

HPLC gyakorlathoz mellékletek

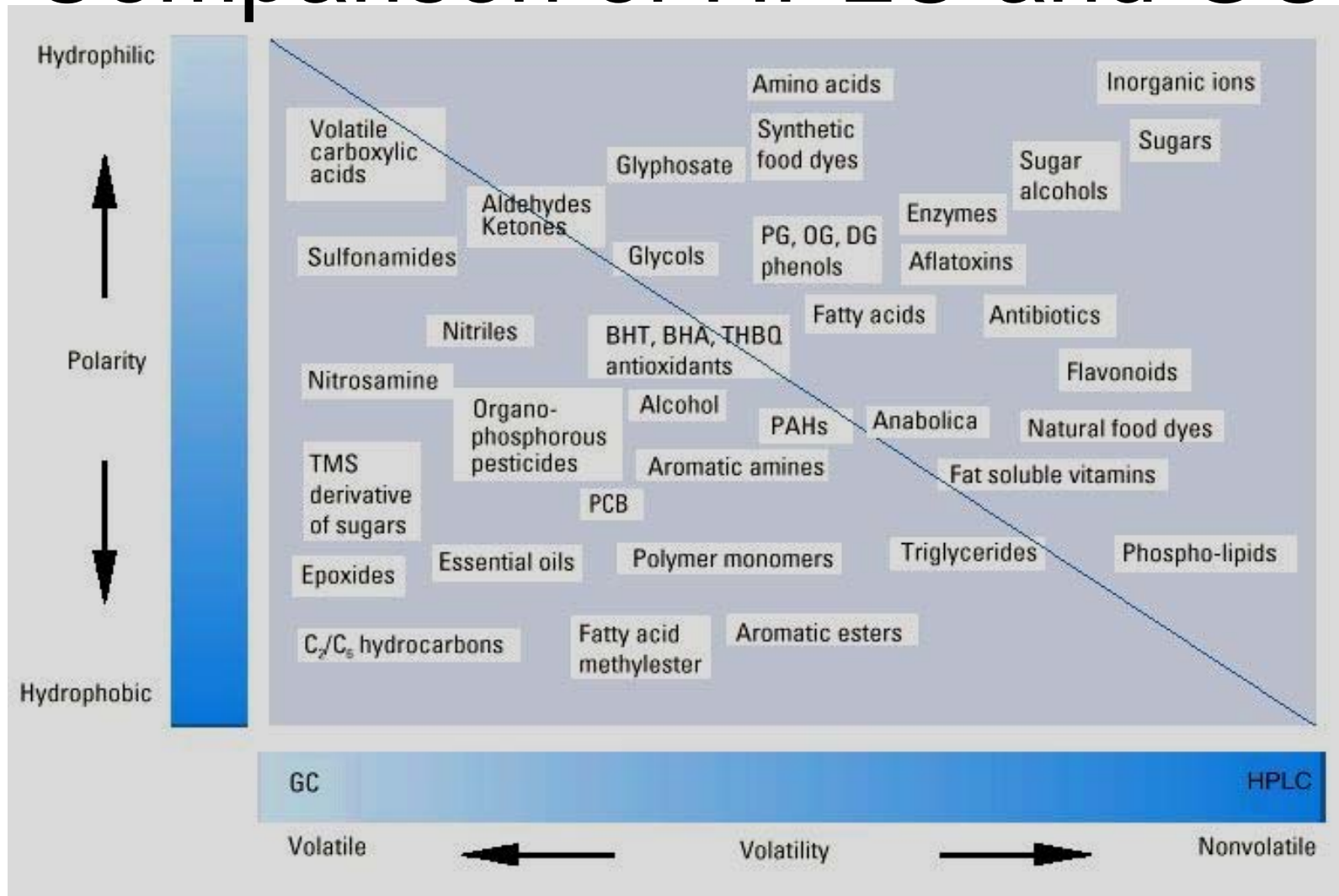
Kémia MSc I. évf.

Solvent UV-Cutoff/Transparency

<u>Solvent</u>	<u>UV Cutoff (nm)</u>
Acetonitrile	190
Water	190
Cyclohexane	195
Hexane	200
Methanol	210
Ethanol	210
Diethyl Ether	220
Dichloromethane	220
Chloroform	240
Carbon Tetrachloride	265
Tetrahydrofuran	280 (220)
Toluene	285

UV cutoff is the wavelength at which absorbance equals 1, measured in a 1 cm cell with air as a reference.

Comparison of HPLC and GC



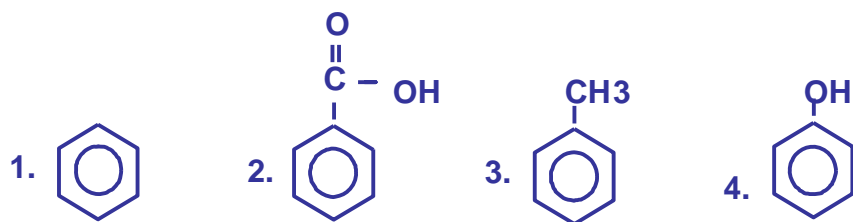
Modes of High Performance Liquid Chromatography

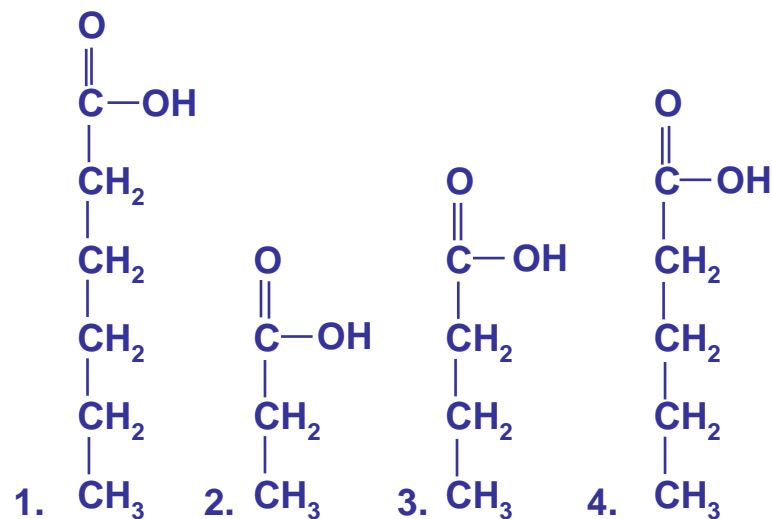
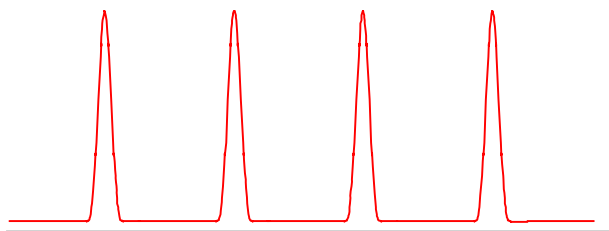


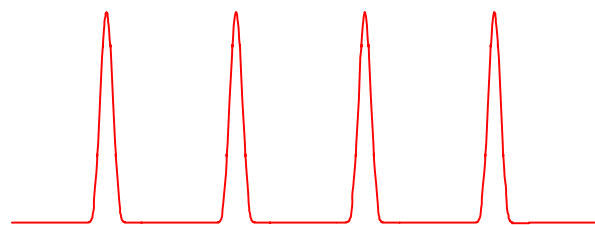
Types of Compounds	Mode	Stationary Phase	Mobile Phase
Neutrals Weak Acids Weak Bases	Reversed Phase	C18, C8, C4 cyano, amino	Water/Organic Modifiers
Ionics, Bases, Acids	Ion Pair	C-18, C-8	Water/Organic Ion-Pair Reagent
Compounds not soluble in water	Normal Phase	Silica, Amino, Cyano, Diol	Organics
Ionics Inorganic Ions	Ion Exchange	Anion or Cation Exchange Resin	Aqueous/Buffer Counter Ion
High Molecular Weight Compounds Polymers	Size Exclusion	Polystyrene Silica	Gel Filtration- Aqueous Gel Permeation- Organic

Self-Assessment

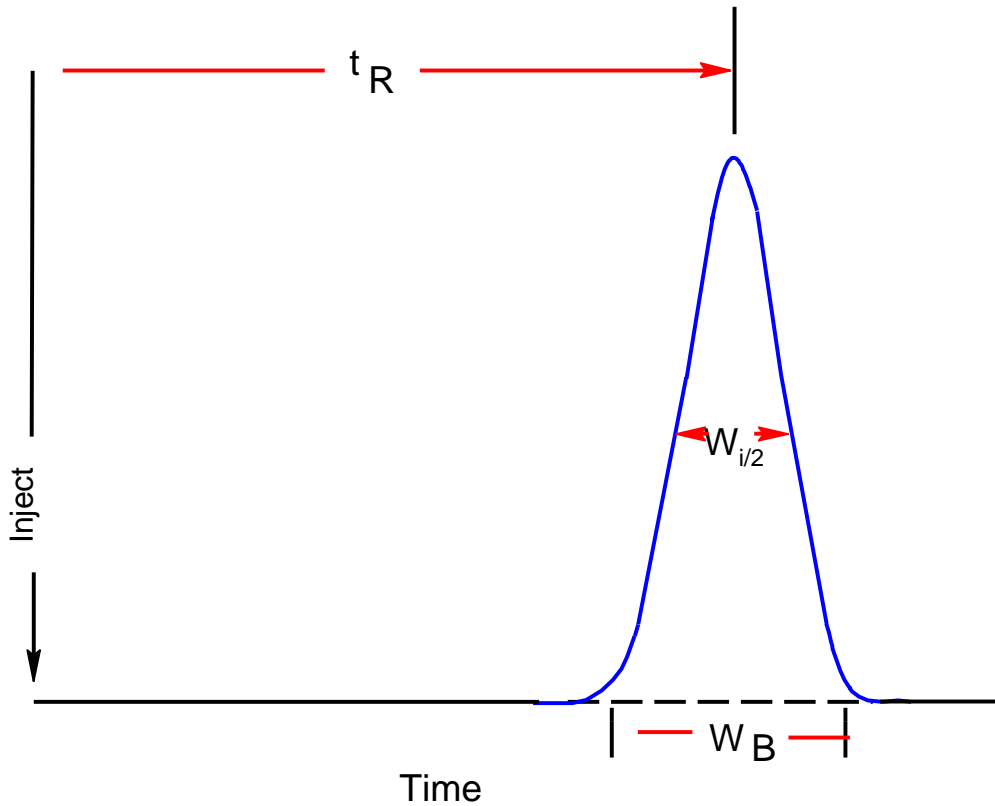
Predict the Elution Order for a Reversed-Phase Analysis







Calculating Efficiency



$$N = 16 \left(\frac{t_R}{W_B} \right)^2 = 5.54 \left(\frac{t_R}{W_{1/2}} \right)^2 = 2\pi \left(\frac{h_p t_R}{A} \right)^2$$

$$\text{HETP} = \frac{L}{N}$$

N = Efficiency; Plate Number
 HETP = Height Equivalent to
a Theoretical Plate

L = Column Length

h_p = Peak height

A = Peak Area

Elution Strength – Reversed Phase

