# Exercises

## Exercise 01

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 02

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.

## Exercise 03

**1-** The following data was obtained from the separation of three constituents using an **18 m** capillary column.

| Constituent | Retention Time ($t_r$) | Peak Width (ω) |
|---|---|---|
| **A** | 7.83 | 0.11 |
| **B** | 8.06 | 0.16 |
| **C** | 8.22 | 0.16 |

**a-** Calculate the number of theoretical plates for each constituent and determine the average number of theoretical plates for the column.

**b-** Calculate the height equivalent to a theoretical plate (HETP) for the column.

**2-** During the chromatographic analysis of low molecular weight acids, butyric acid was eluted with a retention time of **7.44 min**. The dead time of the column is **0.27 min**. Calculate the retention factor (capacity factor) of butyric acid.

**Table**: Important Experimental Parameters in Chromatography

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