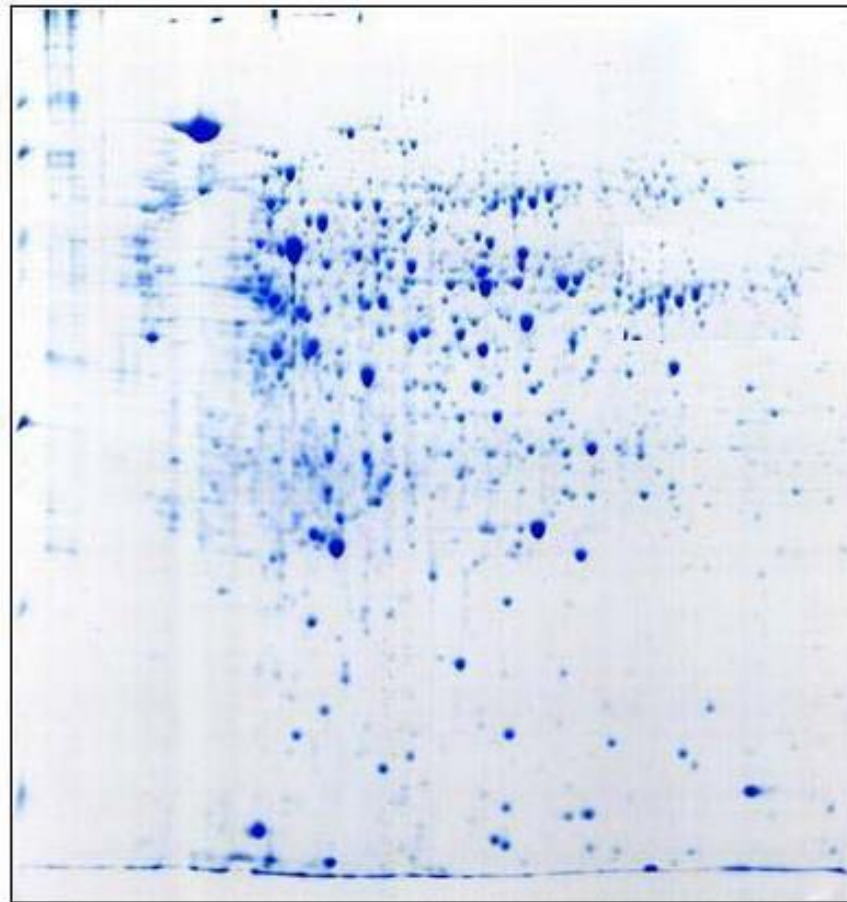


VÍCEROZMĚRNÉ SEPARAČNÍ TECHNIKY

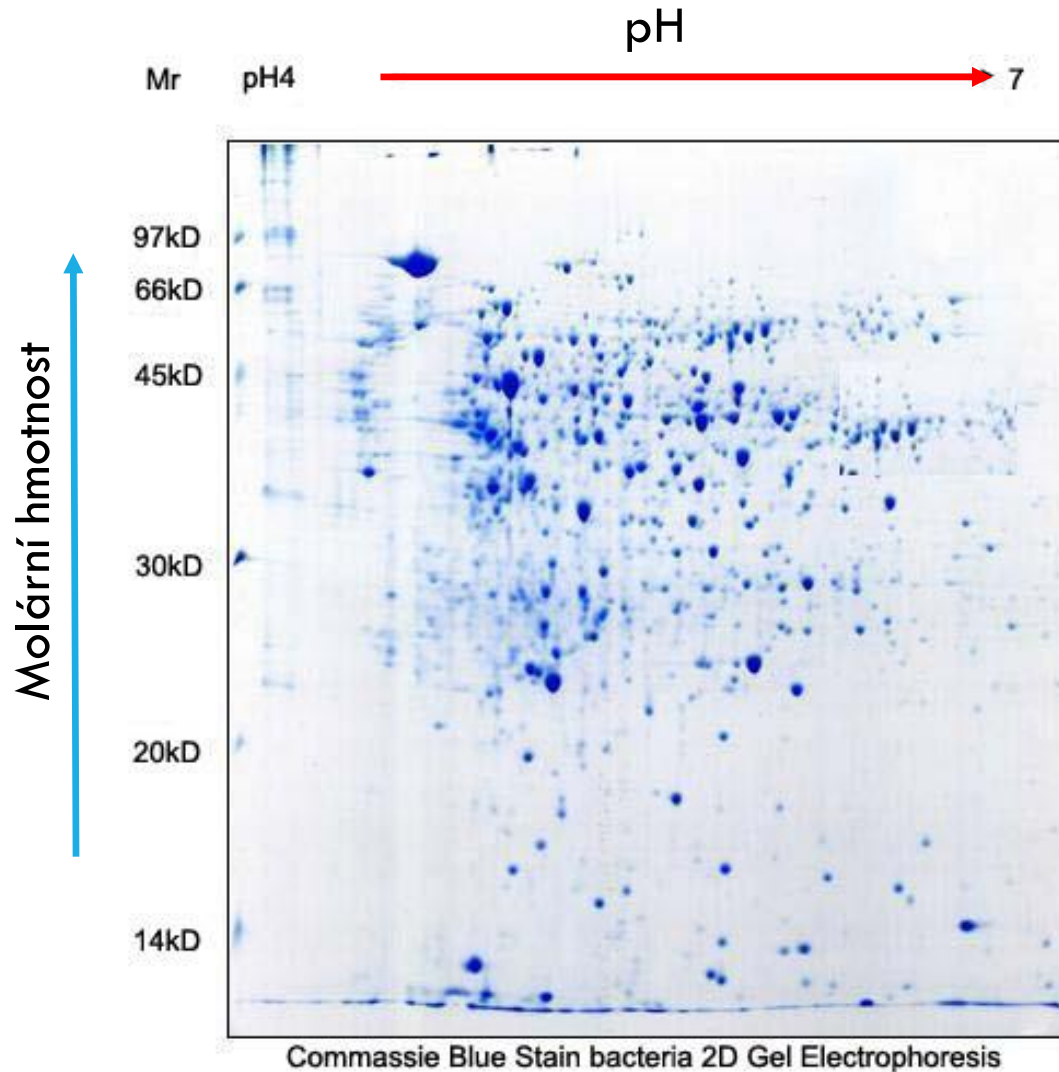
Pokročilá kapalinová
chromatografie



MODERNÍ UMĚNÍ (?)



(2D) GELOVÁ ELEKTROFORÉZA



BYLO NEBYLO ...

„...but the two-dimensional chromatogram is especially convenient, in that it shows at a glance information that can be gained otherwise only as the result of numerous experiments“.



A.J.P. Martin

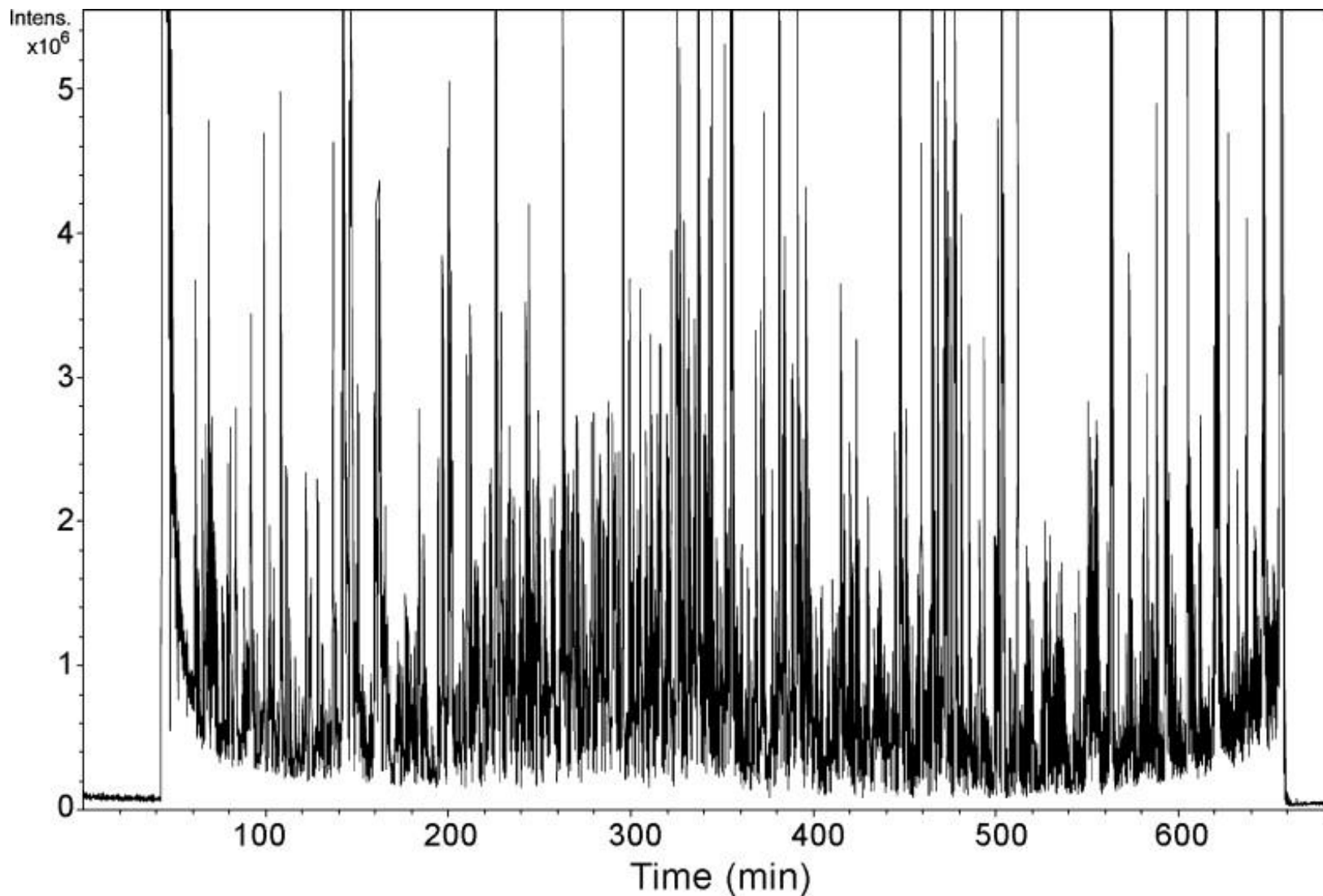
Biochem J. 38 ([1944](#)) 224.



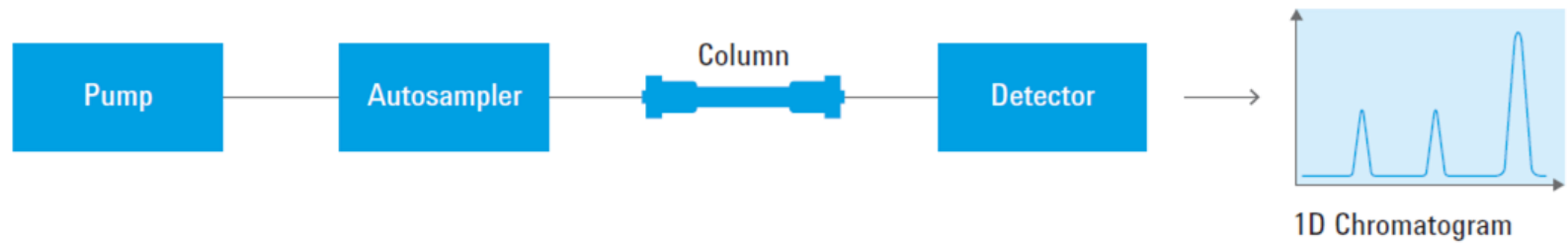
Aminokyseliny z hydrolyzátu vlny
První dimenze: 3 dny
Druhá dimenze: 27 hodin

E. COLI DIGEST

~ 1000 píků za 11 hodin

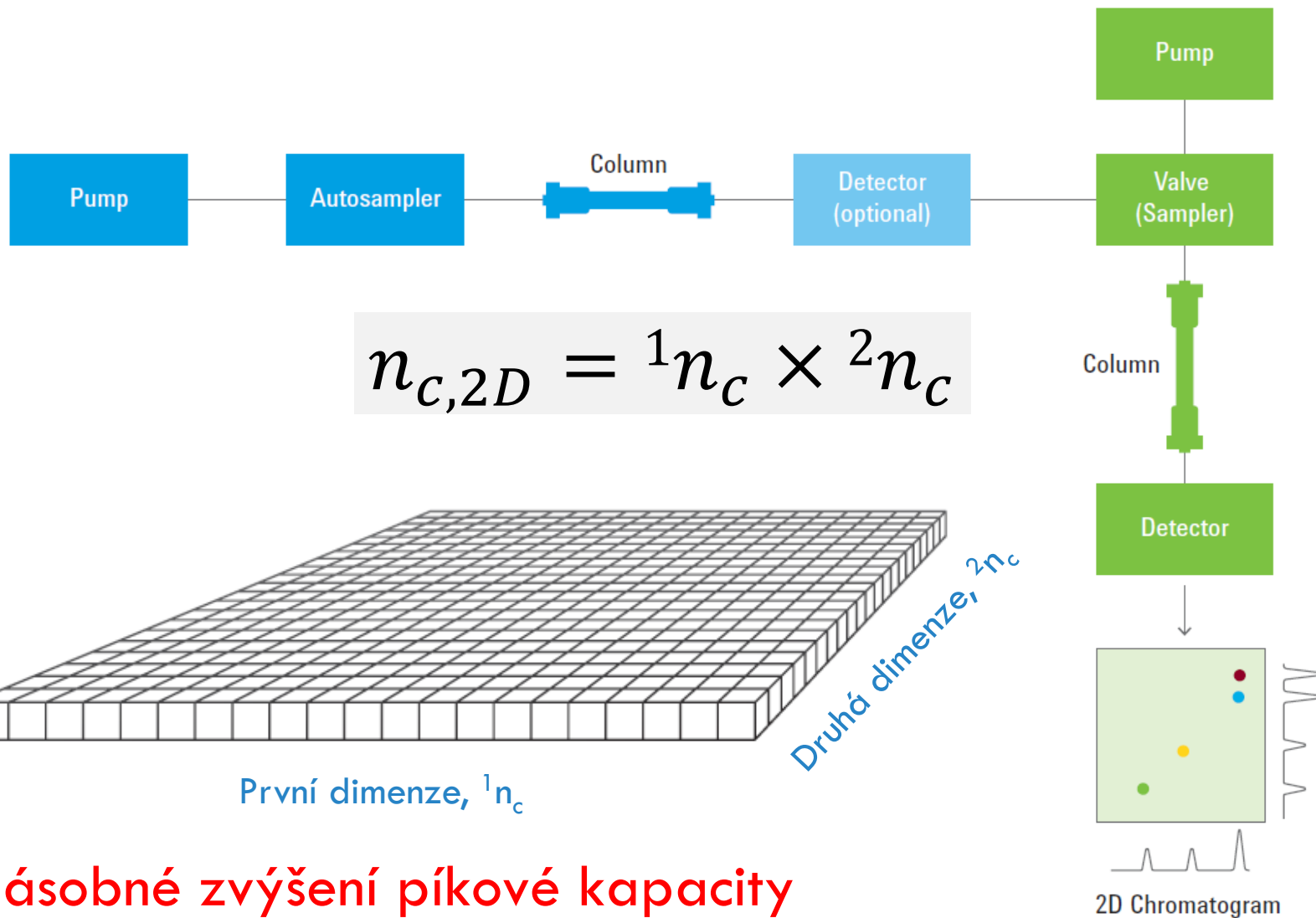


1D HPLC



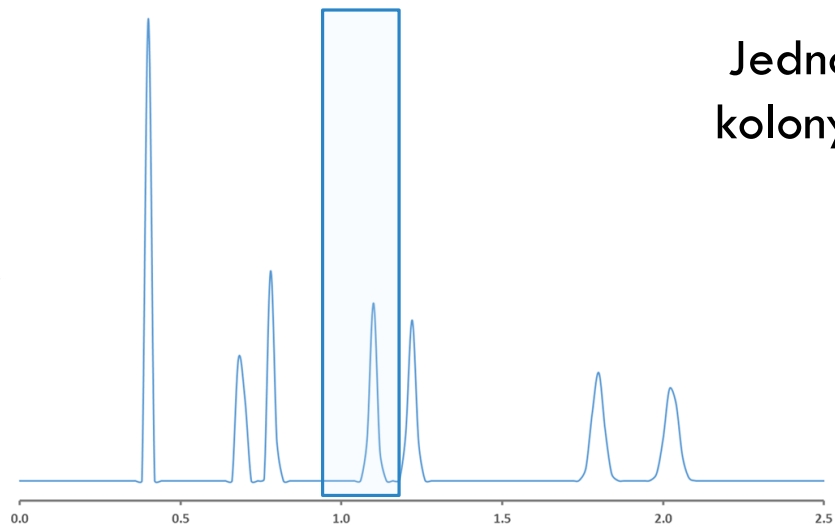
První dimenze, 1n_c

2D HPLC



Násobné zvýšení píkovej kapacity

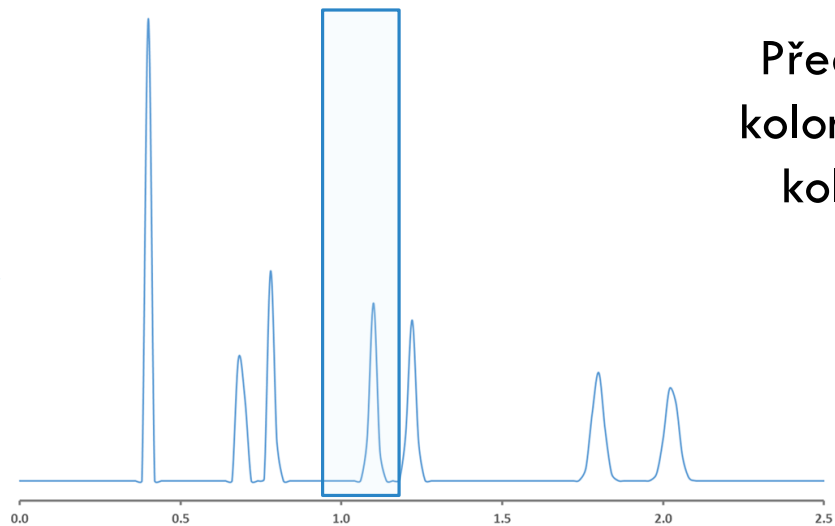
OFF-LINE



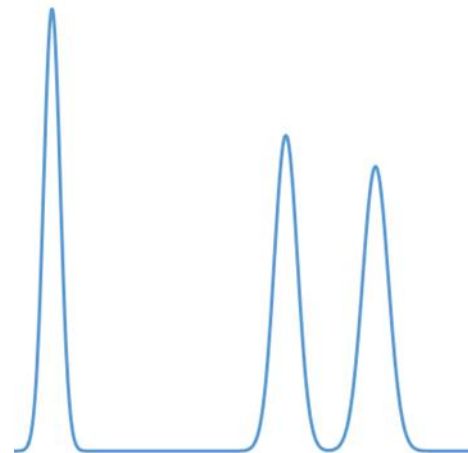
Jednotlivé frakce se sbírají z D1 kolony, zakoncentrují se a dávkují se na D2 kolonu.



ON-LINE: HEART-CUT

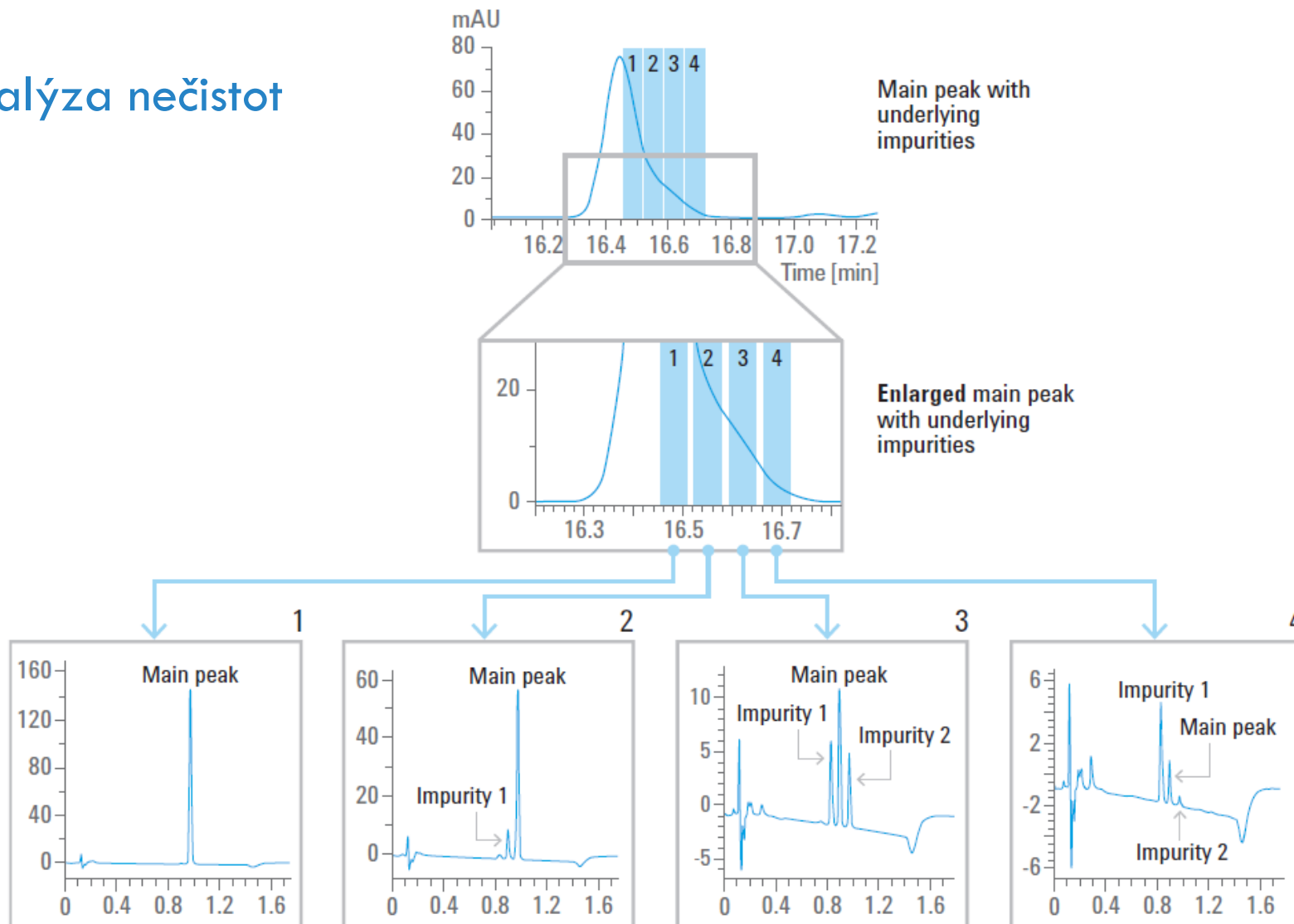


Předem vybrané frakce z D1 kolony se přímo převádí na D2 kolonu přepínacím ventilem



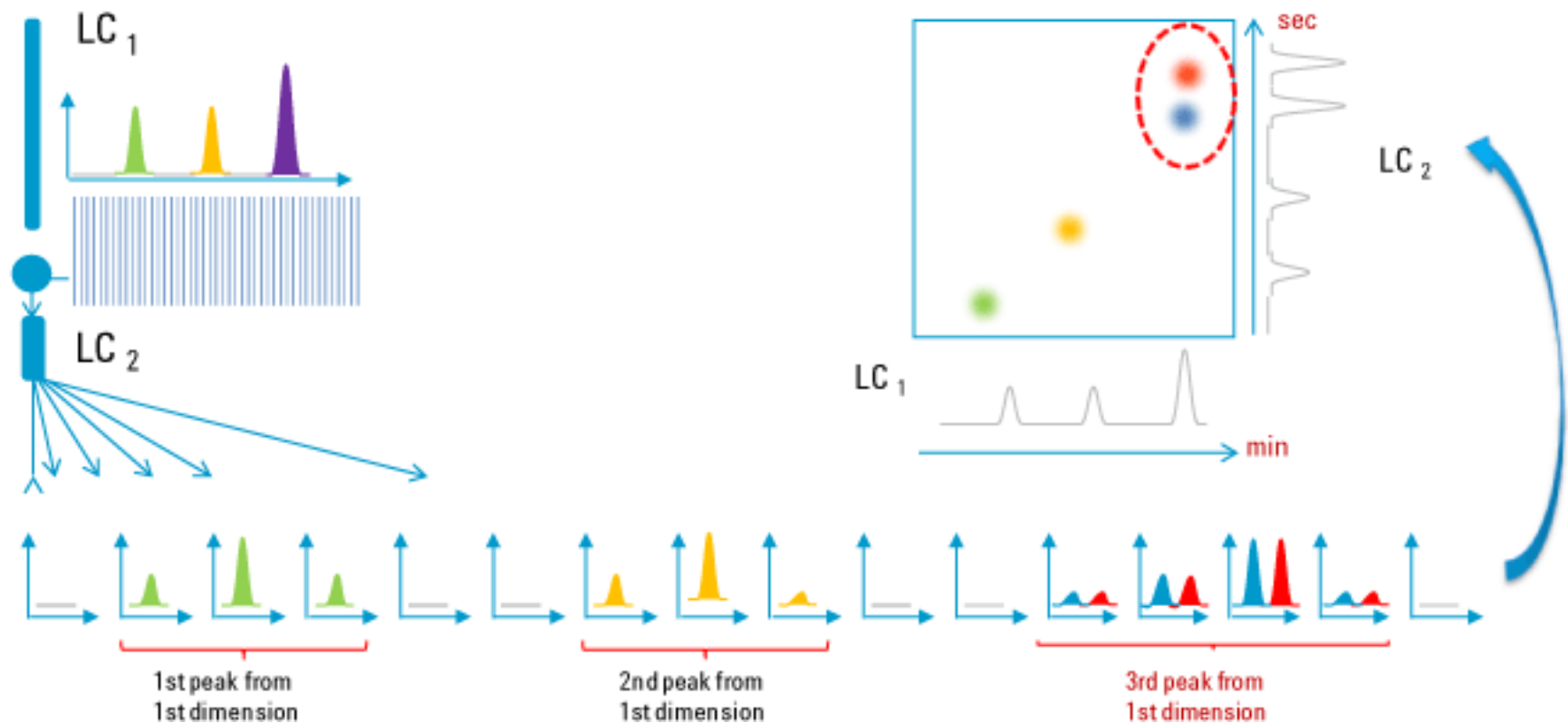
ON-LINE: HEART-CUT

Analýza nečistot



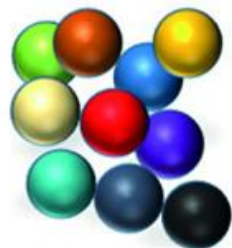
COMPREHENSIVE 2D-LC

„Bezztrátová“ 2D-LC

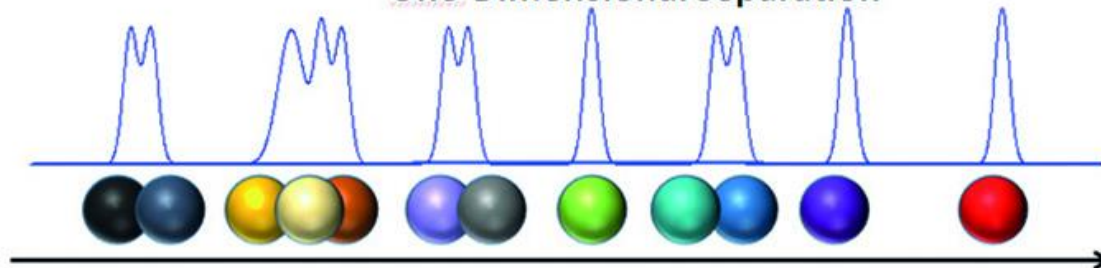


COMPREHENSIVE 2D-LC

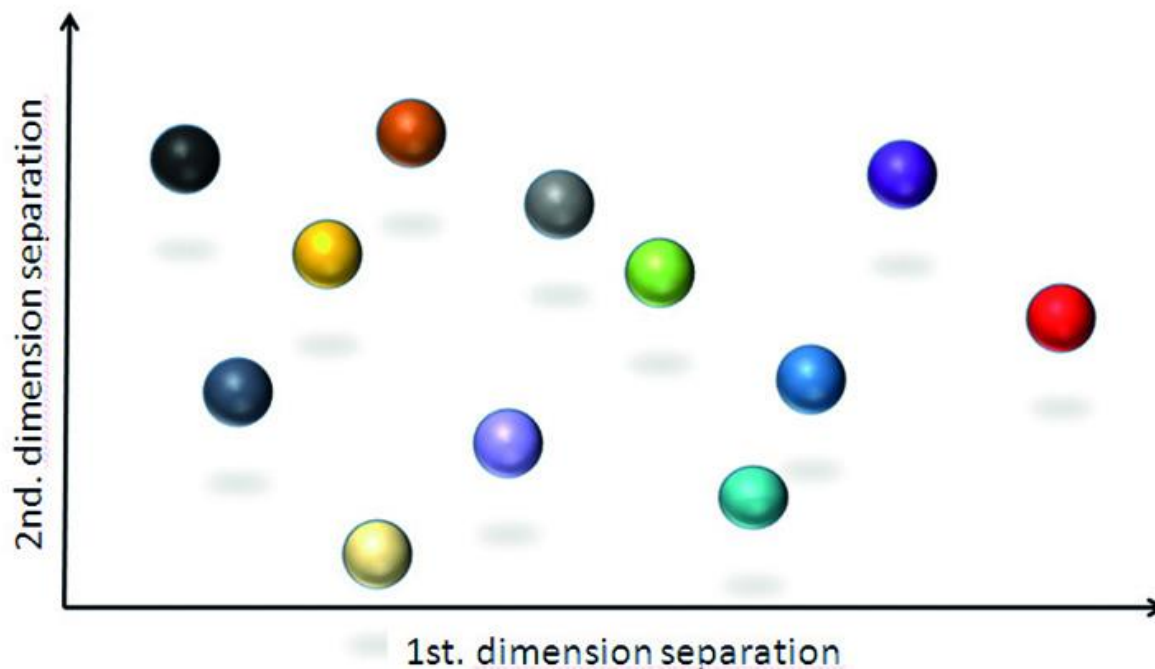
Complex sample



One-Dimensional Separation

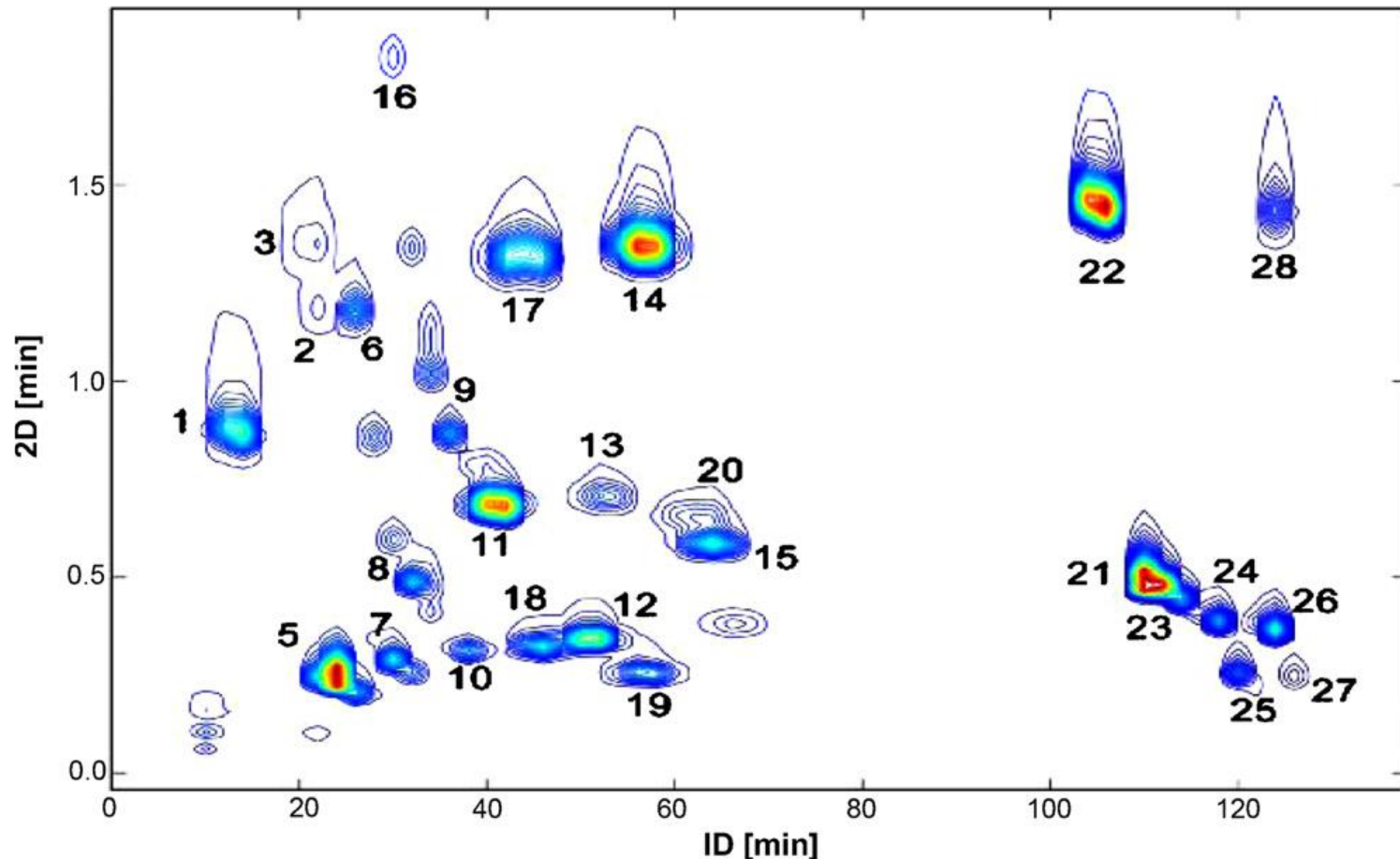


Two-Dimensional Separation



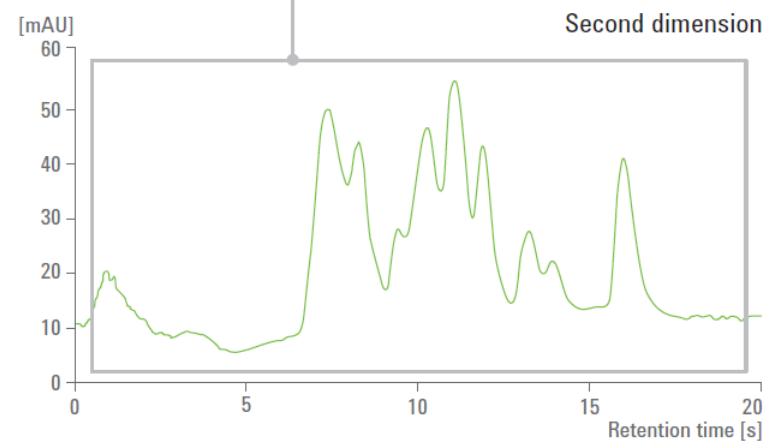
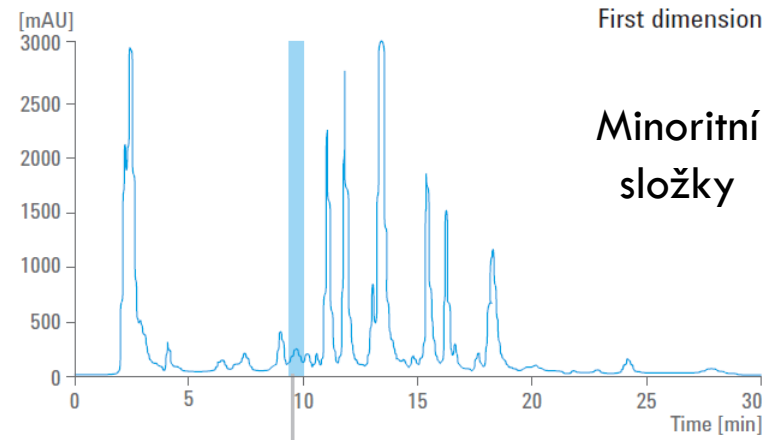
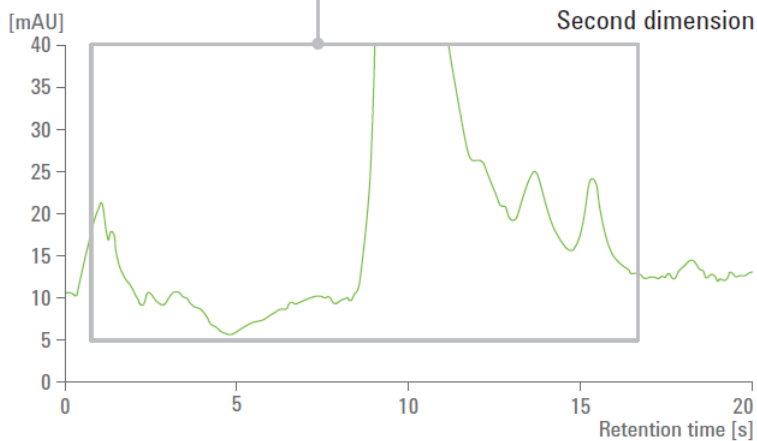
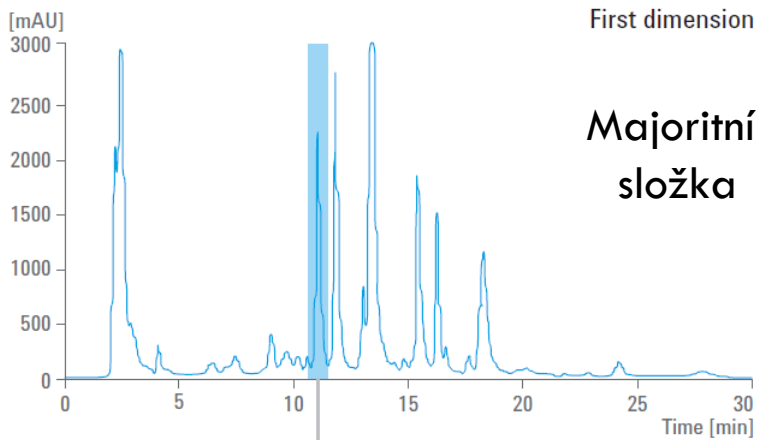
DVOUROZMĚRNÁ CHROMATOGRRAFIE

Fenolické látky a flavony

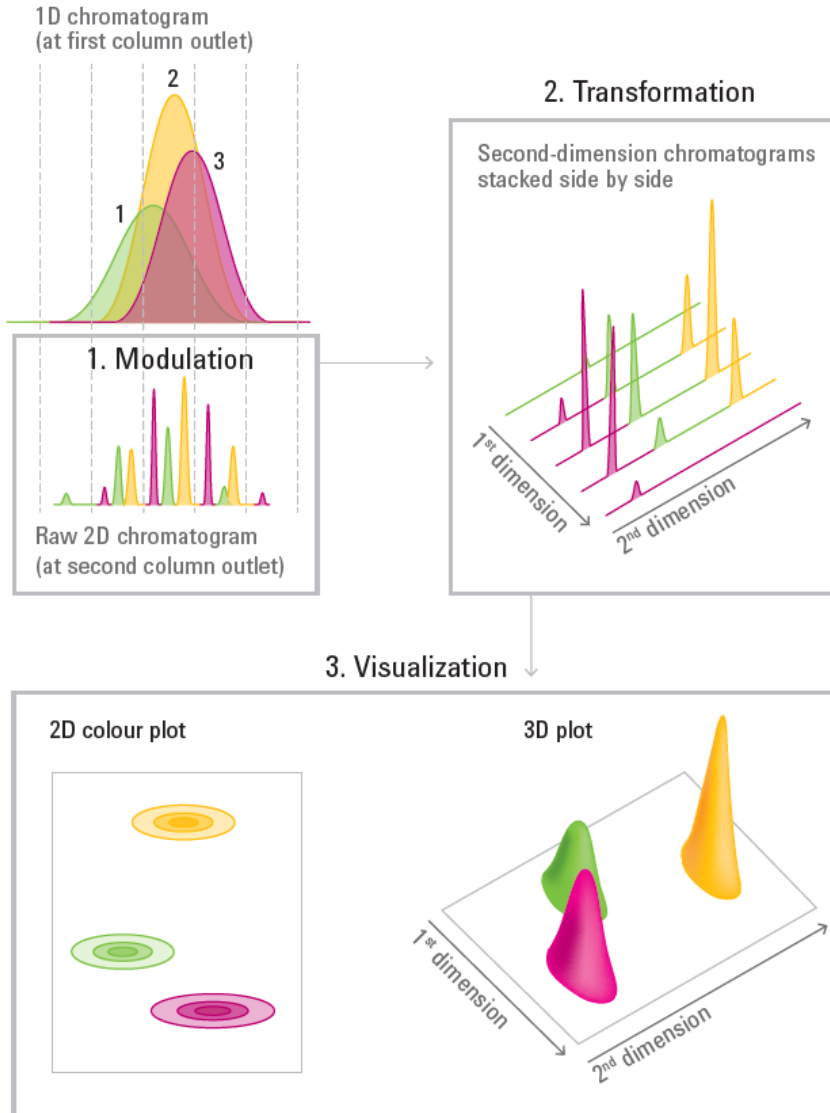


PODROBNĚJŠÍ INFORMACE O VZORKU

Sazenice kukuřice



ZPRACOVÁNÍ DAT



1) Modulace

Kompletní převod D1 eluátu do druhé dimenze (D2)

2) Transformace

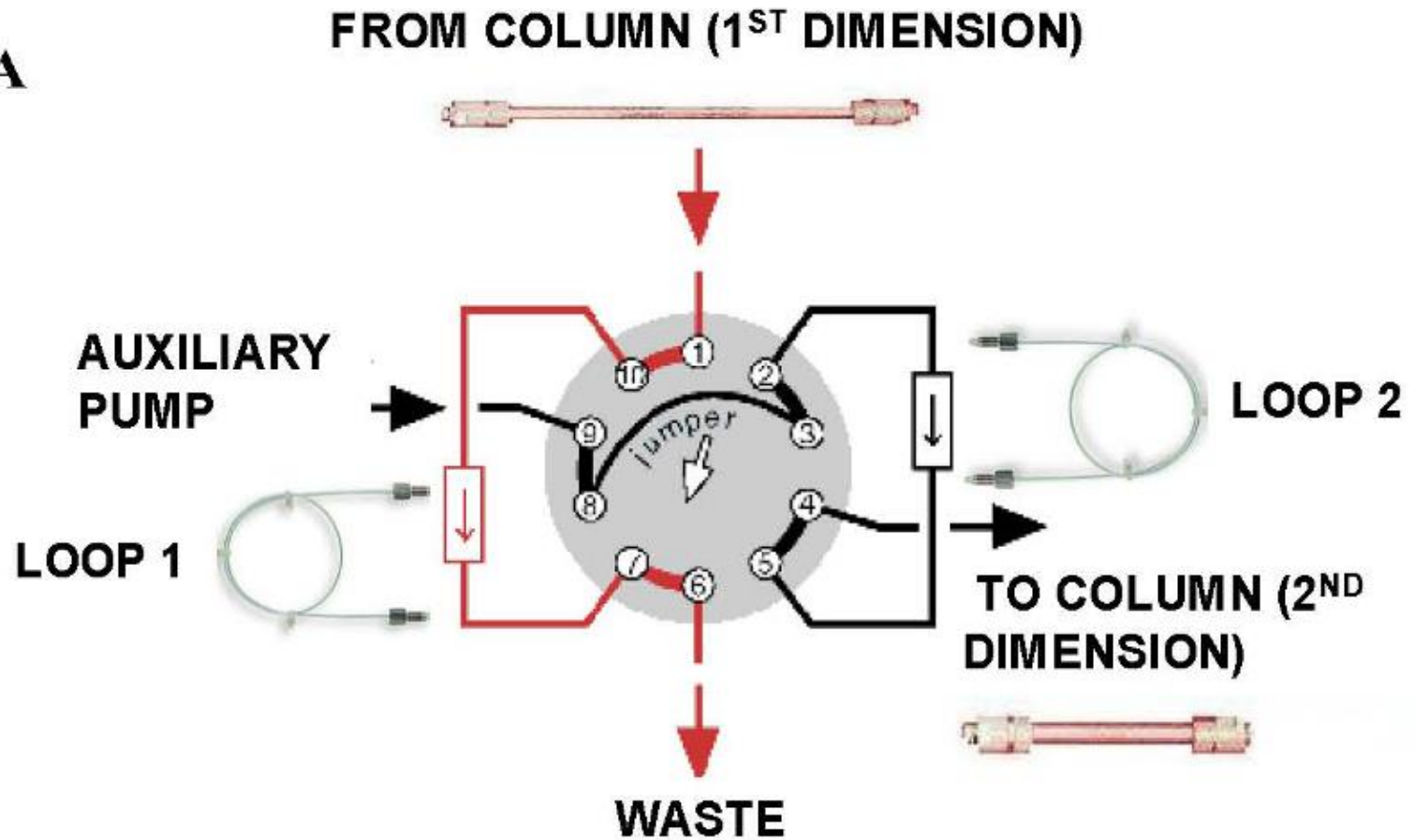
Zpracování dat poskytnutých detektorem druhé dimenze.

3) Vizualizace

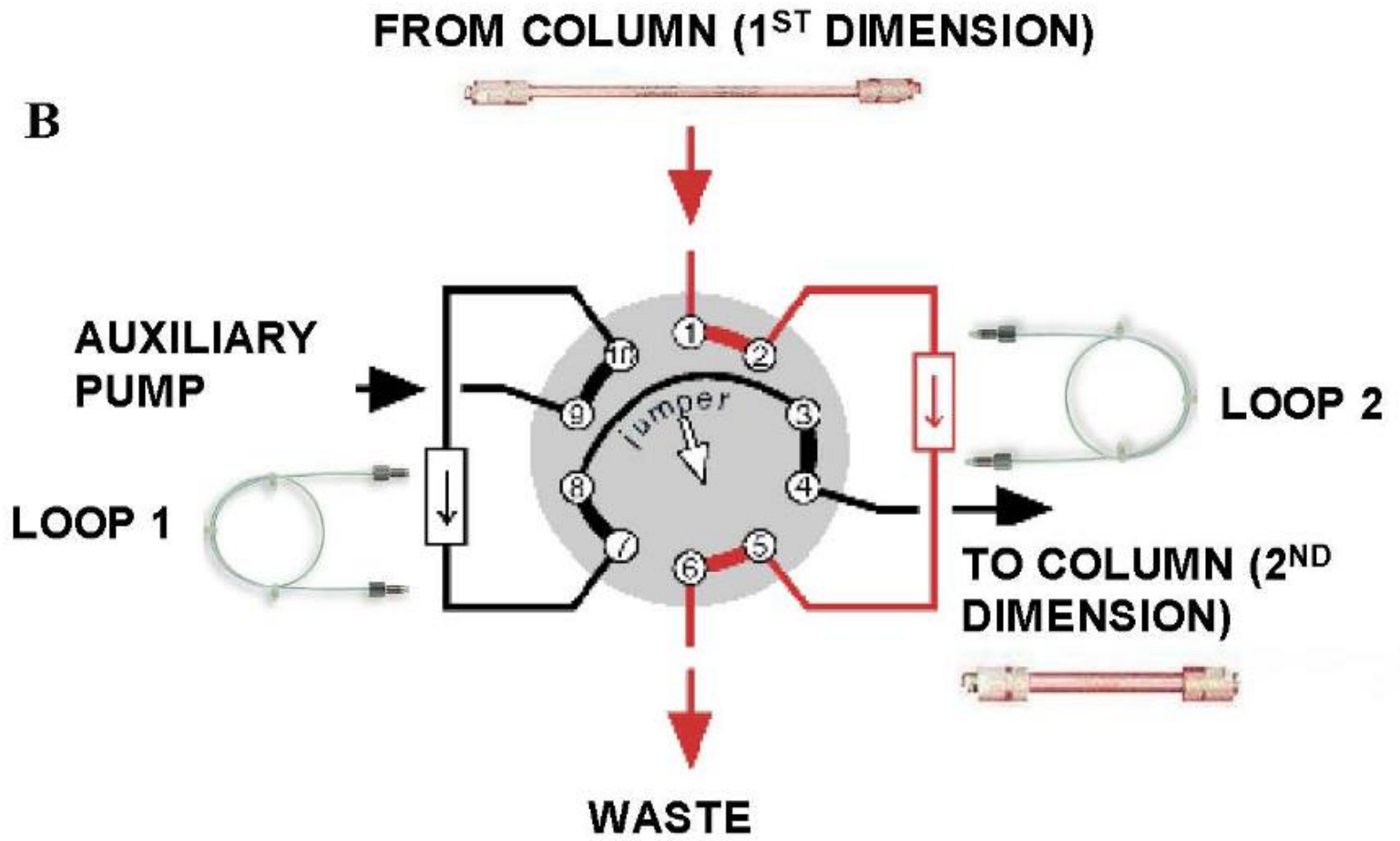
Prezentace dat pomocí obrysového nebo prostorového grafu.

PŘEVOD FRAKCÍ

A



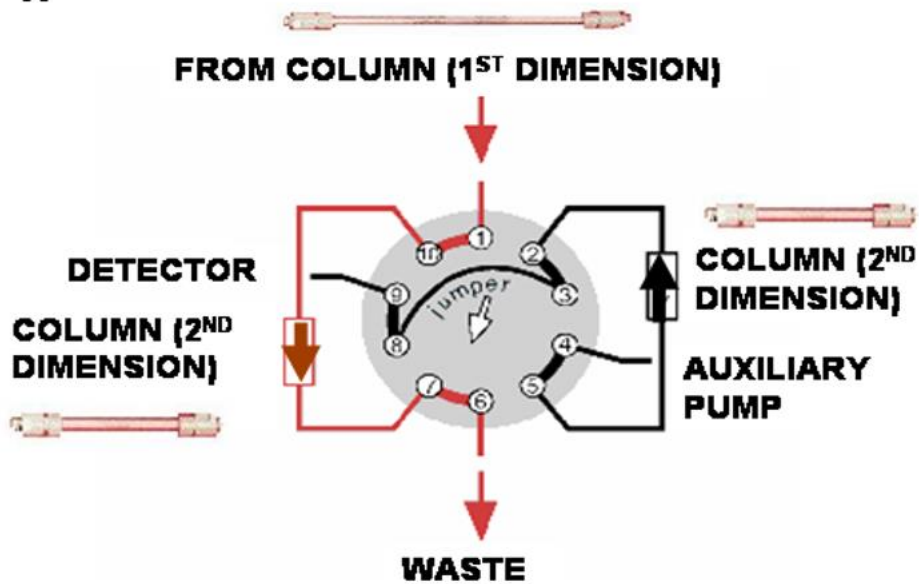
PŘEVOD FRAKCÍ



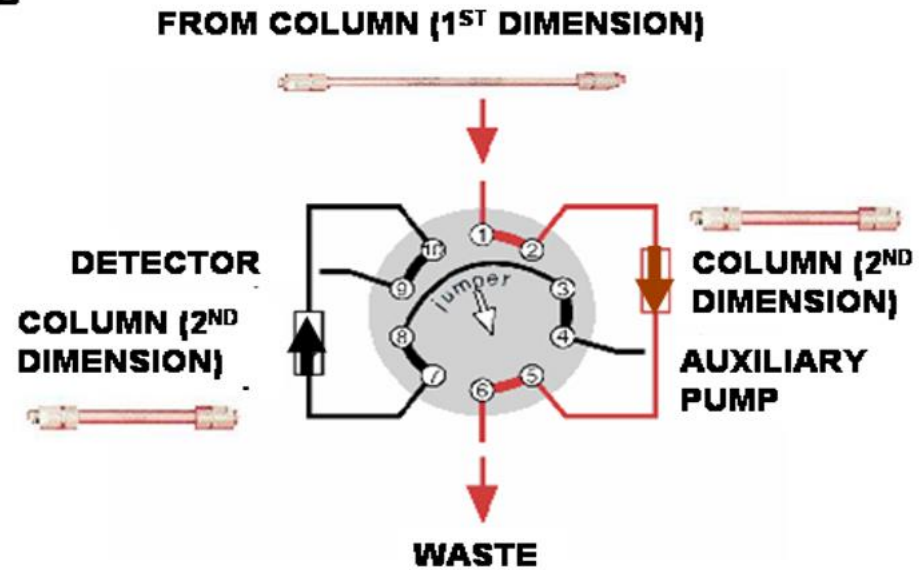
PŘEVOD FRAKČÍ

Dvě 2D kolony

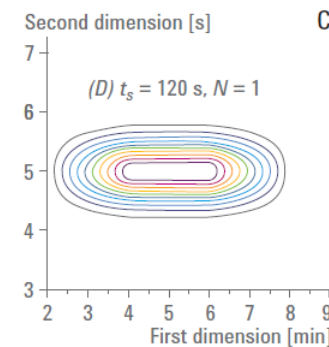
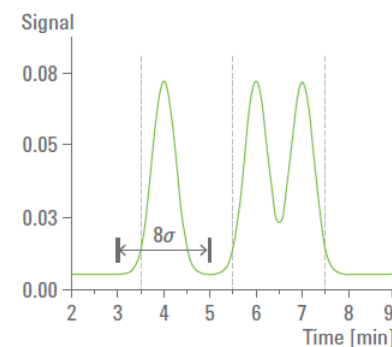
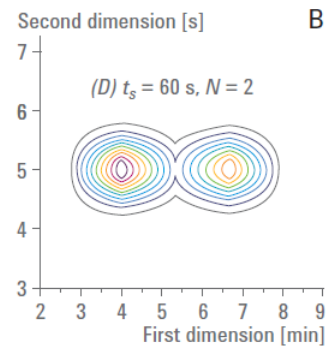
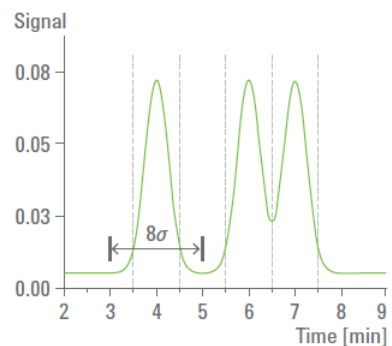
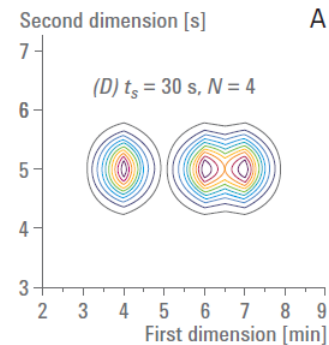
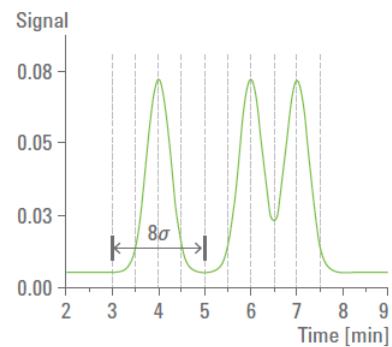
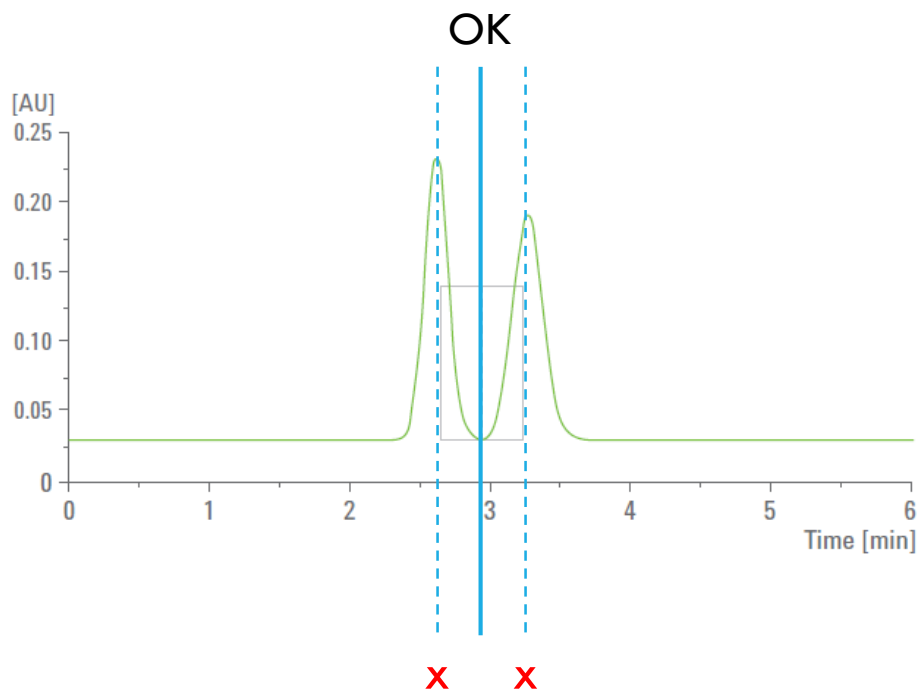
A



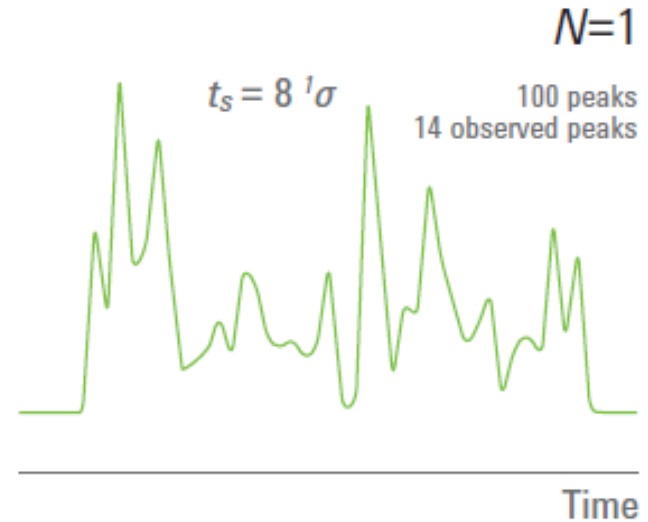
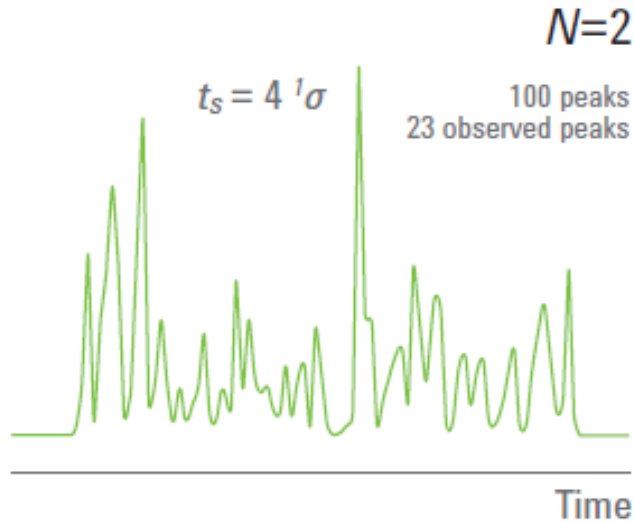
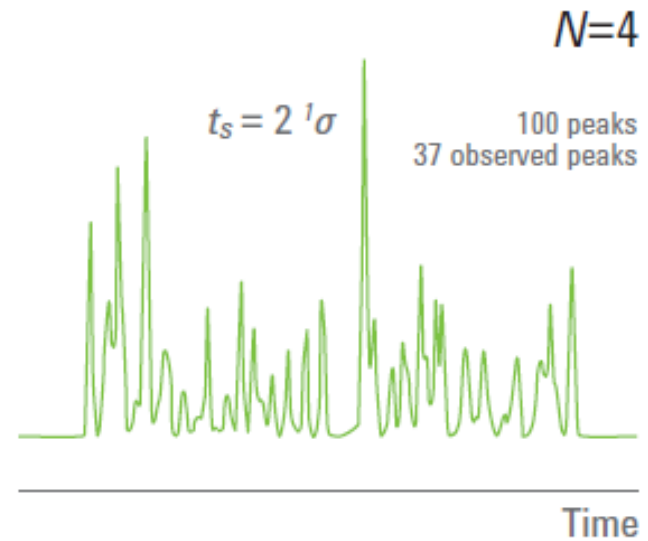
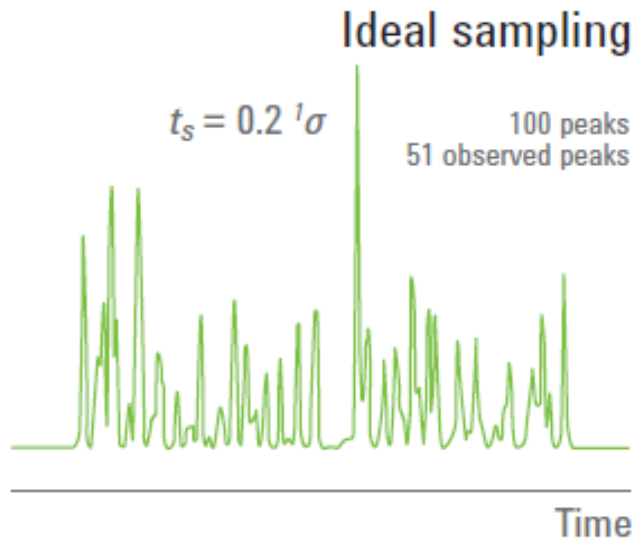
B



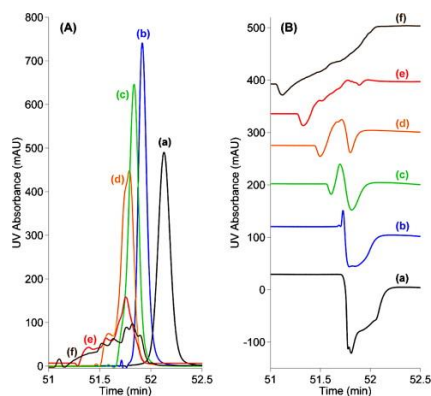
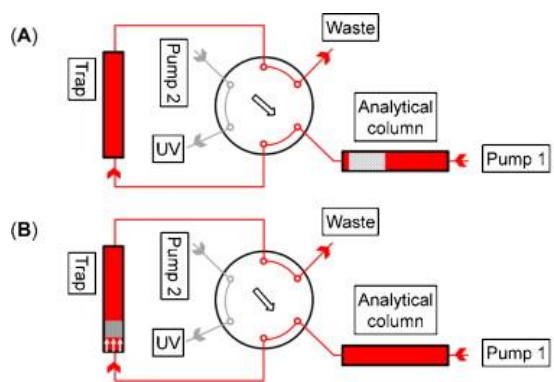
FREKVENCE PŘEVODU FRAKCÍ



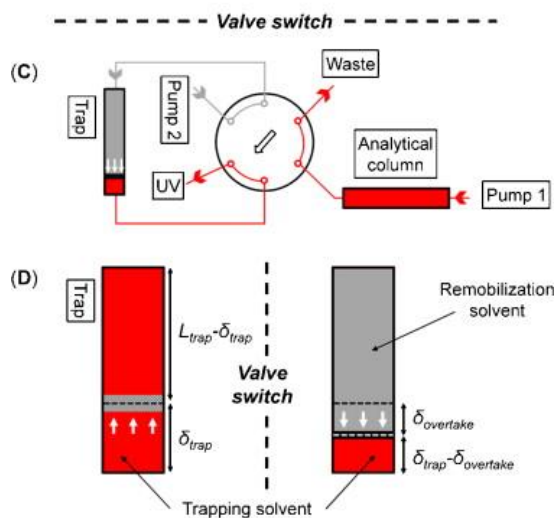
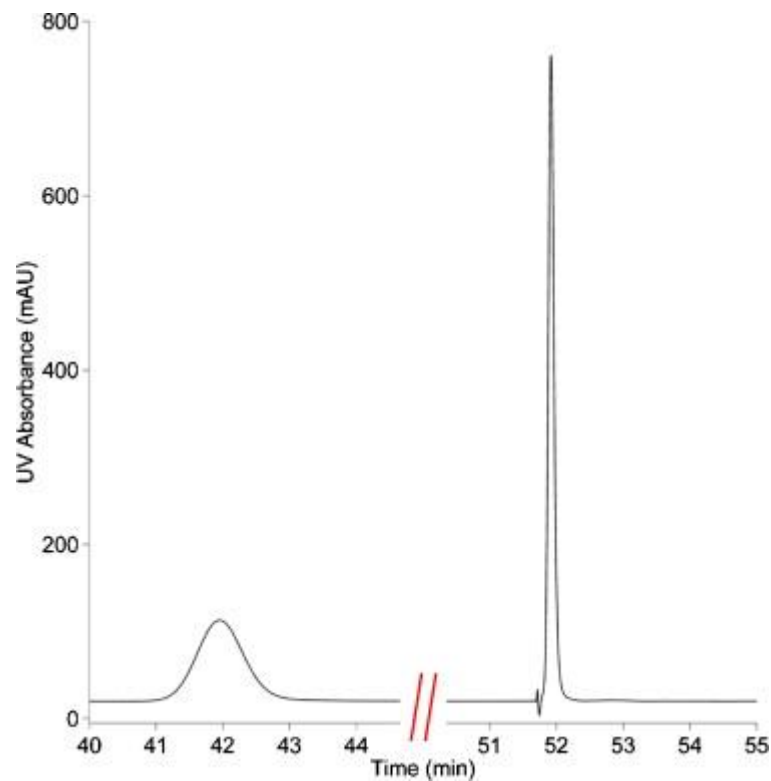
FREKVENCE PŘEVODU FRAKCÍ



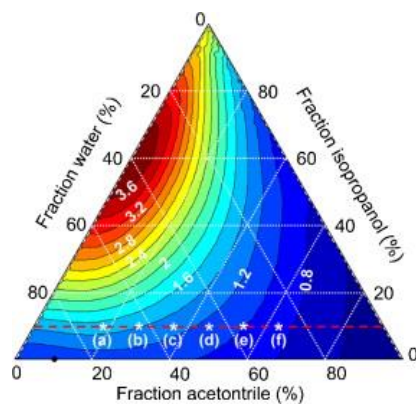
OPTIMALIZACE SLOŽENÍ MOBILNÍ FÁZE



8x vyšší signál



Isopropanol



AKTIVNÍ MODULACE

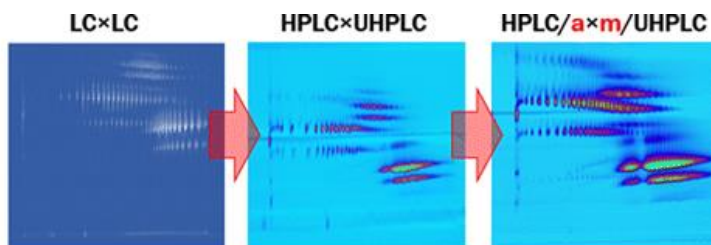
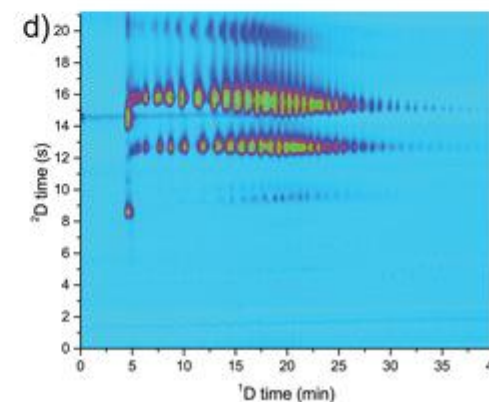
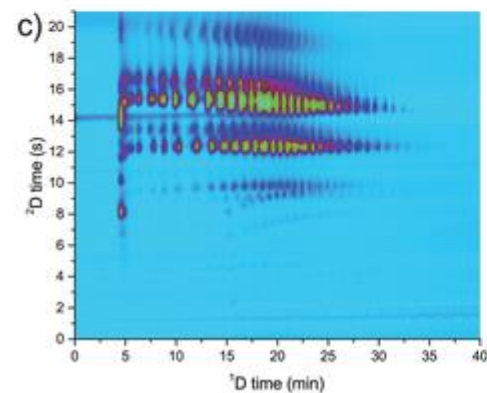
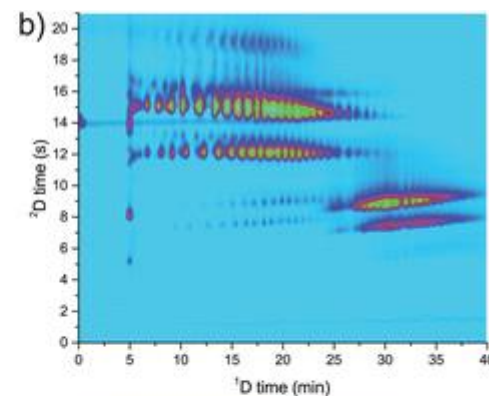
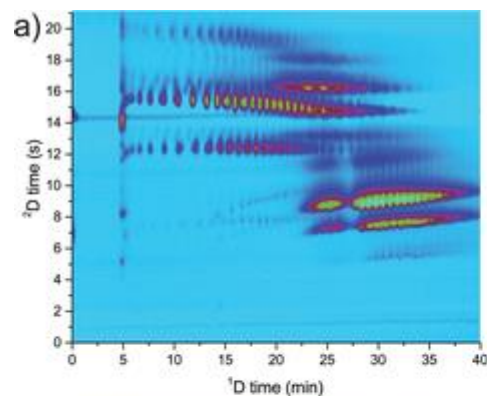
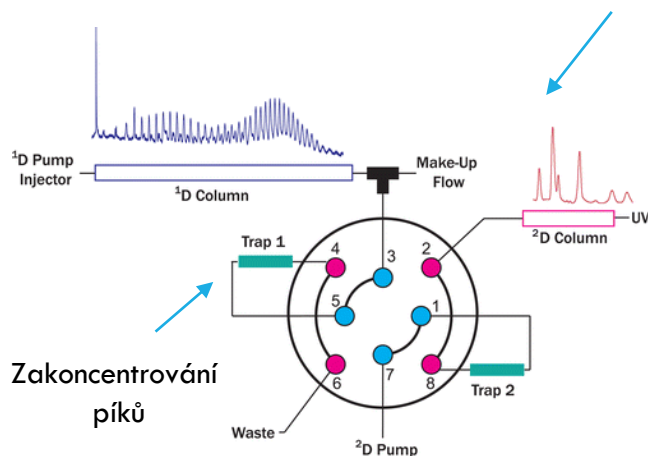
HILIC x RP

Surfaktanty (tristyrylfenol ethoxylátfosfát (TSP))

HILIC: délka etoxylátového řetězce

RP: míra substituce (styren, fosfát)

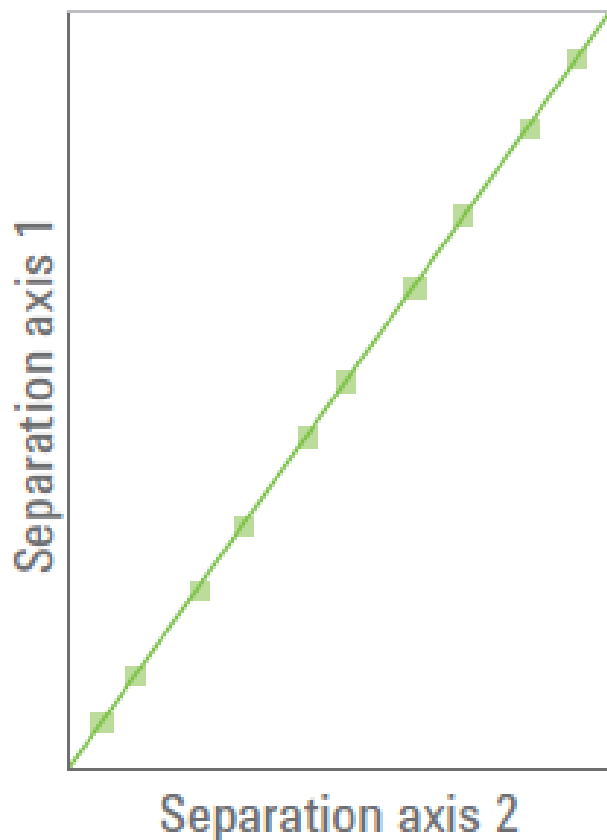
Snížení eluční síly
mobilní fáze
(7 x 1D F_m)



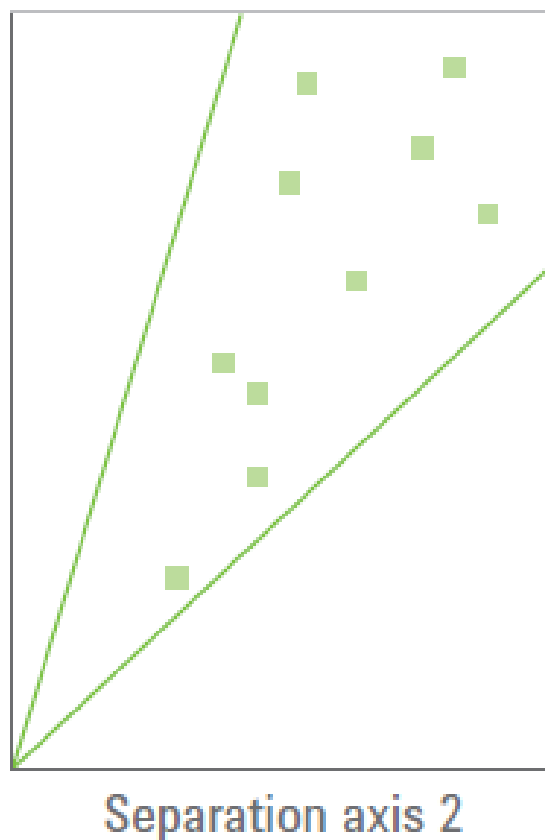
	LC x LC	HPLC x UHPLC	HPLC/a x m/UHPLC
Analysis Time (min)	200	80	40
Dilution Factor	1529	299	142
Eluent Volume (mL)	800	120	78

ORTOGONALITA

Úplná
korelace



Částečná
korelace

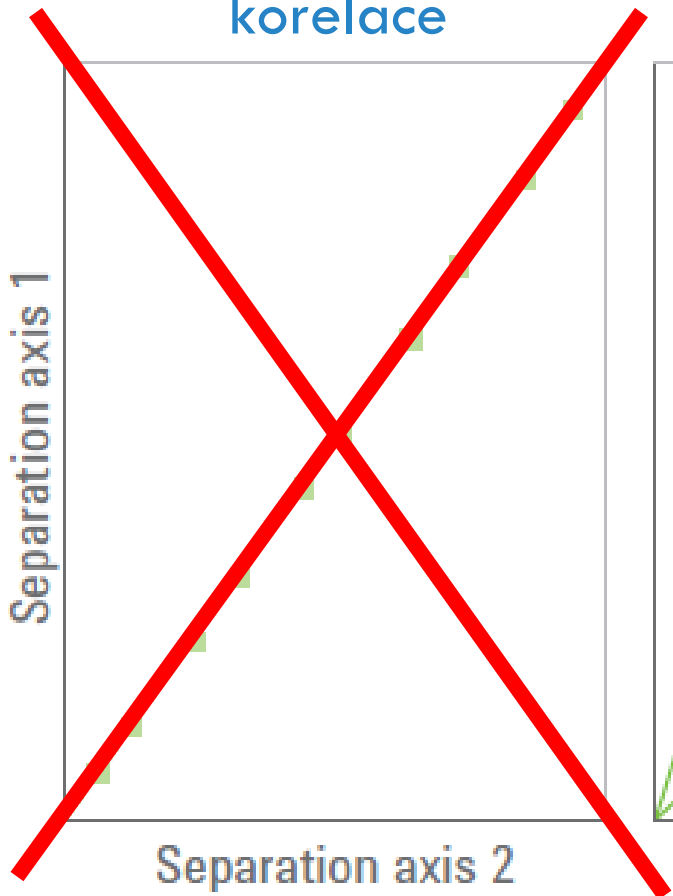


Ortogonalní
separace



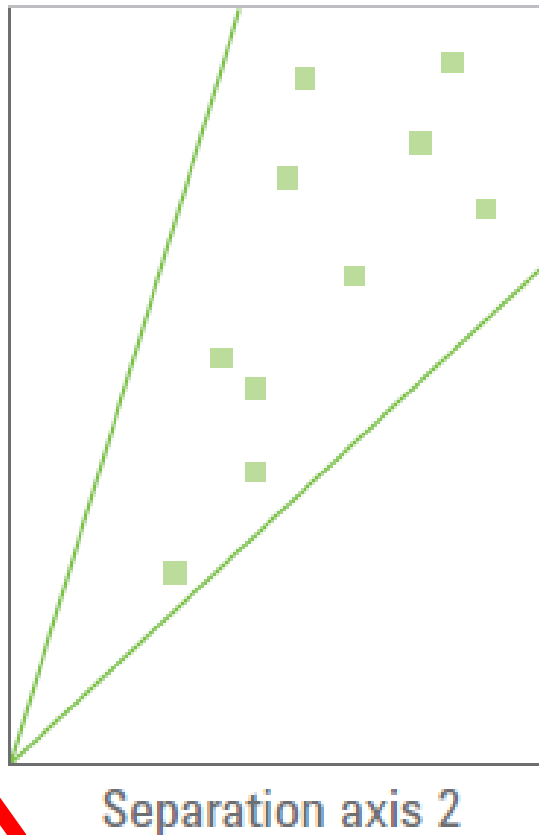
ORTOGONALITA

Úplná
korelace

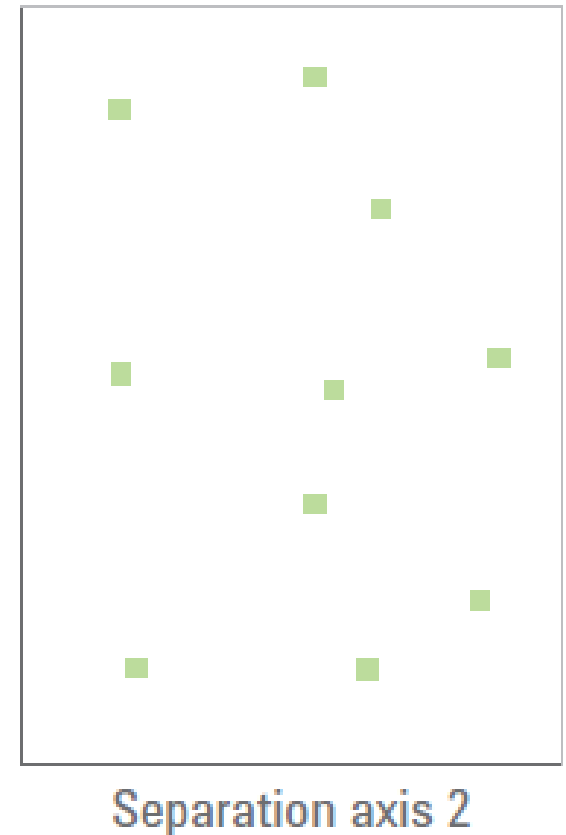


Žádná přidaná informace

Částečná
korelace

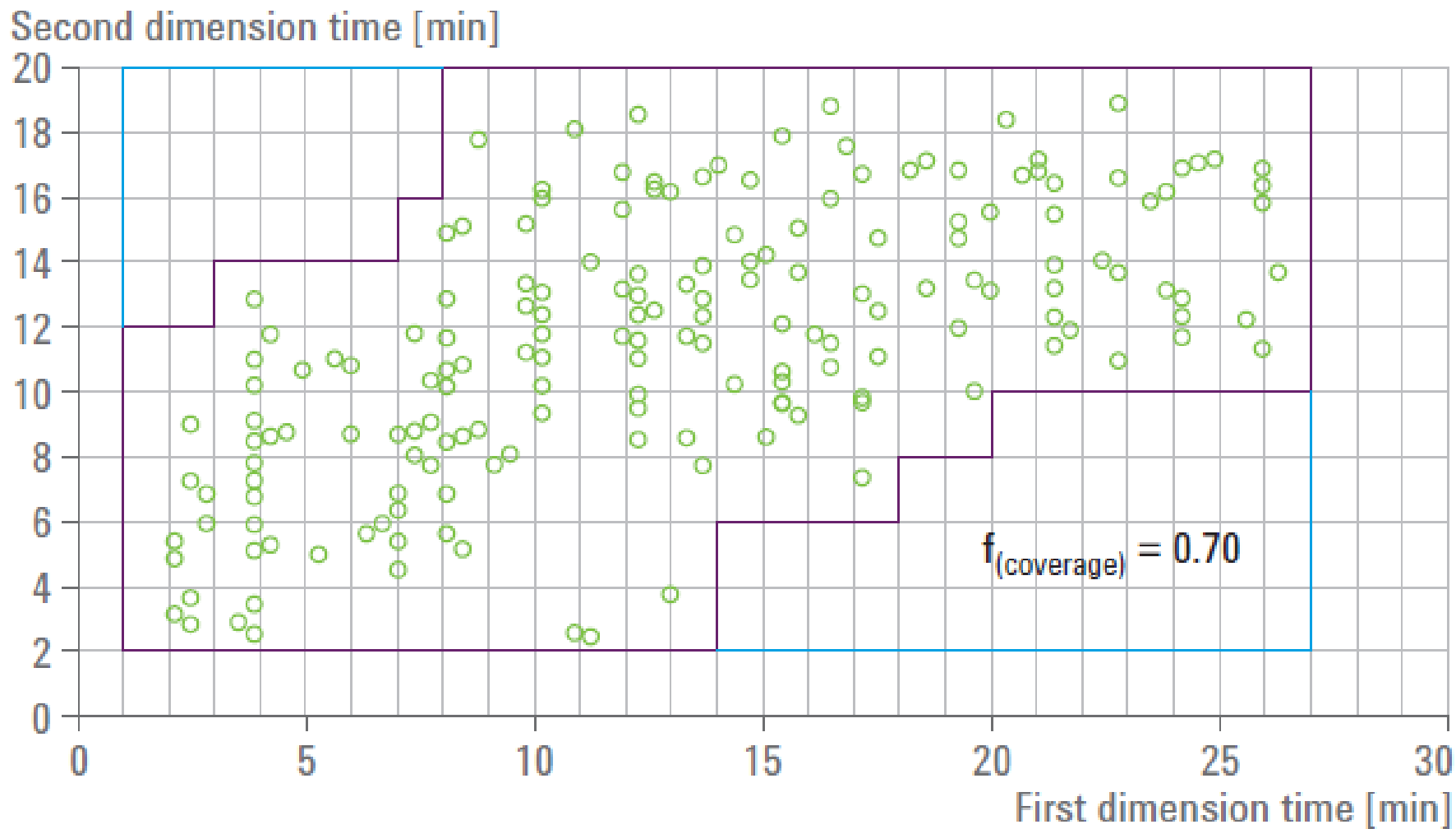


Ortogonalní
separace



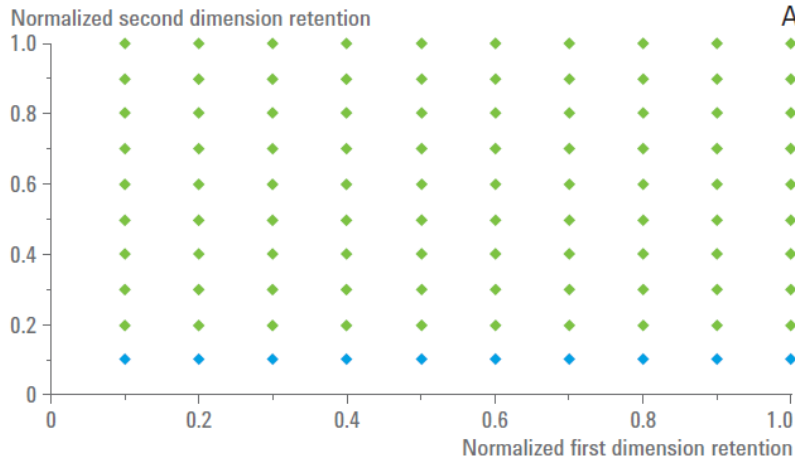
Nejlepší pokrytí prostoru

KVANTIFIKACE ORTOGONALITY



TYPY 2D-LC SEPARACÍ

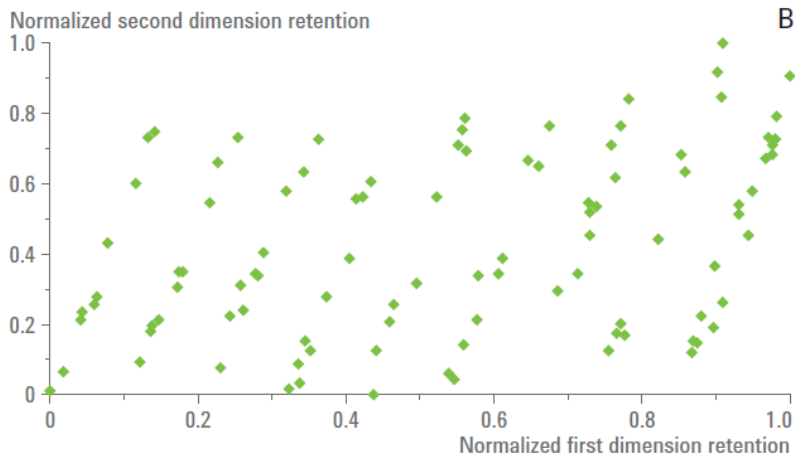
Teorie



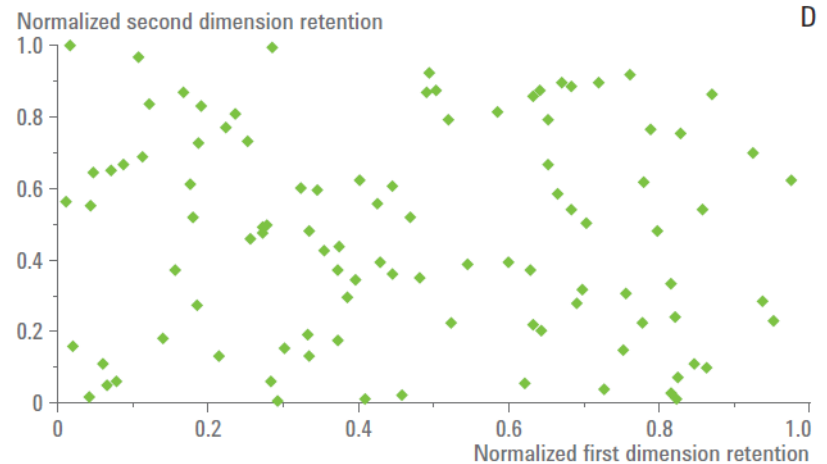
Shluky



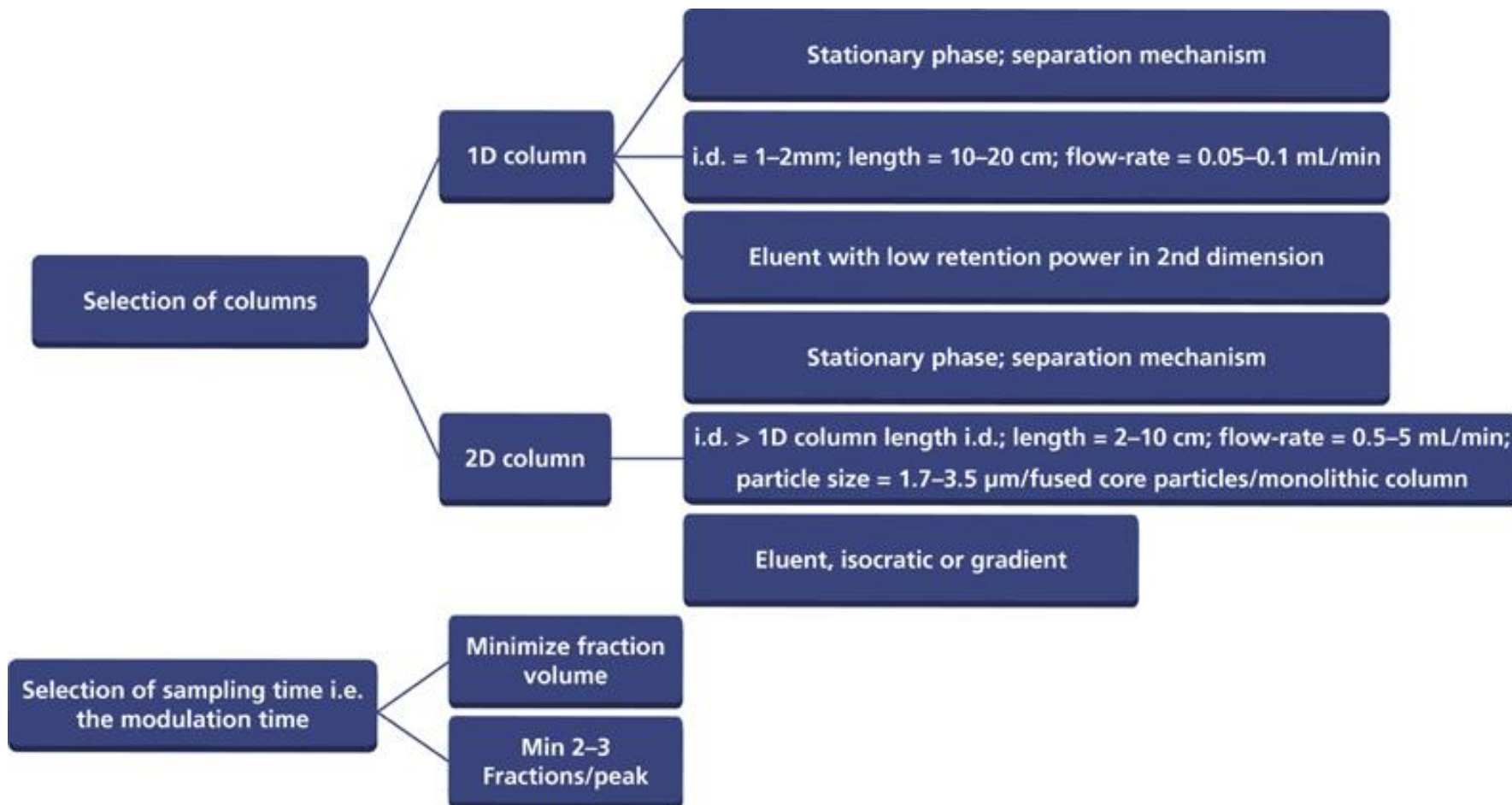
„Banagrams“



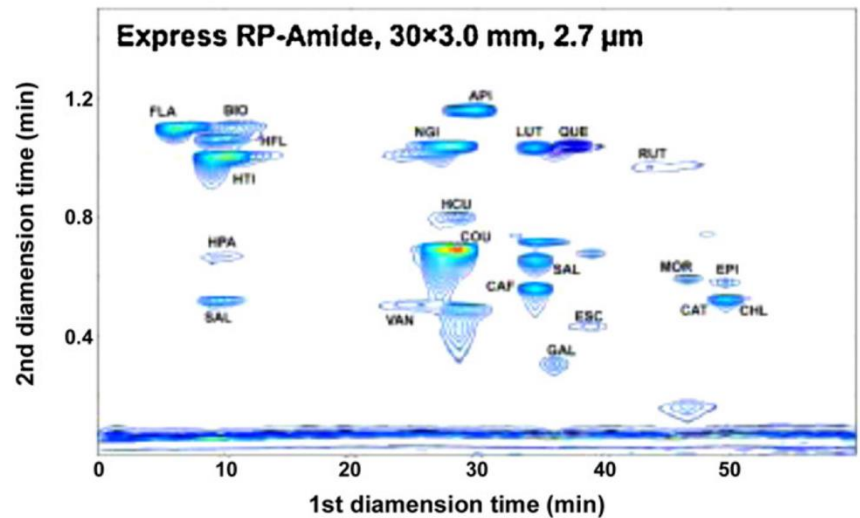
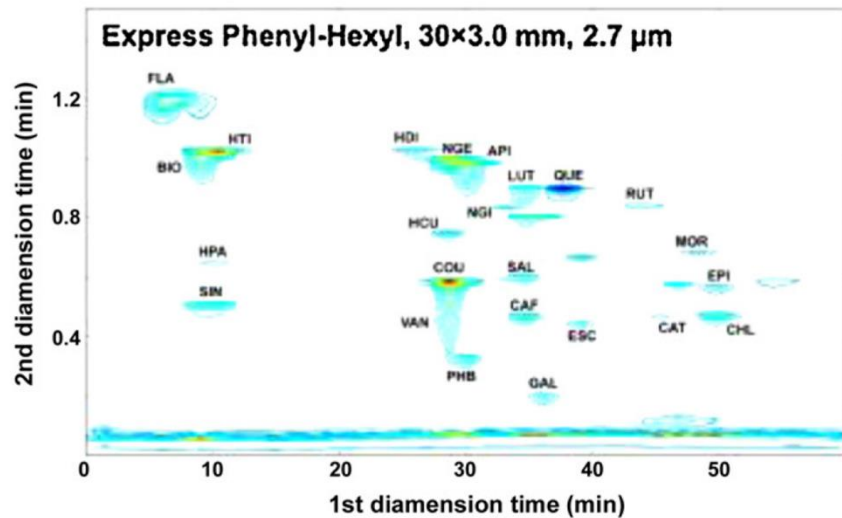
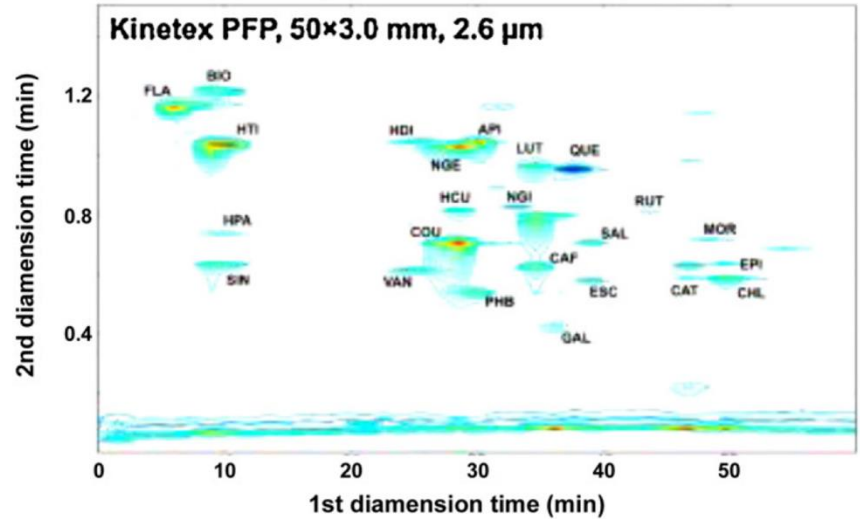
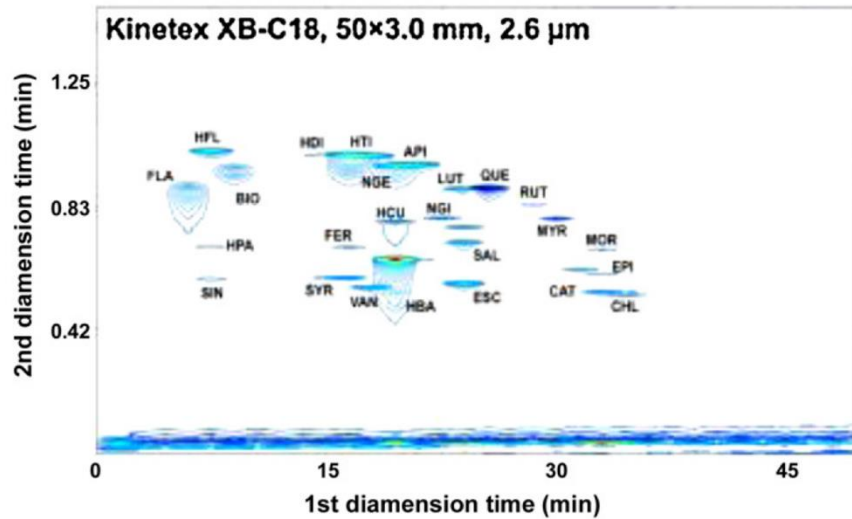
Ideální ortogonalita



OPTIMALIZACE PODMÍNEK



VLIV KOLONY VE DRUHÉ DIMENZI



KOMPATIBILITA PODMÍNEK

	NPLC	RPLC	HILIC	IEX	SEC	GFC
NPLC	+++	+	++	+	+++	+
RPLC	+	+++	+++	+++	+	+++
HILIC	+	++	+++	+	++	++
IEX	-	+++	+	+++	-	+++
SEC	+++	+	++	+	+++	+
GFC	+	+++	++	+++	+	+++

+++ velmi kompatibilní

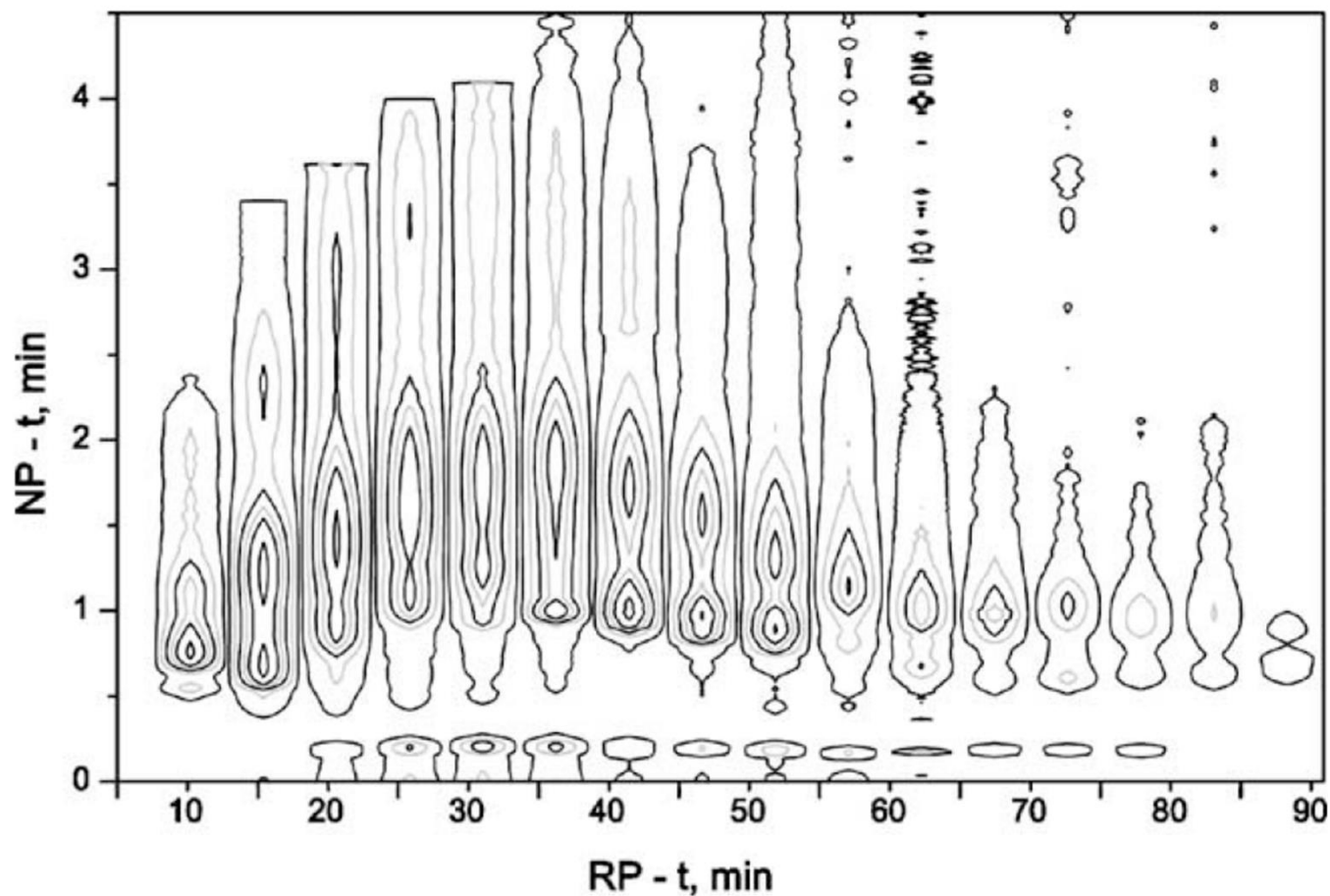
++ relativně kompatibilní

+ možné, ale nesnadné

- obtížné

NPLC – normální fáze, RPLC – reverzní fáze, HILIC – hydrofilní interakce, IEX – iontová výměna,
SEC – size-exclusion, GFC – gelová filtrace

KOMPATIBILITA PODMÍNEK



Reverzní fáze (D1)

Zorbax Extend C18 column, 5 μm ,
150 mm \times 3.3 mm, 50–100% ACN
za 110 min při 0.2 ml/min, 40°C.

Normální fáze (D2)

Separon SGX Amin, 5 μm ,
150 mm \times 1mm i.d., 5% 2-propanol
v hexanu, 0.5 ml/min, 40°C.

$$V_{f(\text{max})} < 5 \mu\text{l}$$

OBJEM PŘEVÁDĚNÉ FRAKCE

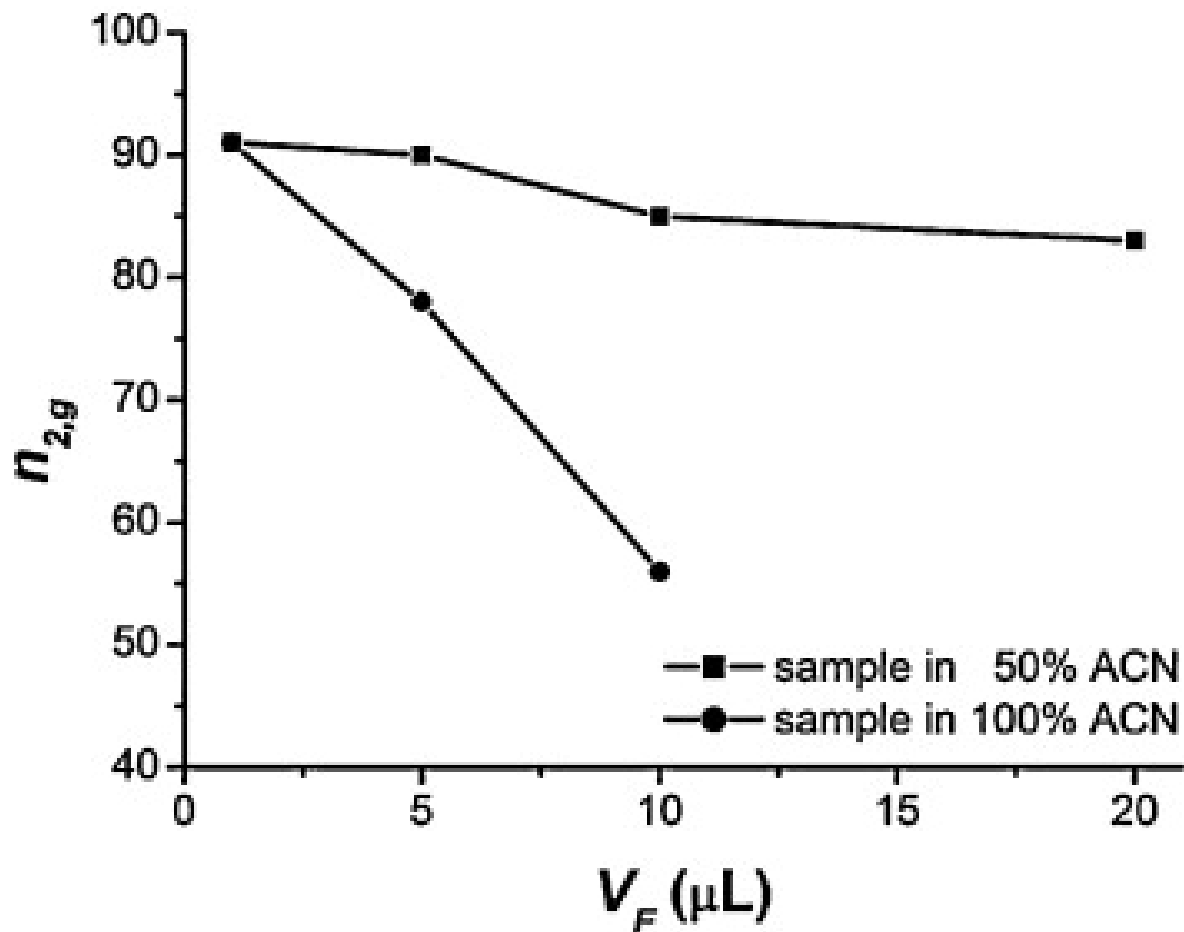
Kolona ve druhé dimenzi	w_{exp} , μL	z , 5 μL	z , 10 μL	z , 20 μL	$V_{f(max)}$, μL
Chromolith Flash RP18-e (25 mm \times 4.6 mm)	77	1.27	1.58	2.68	4
Poroshell 120 SB-C18 (30 mm \times 3.0 mm, 2.7 μm)	49	1.14	–	–	4
Ascentis Express C18 (30 mm \times 3.0 mm, 2.7 μm)	51	1.17	2.05	–	4
Kinetex 2.6 μ C18, 100A (50 mm \times 3.0 mm, 2.6 μm)	33	1.15	1.94	–	4
Kinetex 2.6 μ XB-C18, 100A (50 mm \times 3.0 mm, 2.6 μm)	33	1.03	1.67	1.79	5
Kinetex 2.6 μ XB-C18, 100A (30 mm \times 3.0 mm, 2.6 μm)	41	1.39	2.58	–	3
Ascentis Express C8 (30 mm \times 3.0 mm, 2.7 μm)	48	1.38	–	–	4
Kinetex 2.6 μ PFP, 100A (50 mm \times 3.0 mm, 2.6 μm)	38	1.16	–	–	4
Kinetex 2.6 μ PFP, 100A (30 mm \times 3.0 mm, 2.6 μm)	54	1.63	2.61	–	2
Ascentis Express Phenyl-Hexyl (30 mm \times 3.0 mm, 2.7 μm)	103	1.17	1.81	–	4
Ascentis Express RP-Amide (30 mm \times 3.0 mm, 2.7 μm)	84	1.19	2	–	3

- štěpení píku

$z < 1.1$

z – faktor rozšiřování píku z důvodu nekompatibility

OBJEM PŘEVÁDĚNÉ FRAKCE



Kinetex C18

50 mm \times 3 mm I.D., 2.6 μm

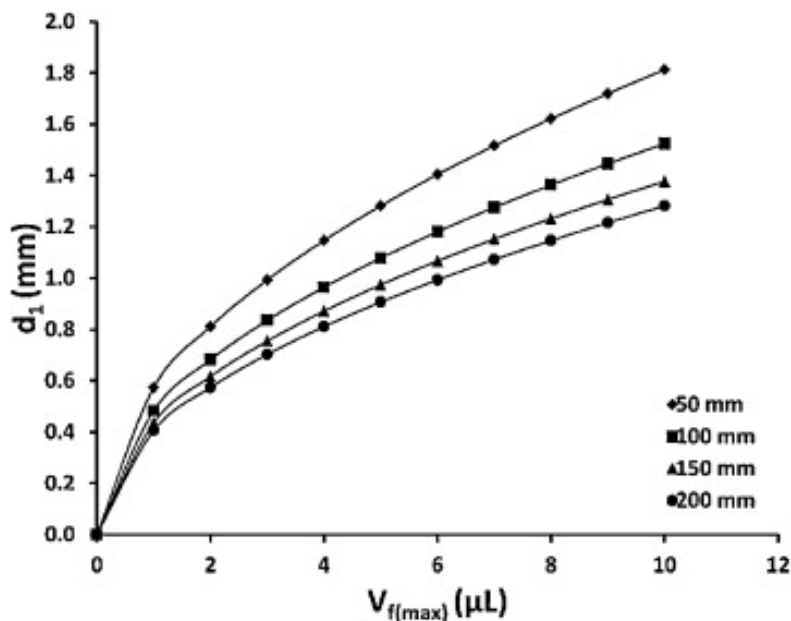
Rychlé gradienty

0 – 100% acetonitrilu

v 1 min při 2.5 mL/min.

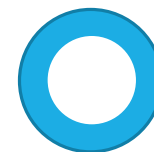
OPTIMÁLNÍ 2D VNITŘNÍ PRŮMĚR

$$d = 2 \sqrt{\frac{3}{4} \frac{V_{f(max)}}{\pi \epsilon_0 (1 + k_e)} (LH)^{-\frac{1}{4}}} = 0.98 \left[\frac{V_{f(max)}}{\epsilon_0 (1 + k_e)} \right]^{\frac{1}{2}} (LH)^{-\frac{1}{4}}$$



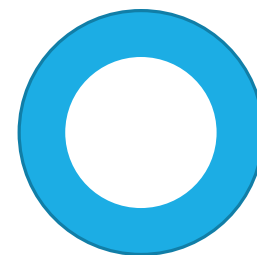
I.D. ~ 0.5 mm

$V_{f(max)} = 4 \mu\text{L}$, $L = 20 \text{ cm}$, $H = 20 \mu\text{m}$,
 $\epsilon_0 = 0.66$, $\alpha k_e = 3$

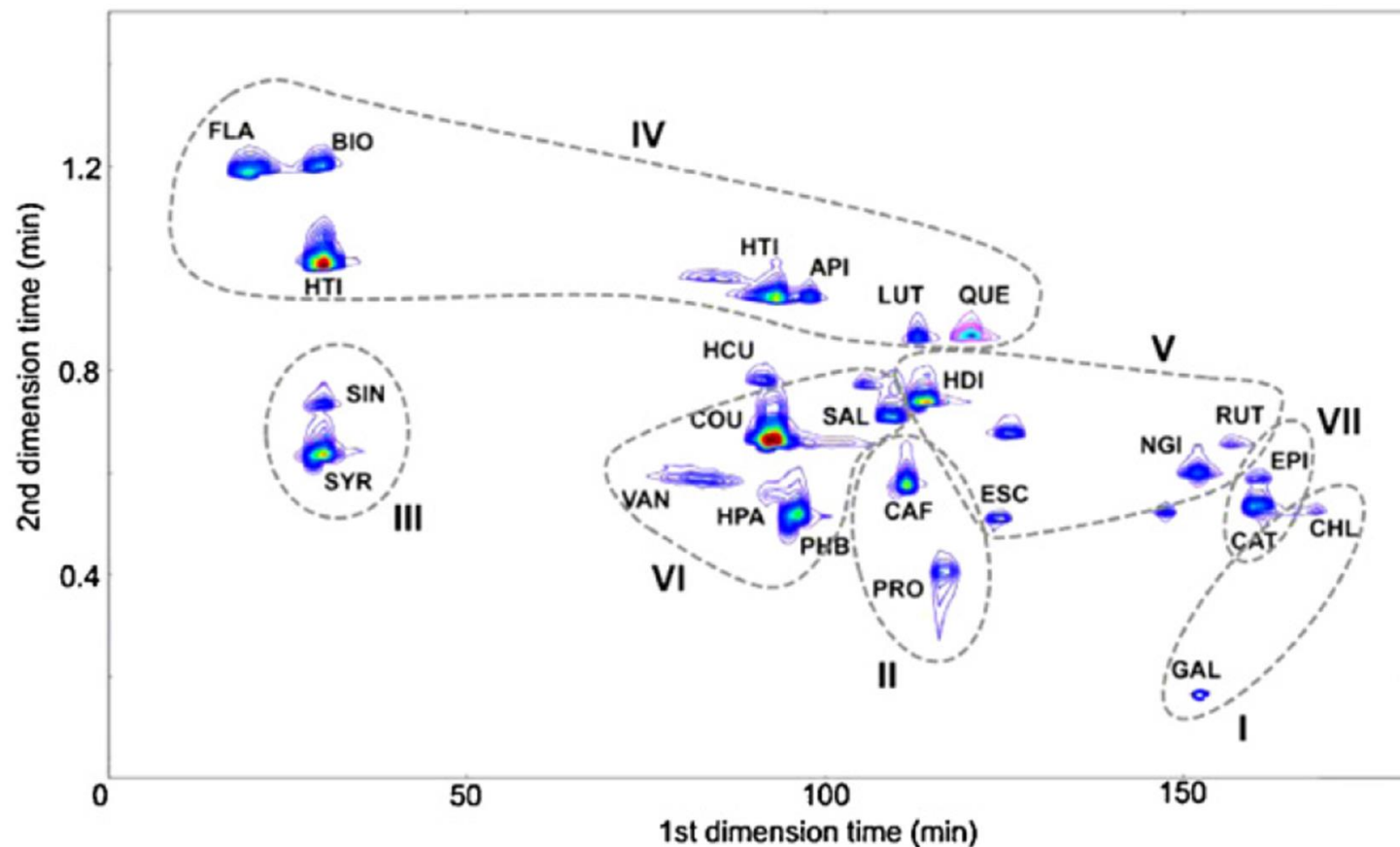


I.D. ~ 1.0 mm

$V_{f(max)} = 4 \mu\text{L}$, $L = 20 \text{ cm}$, $H = 15 \mu\text{m}$,
 $\epsilon_0 = 0.75$, $\alpha k_e = 3.5$

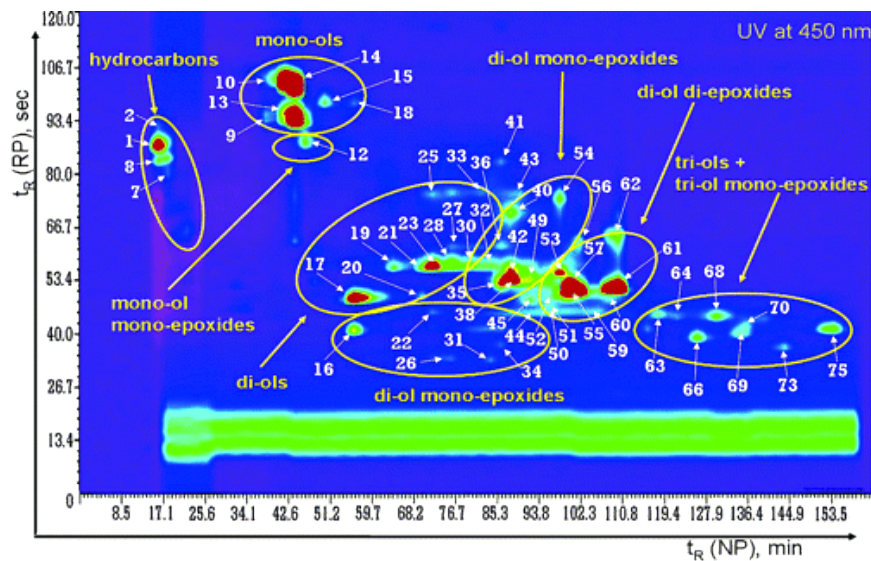
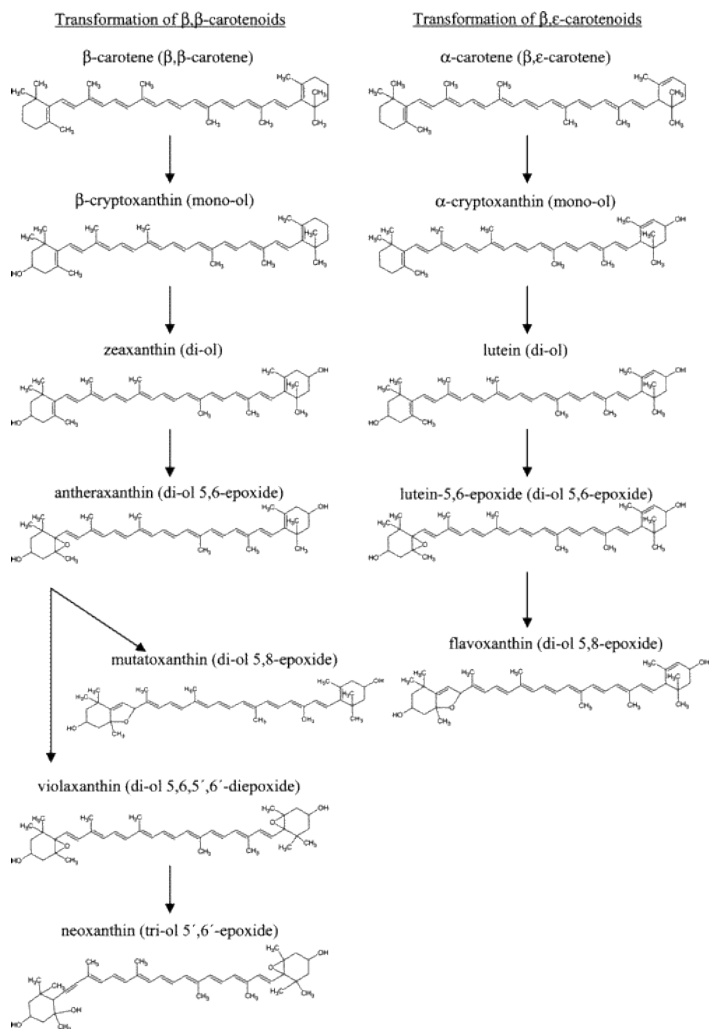


2D KLASIFIKACE

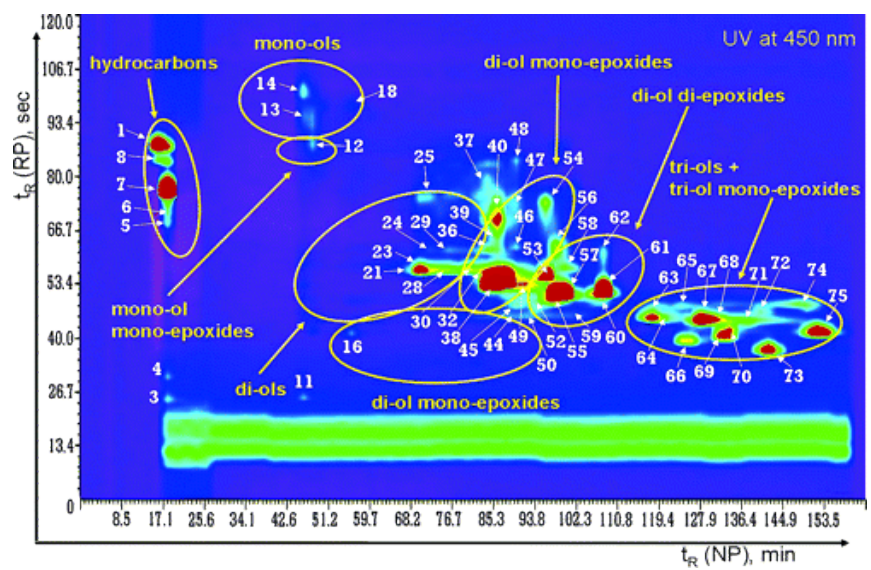


I – polární fenolické kyseliny, II – fenolické kyseliny s dvěma –OH skupinami, III – fenolické kyseliny s jednou –OH skupinou a dvěma metoxy skupinami, IV – aglykosidové flavony, V – glykosidové flavony, VI – ostatní fenolické kyseliny s jednou –OH skupinou, VII – katechiny

2D KLASIFIKACE



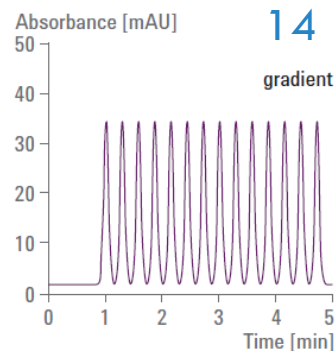
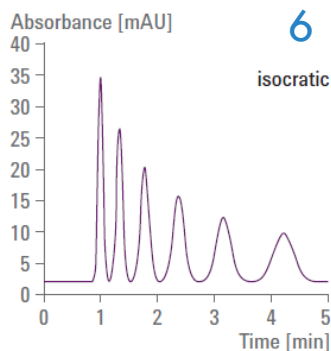
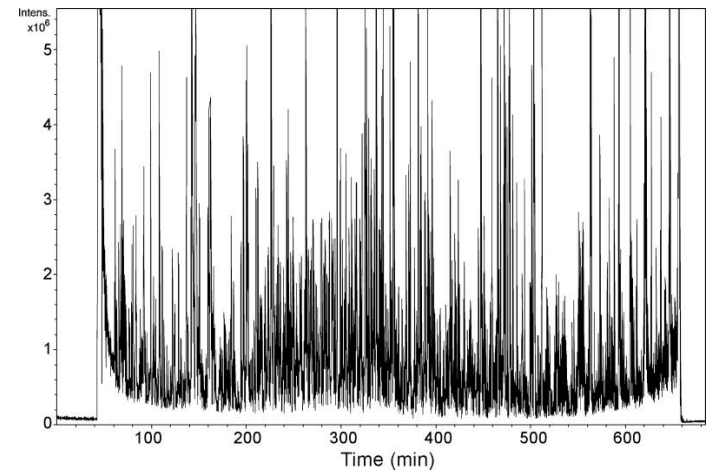
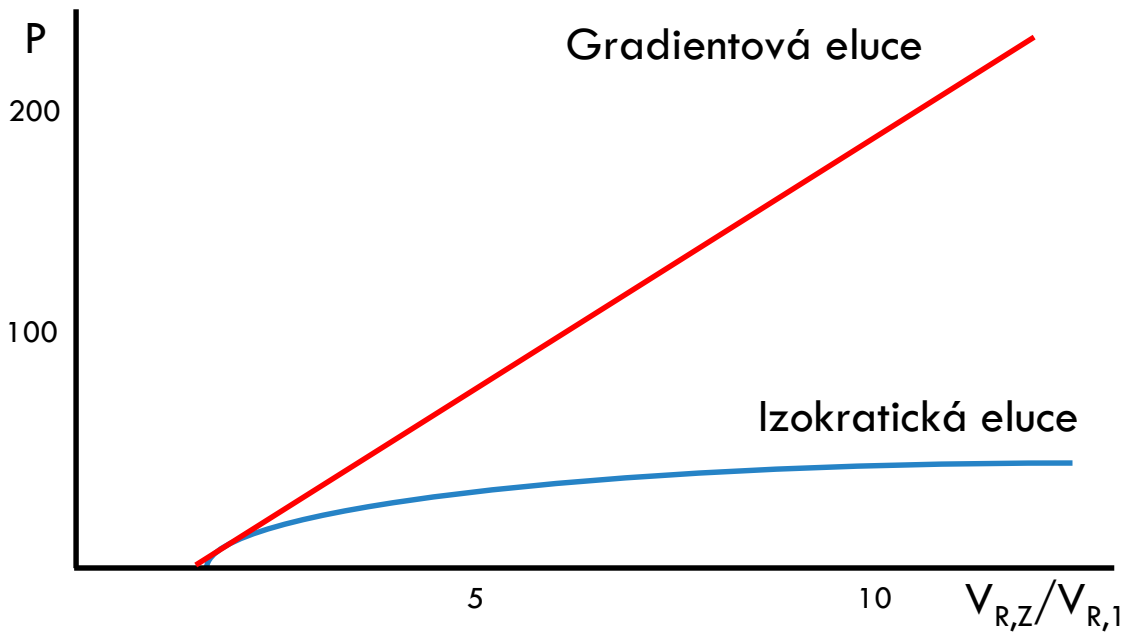
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PÍKOVÁ KAPACITA (IZOKRATIKA/GRADIENT)

Maximální počet píků, které lze rozdělit v rámci jedné analýzy



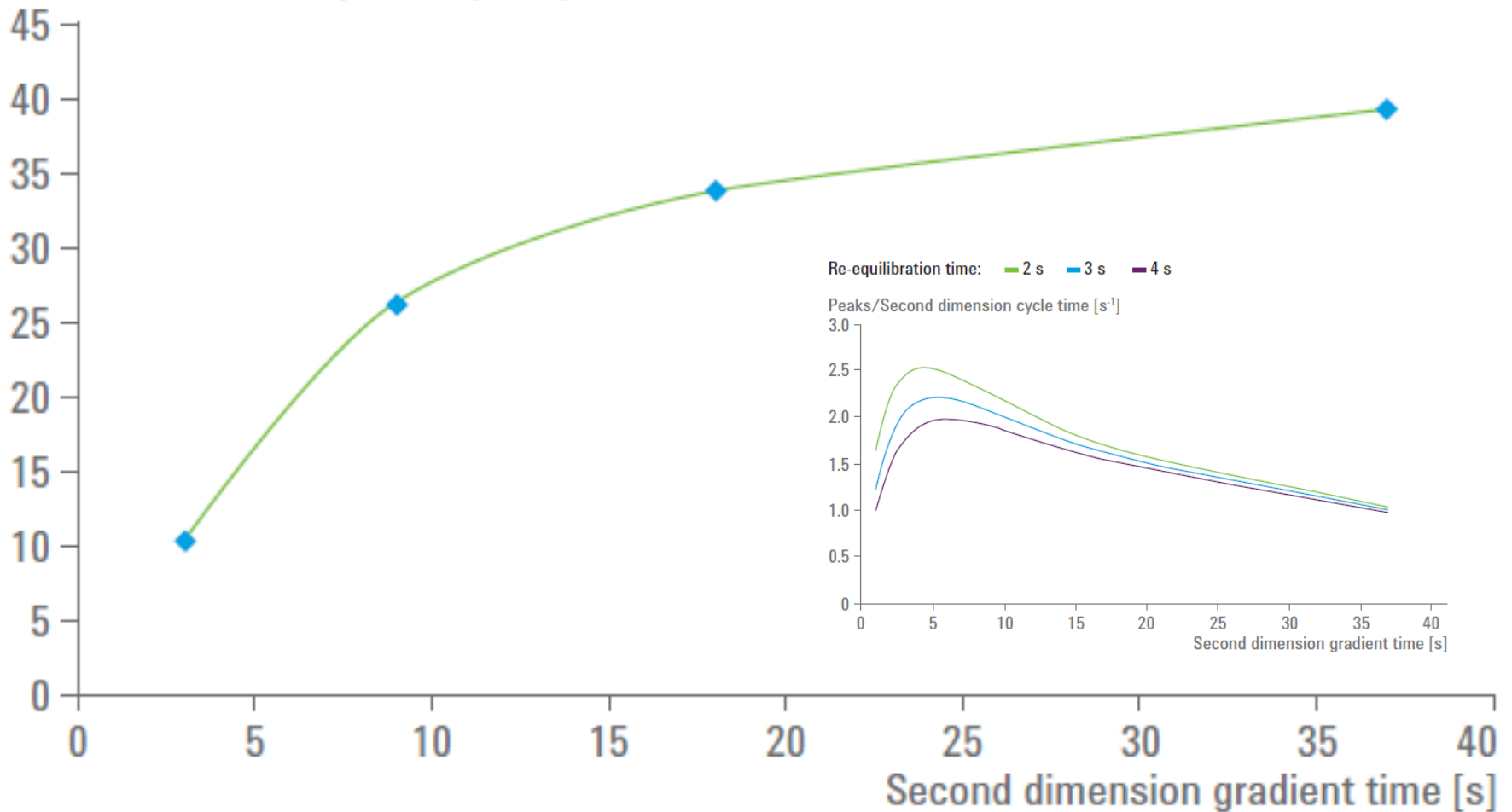
$$P = \frac{\sqrt{N}}{4} \left(\frac{t_{R,Z}}{t_{R,1}} - 1 \right) + 1$$

$$P = 1 + \frac{t_G - t_m}{w_g} = 1 + \frac{\sqrt{N}}{4} \left(\frac{t_G}{t_m} - 1 \right) \frac{1}{(1 + k_e)}$$

$$n_c = \frac{t_g}{w}$$

GRADIENT V DRUHÉ DIMENZI

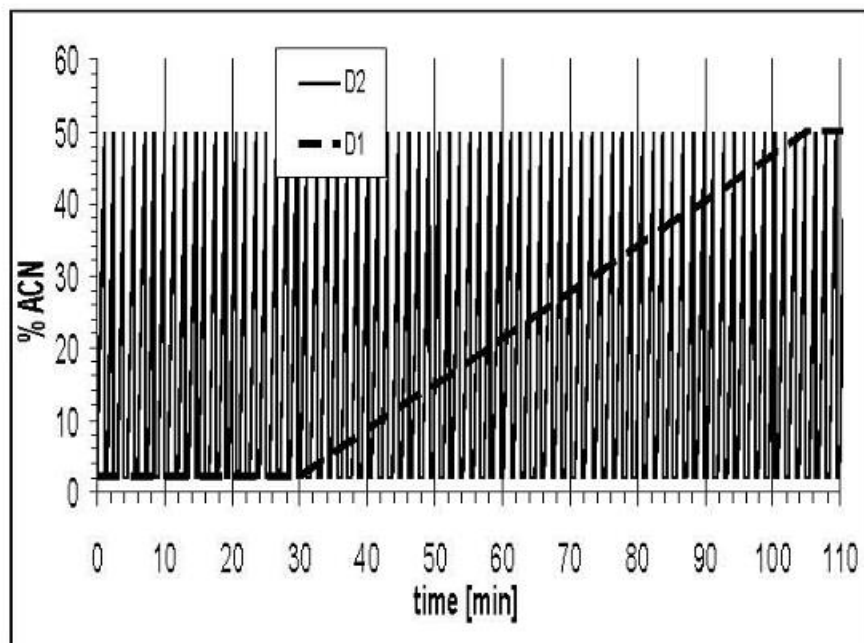
Second dimension peak capacity



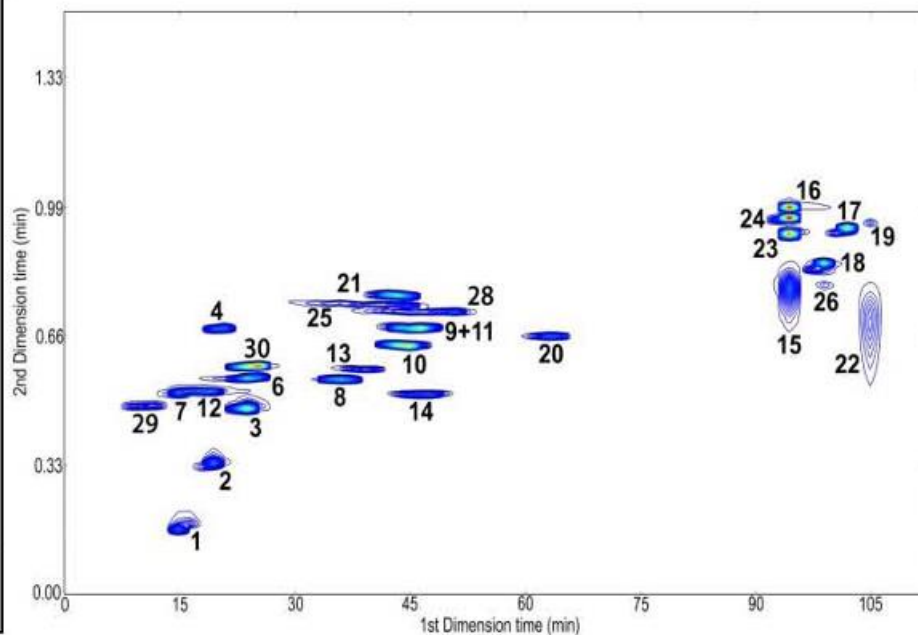
GRADIENT V DRUHÉ DIMENZI

Full in fraction

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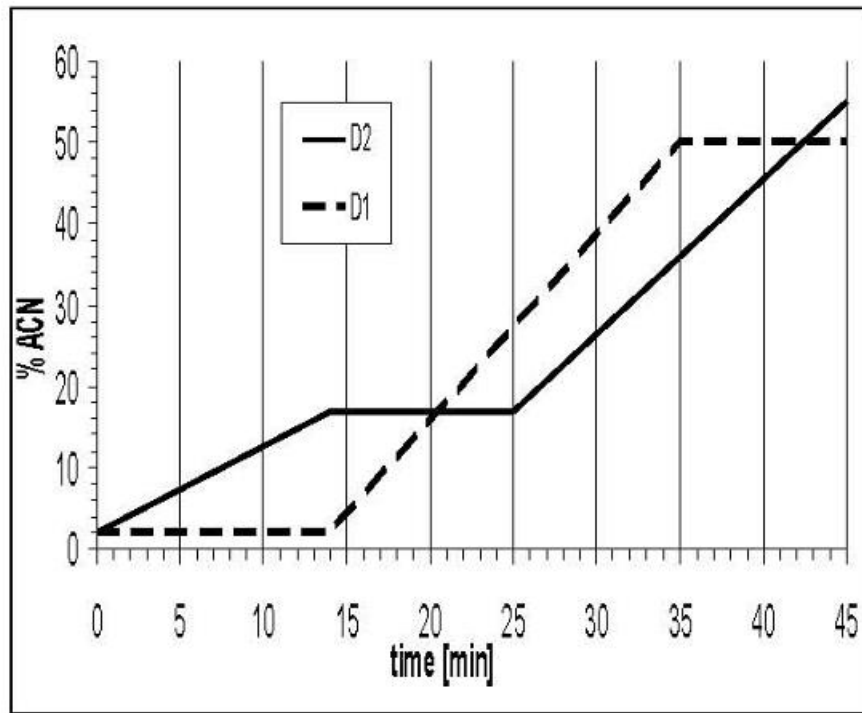
A



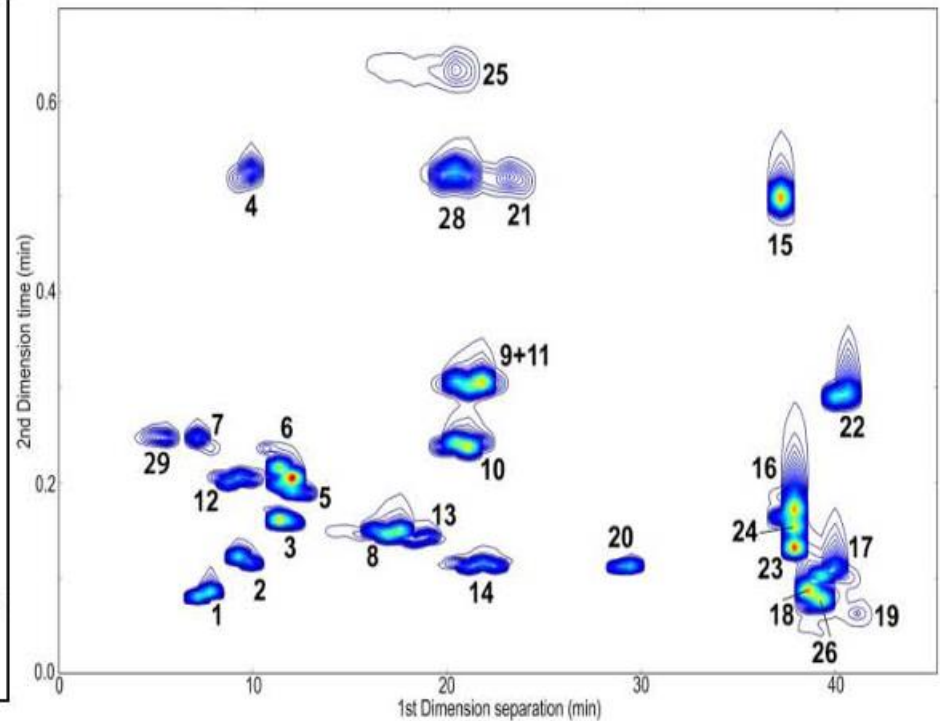
D1 – HS PEG, 150×2.1 mm, 5 μ m, D2 – Ascentis Express C18, 30×3 mm, 2.7 μ m, 100 μ L sampling loops.

GRADIENT V DRUHÉ DIMENZI

Continuously shifting



B

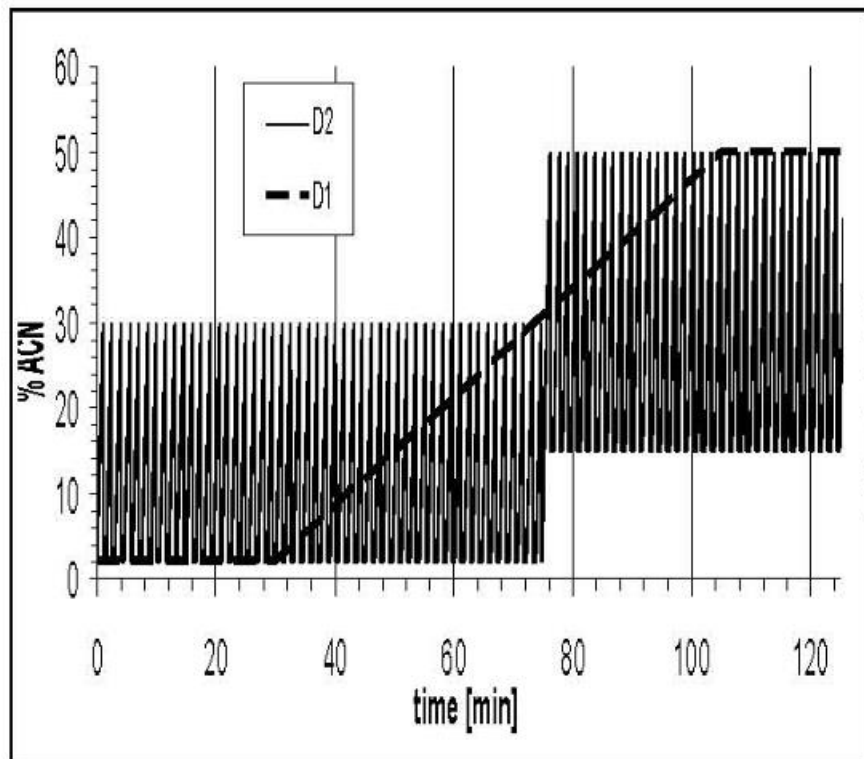


D1 – HS PEG, 150×2.1 mm, 5 μ m, D2 – Ascentis Express C18, 30×3 mm, 2.7 μ m, 100 μ L sampling loops.

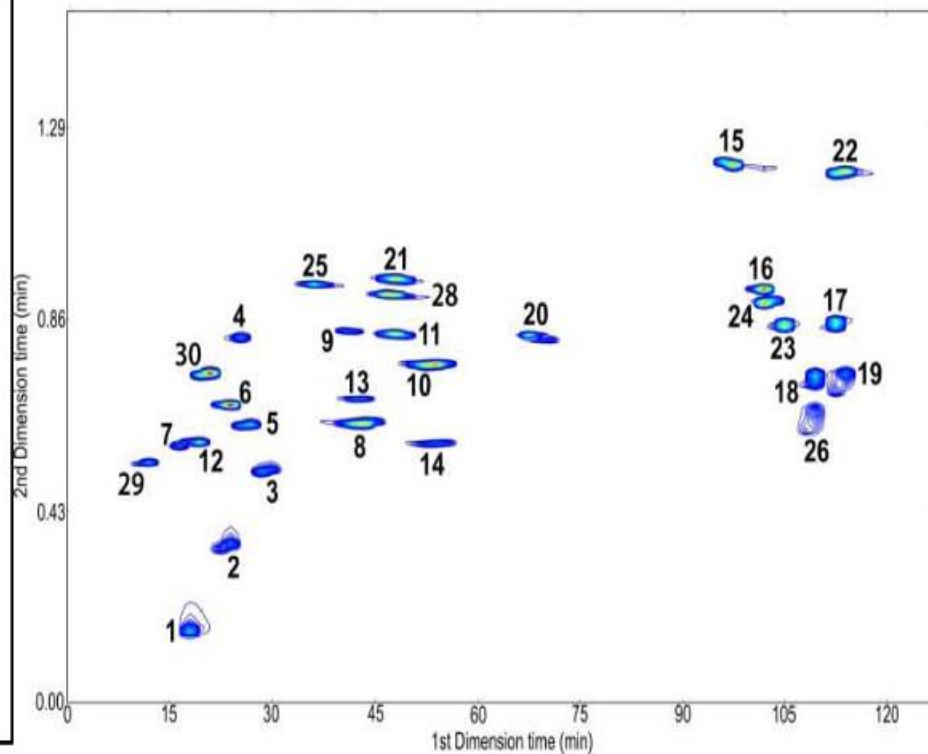
GRADIENT V DRUHÉ DIMENZI

Cent. Eur. J. Chem. 10 (2012) 844-875.

Segment in fraction

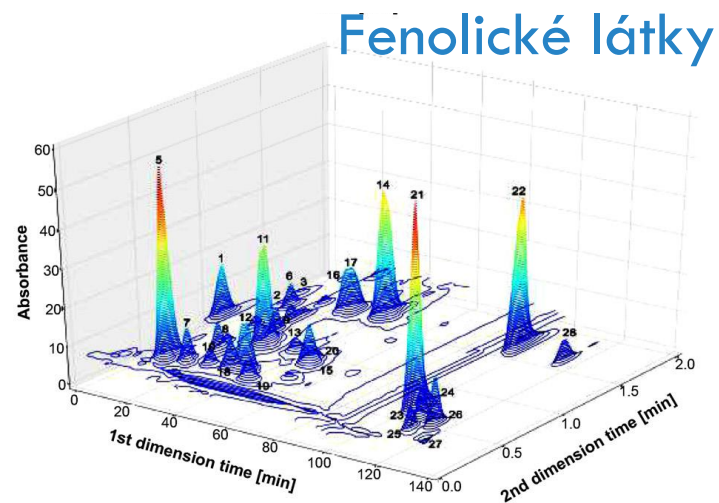
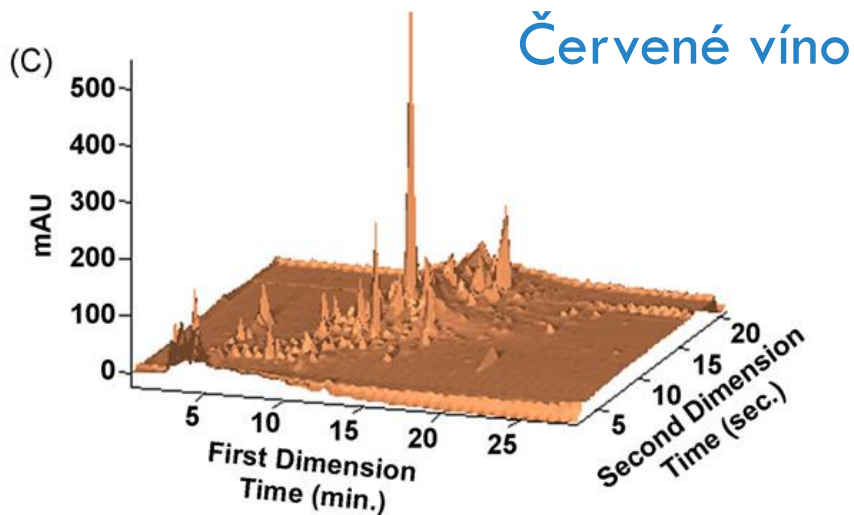
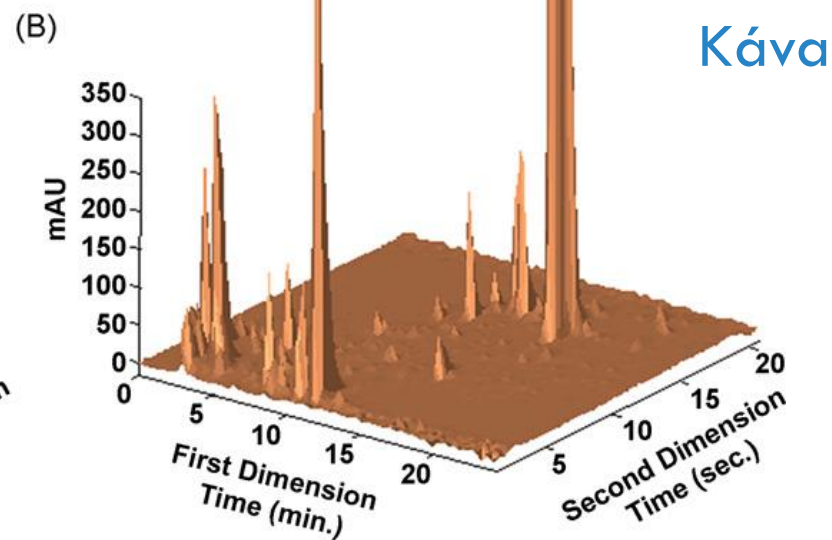
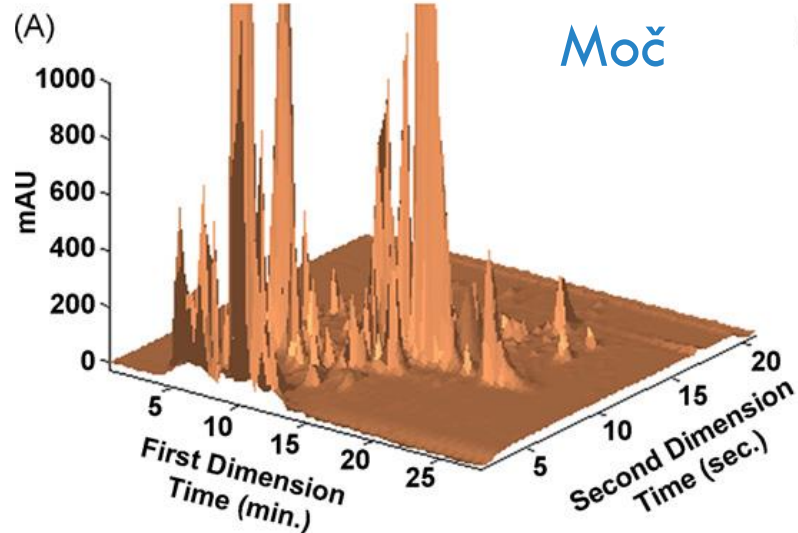


C

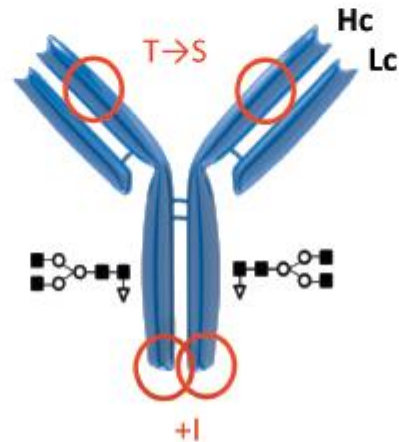
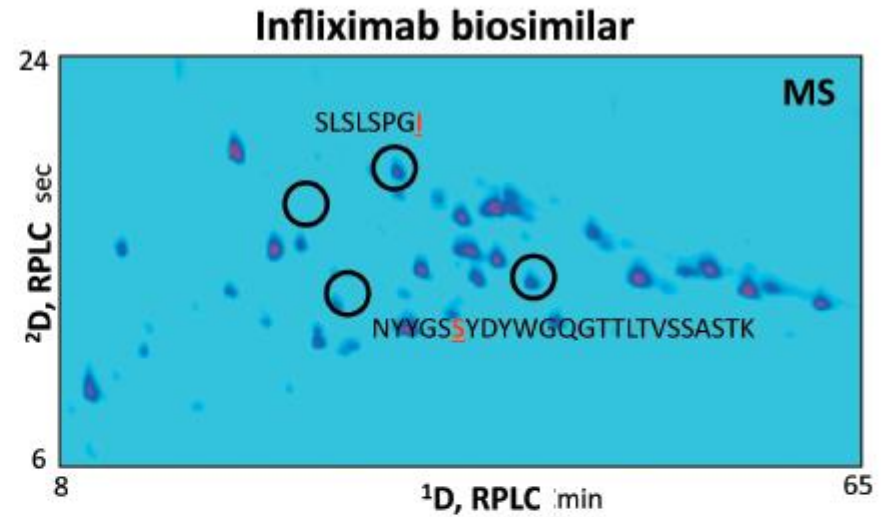
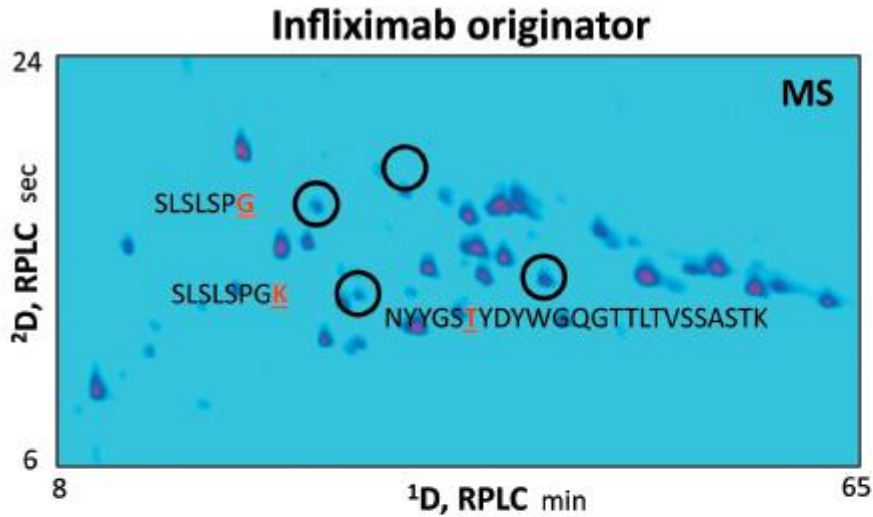


D1 – HS PEG, 150×2.1 mm, 5 μm , D2 – Ascentis Express C18, 30×3 mm, 2.7 μm , 100 μL sampling loops.

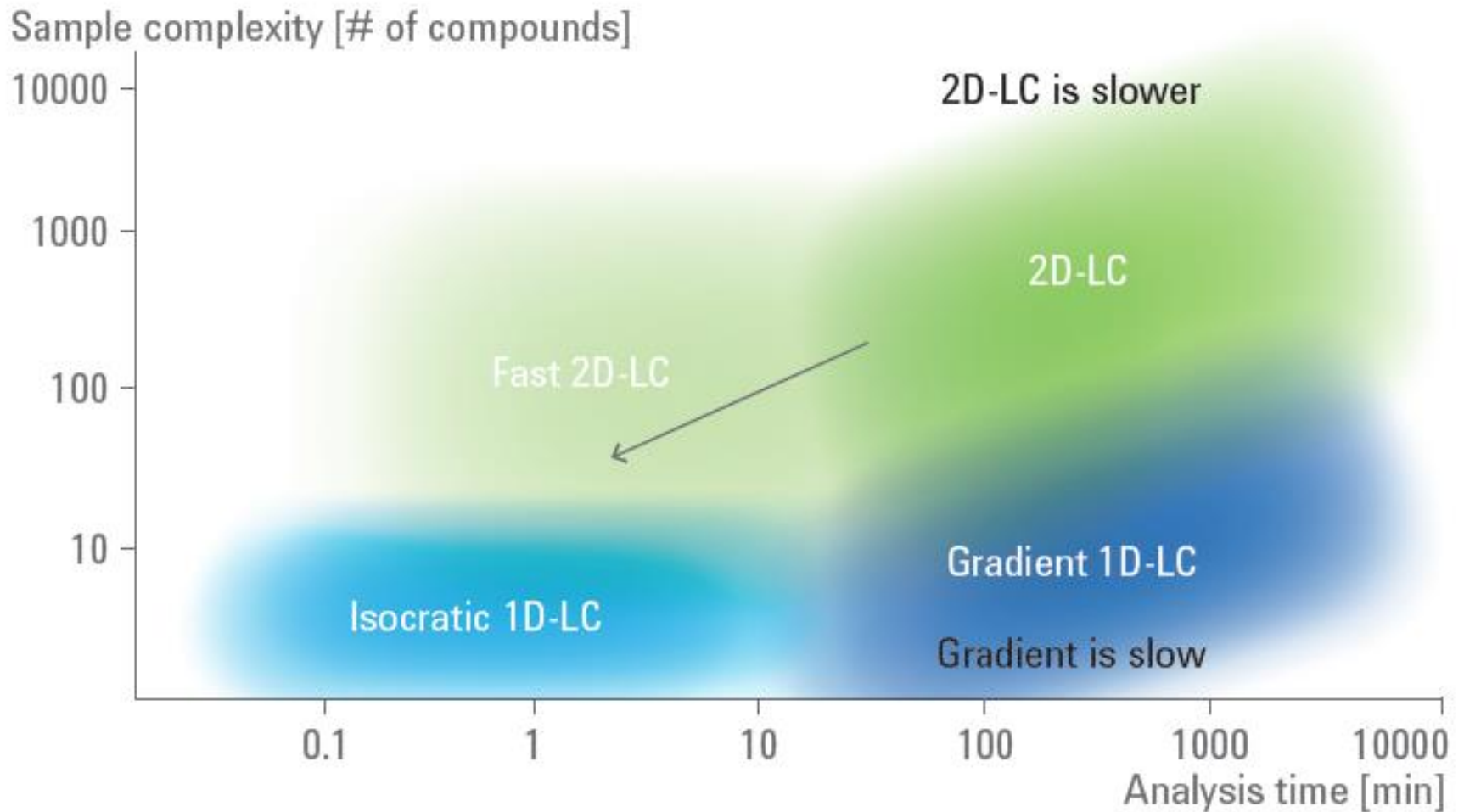
VÍCEROZMĚRNÉ SEPARACE



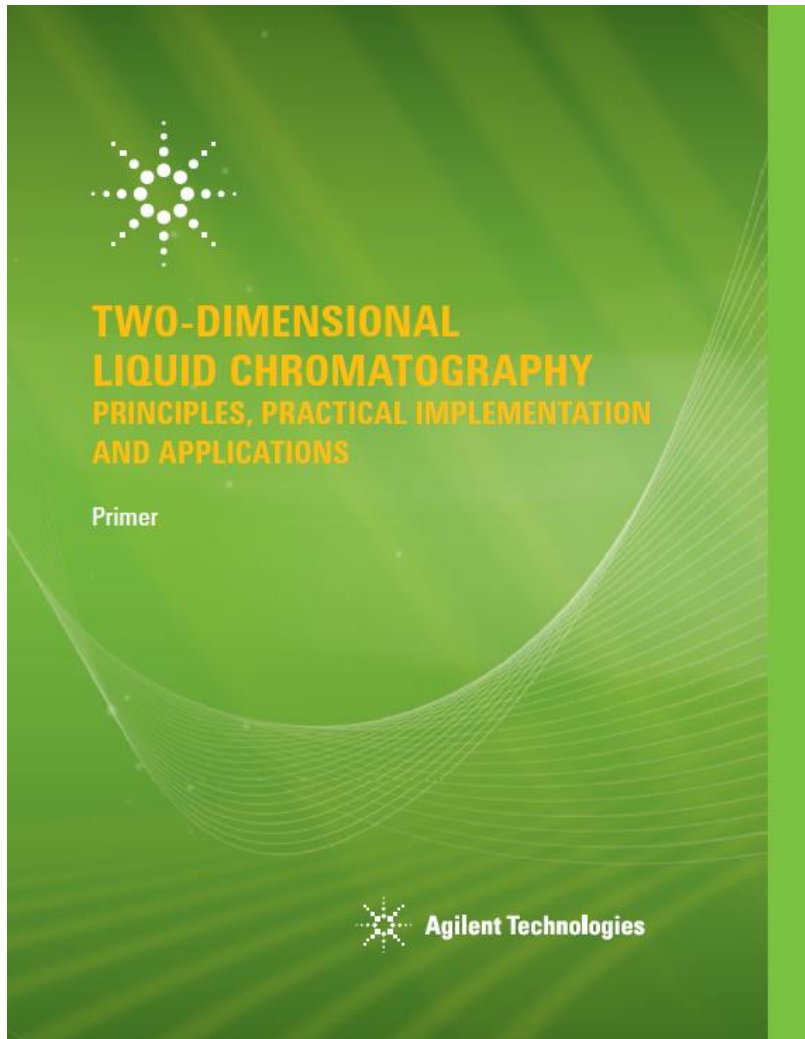
ANALÝZA PROTILÁTEK



BUDOUNOST 2D LC



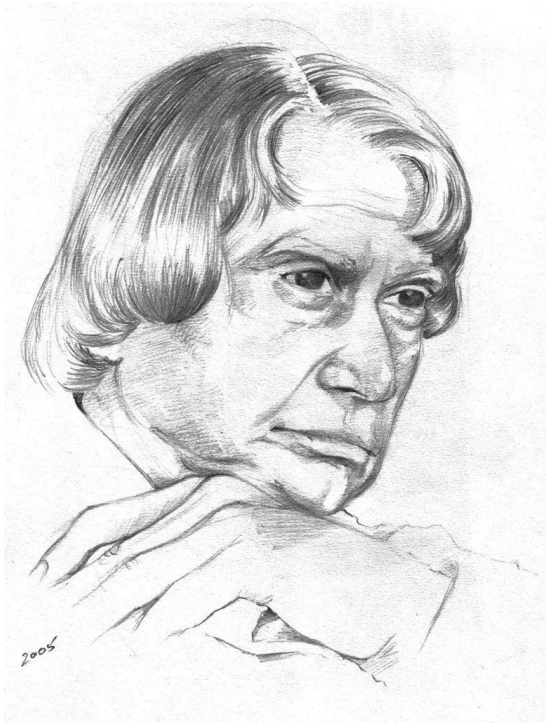
PRO ZÁJEMCE



Peter W. Carr and Dwight R. Stoll

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“Once your mind stretches to a new level it never goes back to its original dimension.”

Abdul Kalam