Simulation Report

Student Name 1: Date: 10/01/25

Student Name 2:

1. Analysis of a mixture of parabens (mix. 1)

Question 1: Predict the elution order of parabens and compare with the obtained chromatogram. Why dead time is evaluated by uracil?

More hydrophobic compounds have higher LogP values and are more retained by C18 (non-polar) stationary phase.

We predict the order of elution according to LogP:

1. Methylparaben 2. Ethyl paraben 3. Propylparaben 4. Butylparaben

The same order is obtained experimentally. The reason is that the four compounds are homologues and have similar polar groups, they only differ in the length of the aliphatic chain.

Uracil is a very polar solute (LogP =-0,86), so unretained by C18 stationary phase, so it is used to evaluate t_M.

Question 2: Explain how you choose the optimum wavelength.

Parabens have similar spectra because they all have the same chromophore group.

We choose as an example ethyl paraben in the spectra window. The spectrum is typical of benzene derivatives. We look for the wavelength of maximum absorption of the B band. We found λ around 260nm.

Question 3: Describe how mobile phase composition in RP-HPLC affects retention and separation: `

	Acetonitrile 50% pH=7	Acetonitrile 40% pH=7	Acetonitrile 30% pH=7	MeOH 40% pH=7	Acetonitrile 40% pH=9
Dead time (min)	1,75	1,75	1,77	1,77	1,75
tR butylparaben (min)	4,92	11,6	32,4	69,01	9,02
k butylparaben	1,82	5,65	17,54	38,55	4,17
Critical Resolution	1,98	5,54	11,57	12,86	4,18
Pressure (bar)	53	57	59	99	57

Conclusion:

a) % of acetonitrile: RP-LC: decrease of the organic solvent induces a decrease of elution because the mobile phase becomes more polar. Retention increases and higher tR and k values are obtained. Resolution increases as a consequence of higher retention. Dead time and back pressure do not change.

b) Methanol: Elution decreases when we replace acetonitrile by methanol. ΔP increases as methanol has a higher viscosity (Darcy equation).

c) pH=9: the increase of pH induces partial ionization of phenol groups. Ionized compounds are more polar and eluted more rapidly (decrease of tR, k and resolution).

Question 4: Describe how flow rate affect retention and separation: `

	D=0,5mL/min	D=1mL/min	D=1,5mL/min	D=2mL/min
Dead time (min)	3,5	1,75	1,17	0,88
tR butylparaben (min)	23,21	11,6	7,74	5,8
k butylparaben	5,65	5,65	5,65	5,65
Critical Resolution	6,91	5,54	4,55	3,85
Pressure (bar)	28	57	85	113

Conclusion:

Increase of flow rate decreases dead time and retention time (both inversely proportional to mobile phase velocity). Retention factors remain unchanged: calculated as the ratio $k=(t_R-t_M)/t_M$. A decrease of resolution is observed related to a loss of efficiency in high mobile phase velocities (see rate theory).

Back pressure increases proportionally to the flow rate according to Darcy equation.

Question 5: Describe how stationary phase type RP-HPLC and column dimensions affect retention and separation: `

	C18 L=150mm dp=5µm	C18 L=50mm dp=5µm	C18 L=150mm dp=3µm	C4 L=150mm dp=5µm	Phenyl L=150mm dp=5µm
Dead time (min)	1,75	0,58	1,75	1,75	1,75
tR butylparaben (min)	11,6	3,87	11,6	9,93	6,64
k butylparaben	5,65	5,65	5,65	4,35	2,81
Critical Resolution	5,54	2,19	6,17	4,75	3,31
Pressure (bar)	57	20	155	57	57
Efficiency propyl paraben(N)	11007	2483	16750	10209	8645

Conclusion:

a) Decrease of column length decreases dead and retention times, number of plates and back pressure. They are all proportional to column length so they are divided by 3. Retention factors remain the same (same explanation as question 4). Resolution decreases as a consequence of N decrease.

b) Decrease of particle diameter did not change dead time, retention times and retention factors. Back pressure increases (Darcy equation). Efficiency (N) increases because plate height (H) decreases (rate theory), so resolution increases.

c) C4 and phenyl phases are less hydrophobic that C18, so retention times and retention factors decrease. N decreases (lower column performance, associated with column manufacturing) so resolution decreases.

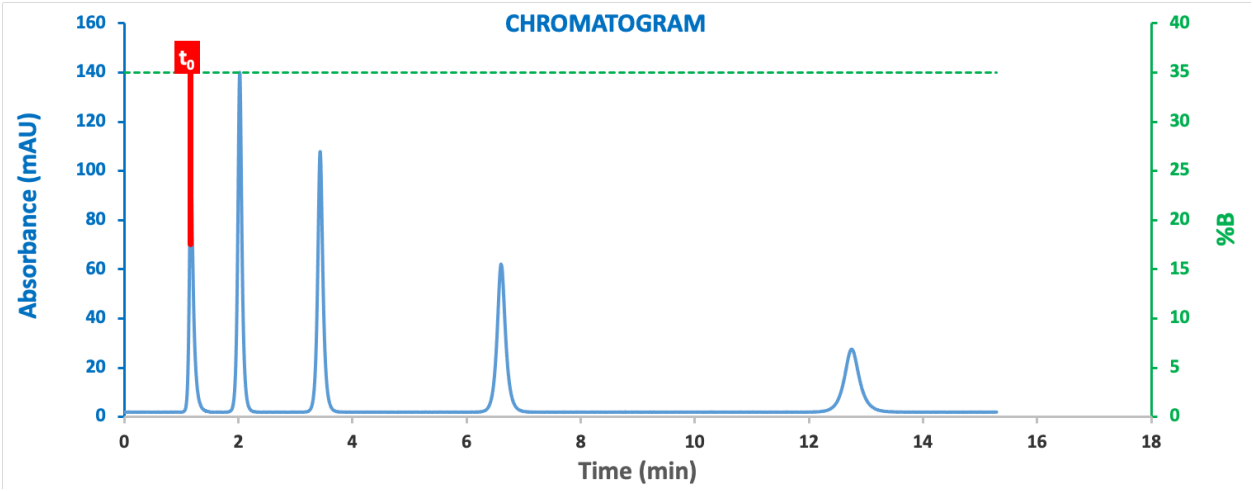
Question 6: Propose optimum operating conditions.

We have to choose operating conditions that give chromatographic results in accordance with : $1<k<10$ and $R>1,5$ and time of analysis <15min.

The initial conditions provide results that fulfill these requirements, except $k<1$ for methyl paraben. We decrease acetonitrile content to 35%, so $k>0,5$ for methyl paraben. Retention times increase but we can decrease the retention times with increasing the flow rate at 1,5 mL/min. The obtained chromatogram is given below. We obtain $0,5<k<10$, $R>1,5$ and time of analysis <15min.

A range $1<k<10$ cannot be obtained for this mixture in isocratic conditions.

Insert Optimized chromatogram (paste special image)*



2. Analysis of a mixture of acids (NAIS Mix.2)

Question 7: Modify mobile phase composition to find isocratic conditions that separate all compounds.

At pH=7 all analytes are ionized (pH>>pKa) except paracetamol and LogP values cannot be used to evaluate polarity.

At pH=7 and 30% acetonitrile, coelution occurs for the first three compounds that are not retained. Good separation occurs for a mobile phase containing only 10% of acetonitrile, but retention factors are too high (k>>20).

At pH=2,7 acids analytes are mainly unionized (pH<pKa) and better retained. Acceptable resolution values are obtained with 38% of acetonitrile, but retention factors exceed the range $0,5 < k < 20$.

Question 8: Predict the elution order at the above chosen conditions and compare with the obtained chromatogram (with acceptable resolution values).

The order of elution is (for unionized compounds at pH=2,7):

Paracetamol, Acetylsalicylic acid, Salicylic acid, Ketoprofen, Ibuprofen, Mefenamic acid.

As predicted by LogP values for unionized compounds.

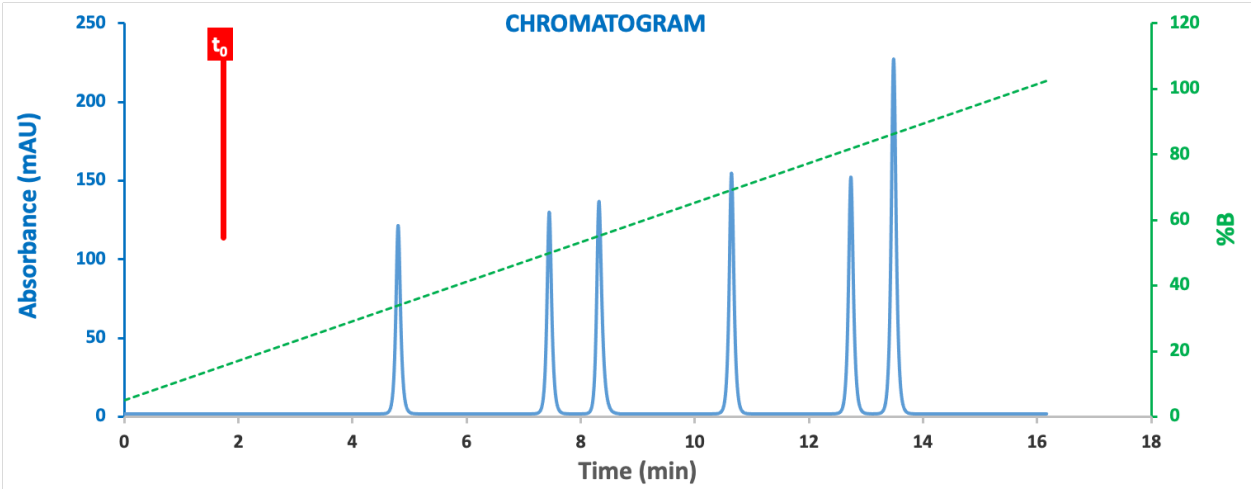
Question 9: Explain why gradient elution is preferred.

At pH=2,7 and 38% acetonitrile retention factor of mefenamic acid is >20 and paracetamol is not retained. If we increase the % of acetonitrile critical resolution of the first compounds also becomes too low.

Good retention for all compounds and acceptable resolution values can be obtained with gradient elution.

Question 10: Optimize gradient elution.

*Insert optimized chromatogram (*paste special image)*



Organic solvent (B): acetonitrile.

Buffer: pH=2,7.

Gradient conditions (gradient elution scouting) :

Gradient time 15 min.

Initial B%=5%

Final B%=100%

We obtain all resolutions > 2 and time of analysis < 15 min.