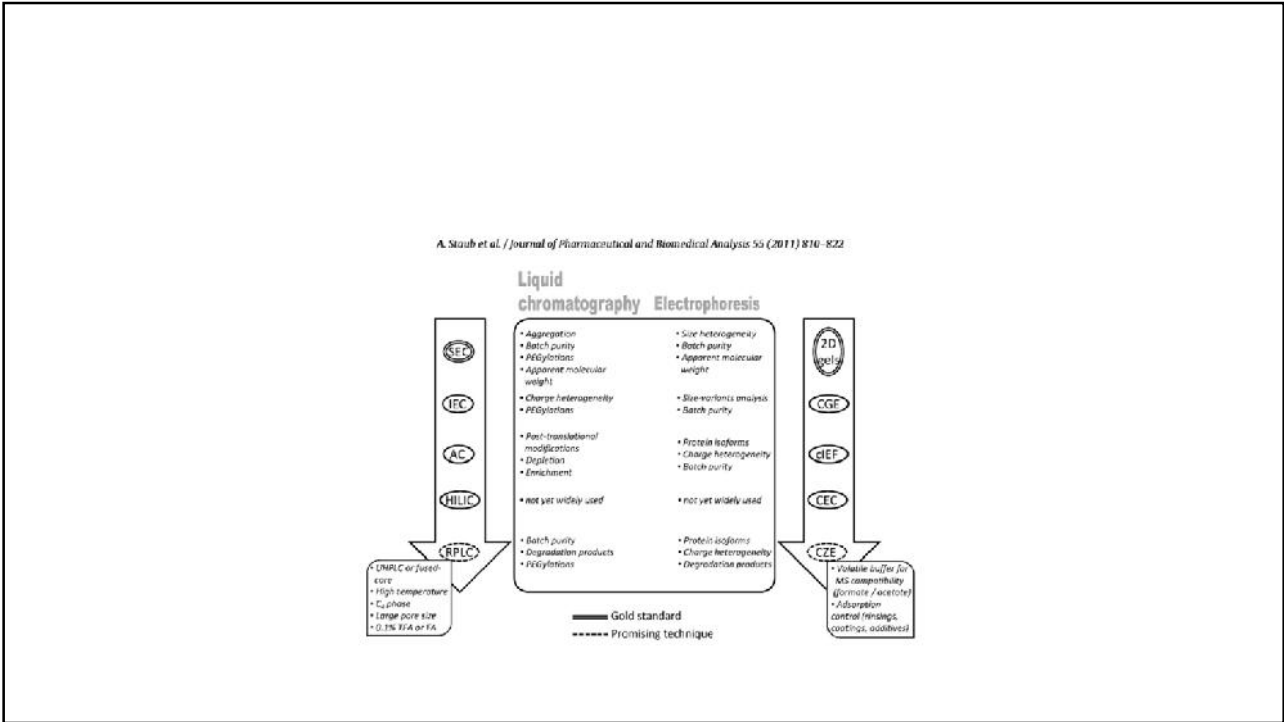


# Biopolimerek elválasztása

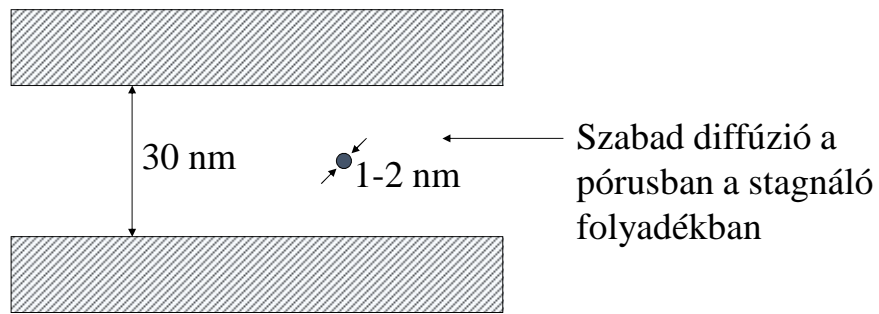
## LC módszerek

- -RPLC
- -IELC
- -SEC
- -HILIC



- RPLC alapjai a biopolimerek elválasztásában

Ha  $d_p/d_m \geq 10 \rightarrow$  nincs jelentős pórus okozta zónaszélesedés  
Makromolekuláknál növelni kell a pórusátmérőt



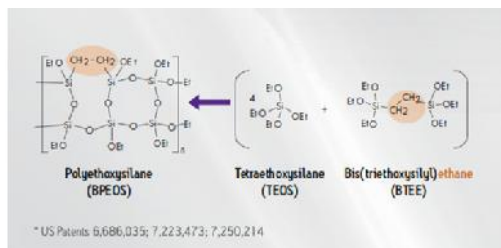
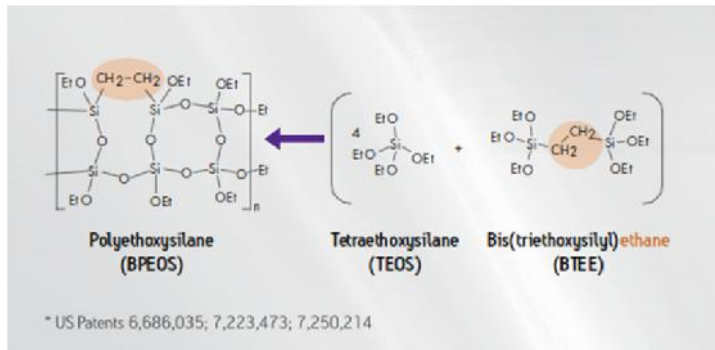
## Kulcsszavak

- Bio töltetek:
- $D_d \geq 20\text{nm}$
- Silica B
- RP 18 utószilanizált,
- Nagy borítottság

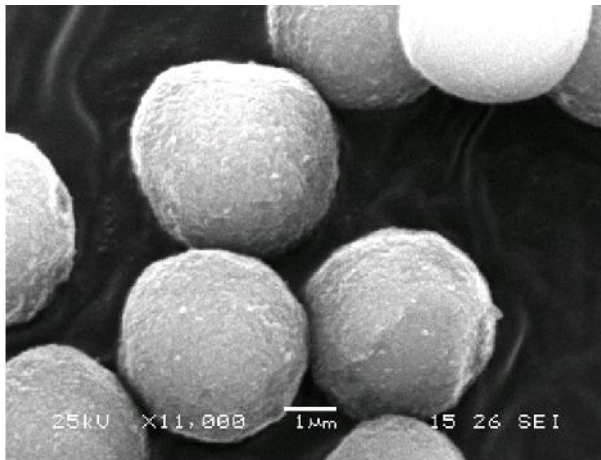
- Morfológia:
- -teljesen porózus
  
- -héjszerű
  
- -monolit
  
- -nem porózus

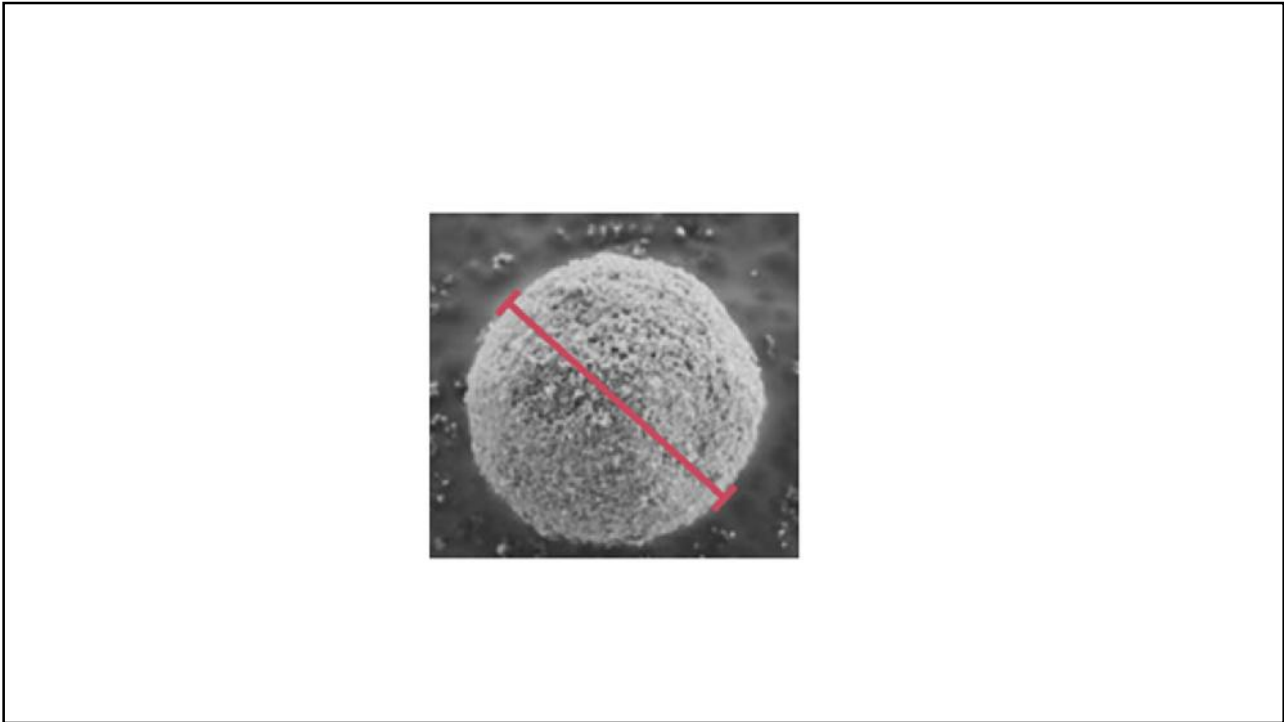
- Teljesen porózus:
  
- -szilikagél, szerves polimer, Silica B
  
- -szerves szilikagél monomer+szerves monomer
  
- Alapvető:pH túrés

# Hibrid töltetek

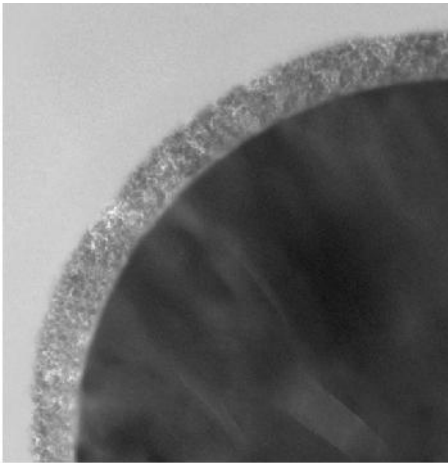


$$H = A \cdot d_p + \frac{B \cdot D_M}{u} + C \frac{d_p^2 \cdot u}{D_M}$$

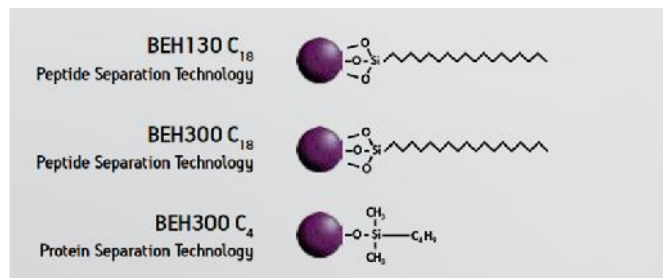
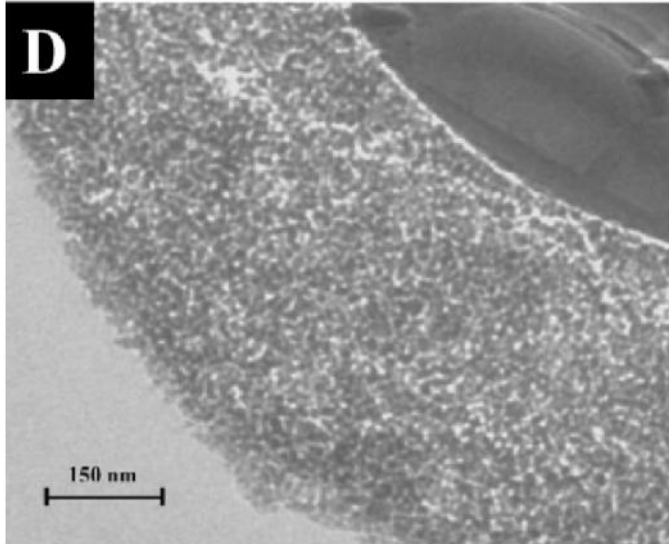




Aeris héjszerű töltet, makromolekulák elválasztására használt töltet



Kis molekula tömegű anyagok elválasztására használt töltet

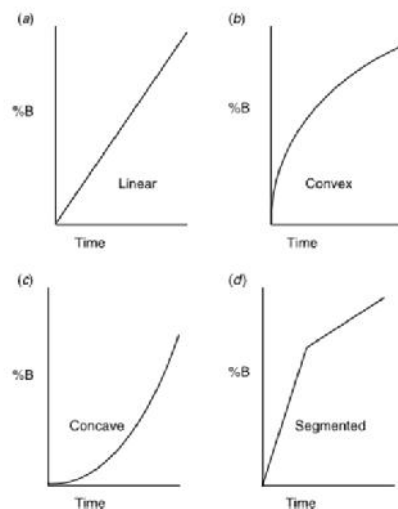




- Alapvető:

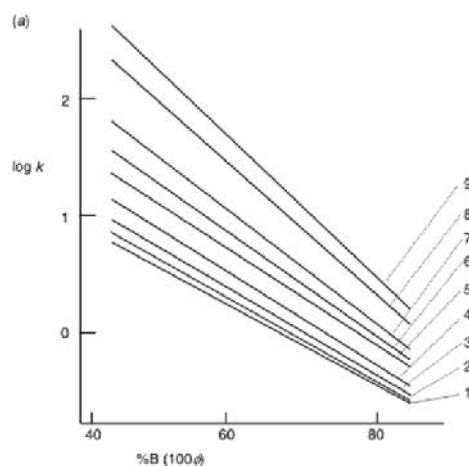
- **A fehérjék elválasztásához mindig gradienselúciót kell alkalmazni!**

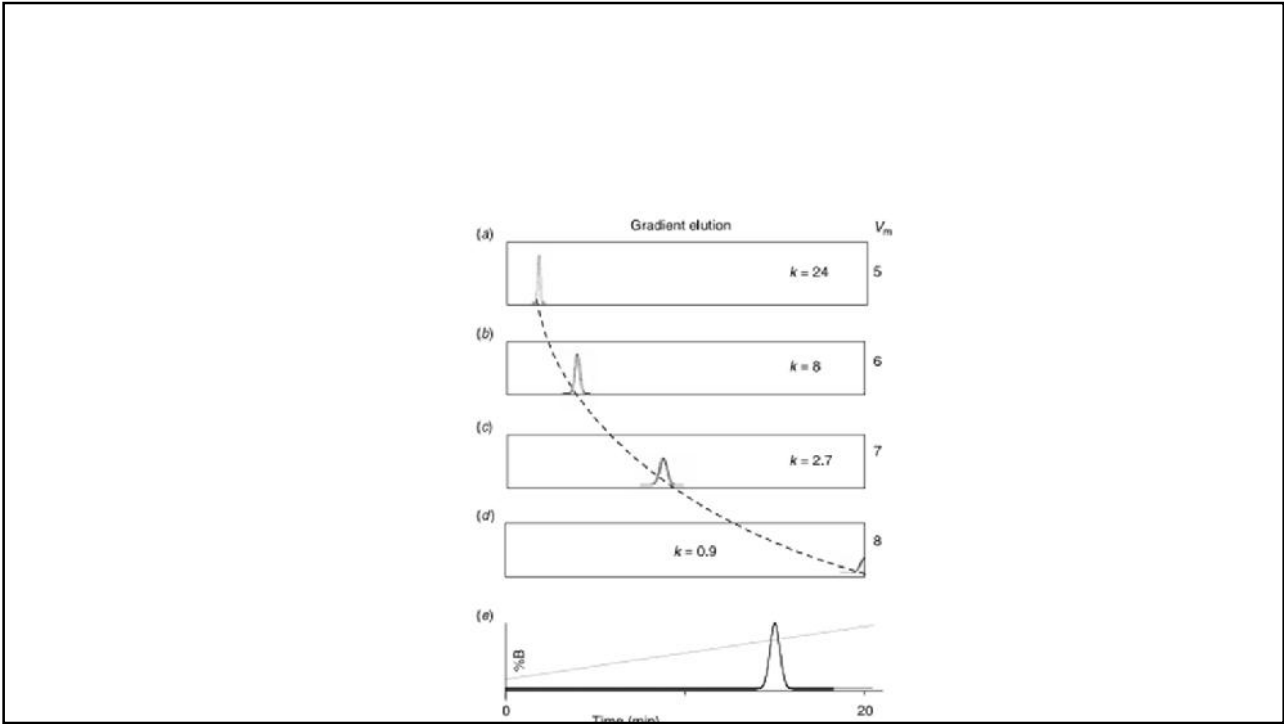
Lehetséges gradiensprofilok

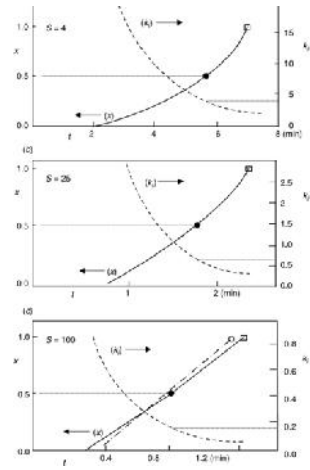


- Kérdés:
  - 1. Milyen legyen a grádiensprofil?
  - 2. Milyen legyen a grádiensidő?

Lineáris grádienselúció(LSS) modellezése izokratikus elválasztással







- Ide a törvényszerűségek beírása egyetem

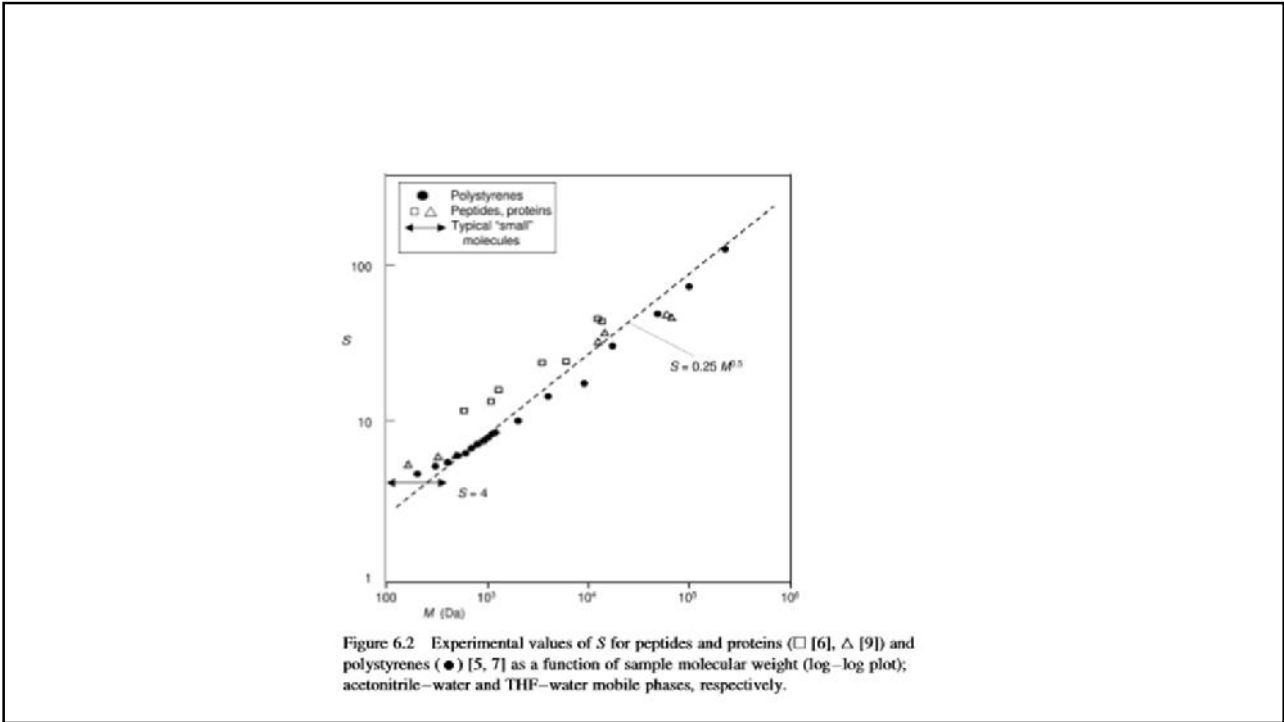
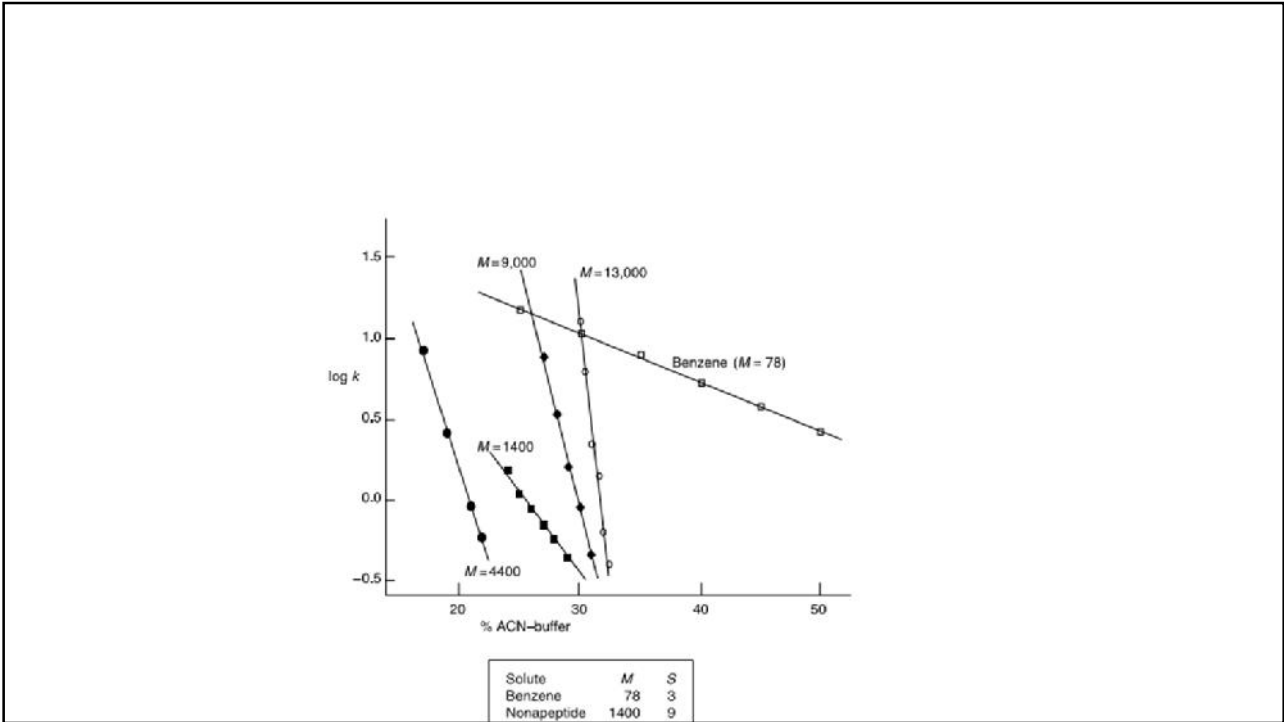


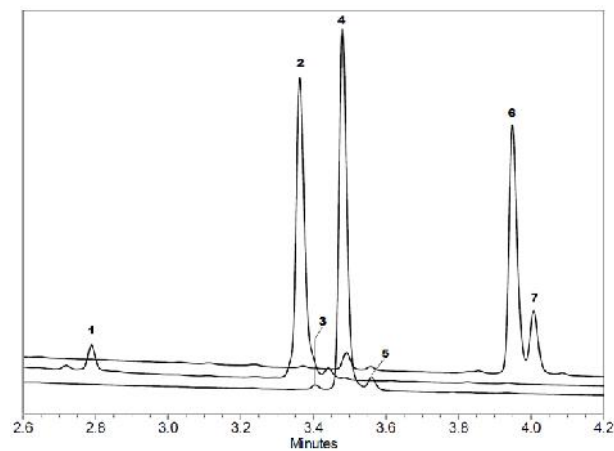
Figure 6.2 Experimental values of  $S$  for peptides and proteins ( $\square$  [6],  $\Delta$  [9]) and polystyrenes ( $\bullet$ ) [5, 7] as a function of sample molecular weight (log-log plot); acetonitrile-water and THF-water mobile phases, respectively.

**Figure 6.3** Migration of different compounds during gradient elution as a function of their molecular size or value of  $S$  and time  $t$ . Hypothetical examples based on identical conditions for each separation: gradient of 0–100 percent B in 15 min at 2 mL/min (150 × 4.6 mm column). The sample has  $S$  equal to 4 in (a, d), 25 in (b, e), and 100 in (c, f);  $k_0 = 100$  in (a–c), and 100 and 120 for the two peaks in (d–f). (—) Fractional band migration  $x$  through the column; (---) instantaneous value of  $k$  ( $k_t$ ) for a band at time  $t$ ; (---) “on–off” behavior in (c). (●) Designates  $k^*$ ; (□) designates  $x = 1.00$ . See text for details.

Representative chromatograms of Interferon related proteins.

Conditions: mobile phase A: 0.1% TFA in water, mobile phase B: 0.1% TFA in ACN, gradient profile: 35 to 60% B in 5 min (then 2 min reequilibrating), flow rate: 0.3 mL/min, T = 60 °C, injected volume = 5 μL (full loop), λ = 210 nm.

Column: Aeris WP C18, 150 × 2.1 mm, 3.6 μm. Peaks: 1: oxidized-1, 2: oxidized-2, 3: related impurity-1, 4: native interferon, 5: related impurity-2, 6: reduced form-1, 7: reduced form-2



S. Fekete et al. / Journal of Pharmaceutical and Biomedical Analysis 70 (2012) 158–168

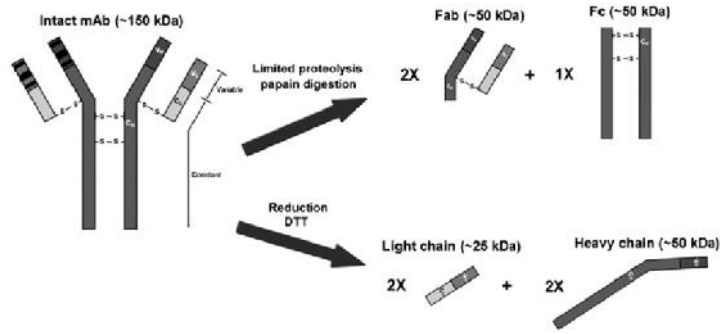


Fig. 1. Schematic view of the limited proteolytic digestion and reduction of monoclonal antibodies.

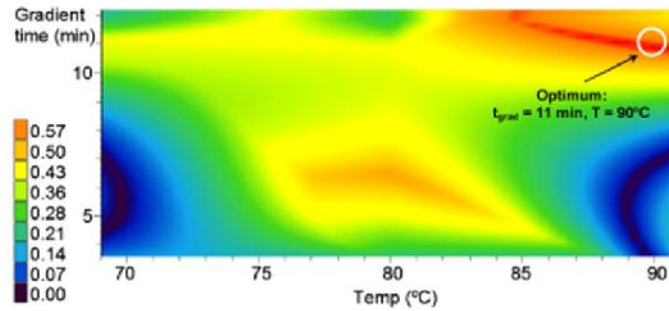
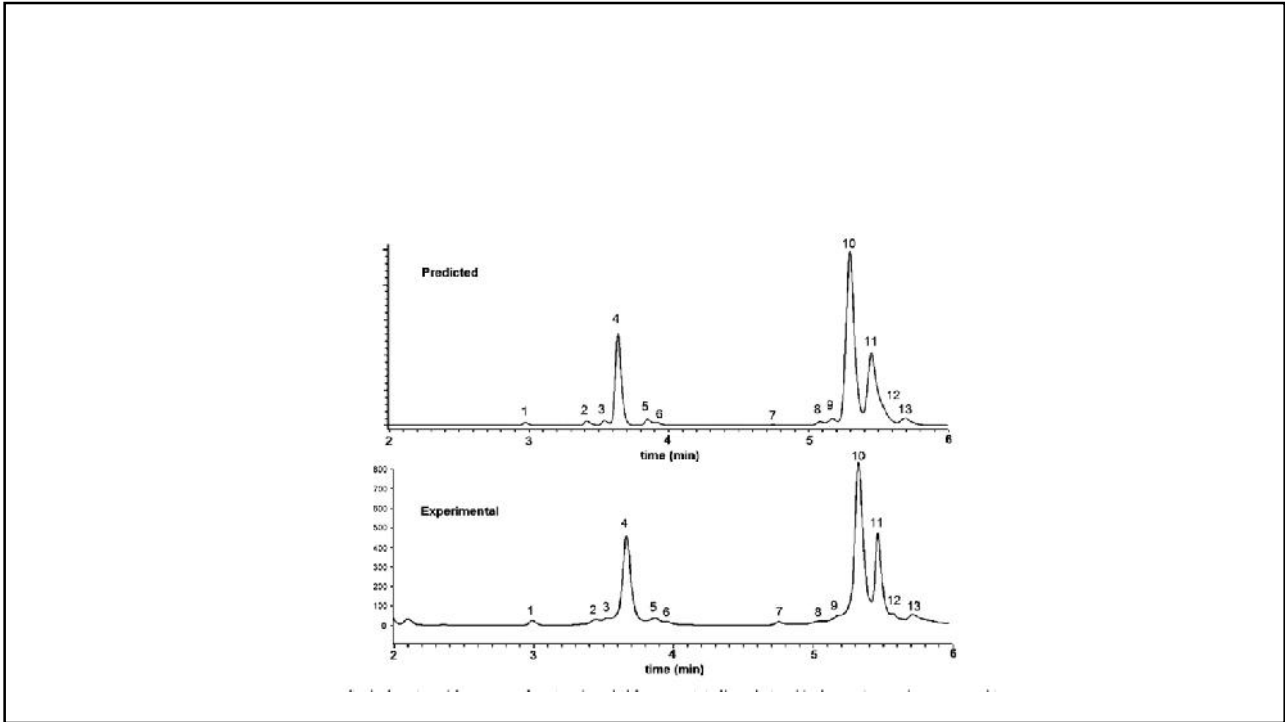


Fig. 6. Two-dimensional resolution map of the column temperature (°C) against gradient time ( $t_g$ , min) for the separation of Bevacizumab Fc and Fab fragments.



**Fig. 7.** Predicted and experimental chromatograms of Bevacizumab Fc and Fab fragments optimized by quadratic model. Column: Aeris WP C18 (150 mm × 2.1 mm), injected volume: 0.5  $\mu$ l, detection: fluorescence (excitation at 280 nm, emission at 360 nm). Mobile phase A: 0.1% TFA in water, mobile phase B: 0.1% TFA in acetonitrile. Gradient: from 30% to 40% B, flow rate: 0.35 ml/min. Gradient time: 11 min,  $T=90^{\circ}\text{C}$ . Peaks: 1–3: pre-Fc peaks, 4: Fc, 5,6: post-Fc peaks, 7–9: pre-Fab peaks, 10: Fab, 11–13: post-Fab peaks.



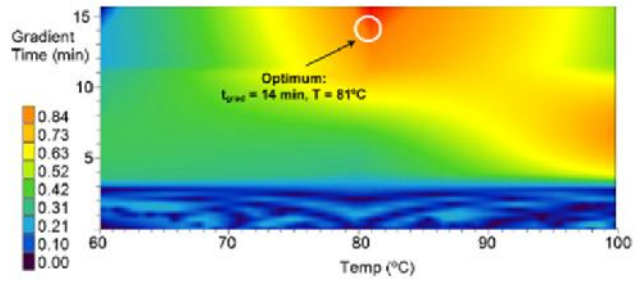
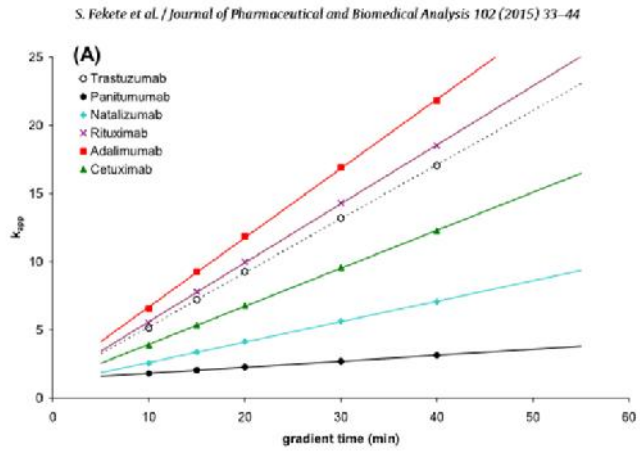
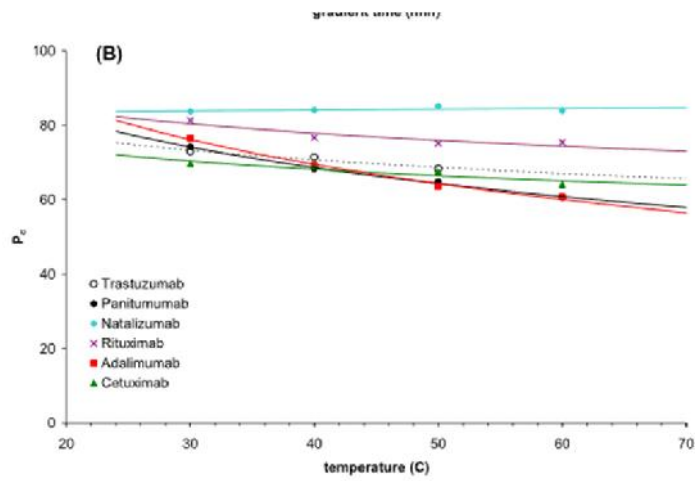
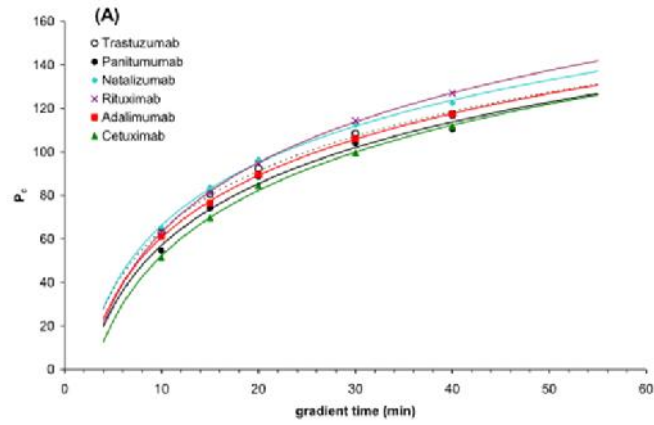
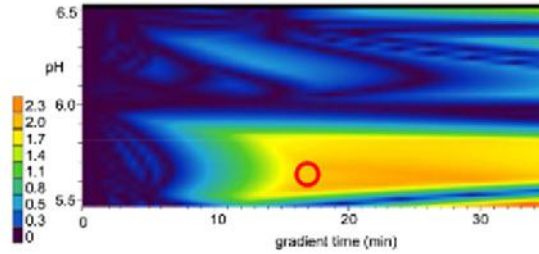


Fig. 9. Two-dimensional resolution map of the column temperature ( $^\circ\text{C}$ ) against gradient time ( $t_g$ , min) for the separation of Rituximab LC and HC fragments.



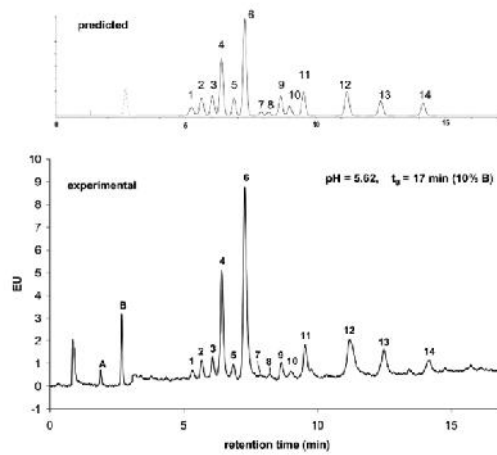
S. Fekete et al. / Journal of Pharmaceutical and Biomedical Analysis 102 (2015) 33–44

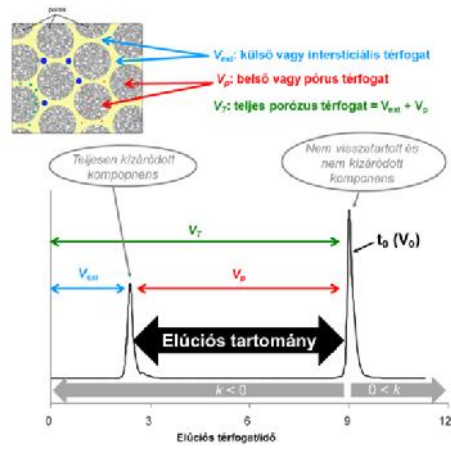
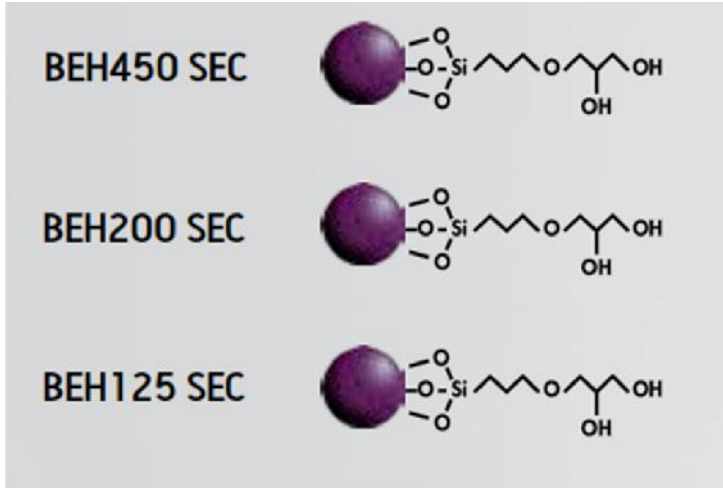




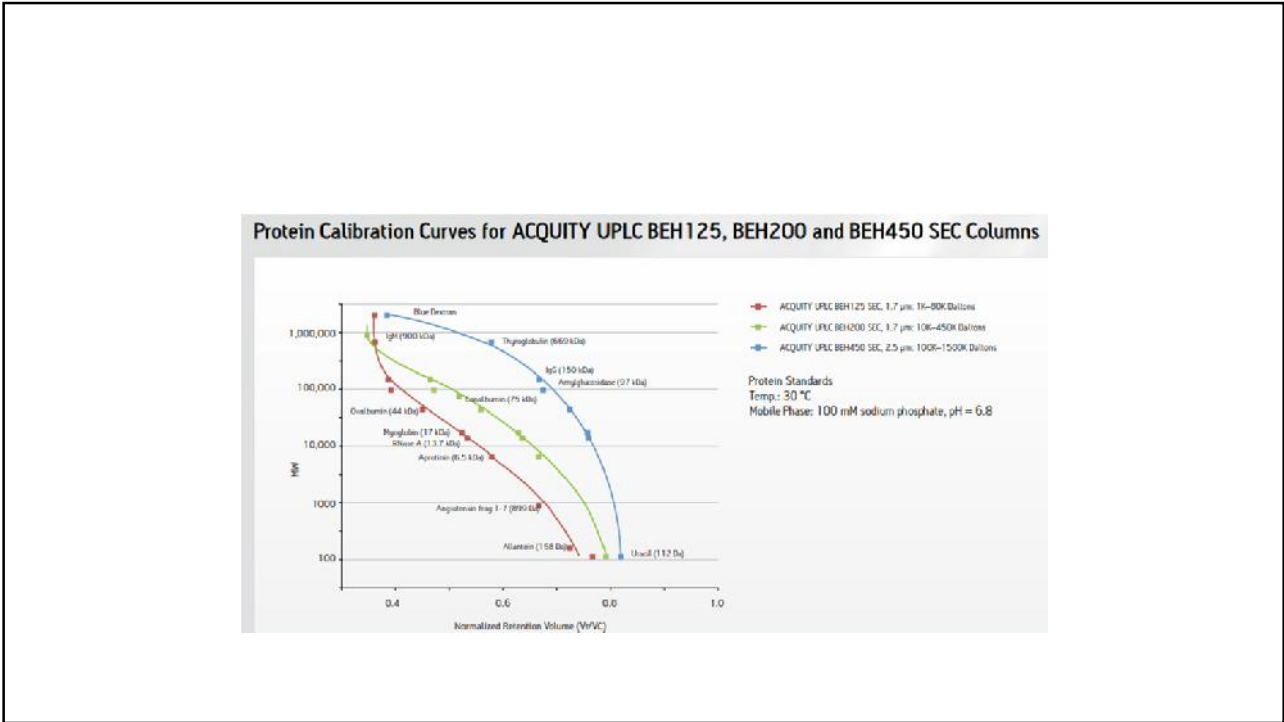
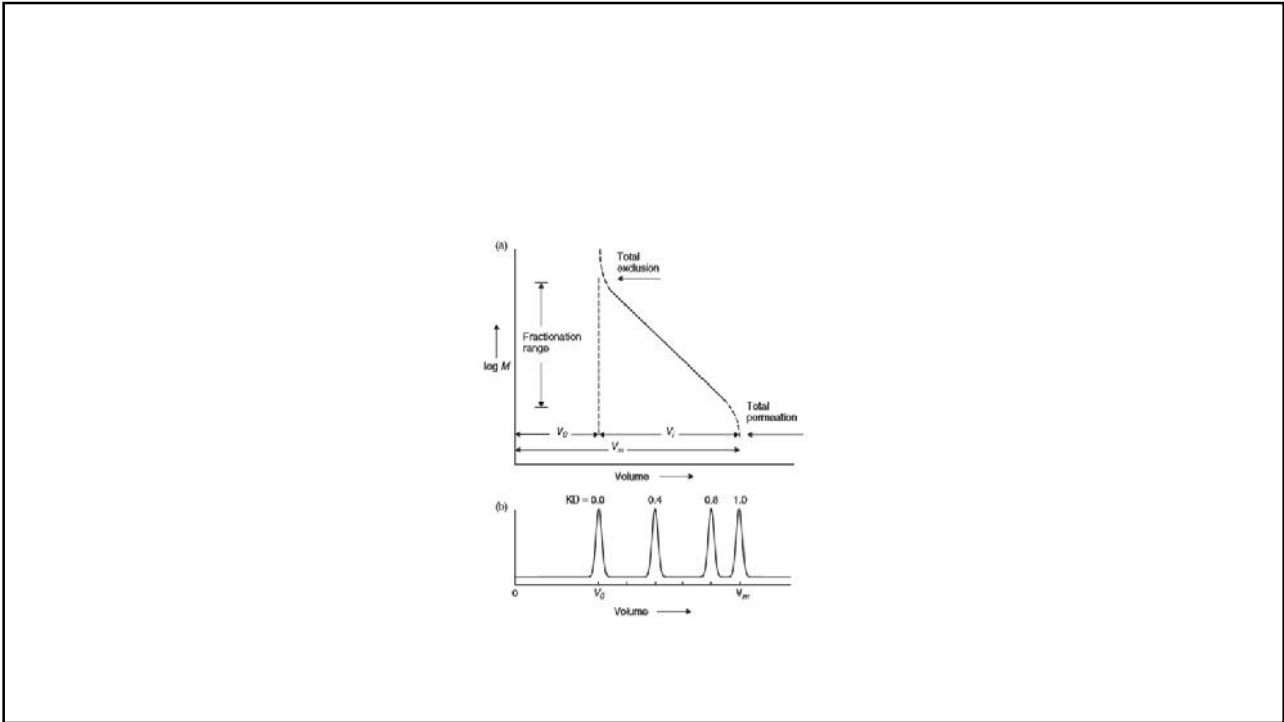
**Fig. 6.** Cetuximab papain digestion resolution map ( $t_R$ -pH model). Column: YMC BioPro SP-F (100 × 4.6 mm). Mobile phase: "A", 10 mM MES; "B", 10 mM MES + 1 M NaCl. Flow rate: 0.6 ml/min; gradient: 0–20% B; temperature: 30 °C; detection: FL (280–360 nm); injected volume: 2  $\mu$ l. Gradient times:  $t_{g1}$  = 10 min,  $t_{g2}$  = 30 min, pH<sub>1</sub> 5.6, pH<sub>2</sub> 6.0, pH<sub>3</sub> 6.4.

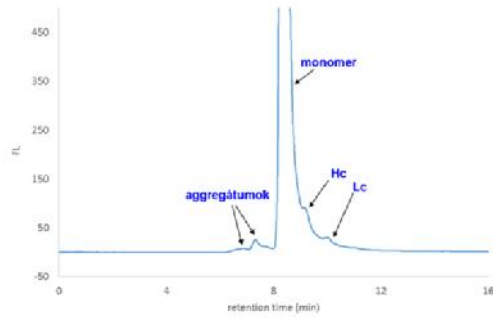
**Fig. 7.** Comparison of predicted and experimental chromatograms. Column: YMC BioPro SP-F (100 × 4.6 mm). Mobile phase: "A", 10 mM MES; "B", 10 mM MES + 1 M NaCl. Flow rate: 0.6 ml/min; gradient: 0–10% B; temperature: 30 °C; detection: FL (280–360 nm); injected volume: 2  $\mu$ l. Gradient times:  $t_g$  = 17 min, pH 5.62.





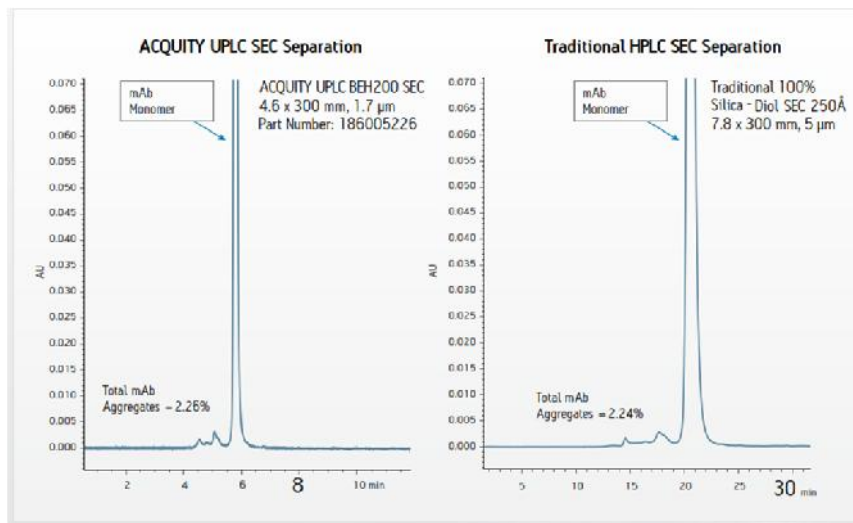
2. ábra: Elúciós tartomány értelmezése a méretkizárási kromatográfiában: külső és belső porozitás szerepe.



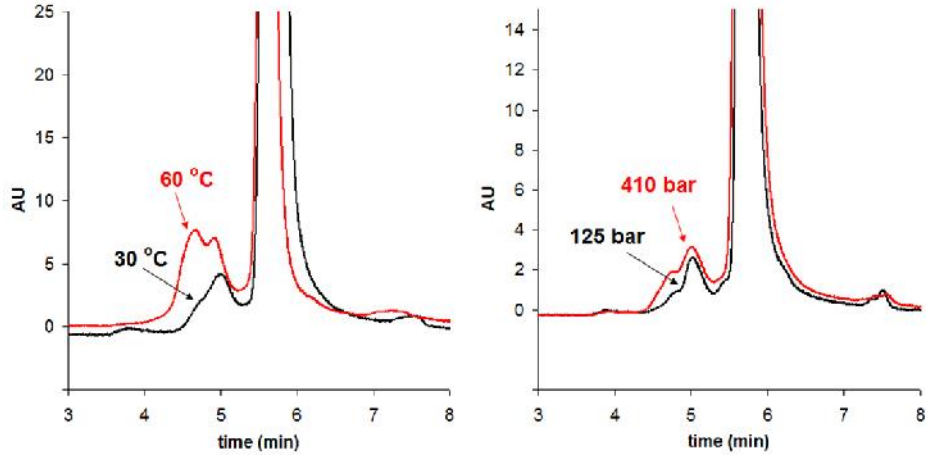


1. ábra: Terápiás fúziós-fehérje aggregátumainak és fragmenseinek elválasztása hagyományos 300 x 4.6 mm kolonnán.

## SEC, HPLC, UHPLC



Hőmérséklet (A) és nyomás (B) okozta oszlopon létrejövő aggregátumok UHP-SEC elválasztás során



Journal of Pharmaceutical and Biomedical Analysis 101 (2014) 161–173



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Review

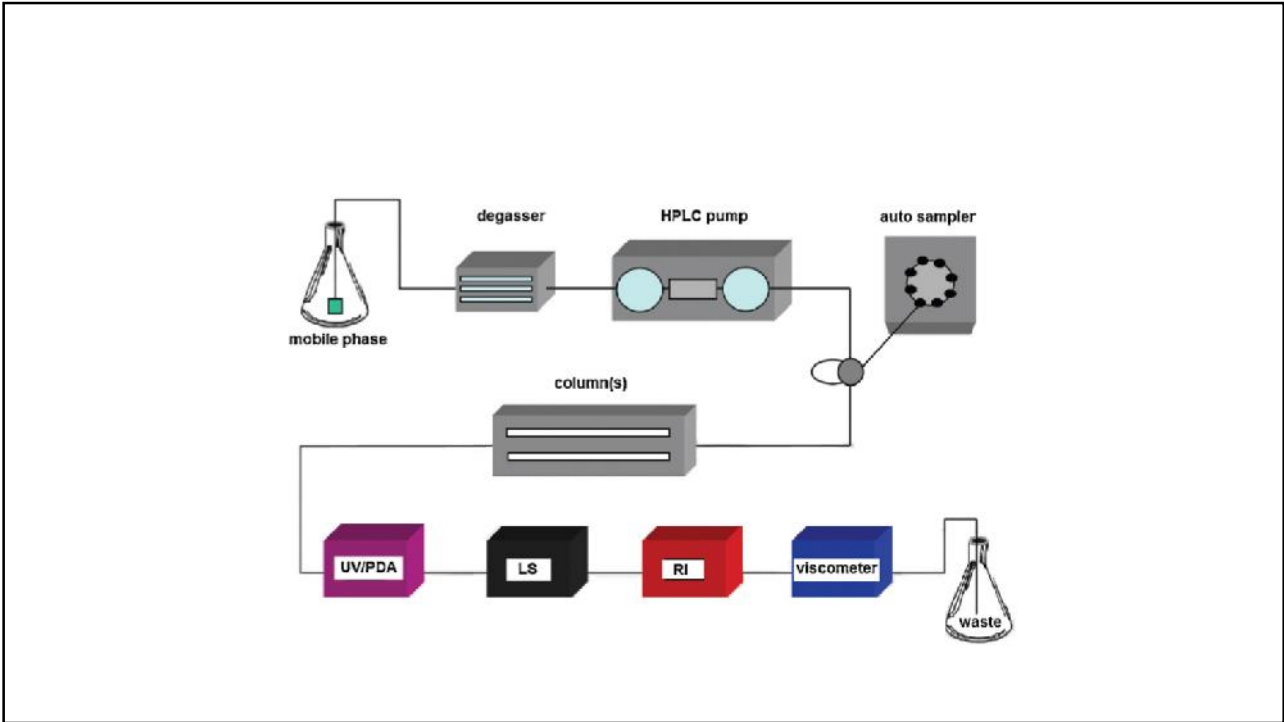
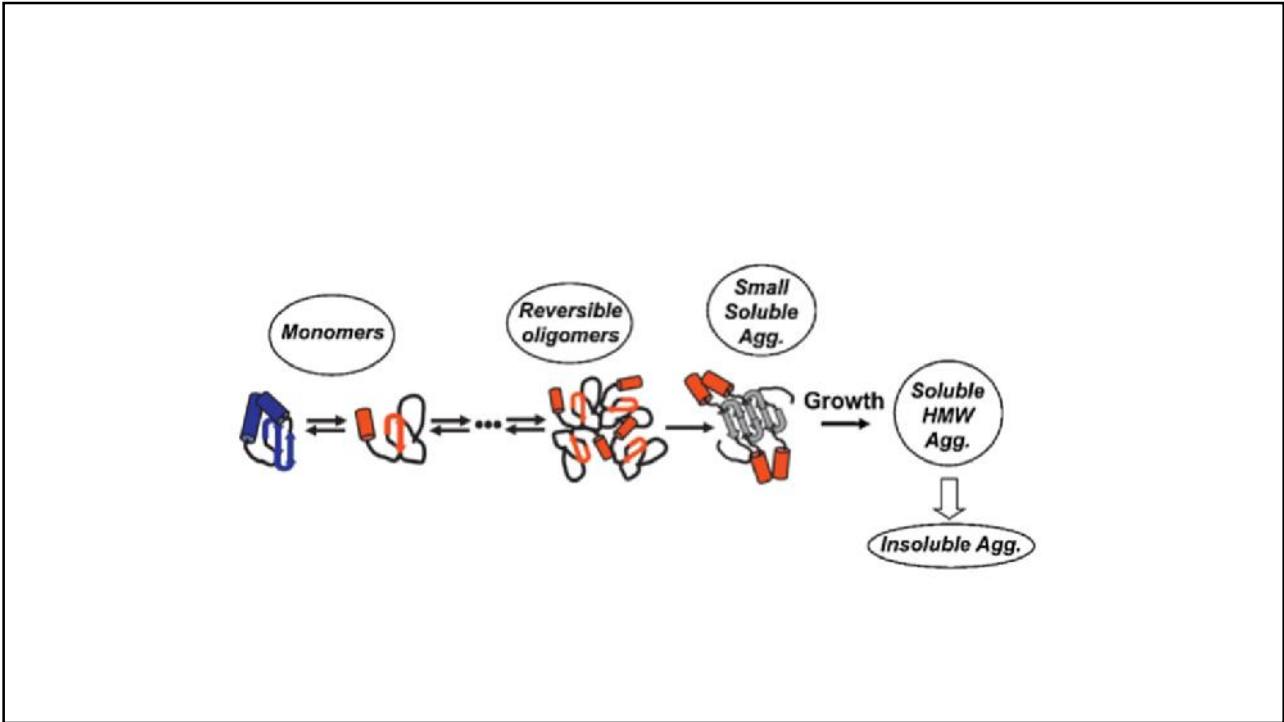
Theory and practice of size exclusion chromatography for the analysis of protein aggregates



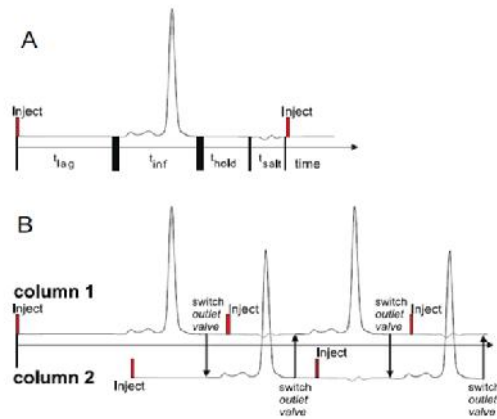
Szabolcs Fekete<sup>a,\*</sup>, Alain Berk<sup>b</sup>, Jean-Luc Veuthey<sup>a</sup>, Davy Cuillarme<sup>a</sup>

<sup>a</sup>School of Pharmaceutical Sciences, University of Geneva, University of Jussieu, Boulevard de Prévost 20, 1211 Geneva 4, Switzerland

<sup>b</sup>Centre of Immunology Pierre Fabre, 5 Avenue Napoléon III, BP 60497, F-13689 Salin-Juilien-en-Cenevins, France







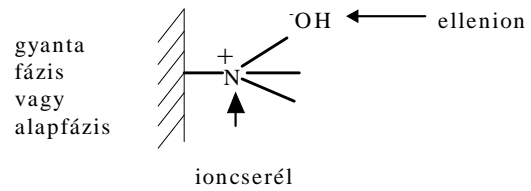
4. ábra: „Párhuzamosan összefont” (parallel interfaced) SEC elválasztás vázlatát két kolonnát alkalmazva.

## Megjelenés alatt: Kromatográfus

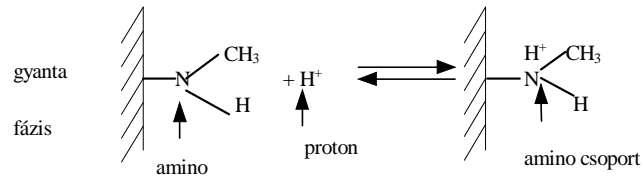
- **Korszerű méretkizárásos kromatográfia: fehérje aggregátumok elválasztása**
- 
- Fekete Szabolcs<sup>1</sup>, Fekete Jenő<sup>2</sup>, Guillaume Davy<sup>1</sup>
- 
- <sup>1</sup> Genfi Egyetem, Gyógyszerészeti Tudományok Tanszék, 1211 Genève, Boulevard d'Yvoy 20.
- <sup>2</sup> BME Szervetlen és Analitikai Kémiai Tanszék, 1111 Budapest, Szent Gellért tér 4.

# Ioncserés kromatográfia

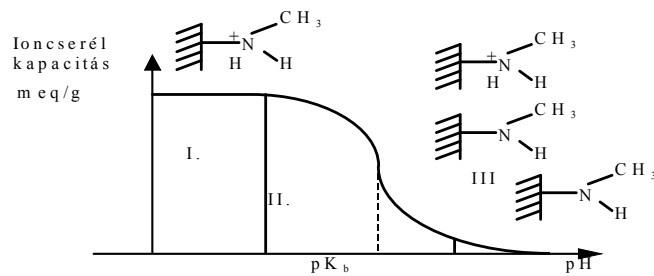
## Erős anioncserélő gyanta (állófázis) szerkezete



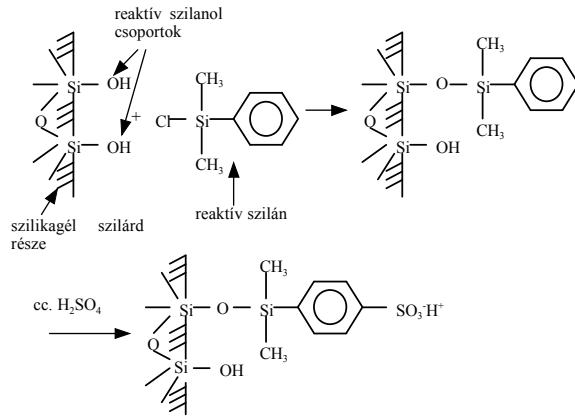
## Gyenge ioncserelő csoport protonálódási folyamata



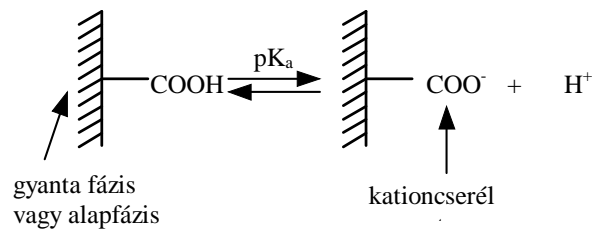
## A gyenge anioncserelő kapacitásának függése a mozgófázis pH értékétől



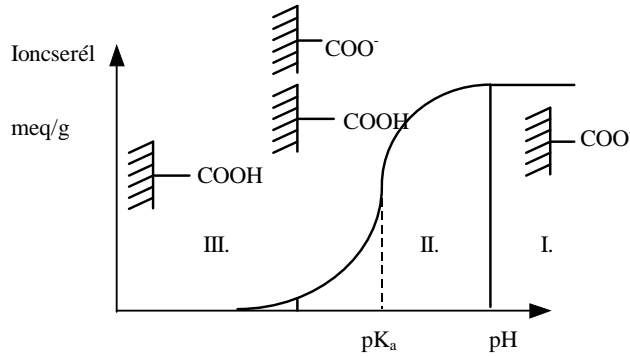
## A szilikagél kémiai módosításával készített kationcserélő



## Gyenge kationcserélő állófázis szerkezete

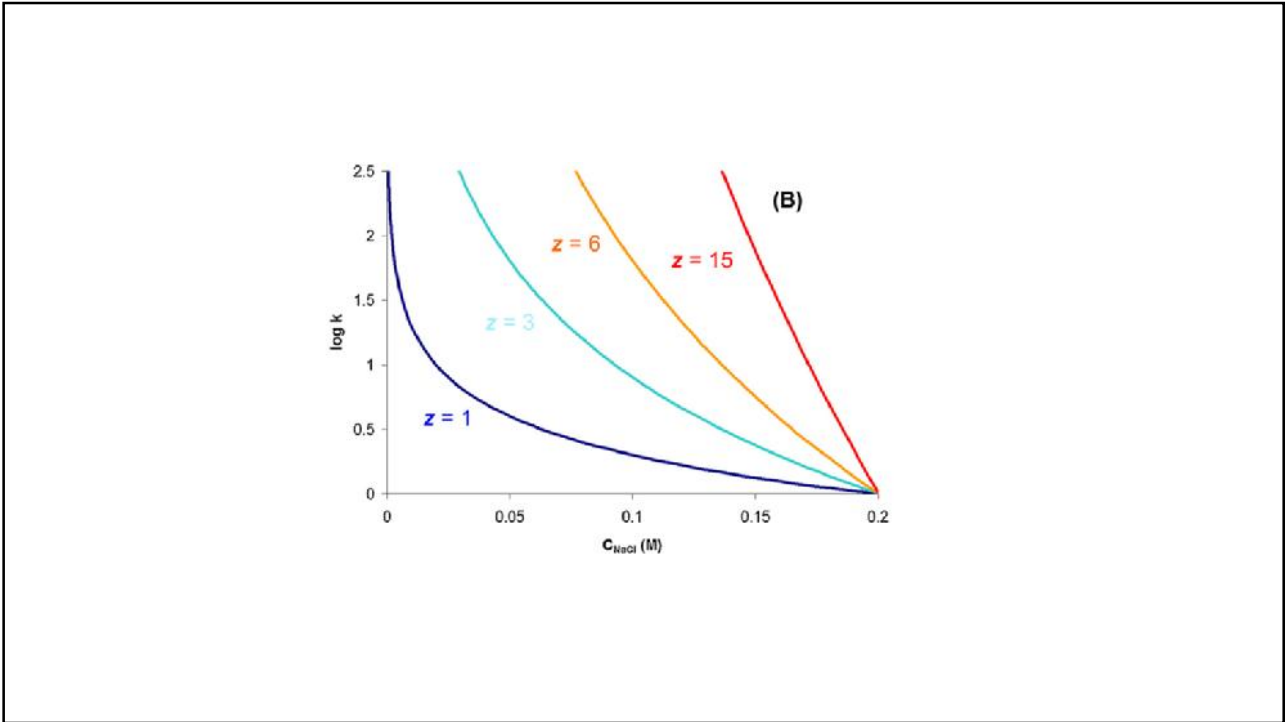


Az ioncserélő kapacitás függése a mozgófázis pH értékétől

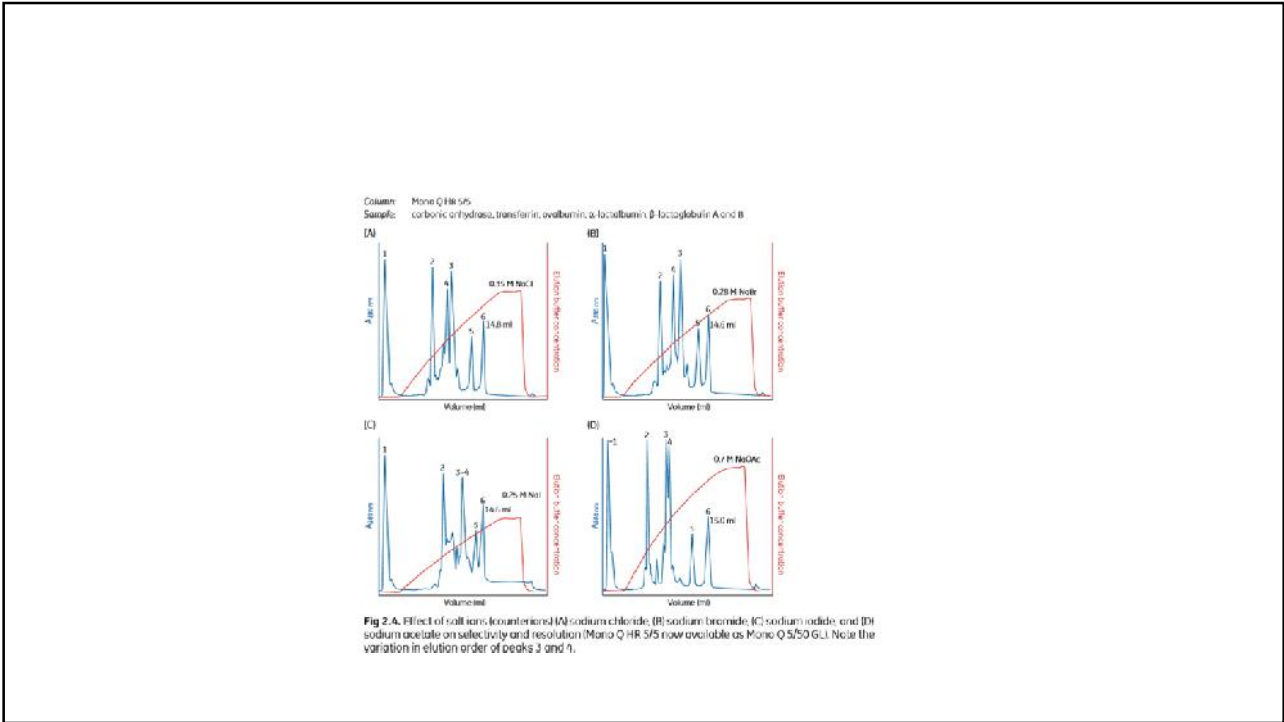


**Table 1**  
Properties of the most popular state-of-the-art IEX columns available for protein separations.

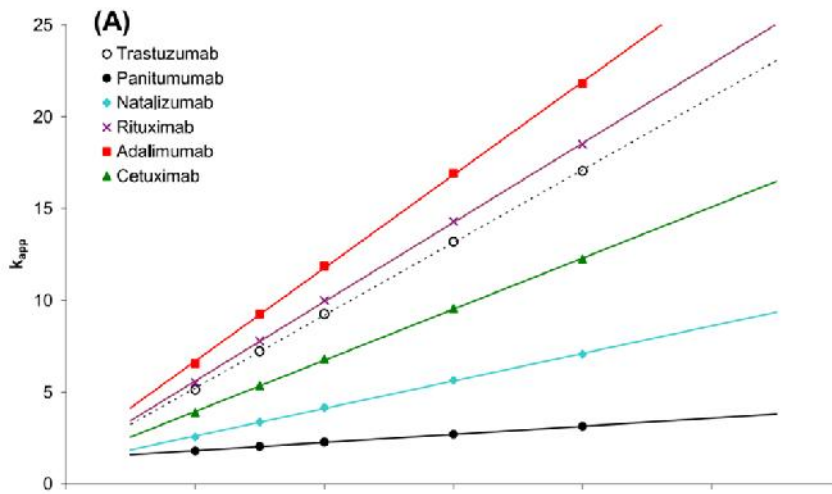
Column name	Chemistry	Particle size/macropore size	Max temperature (°C)	pH range	Max pressure (bar)
Monoliths	SAX-15	Strong anion exchange (quaternary amine)	70	2-12	70
	Proswift (Thermo) WAX-15	Weak anion exchange (tertiary amine)	60		
	WCX-15	Weak cation exchange (carboxylic acid)	60		
	SCX-15	Strong cation exchange (polymethacrylate)	60		
Packed	SCX	Strong cation exchange (sulfonic acid)	5	2-14	50
	TSKgel (Tosoh) SuperQ-5PW	Strong cation exchange (trimethylamino)	10	2-12	
	SP-STAT	Strong cation exchange (sulfopropyl)	7, 10	3-10	
	Q-STAT	Strong anion exchange (quaternary ammonium)	7, 10	3-10	
	Bio Mab (Agilent)	Weak cation exchange (carboxylate)	1.7, 3, 5, 10	80	
Antibodix (Supelco, Sepax)	Weak cation exchange (carboxylate)	1.7, 3, 5, 10	80	2-12	270, 410, 550, 680
Protein-Pak Hi Res IEX (Waters)	SP	Strong cation exchange (sulfopropyl)	7	3-10	100
	CM	Weak cation exchange (carboxymethyl)	7		100
	Q	Strong anion exchange (quaternary ammonium)	5		150
MABPac SCX-10 (Thermo)	Strong cation exchange (sulfonic acid)	3, 5, 10	60	2-12	480, 480, 200

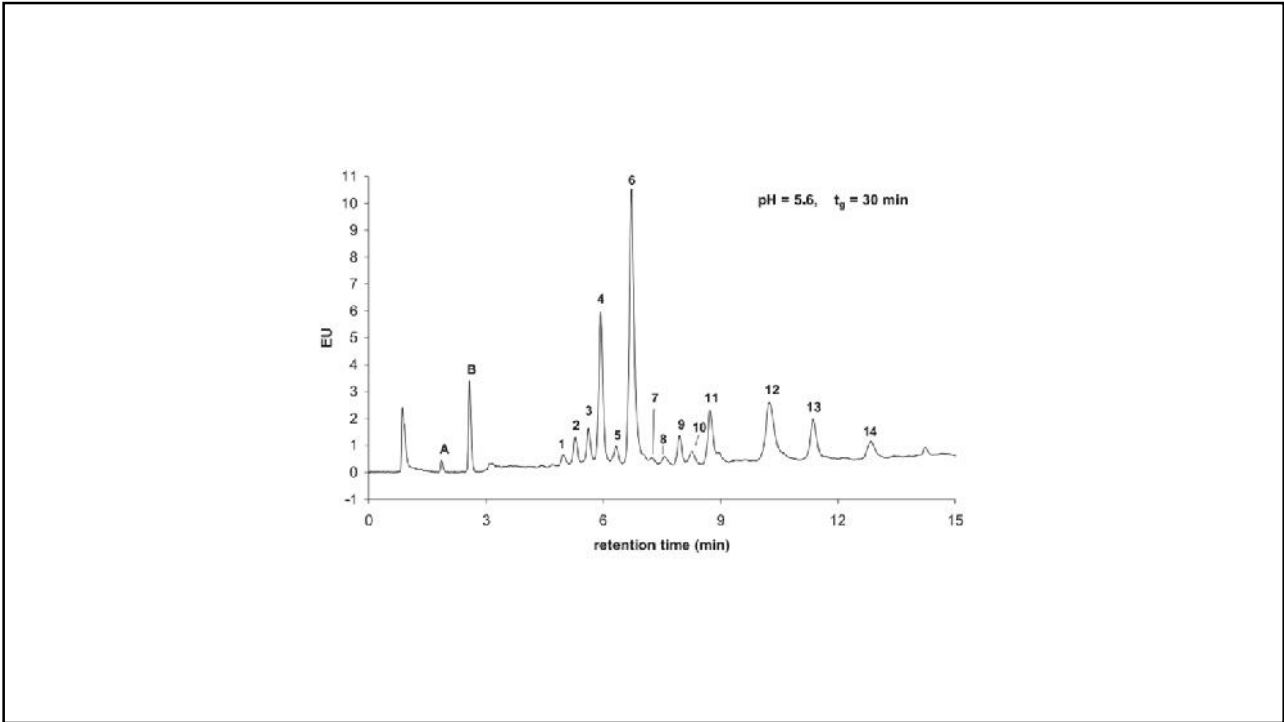
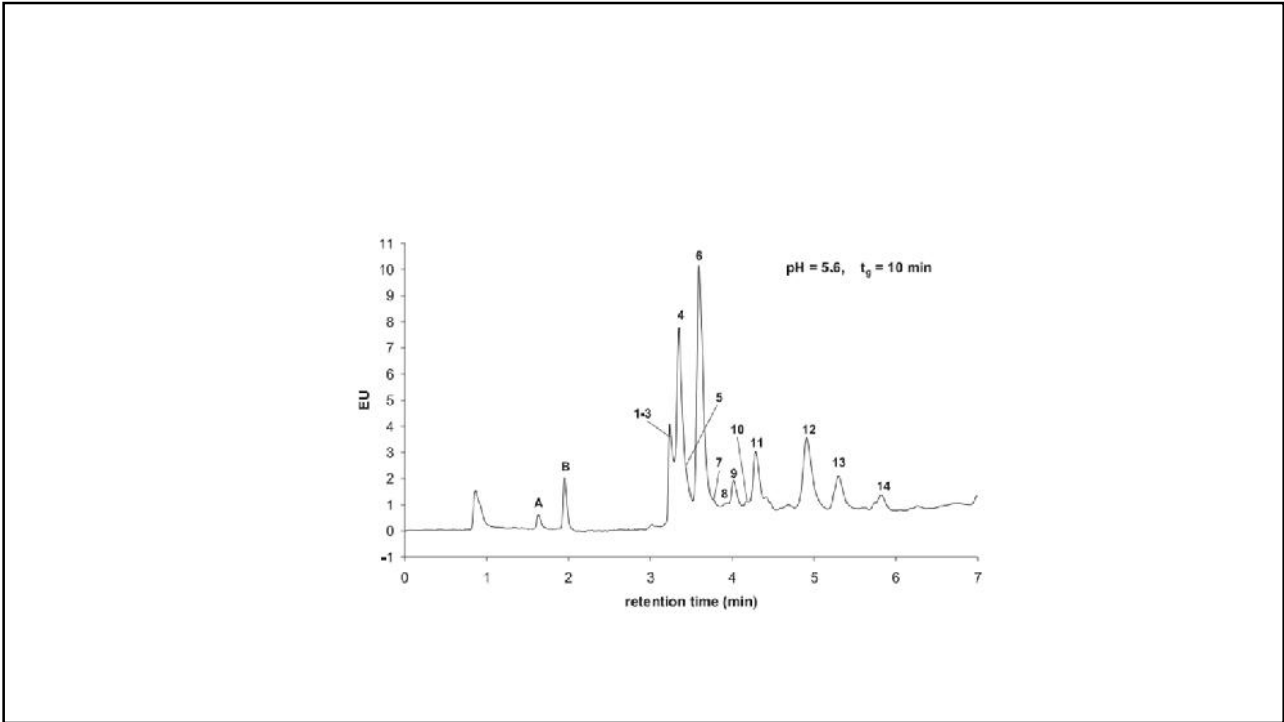


$$\log k = \log K - z \log \left( C_0 + \frac{\Delta C}{r_g} \right)$$

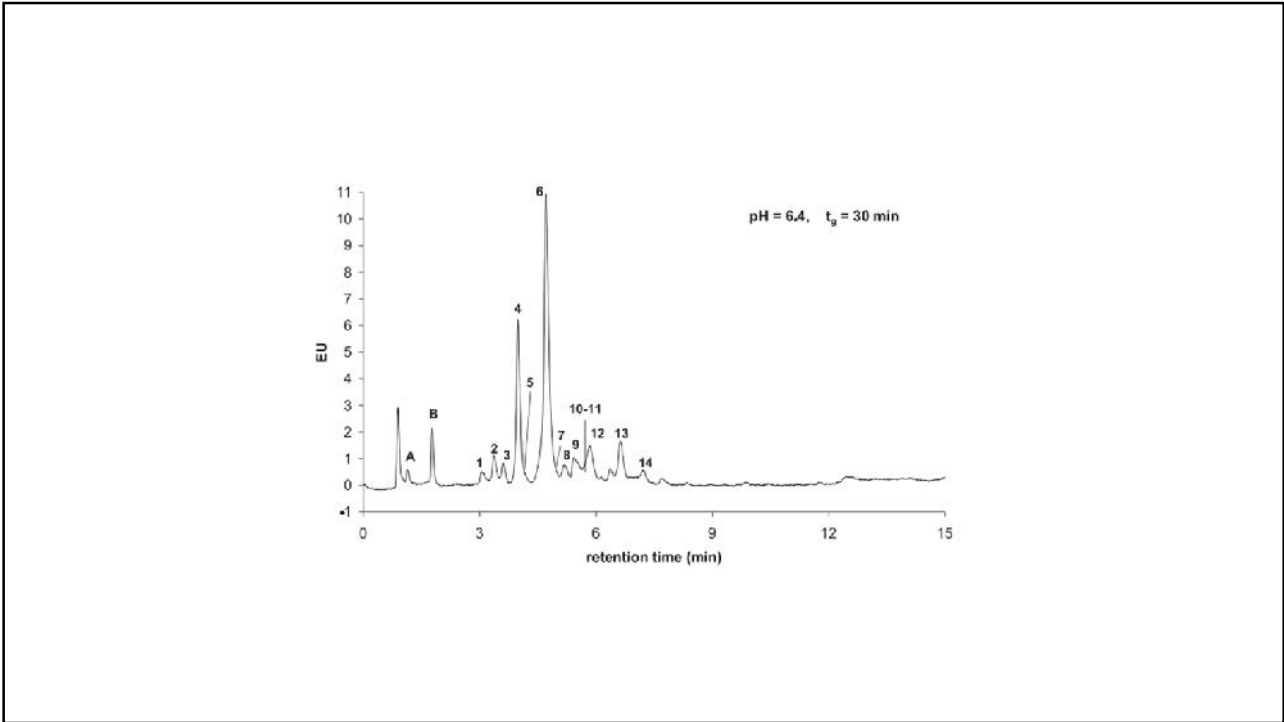
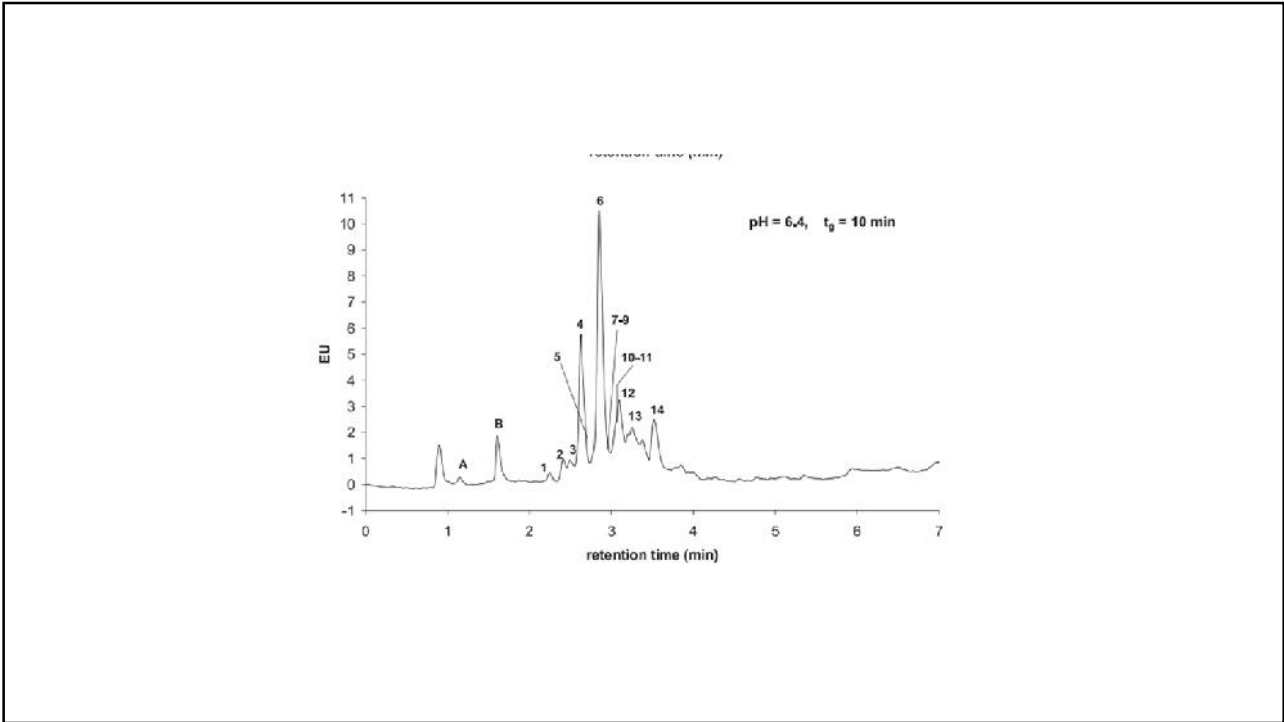


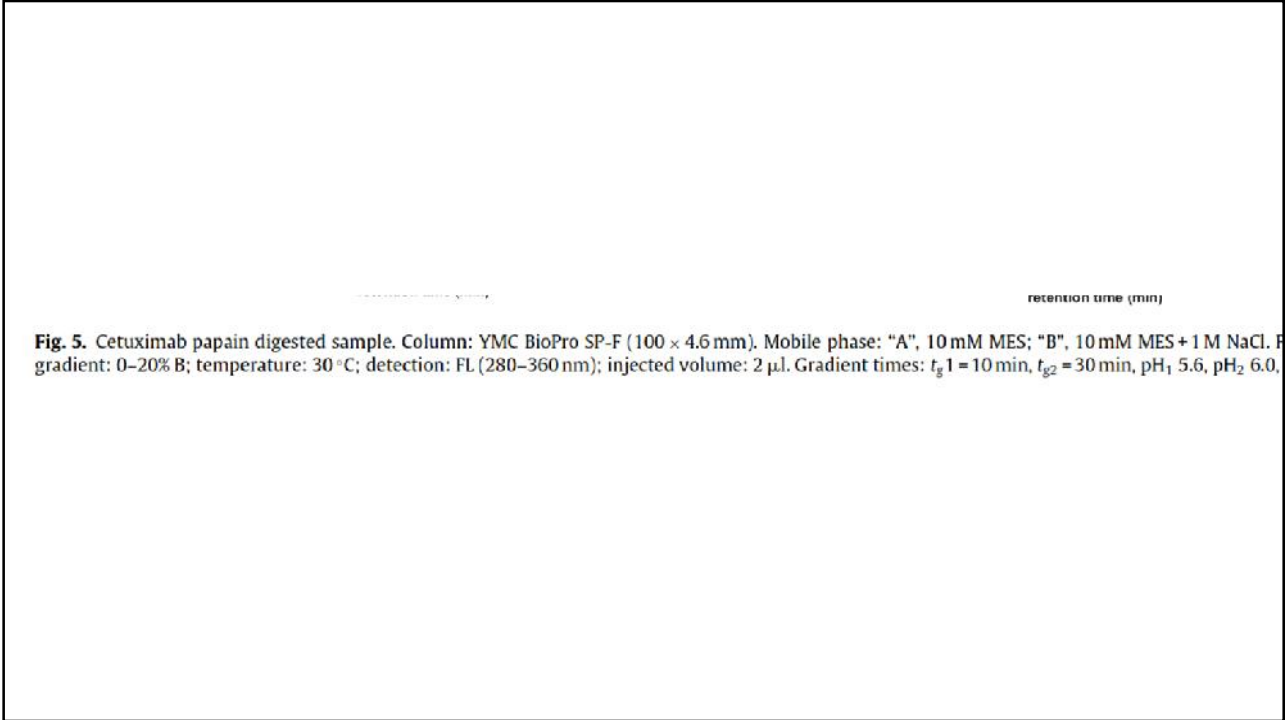
S. Fekete et al. / Journal of Pharmaceutical and Biomedical Analysis 102 (2015) 33–44











**Fig. 5.** Cetuximab papain digested sample. Column: YMC BioPro SP-F (100 × 4.6 mm). Mobile phase: "A", 10 mM MES; "B", 10 mM MES + 1 M NaCl. Gradient: 0–20% B; temperature: 30 °C; detection: FL (280–360 nm); injected volume: 2 μl. Gradient times:  $t_{g1}$  = 10 min,  $t_{g2}$  = 30 min, pH<sub>1</sub> 5.6, pH<sub>2</sub> 6.0.