COMPUTER SIMULATION OF ORGANIC SUBSTANCES ANALYSIS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Introduction

Principle of chromatographic methods is separation of substances based on distribution between two phases, mobile and stacionary. Distribution is chracterized by distribution coefficient:

$K = c_S/c_m$ where c_S and c_m are concentrations of analyzed substance in stationary and mobile phase.

Elution time is a qualitative parameter of separation, it is the position of analyzed substance´s peak on the chromatogram. Height or area of the peaks represent quantitative parameter.

1. Qualitative analysis. Optimalization of organic substances mixture´s separation

a) Run the program HPLCESIM.exe. Copy all basic parameters (Column, Eluent, Inject, Sample, Detect) of given chromatographic separation of mixture of 5 organic substances into table 1.

Table 1

Type of column (type of stationary phase)	
Lenght of column (mm)	
Inner diameter of column (mm)	
Particles size of adsorbent (μm)	
Temperature (ºC)	
Eluent (type of mobile phase) and concentration (%)	
Flow rate (ml/min)	
Pressure (bar)	
Viscosity (N/m^2)	
Injected volume V (μl)	
Analysed substances	
Type of detector	
Width of detection cell (mm)	

b) Observe the influence of stationary phase on the separation of organic substances (Column)

Change the type of stationary phase gradually from C18 to C8 and to C2 (Column, Type). Observe the changes on the chromatogram (change the scale of time ax in aid of Detect, Interv). Copy the elution times of analyzed substances (Sample, Time) in table 2.

Table 2

Analyte	Elution time t (min)		
	C18	C8	C2
benzyl alcohol			
benzaldehyde			
benzonitrile			
benzene			
ethylbenzoate			

Draw a graphic representation of dependence of elution time (ax y) on lenght of the stationary phase carbon chain (ax x) and explain these dependencies.
After finishing the task, return to the original chromatographic record by pressing Esc or File, Restart.

c) Observation of the mobile phase influence on the separation of organic substances (Eluent)
Change the type of mobile phase: acetonitrile, methanol, tetrahydrofuran (Eluent, Modifier). Observe the changes on chromatogram and explain them.
Change the concentration (in %) of acetonitrile and water in mobile phase from 35% to 55% (w/v) of acetonitrile (Eluent, Begin). Copy elution times of analyzed substances (Sample, Time) into table 3.

Table 3

Analyte	Elution time t (min)		
	35% acetonitrile	45% acetonitrile	55% acetonitrile
benzyl alcohol			
benzaldehyde			
benzonitrile			
benzene			
ethylbenzoate			

Draw a graphic representation of dependence of elution time (ax y) on concentration of acetonitrile in mobile phase (ax x) and explain these dependencies. Relatice permitivity of acetonitrile is 37,5 and of water 80,37.

2. Quantitative analysis. Determination of uknown concentration of chosen substance in mixture

a) Observe the influence of the wavelenght of adsorption photometric detection on the size of detection signals (peaks) of separated substances. Change the wavelenght from 220-300 nm (Detect, Wave). Copy areas of peaks of analyzed substance (Sample, Area) in table 4.

Table 4

	Area of peak (mAUs)					
Analyte (100 mg/ml)	220 nm	240 nm	254 nm	260 nm	280 nm	300 nm

Draw a graphic representation of peak area of analyte (ax y) on wavelenght (ax x) and name this dependence. According to this dependency choose the otimal wavelenght for the determination of your analyte.

b) Observe the influence of the concentration of analyzed substance on the size (height, area) of the peak. Change the concentration from 10 to 100 mg/l (Sample, Concentration, number of analyte). Copy areas of peaks of analyzed substance (Sample, Area) in table 5.

Table 5

	Area of peak (mAUs)									
Analyte (mg/l)	10	20	30	40	50	60	70	80	90	100

Draw a graphic representation of peak area of analyte (ax y) on concentration (ax x) and name this dependence. Determine the concentration of the substance with peak area 378 mAUs using this dependence.
End the program (Files, Quit, Yes)