

M1 D²HP – TU 09

TUTORIALS ELECTROPHORESIS

I. Analysis of molecules of pharmaceutical interest by capillary zone electrophoresis

The structure, molar mass and pKa of the molecules of interest are presented in Table 1.

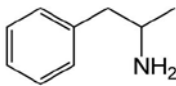
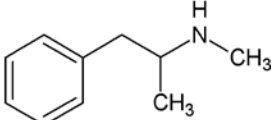
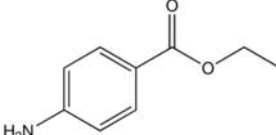
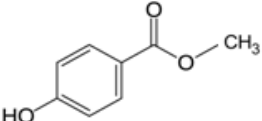
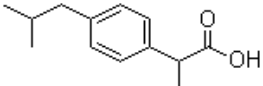
Amphetamine (A)  pKa = 10,1 M = 135 g/mol	Metamphetamine (MET)  pKa = 9,9 M = 149 g/mol	Benzocaine (BZ)  pKa = 4,9 M = 165 g/mol
Methyl parahydroxybenzoate (PHM)  pKa (OH) = 8,2 M = 152 g/mol	Ibuprofen (I)  pKa = 4,5 M = 206 g/mol	

Table 1

The analysis of a solution containing amphetamine (A), metamphetamine (MET) and benzocaine (BZ) was performed by capillary zone electrophoresis (CZE) in conditions below.

Separation capillary: bare-fused silica, internal diameter 75 μ m, total length 57 cm, effective length 50 cm

Electrolyte : sodium phosphate buffer, pH 2,3 ; ionic strength 50 mM

Detection : UV 254 nm, cathodic end

Hydrodynamic injection : 10 s, 4 kPa, anodic end

Applied voltage : 22 kV

Temperature : 25°C

The obtained electrophoregram is presented in figure 4.
Migration times are collected In Table 2.

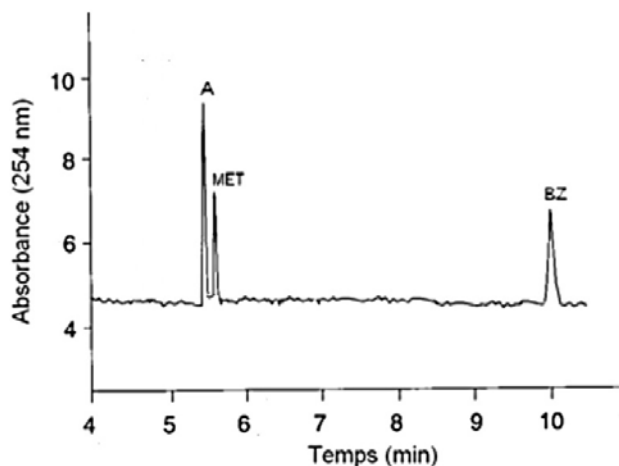


Figure 4

Peak	Migration time (min)
A	5.41
MET	5.50
BZ	9.96

Table 2

- 1- Draw a schema of the experimental device and the charge distribution inside the capillary. Explain the CZE mechanism.
- 2- Define the ionization state of each of the 5 compounds at pH 2.3 and 8.8.
- 3- Interpret the migration order of compounds A, MET and BZ.
- 4- Would it be possible to separate compounds PHM and I in these experimental conditions?
- 5- The use of an electrolyte at pH 8,8 showed a $\mu_{eo} = 5,8 \cdot 10^{-4} \text{ cm}^2 \cdot \text{s}^{-1} \cdot \text{V}^{-1}$. In these conditions, the migration time of PHM is 6,80 min.
Calculate the μ_{app} and μ_{ep} of PHM ($\text{cm}^2 \cdot \text{s}^{-1} \cdot \text{V}^{-1}$).
- 6- Which will be the migration order of compounds PMH, MET and BZ in these new conditions (pH 8.8)?
- 7- What's about the migration of compounds A and I in these conditions ?