Series N° 1

Exercise 1

The retention times of two natural products **A** and **B** in a mixture to be separated are 16.40 and 17.63 minutes, respectively, on a 30.0 cm column. A non-retained species passes through the column in 1.30 minutes. The peak widths (at the base) of A and B are 1.11 and 1.21 minutes, respectively.

Calculate:

a) The resolution of the column.

b) The average number of theoretical plates in the column.

c) The height equivalent to a theoretical plate.

d) The length of the column required to achieve a resolution of 1.5.

e) The time required to elute substance B from this column.

Exercise 2

We have a C18 silica column with the following characteristics:

Pore volume	0.25 mL/g
Specific surface area (S)	96.8 m^2
Average particle diameter (dp):	3µm
Maximum pressure drop	300 bars (30 106 Pa)
pH range	2-7.5
Temperature range	10-50 °C
Length	10 cm
Internal diameter	4.6 mm
Total porosity ε	0.8
Flow resistance	500

We are studying the separation of a series of drugs with a diffusion coefficient in the mobile phase $\mathbf{D_m=10^{-9} m^2/s}$. Two of the drugs we are particularly interested in have retention factors **k′ =2.8** and **k′=3.1**. We have established the Knox curve for these drugs, and it shows a minimum at **H=4** and **ν = 4**.

1. Calculate the dead volume of the column.
2. Calculate the optimal flow rate for this column when analyzing the drugs.
3. Calculate the dead time at this flow rate.
4. Calculate the number of theoretical plates and the column length with the same packing required to achieve a resolution of 1.5.

Table 1: Important Experimental Parameters in Chromatography

Name	Symbol of Experimental Parameter	Determined from
Dead time	t_m	Chromatogram
Retention time of species 1 and 2	t_{r1}, t_{r2}	Chromatogram
Corrected or Reduced Retention Time	$t_{r'}$	$t'_r = t_r - t_m$
Peak Width, Species 1 and 2	l_1, l_2	Chromatogram
Column Length	L	Direct Measurement
Flow Rate	F	Direct Measurement
Stationary Phase Volume	V_s	Filler Data
Column Volume	V_c	Data
Solute Concentration in Stationary and mobile Phases	C_m, C_s	Data
Diffusion Coefficient	D_m	Data
Particle Diameter	d_p	Data
Total Porosity	ε	Data

Table 2: Most Important Derived Parameters

Name	Calculation	Relation with Derived Parameters
Linear Velocity of the Mobile Phase	$\mu = \frac{L}{t_m} = \frac{v\,D_m}{d_p}$	
Mobile Phase Flow Rate	$D = \varepsilon . S . \mu$	
Total Porosity	$\varepsilon = \frac{V_m}{V_c}$	
Mobile Phase Volume	$V_m = \frac{t_m}{D}$	
Retention Factor	$K' = \frac{t_r - t_m}{t_m}$	$K' = K\,\frac{V_s}{V_M}$
Distribution Constant	$K = K'\,\frac{V_M}{V_s}$	$K = \frac{C_s}{C_m}$
Selectivity Factor	$\alpha = \frac{t'_{r_2}}{t'_{r_1}}$	$\alpha = \frac{k'_2}{k'_1}$
Resolution	$R = \frac{2(t_{r_2} - t_{r_1})}{\omega_1 + \omega_2}$	$R = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{K'_2}{1 + K'_2}\right)$
Number of Theoretical Plates	$N = 5.54\,\frac{t_r^2}{\delta^2}$ $N = 16\,\frac{t_r^2}{\omega^2}$	$N = 16\,R^2\left(\frac{\alpha}{\alpha - 1}\right)^2\left(\frac{1 + K'_2}{K'_2}\right)^2$
Theoretical Plate Height (HEPT or H)	$H = \frac{L}{N}$	$h = \frac{H}{d_p}$
Retention time	$t_r = \frac{16R^2H}{\mu}\left(\frac{\alpha - 1}{\alpha}\right)^2\left(\frac{1 + k'}{K'_2}\right)^3$	