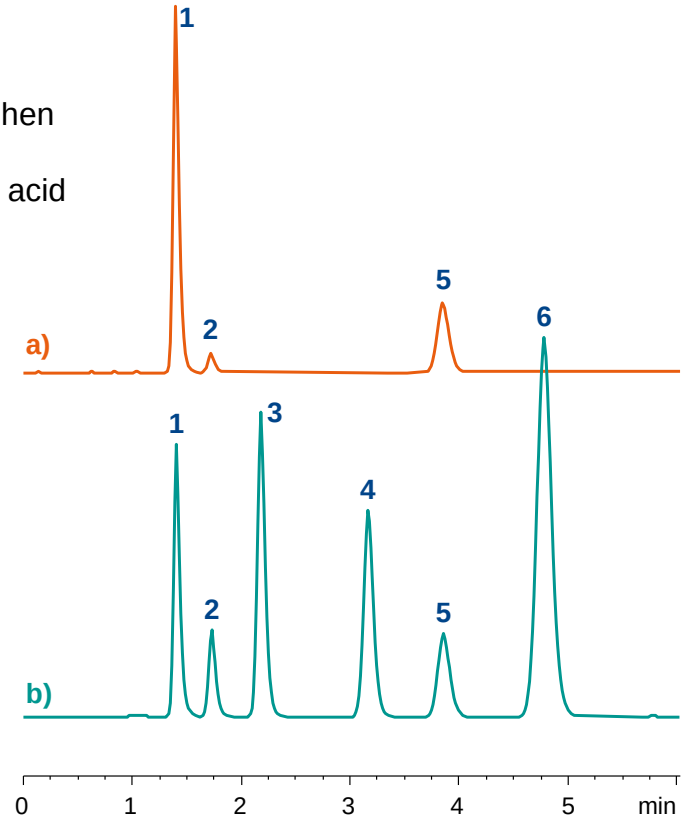


Figure 2 (MN Appl. No. 118600, Macherey-Nagel, 2011, www.mn-net.com**)**

The chromatogram of the mixture of standards (b) provided the following data:

	t_R (min)	$\omega_{0.5}$ (min)
1. Paracetamol	1,40	0,057
2. Caffeine	1,75	0,071
3. 2-Acetamidophenol	2,20	0,072
4. Acetanilide	3,17	0,086
5. Acetylsalicylic acid	3,83	0,116
6. Phenacetin	4,77	0,143

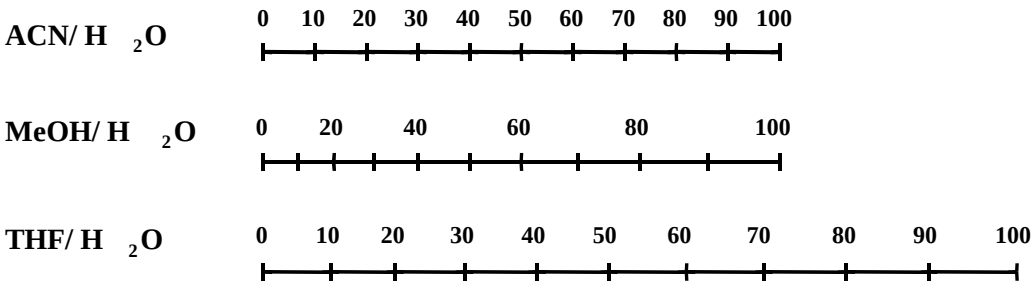
t_R is the retention time and $\omega_{0.5}$ is the width of the peak at half of its maximum height.

1. What is the type of HPLC used ? (stationary phase, mobile phase, retention process).
2. Explain why phosphoric acid is added in the mobile phase.
3. Explain the order of elution of the studied drugs.
4. Indicate which drugs are present in Thomapyrin® tablets.
5. Calculate column dead volume and dead (void) time, taking into account that column porosity is ε=0,73.
6. Calculate the retention factors (k) for the 6 drugs and as well as the selectivity (α) and resolution (R) between consecutive peaks.
7. Calculate from the peak of acetylsalicylic acid (5) the number of theoretical plates (N) and the plate height (H) for the column.
8. Flow rate is increased to 1,5 mL/min. How will this increase affect the retention times, retention factors, resolution and back pressure values?
9. We change the solvent strength by decreasing methanol content to 30%. Calculate the effect on retention factors (k).
10. We want to change solvent selectivity (without changing solvent strength). List two mobile phase compositions that can be used.
11. Acetylsalicylic acid concentration in the mixture of standards is 60mg/L and the corresponding peak area (peak 5) in the chromatogram of standards is 689. Twenty Thomapyrin® tablets were ground and well mixed, and 20mg of the powder mixture was dissolved into 20mL of water. 1 mL of this solution was diluted in a 10mL volumetric flask. The solution was analyzed and the peak area of acatylsalicylic acid (5) is 591. Calculate the quantity of acetylsalicylic acid contained in one Thomapyrin® tablet, knowing that a Thomapyrin® tablet weights 500mg .

   Note : Previously, it has been established, that calibration curve is a straight line passing through the origin. Quantitation then can be performed using a single standard.

Data:

Solvent-strength nomograph for reversed phase HPLC



List of equations:
(The list below will be given for the exam)

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

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

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

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

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