

**M U N I**  
**S C I**

# Separáční vědy, jak je (ne)znáte

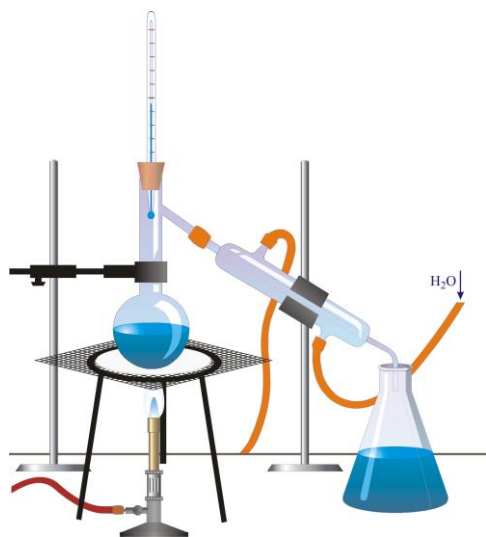
**Jiří Urban**

Ústav chemie, Přírodovědecká fakulta, Masarykova univerzita

# Separáčn metody?

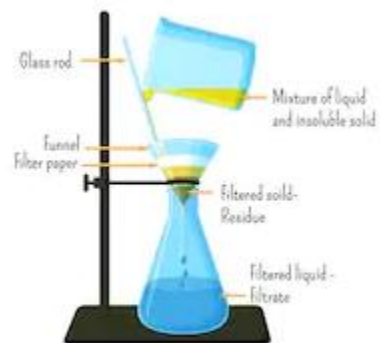
# Separační metody

## Destilace



<https://glossary.periodni.com>

## Filtrace



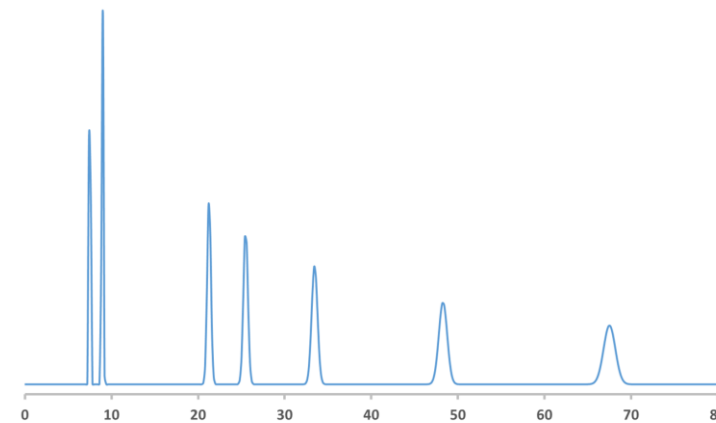
<https://shutterstock.com>

## Extrakce



<https://chemed.chem.purdue.edu>

## Chromatografie



## Sublimace



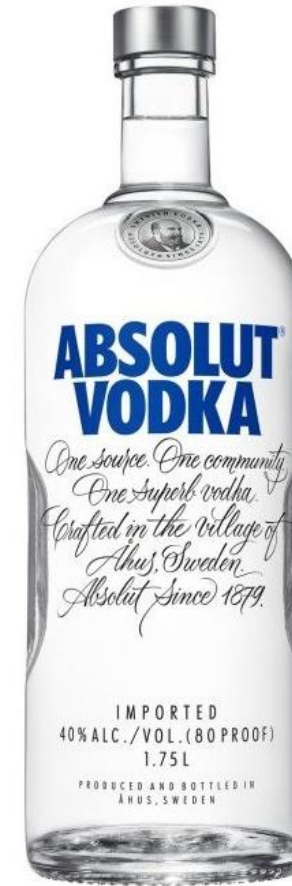
<https://education.seattlepi.com>

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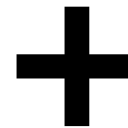
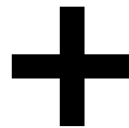
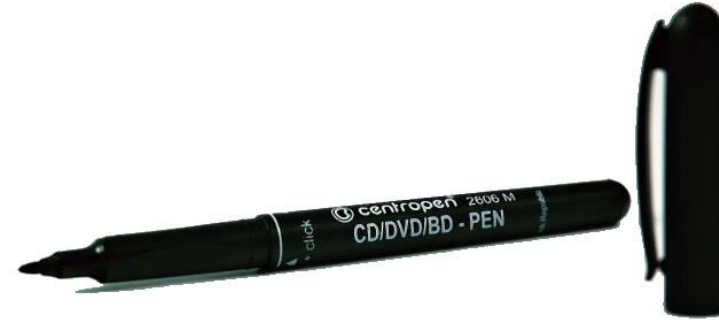
# **Co je to chromatografie?**



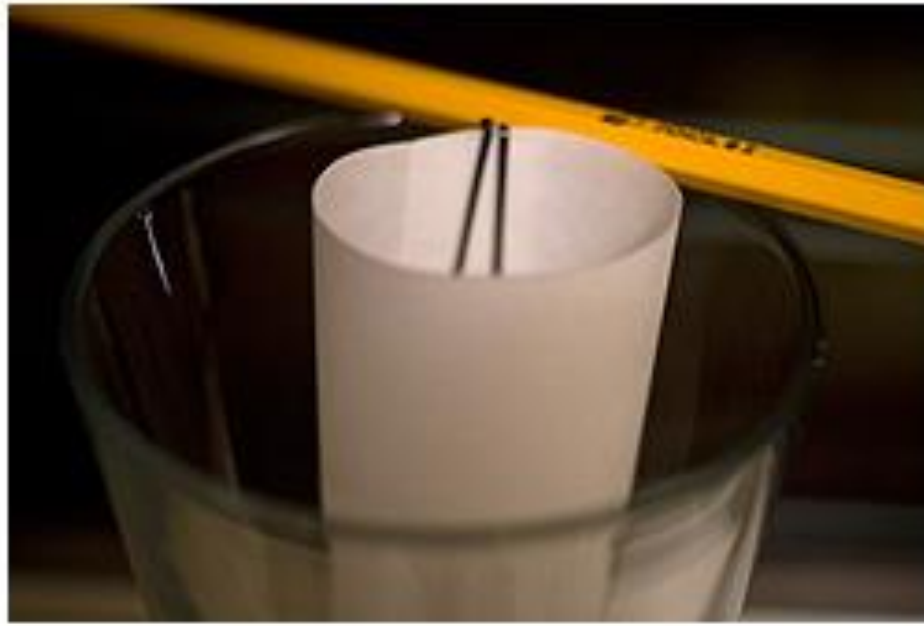
# Co s načatým večerem?







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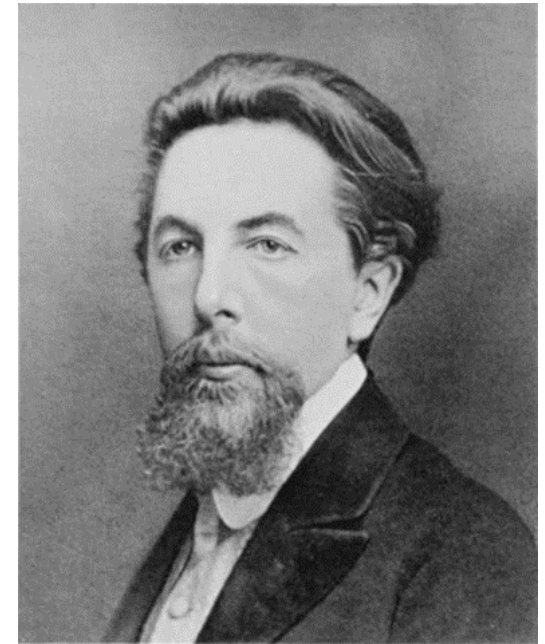




# Chromatografie



# Chromatografie



Michail Semjonovič Cvět

1872 - 1919

χρώμα [chróma] – barva

γραφειν [grafin] – psát

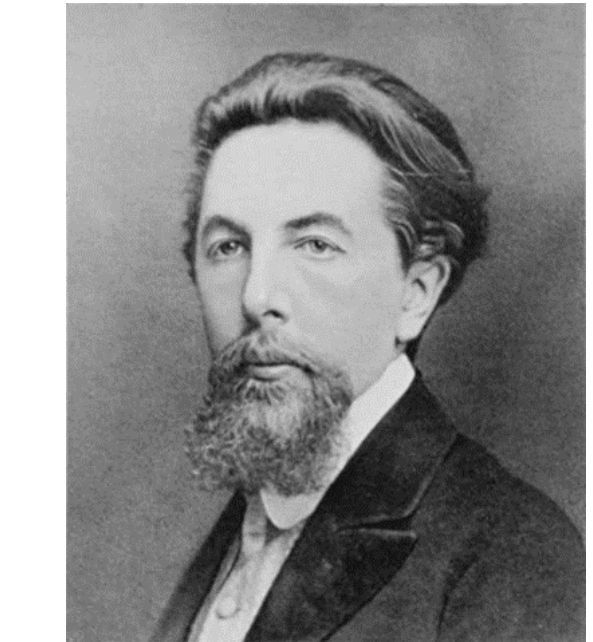
M U N I  
S C I

# Chromatografie



Martin Prince

S03E10



Michail Semjonovič Cvět

1872 - 1919

χρώμα [chróma] – barva

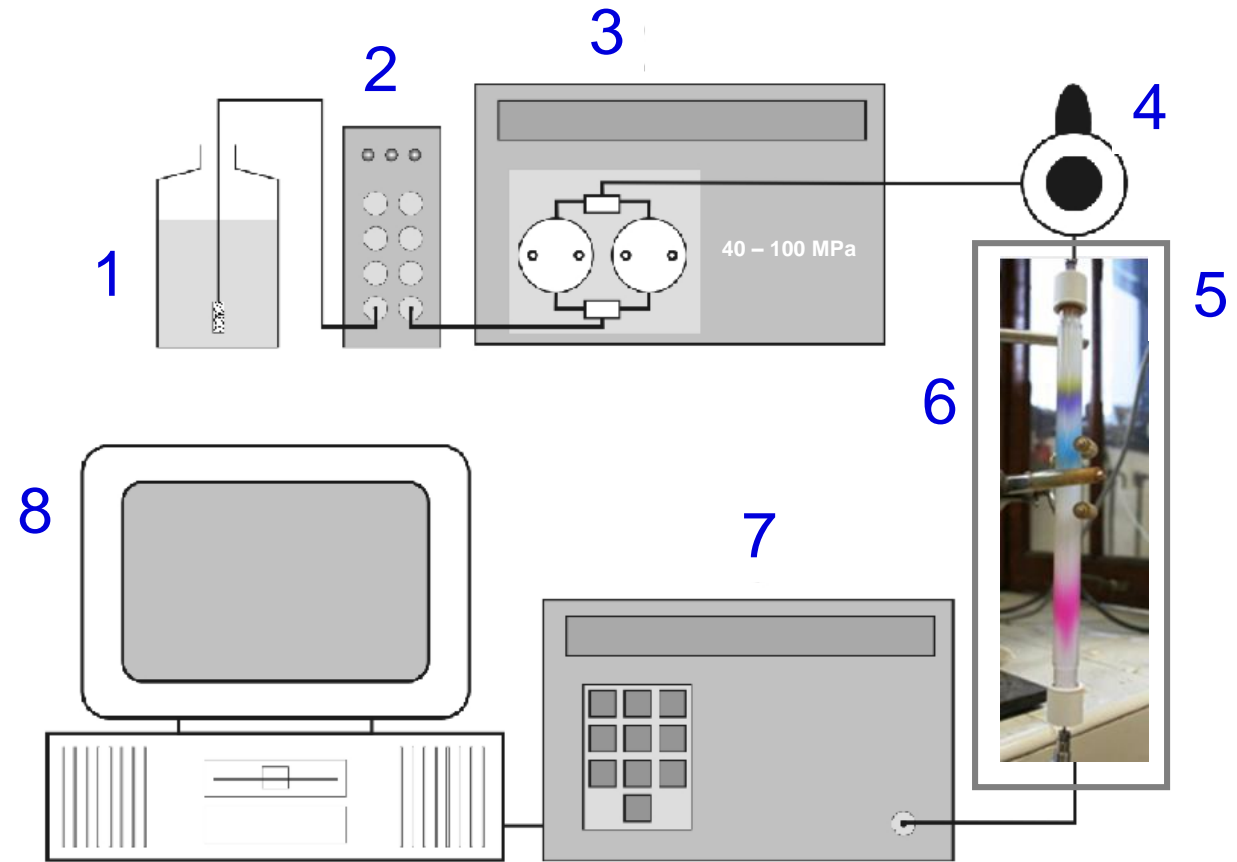
γραφειν [grafin] – psát

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# Kapalinová chromatografie



# Kapalinový chromatograf



- 1 – zásobník mobilní fáze
- 2 – odplyňovač mobilní fáze
- 3 – vysokotlaké čerpadlo
- 4 – dávkovač

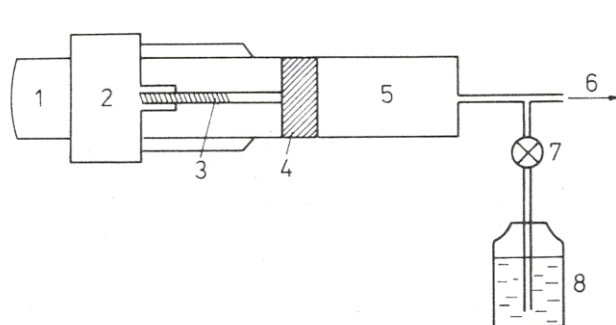
- 5 – separační kolona
- 6 – termostat kolony
- 7 – detektor
- 8 – datová stanice

# Vysokotlaká čerpadla

30 – 120 MPa, odolnost vůči korozi, stabilní a reprodukovatelný průtok < 0,5 – 1%

Aplikace	Průměr kolony	Průtok
Konvenční LC	4,6 mm	0,5 – 1,0 ml/min
Mikrokolonová LC	1 mm – 500 $\mu$ m	40 $\mu$ l/min – 20 $\mu$ l/min
Kapilární LC	500 $\mu$ m – 100 $\mu$ m	20 $\mu$ l/min – 300 nl/min
Nano LC	100 $\mu$ m – 75 $\mu$ m – 50 $\mu$ m	300 nl/min – 180 nl/min – 80 nl/min

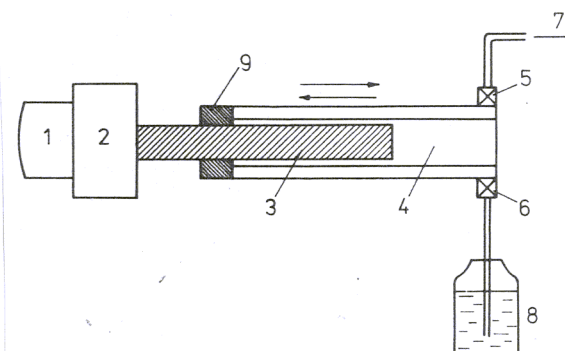
## Velkoobjemová stříkačka



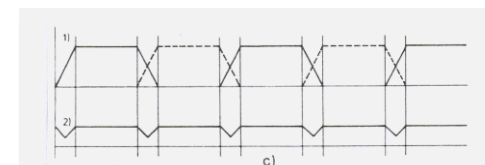
Čerpadlo na principu velkoobjemové injekční stříkačky

1 – elektromotor s krokovým chodem, 2 – převodovka, 3 – závit na táhle pístu, 4 – píst, 5 – válec naplněný mobilní fází, 6 – směr toku mobilní fáze ke koloně, 7 – jednosměrný ventil umožňující plnění čerpadla mobilní fází, 8 – zásobník mobilní fáze

## Pístové čerpadlo



Jednočinné pístové čerpadlo s malým objemem činné části  
 1 – elektromotor,  
 2 – převodový mechanismus,  
 3 – píst, 4 – pracovní prostor válce, 5 – výtláčný ventil, 6 – nasávací ventil, 7 – připojení ke koloně, 8 – zásobník mobilní fáze, 9 – těsnění pístu



Průtokové profily v závislosti na čase a na poloze pístu u čerpadel s jednou a dvěma hlavami

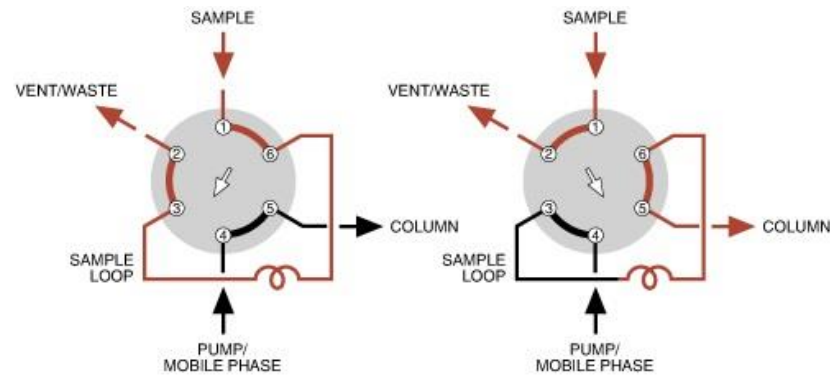
# Nanášení vzorku – Dávkování

“Bez prottlaku”

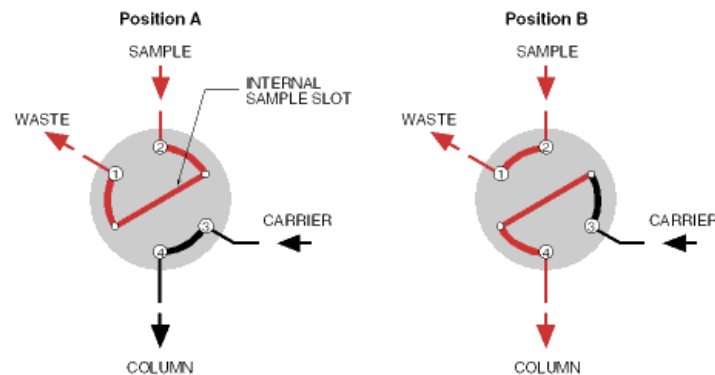
“S prottlakem”



Vnější smyčka



Vnitřní smyčka



- Nutno překonat prottlak mobilní fáze (40 – 100 MPa)
- Automatické dávkovače (úprava vzorku, derivatizace,...)



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# Chromatografická kolona



www.prozyme.com

Délka, průměr, velikost částic

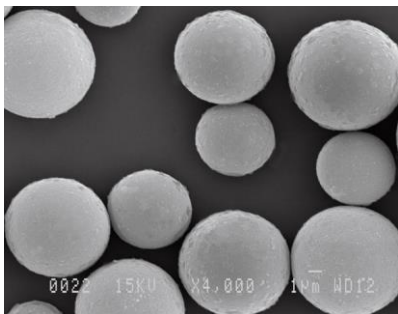


www.analytical-sales.com

Konvenční LC	Mikrokolonová LC	Kapilární LC	Nano LC
4.6 mm	1 mm – 500 µm	500 µm – 100 µm	100 µm – 50 µm

## Silikagel

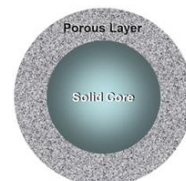
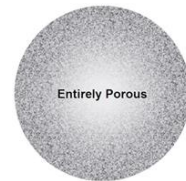
Porézní oxid křemičitý, SiO<sub>2</sub>



www.silicycle.com

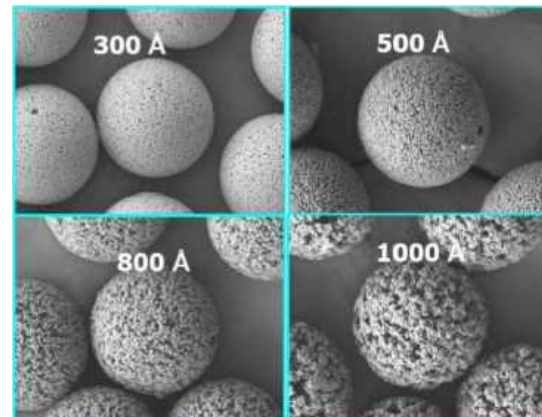


Tvar částic

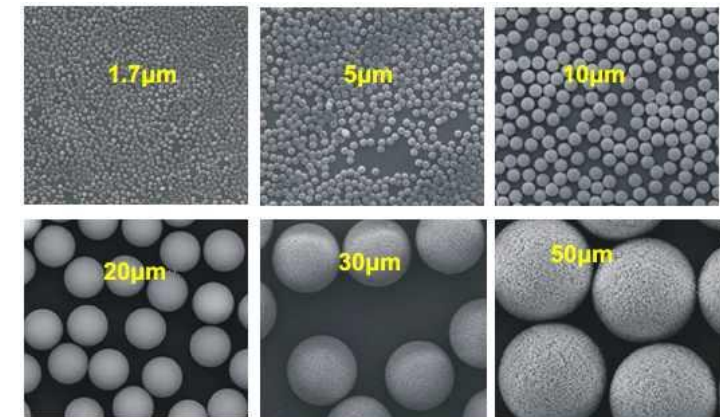


www.chromnet.net

Porozita



Velikost částic



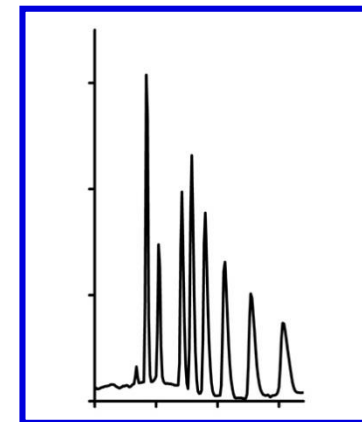
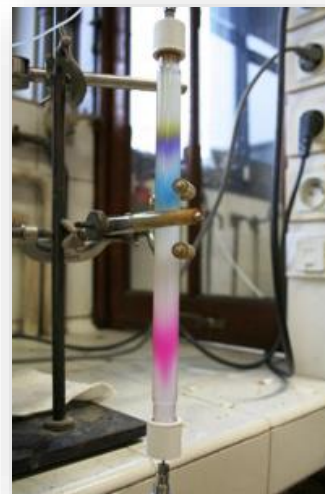


# Detekce jednotlivých analytů



## Detektor

- hmotnostní (HPLC/MS)
- spektrofotometrický (UV – Vis)
- fluorimetrický
- elektrochemický
- vodivostní
- rozptyl světla



# Separace



[www.analytical-sales.com](http://www.analytical-sales.com)



[www.amazon.co.uk](http://www.amazon.co.uk)



# Separace



[www.analytical-sales.com](http://www.analytical-sales.com)



[www.amazon.co.uk](http://www.amazon.co.uk)



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# **Jak chromatografie funguje?**

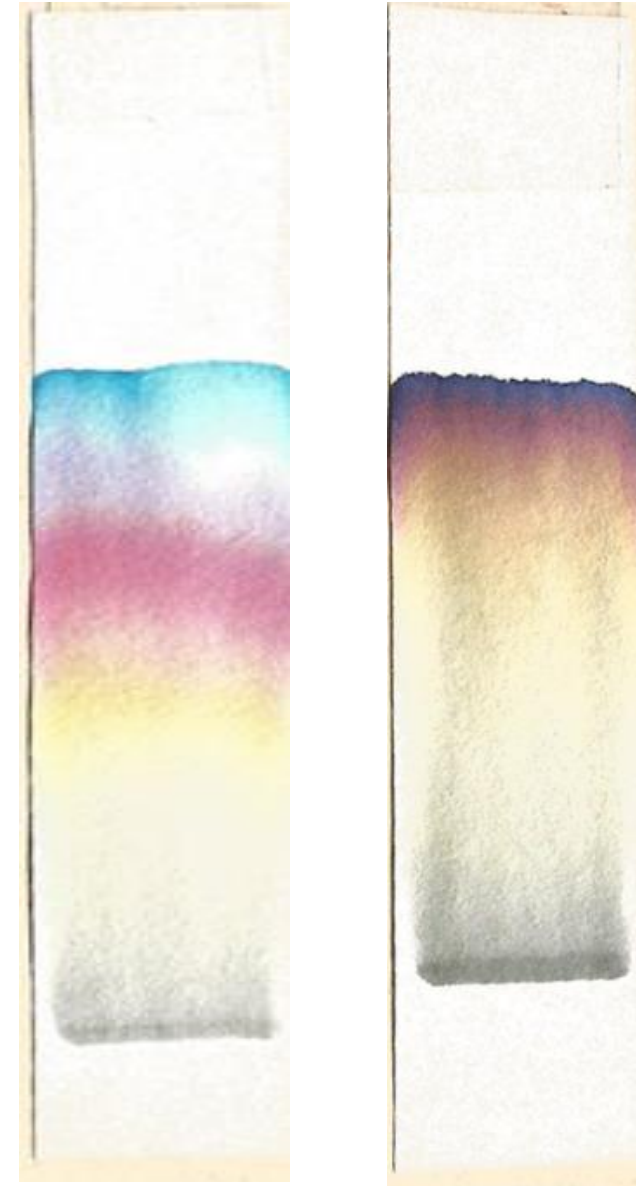


**KEEP  
CALM  
AND**

**DON'T BE AFRAID  
OF CHEMISTRY**

# Princip chromatografie

Aneb co má Usain Bolt společného s chromatografií?



ZDF | WM09

OK TDK

TOYOTA TOYOTA TOYOTA



# Separace – Rozdělení

Usain Bolt

9.58 s

Mistrovství světa v atletice, Berlín

16. srpen 2009



# Chromatografické interakce

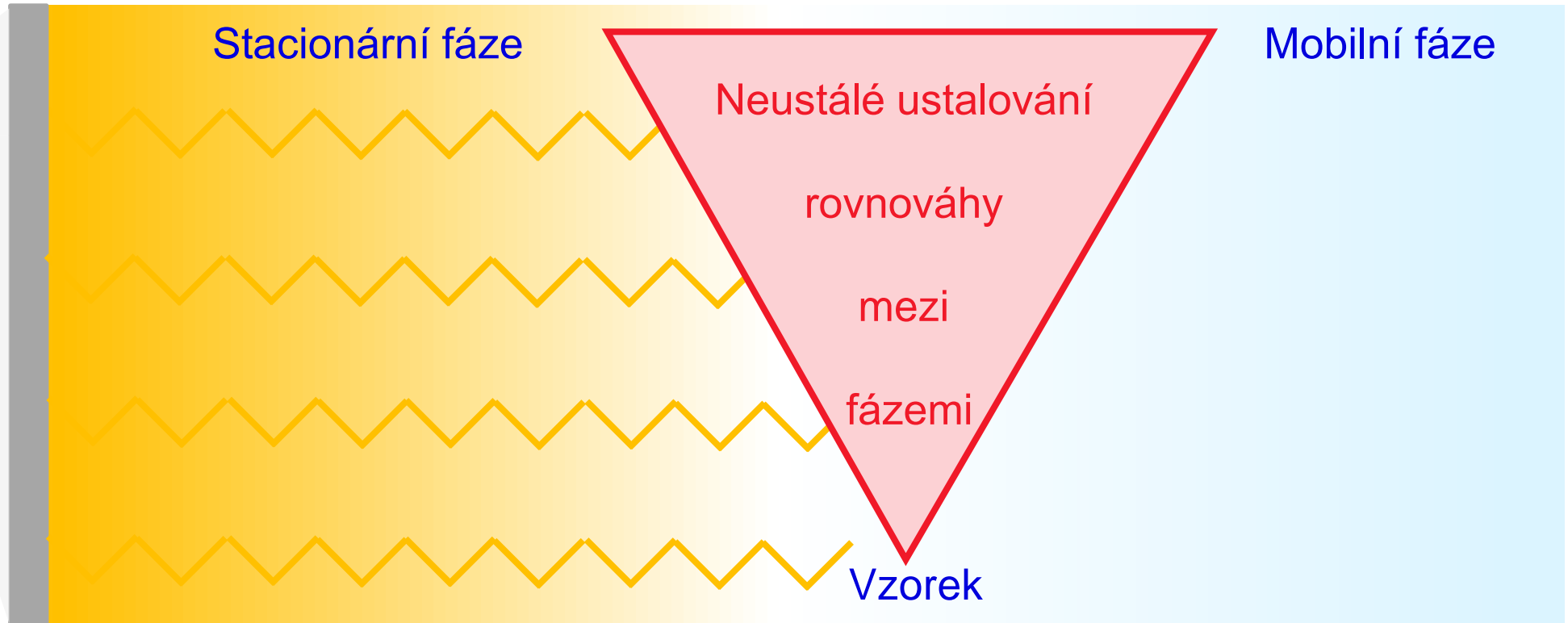
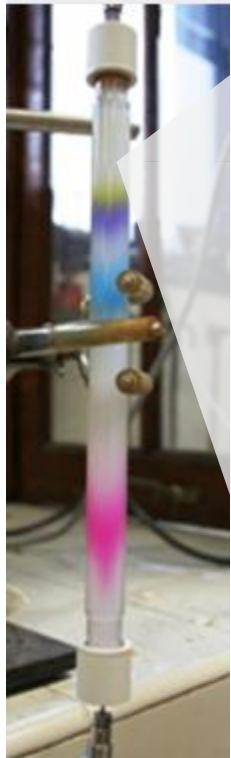




# Chromatografické interakce

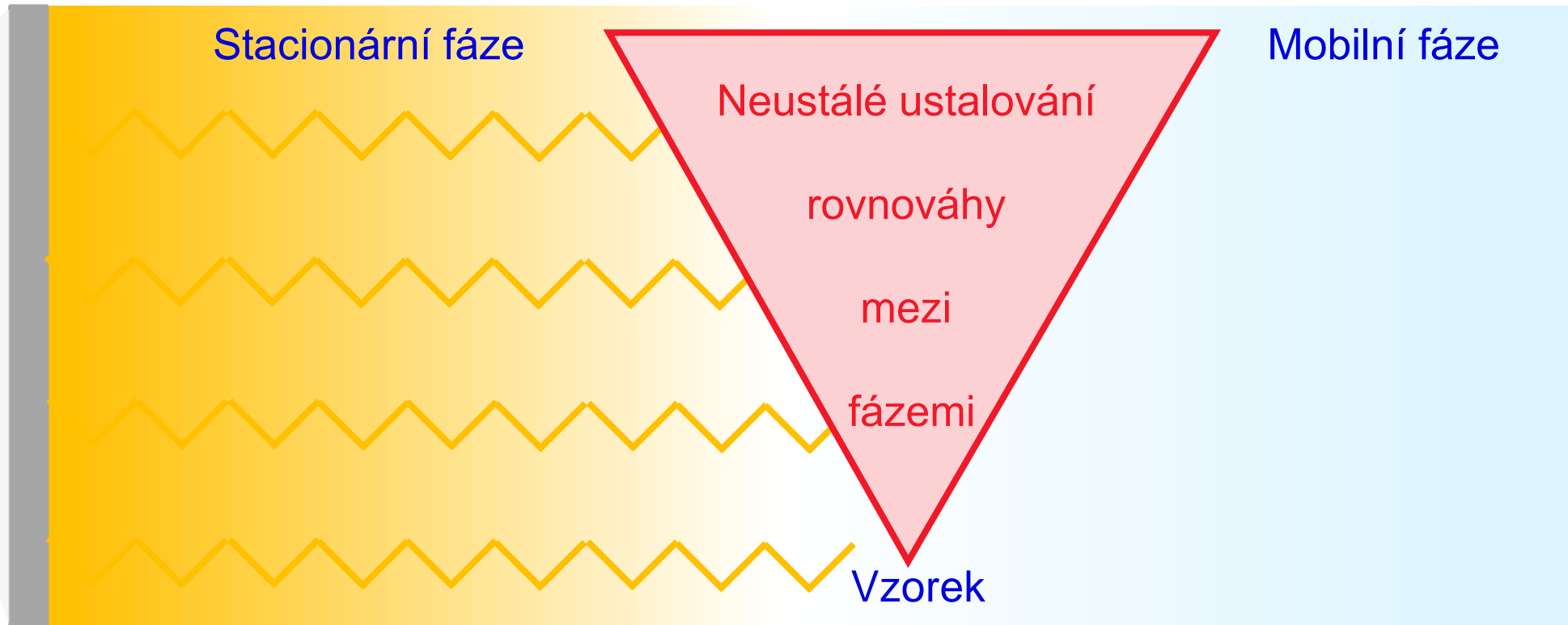
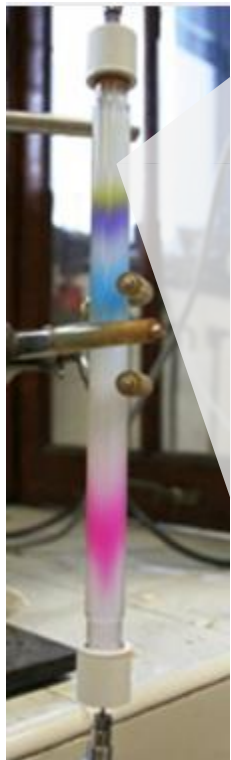


# Chromatografické interakce



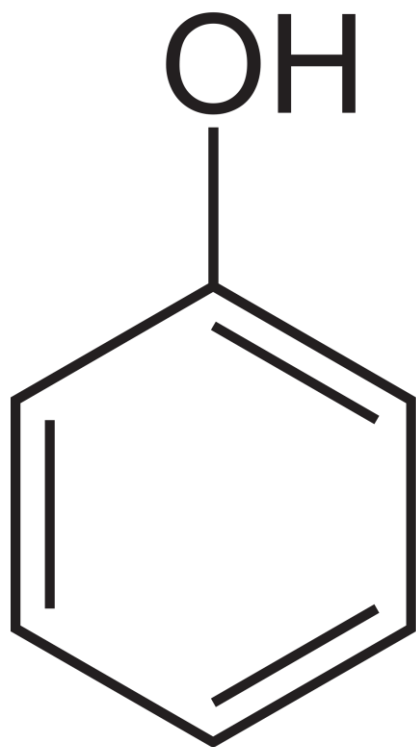
# Chromatografické interakce

Míra interakcí je ovlivněna **polaritou vzorku a obou fází**

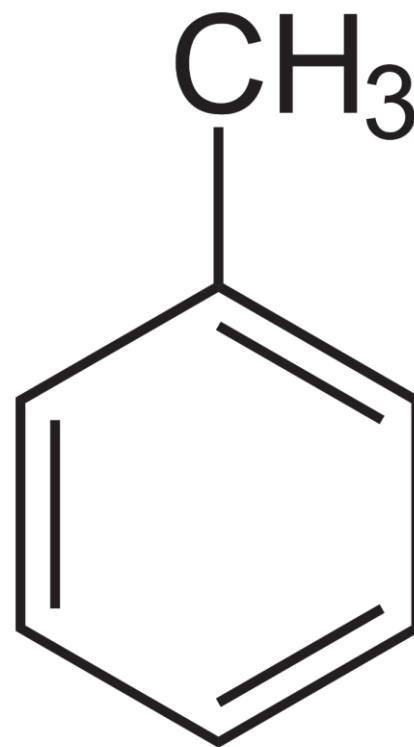


# Co je polárnější

Fenol

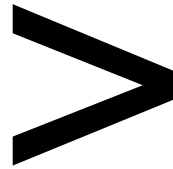
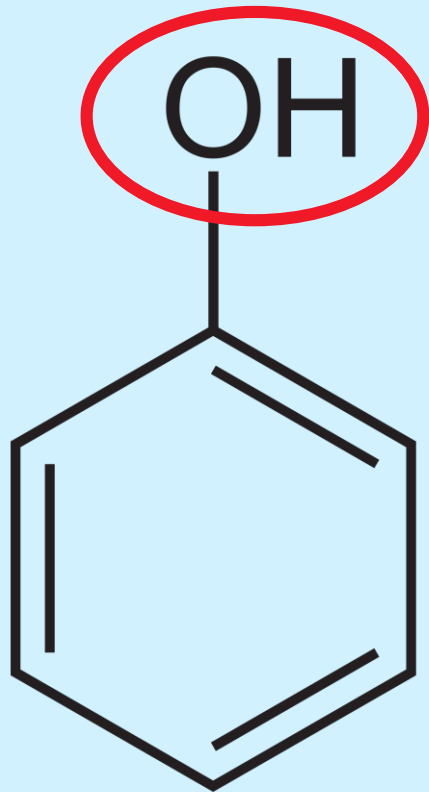


Toluen

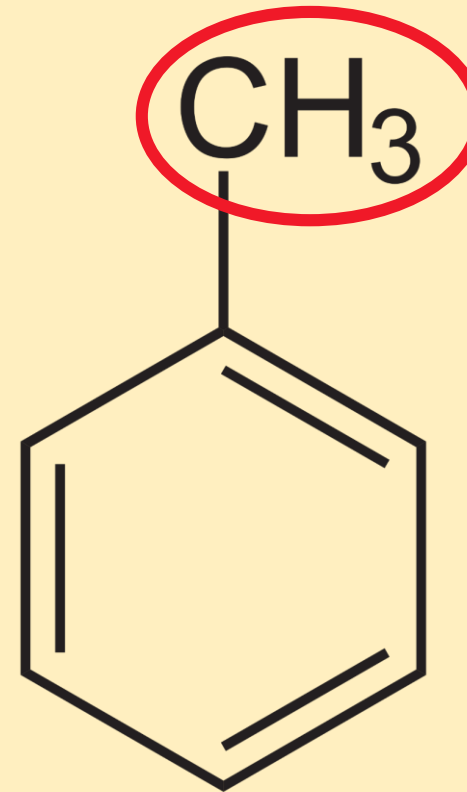


# Co je polárnější

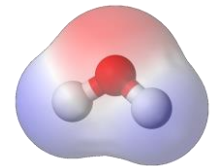
Fenol



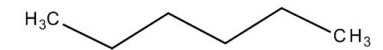
Toluen



A small periodic table of elements is shown, with the element Iron (Fe) highlighted in red. The table includes element symbols, atomic numbers, and names.



Voda

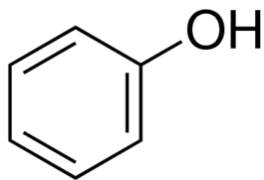


Hexan

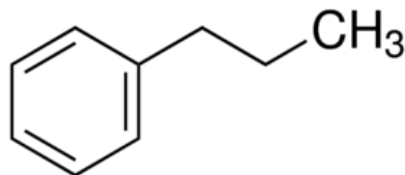
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Polarita  $\approx$  Rozdíl elektronegativity jednotlivých atomů

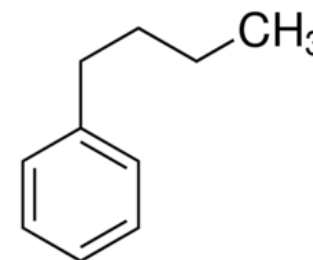
# Seřad'te podle polarity



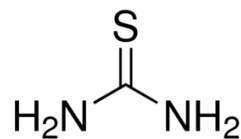
Fenol



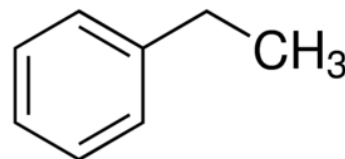
Propylbenzen



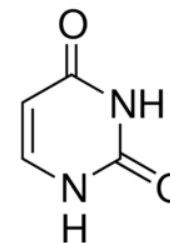
Butylbenzen



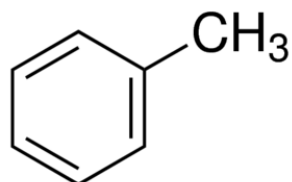
Thiomočovina



Ethylbenzen

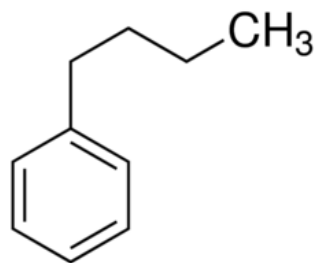


Uracil

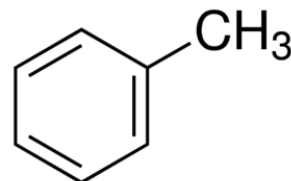


Toluen

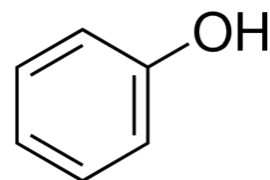
# Seřad'te podle polarity



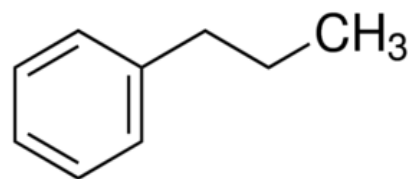
Butylbenzen



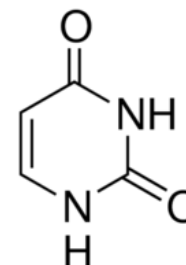
Toluen



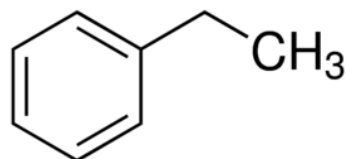
Fenol



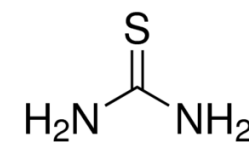
Propylbenzen



Uracil



Ethylbenzen



Thiomočovina

NEPOLÁRNÍ

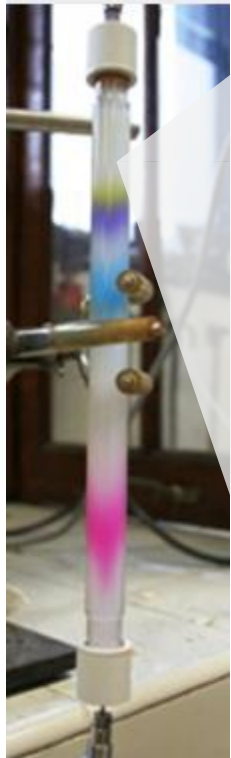


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# Retenční mechanismy

Stacionární fáze

Mobilní fáze



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NEPOLÁRNÍ

NEPOLÁRNÍ

POLÁRNÍ

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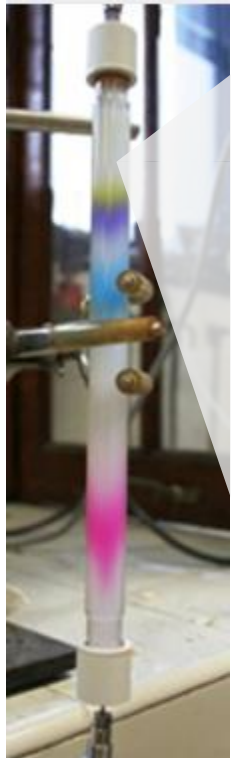
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# Retenční mechanismy

Stacionární fáze

Mobilní fáze



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**NORMÁLNÍ FÁZE**

NEPOLÁRNÍ

NEPOLÁRNÍ

**OBRÁCENÉ FÁZE**

POLÁRNÍ

POLÁRNÍ

**HYDROFILNÍ INTERAKCE**

POLÁRNÍ

# Normální fáze (Normal Phase, NP)

Stacionární fáze

Mobilní fáze

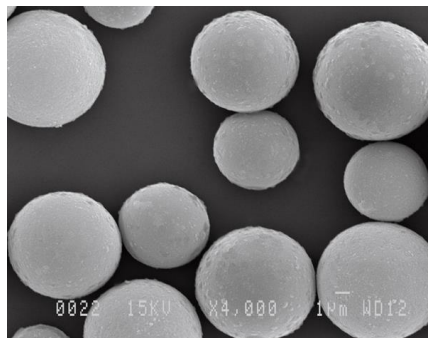
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**NORMÁLNÍ FÁZE**

NEPOLÁRNÍ

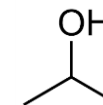
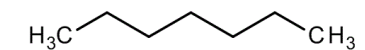
Silikagel

Porézní forma oxidu křemičitého (SiO<sub>2</sub>)



- Izomery ( tokoferol)
- Cukry
- Ve vodě nestabilní látky

Hexan, Heptan  
s přídavkem  
izopropanolu,



# Normální fáze (Normal Phase, NP)

Stacionární fáze

Mobilní fáze

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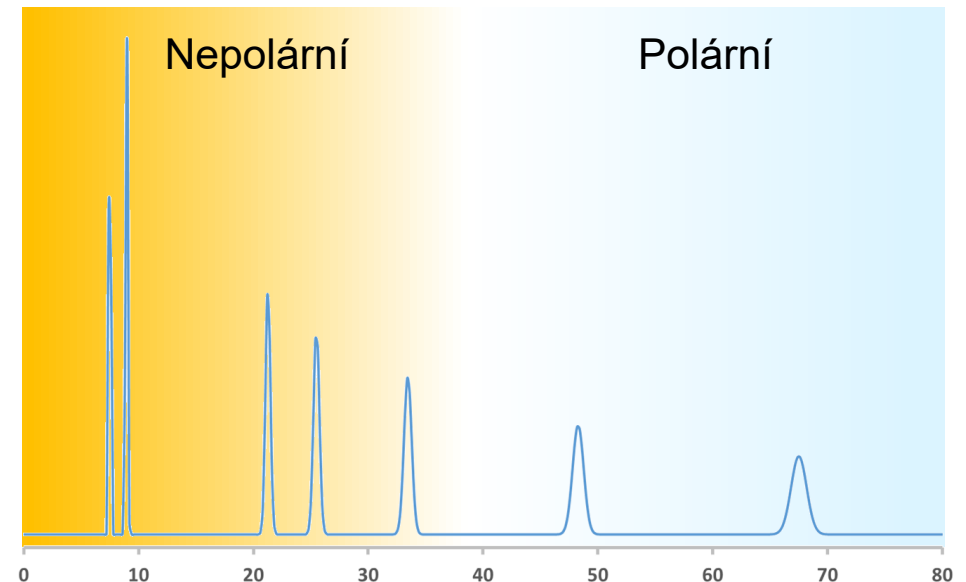
**NORMÁLNÍ FÁZE**

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Pořadí eluce

*Similia similibus solvuntur.*

Podobné v podobném se rozpouští.



# Obrácené fáze (Reversed-Phase, RP)

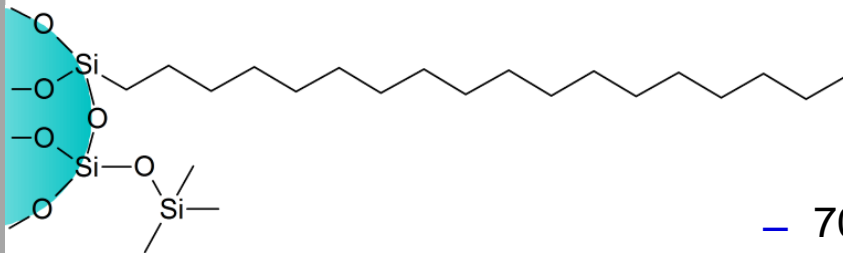
Stacionární fáze

Mobilní fáze

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**OBRÁCENÉ FÁZE**

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Chemicky vázané fáze

-C4, -C8, -C18

- 70 – 80 % všech aplikací
- Lékařství
- Životní prostředí
- Průmysl
- ...

Acetonitril

Metanol

Tetrahydrofuran

Voda

# Obrácené fáze (Reversed-Phase, RP)

Stacionární fáze

Mobilní fáze

NEPOLÁRNÍ

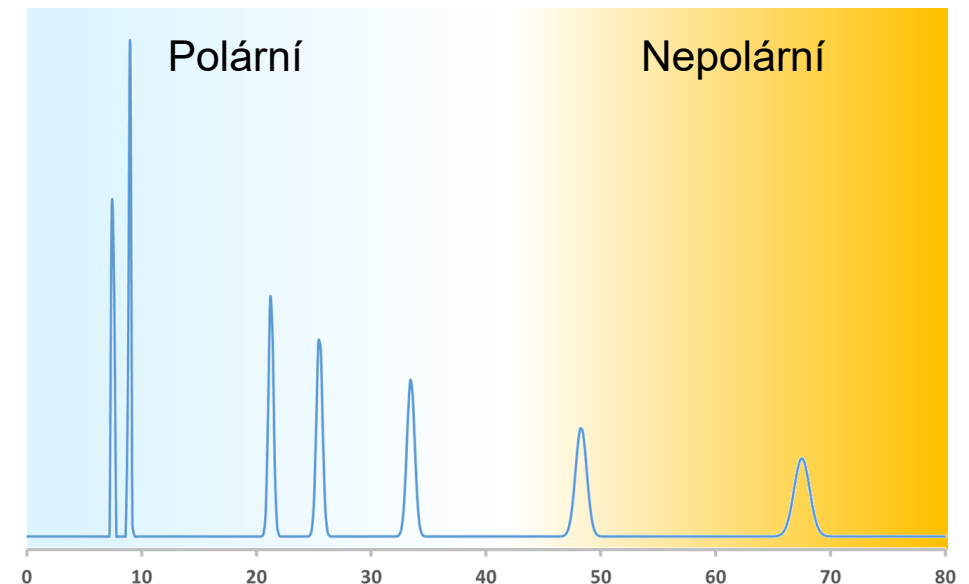
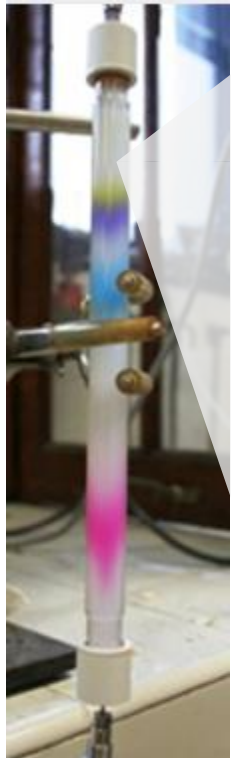
**OBRÁCENÉ FÁZE**

POLÁRNÍ

Pořadí eluce

*Similia similibus solvuntur.*

Podobné v podobném se rozpouští.



# Hydrofilní interakce (HILIC)

Stacionární fáze

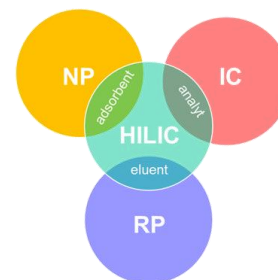
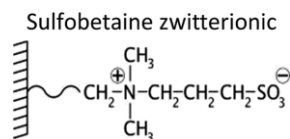
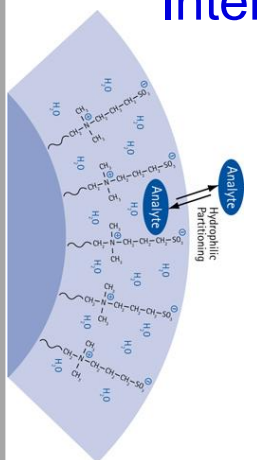
Mobilní fáze

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**HYDROFILNÍ INTERAKCE**

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Interakce ve vodné vrstvě



- Lékařství
- Životní prostředí

Acetonitril

Metanol

Voda/Pufř

(nízké koncentrace)

[www.merckmillipore.com](http://www.merckmillipore.com)

# Hydrofilní interakce (HILIC)

Stacionární fáze

Mobilní fáze

POLÁRNÍ

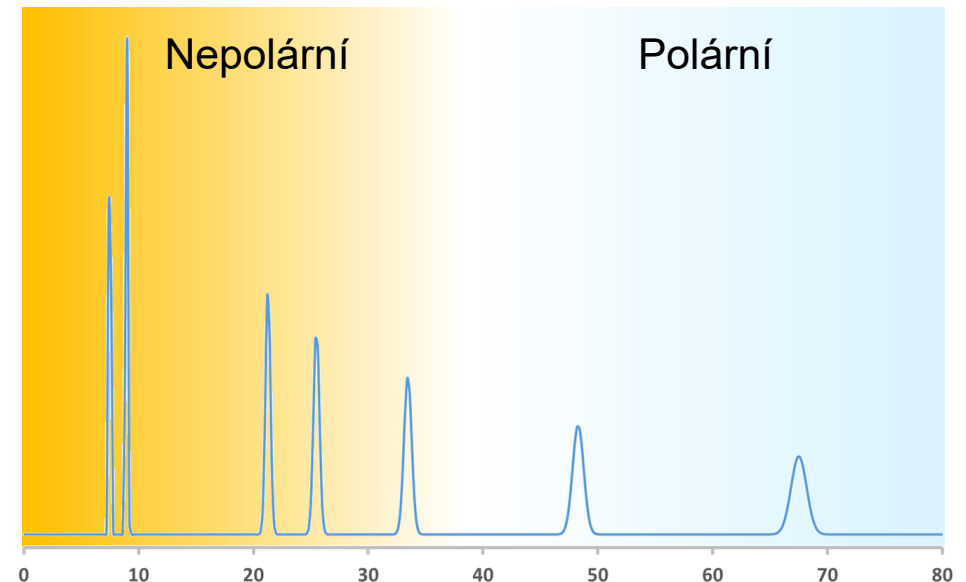
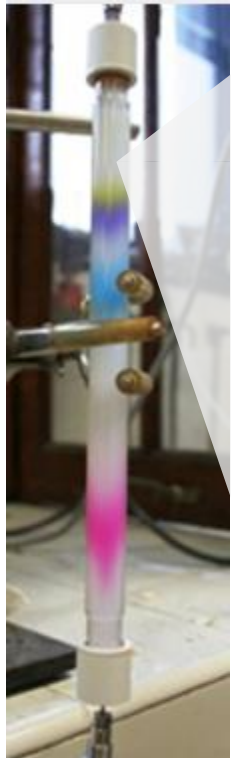
**HYDROFILNÍ INTERAKCE**

POLÁRNÍ

Pořadí eluce

*Similia similibus solvuntur.*

Podobné v podobném se rozpouští.



# Srovnání retenčních mechanismů

	<b>Normální fáze</b>	<b>Obrácené fáze</b>	<b>HILIC</b>
Stacionární fáze	Polární	Nepolární	Polární
Mobilní fáze	Nepolární	Polární	Polární
Vzorky	Méně polární	Ne/Polární	Polární



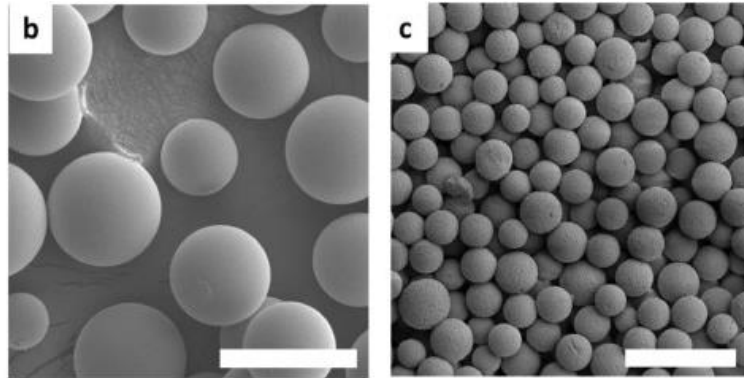
M U N I  
S C I

# Monolitické stacionární fáze



# Stacionární fáze

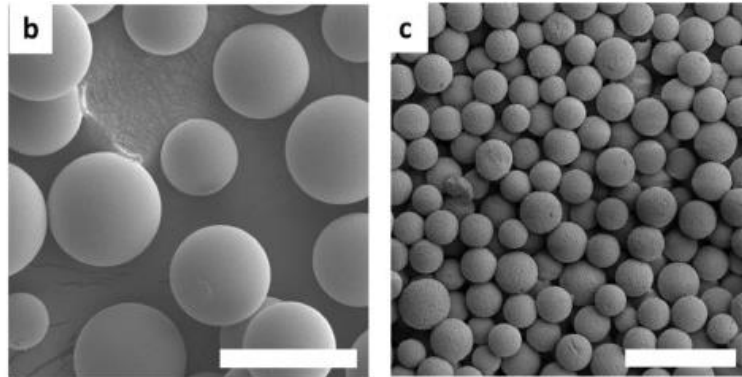
Sférické částice



<https://doi.org/10.1016/j.chroma.2019.460688>

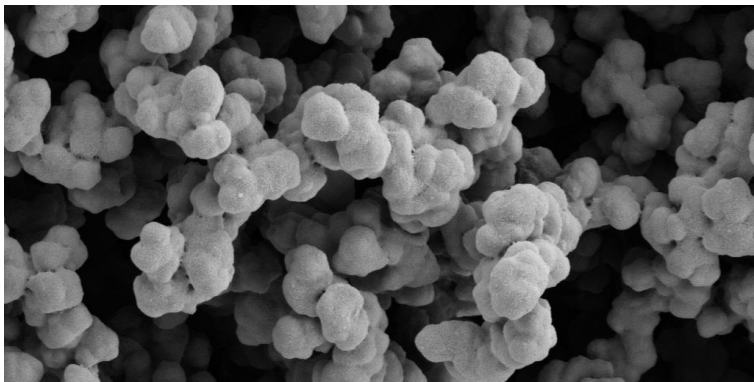
# Stacionární fáze

## Sférické částice

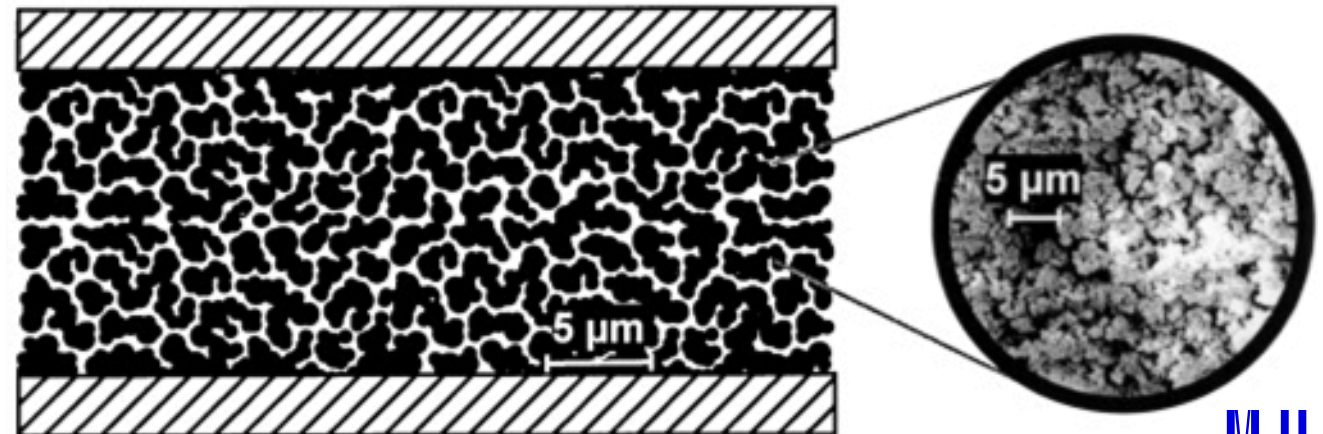
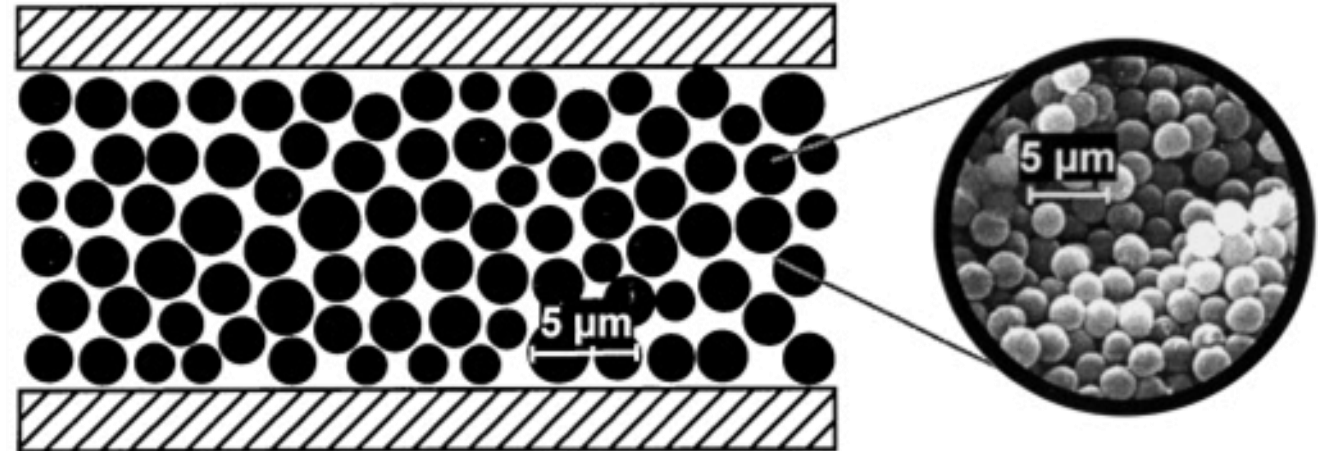


<https://doi.org/10.1016/j.chroma.2019.460688>

## Monolitické stacionární fáze



<https://doi.org/10.1002/jssc.201901331>



[https://doi.org/10.1016/S0165-9936\(02\)00304-7](https://doi.org/10.1016/S0165-9936(02)00304-7)

# Světové monolity



Peter Polichronis



[www.crystalinks.com](http://www.crystalinks.com)



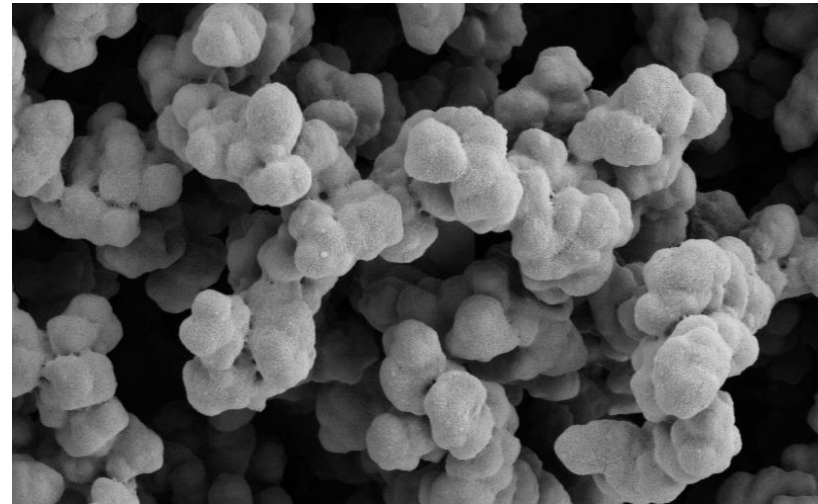
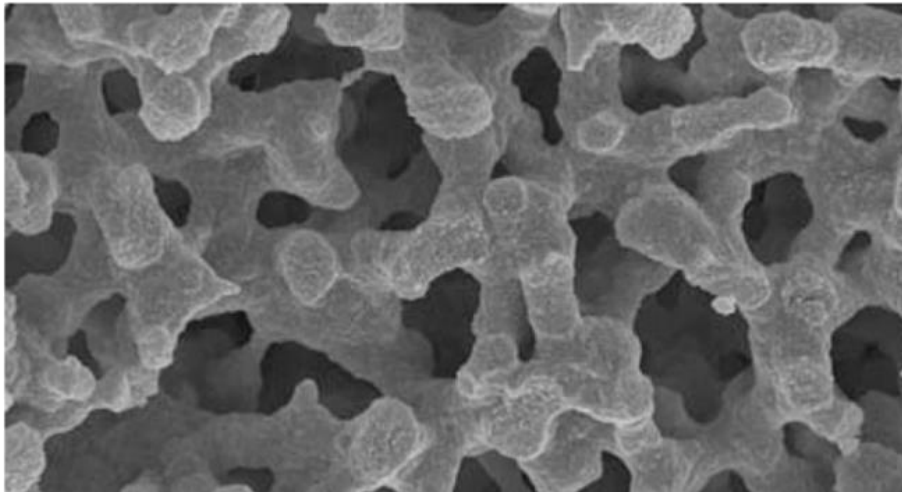
# Světové monolity



Peter Polichronis



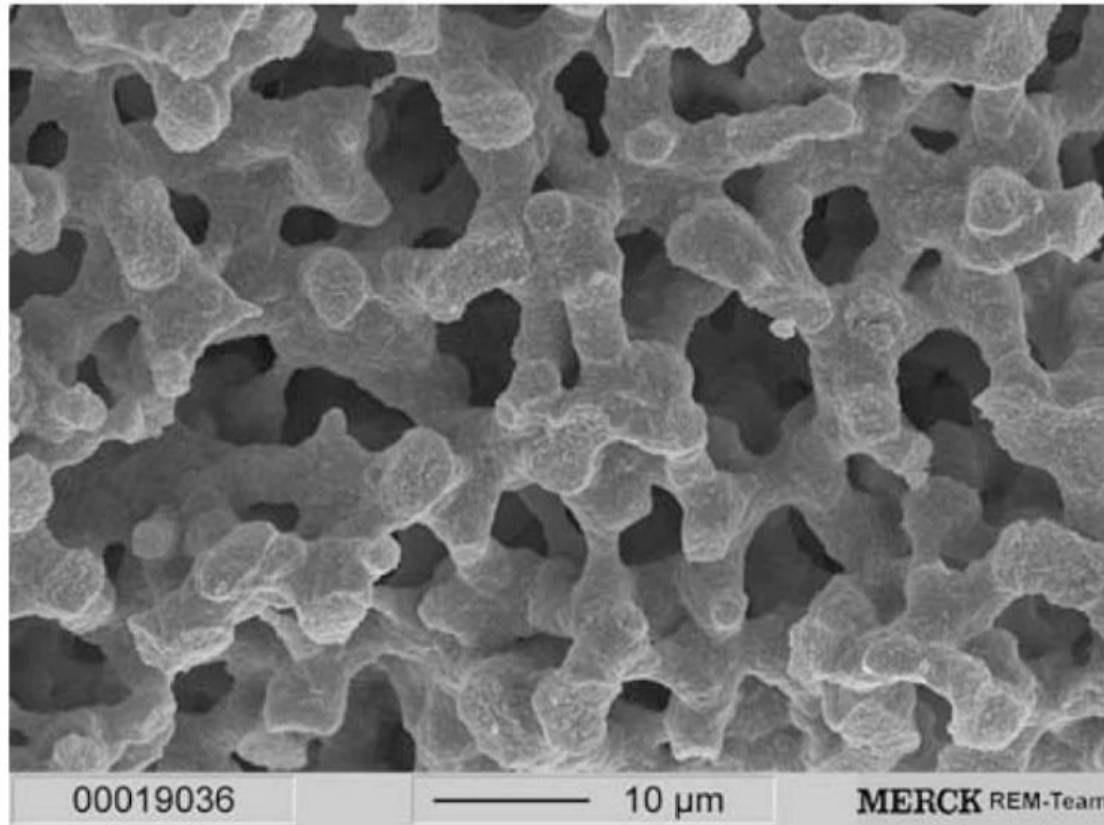
[www.crystalinks.com](http://www.crystalinks.com)



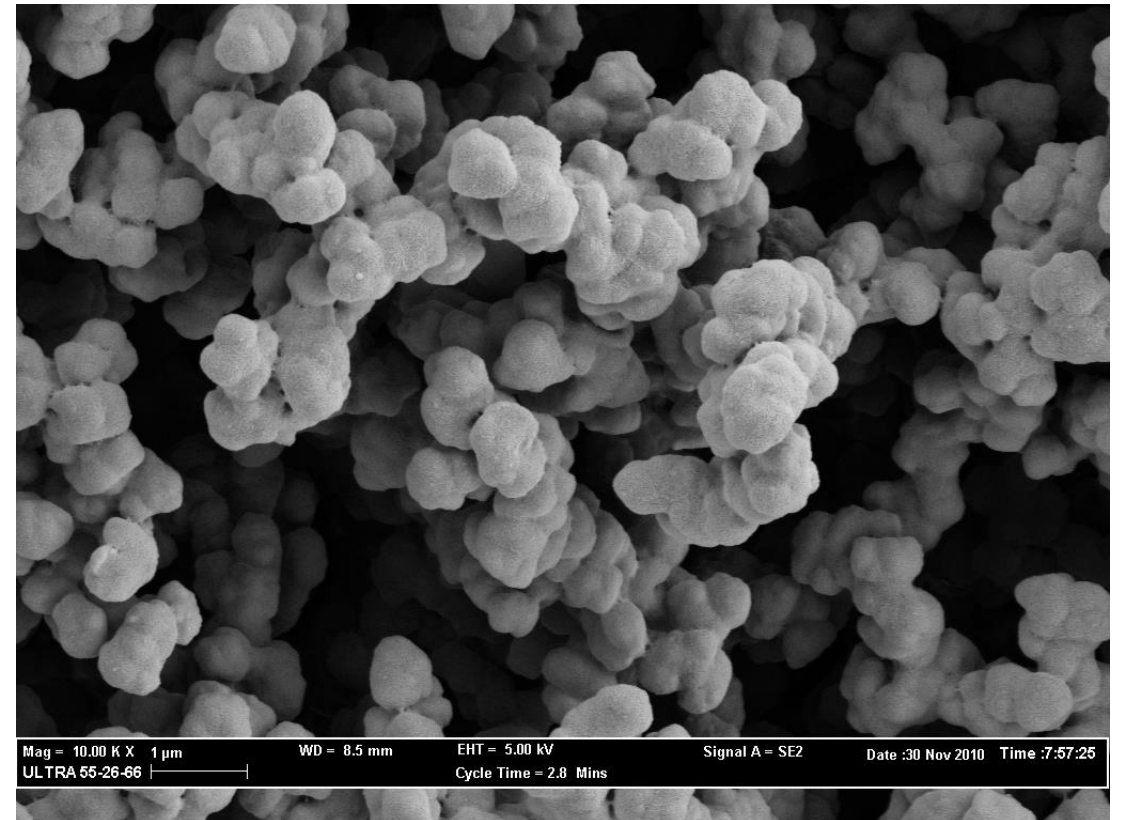
<https://www.studentagencybus.com>

# Monolitické stacionární fáze

Anorganické

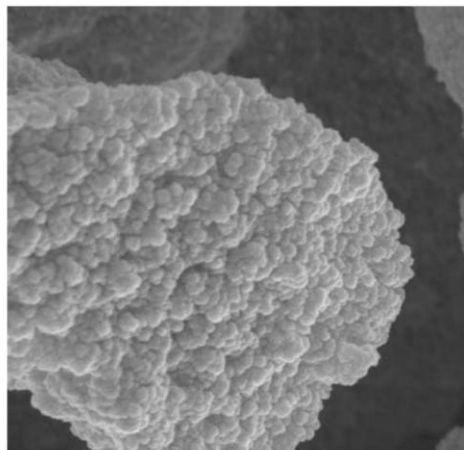
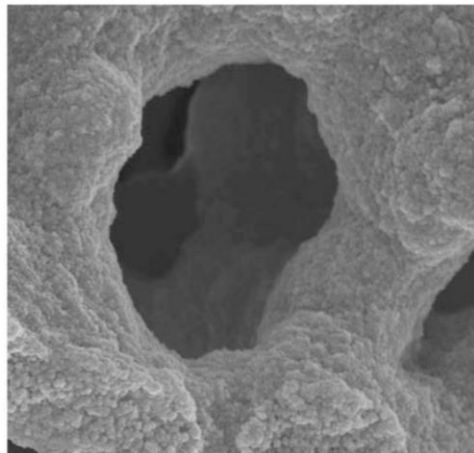
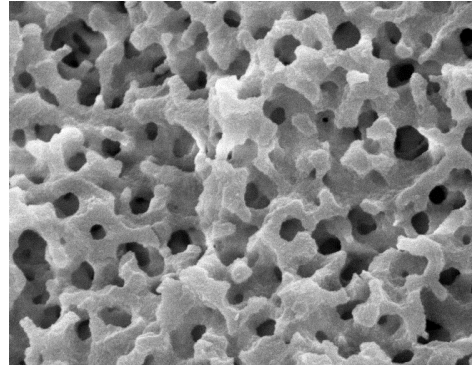
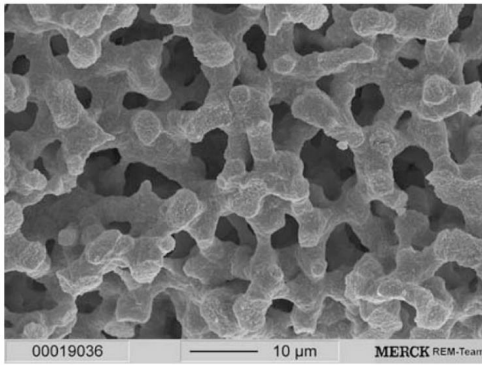


Polymerní

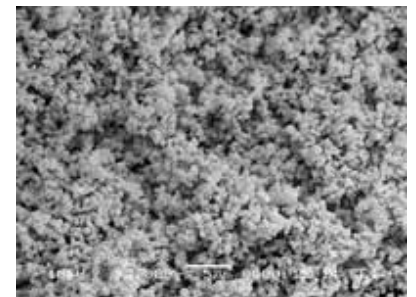
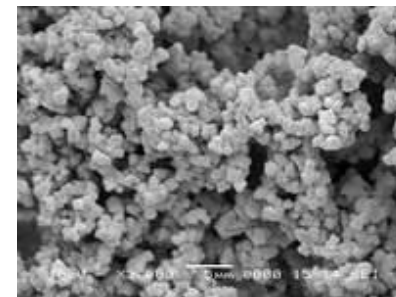
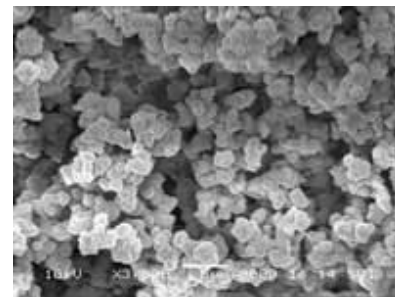
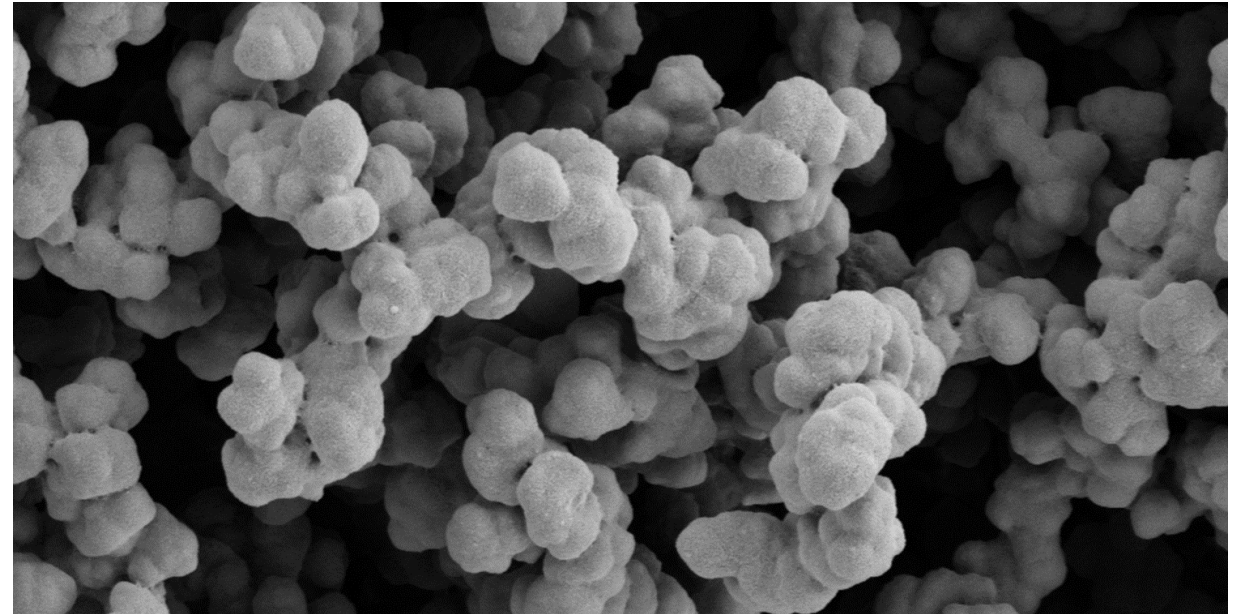


# Monolitické stacionární fáze

Anorganické

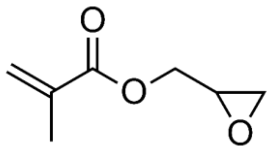


Polymerní

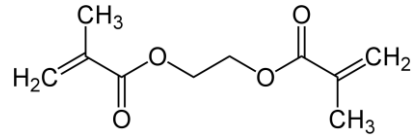


# Polymerní monolity

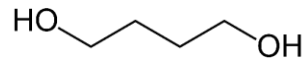
## Metakrylátové



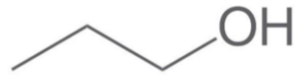
glycidyl metakrylát



ethylen dimetakrylát



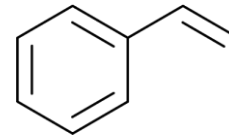
1,4-butandiol



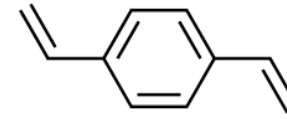
1-propanol



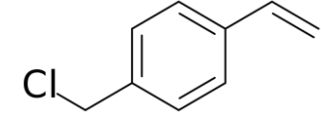
## Styren-divinylbenzenové



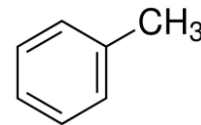
styren



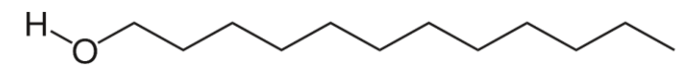
divinylbenzen



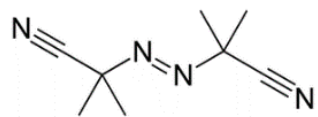
vinylbenzyl chlorid



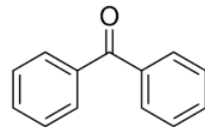
toluen



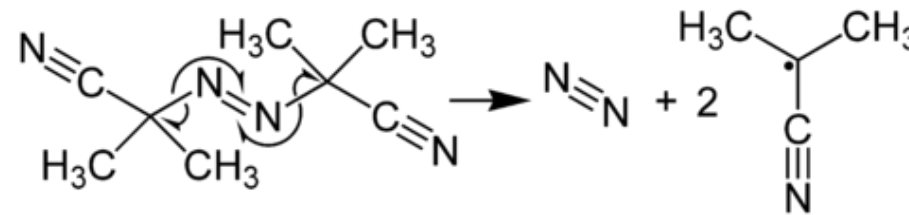
1-dodekanol



azobisisobutyronitril

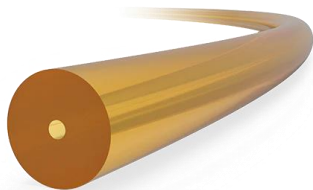
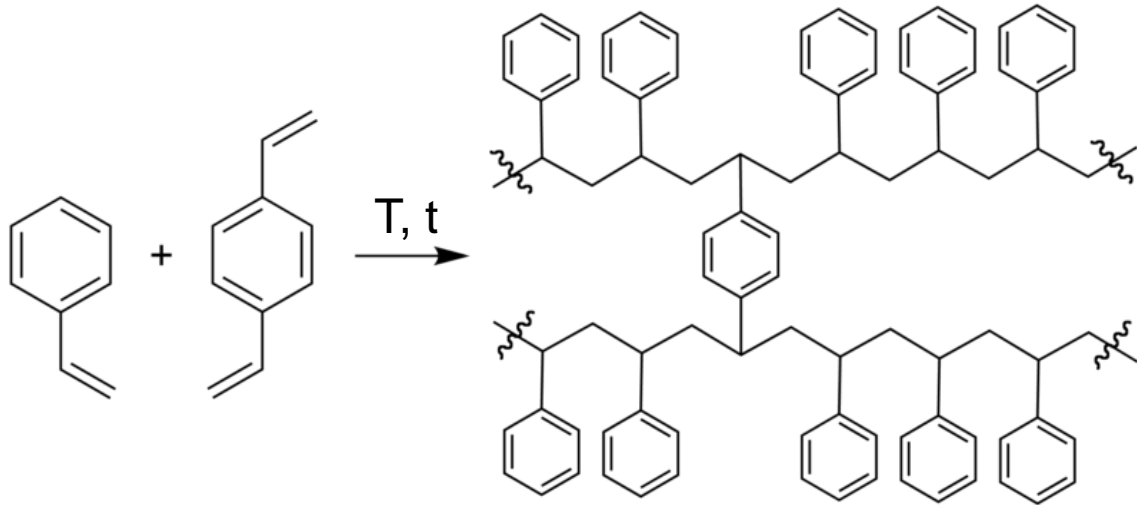


benzofenon





# Polymerační reakce

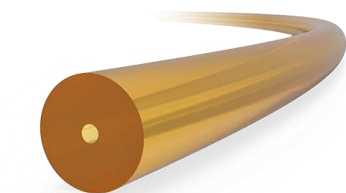
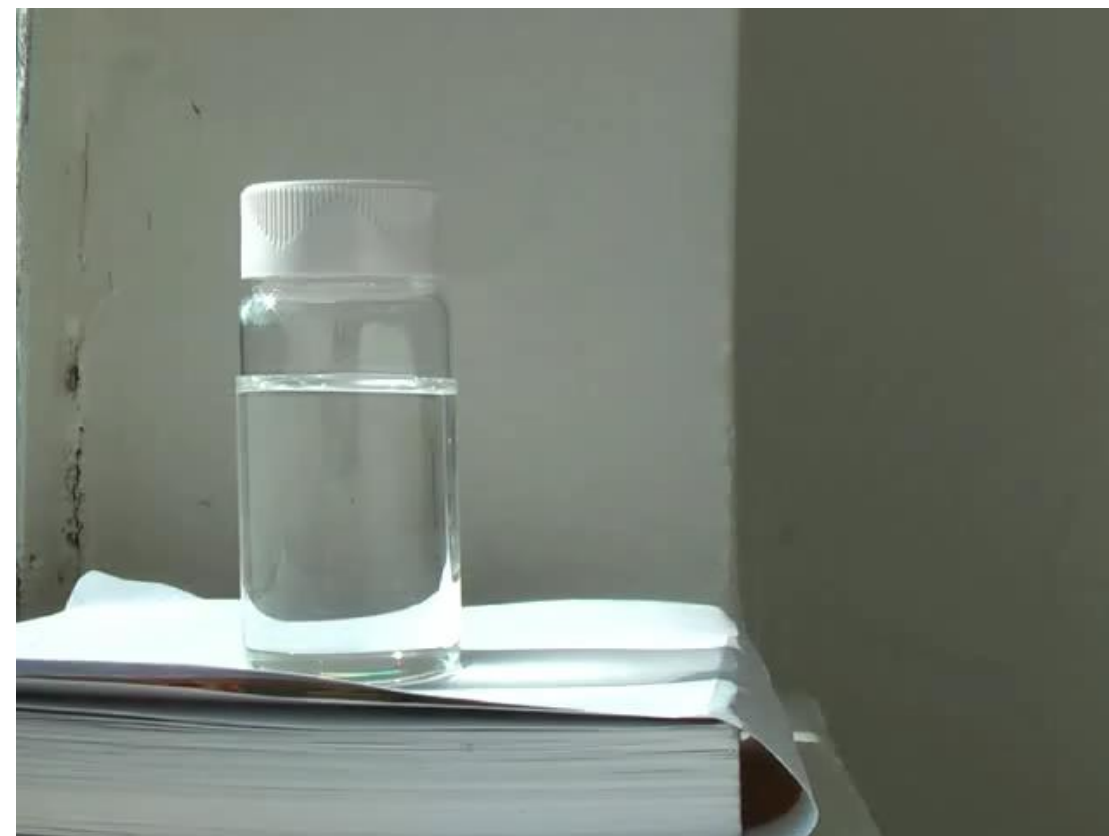
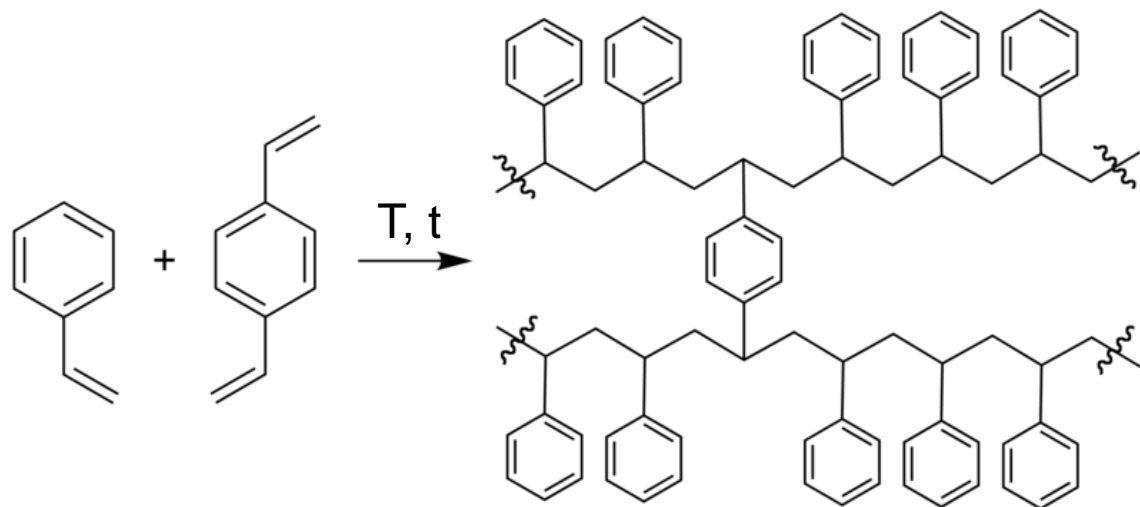


[www.vicijour.com](http://www.vicijour.com)

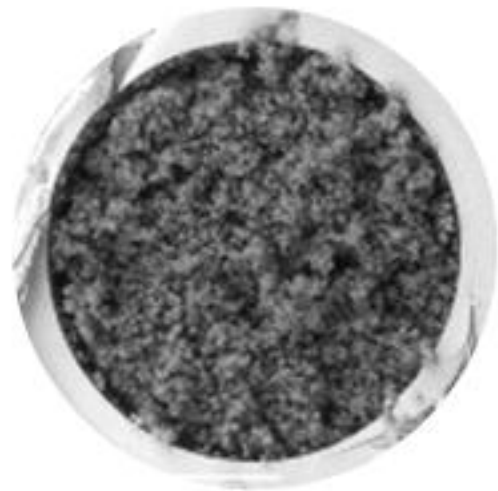


[www.fishersci.se](http://www.fishersci.se)

# Polymerační reakce

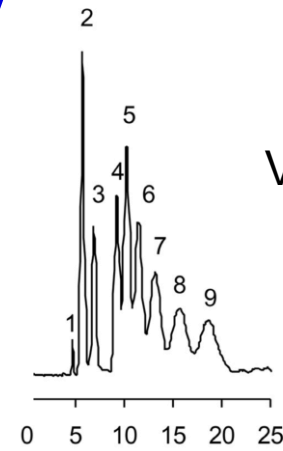
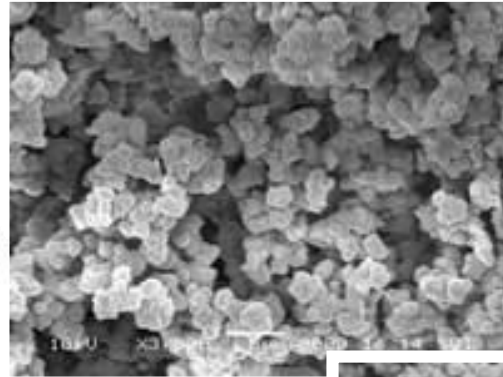


# Monolitické kapilární kolony



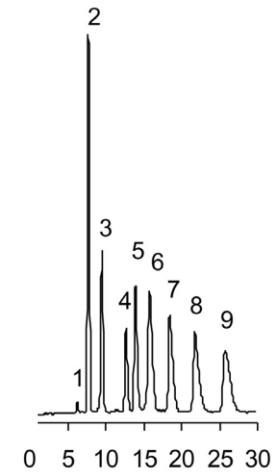
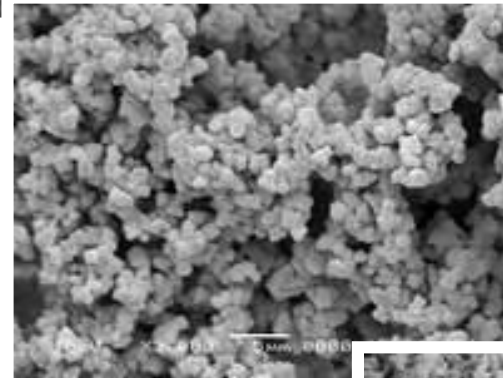
50 – 320  $\mu\text{m}$

30%

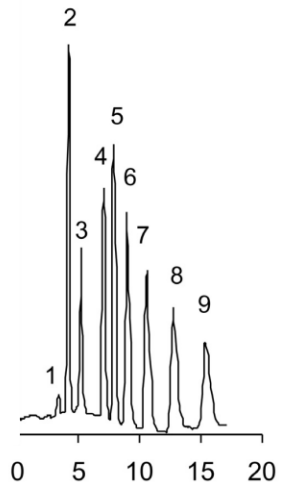
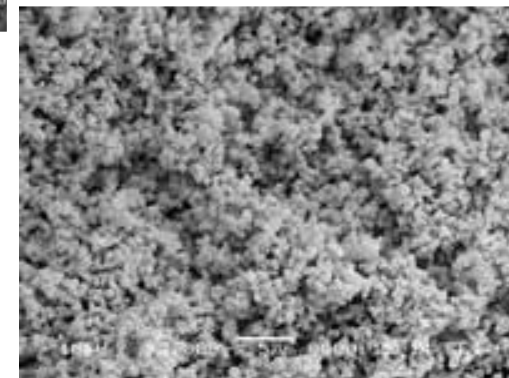


Vliv koncentrace 1,4-butandiolu  
v polymerační směsi

29%

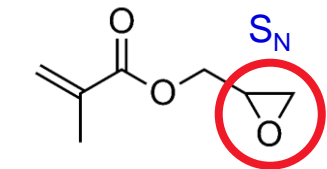


28%

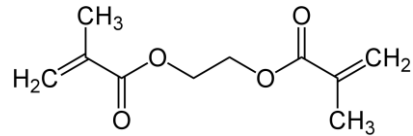


# Snadná modifikace

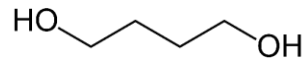
## Metakrylátové



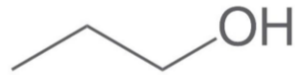
glycidyl metakrylát



ethylen dimetakrylát



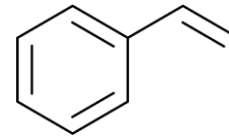
1,4-butandiol



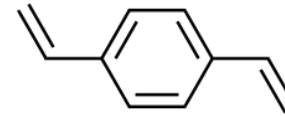
1-propanol



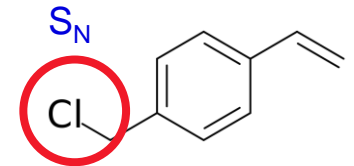
## Styren-divinylbenzenové



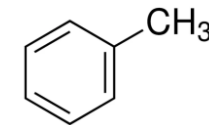
styren



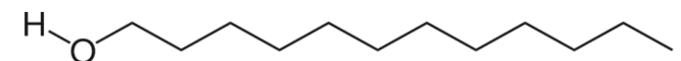
divinylbenzen



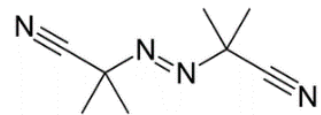
vinylbenzyl chlorid



toluen



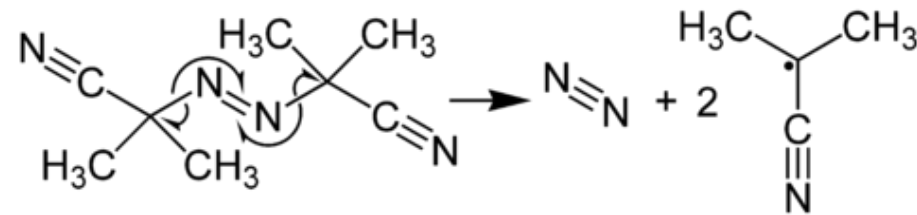
1-dodekanol



azobisisobutyronitril

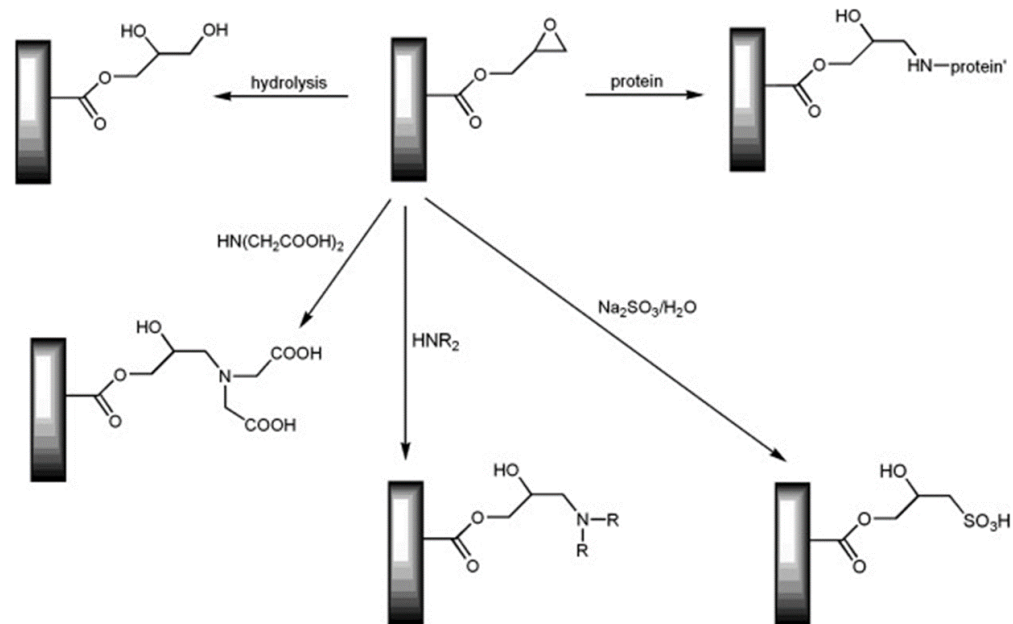


benzofenon

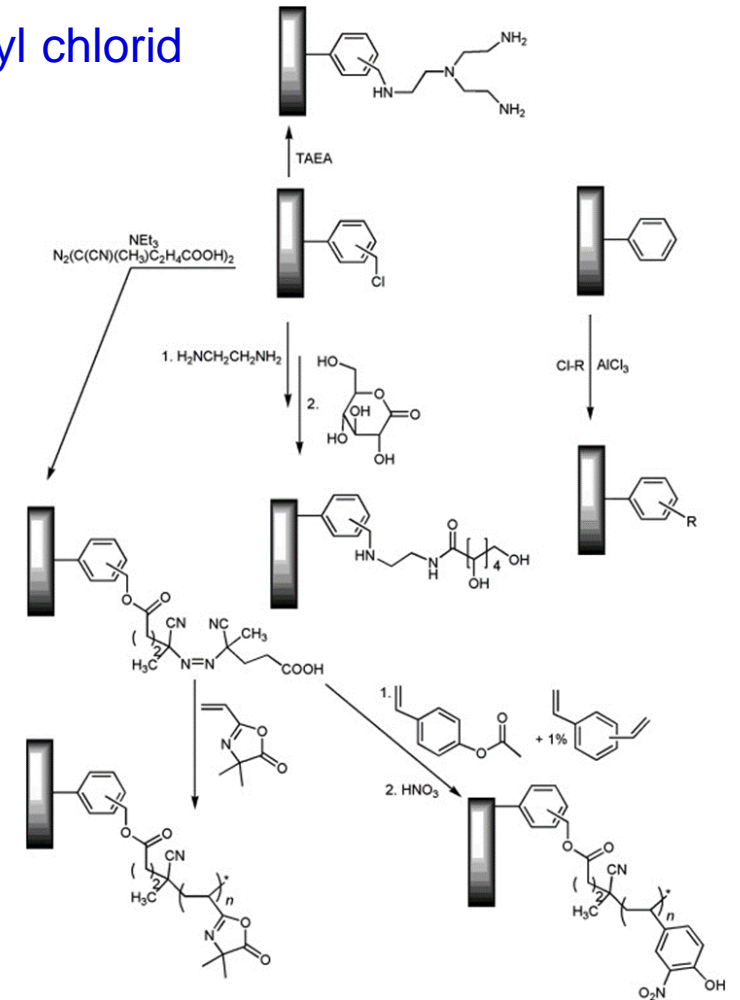


# Chemická modifikace

## Glycidyl metakrylát

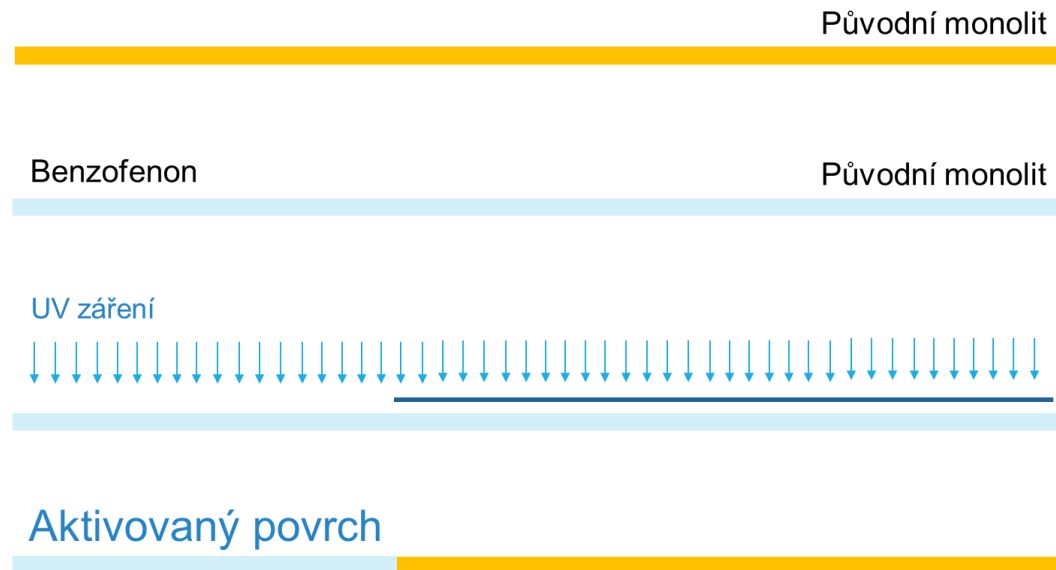


## Vinylbenzyl chlorid

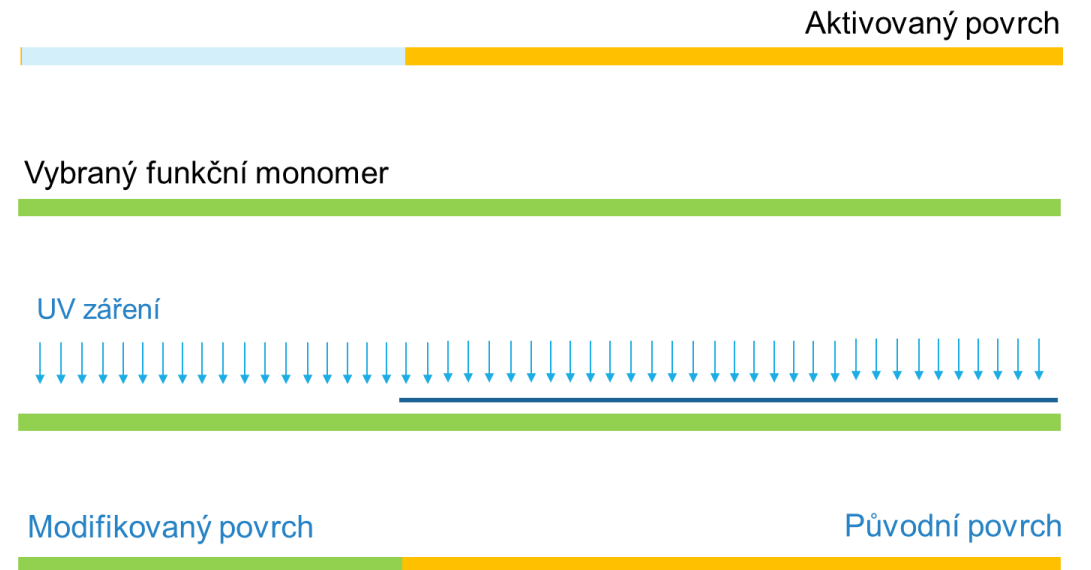


# Roubování (Grafting)

1. krok

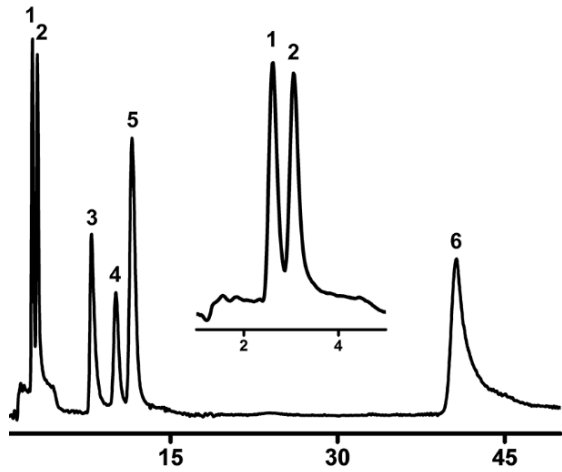


2. krok

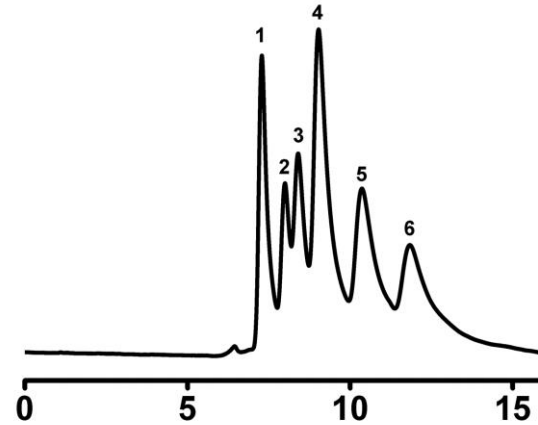


# Stacionární fáze

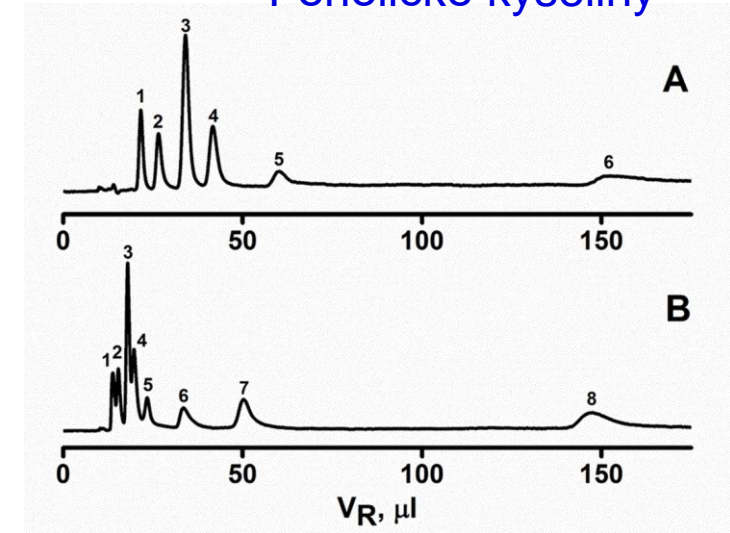
Neurotransmitery



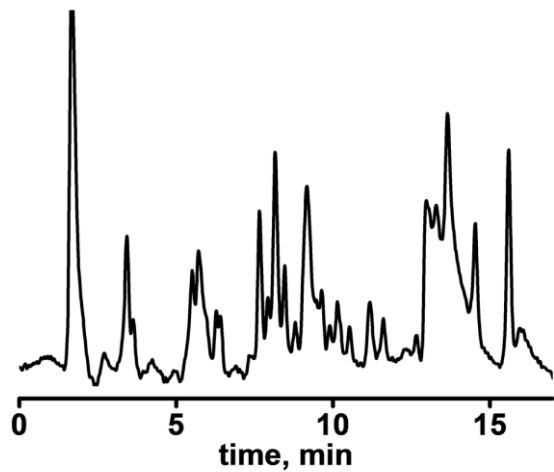
Barbituráty



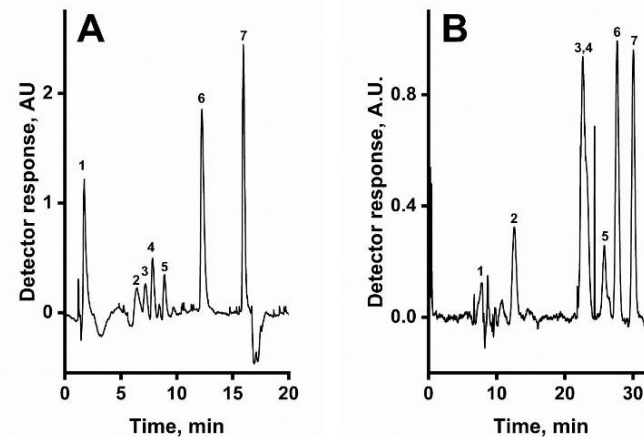
Fenolické kyseliny



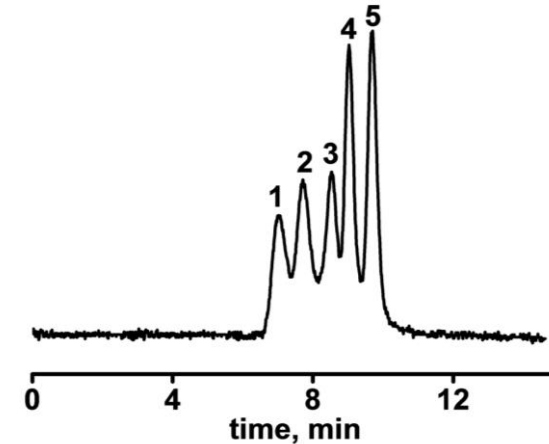
Tryptický digest



Peptidy



Polystyreny



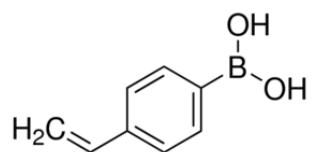
# Multifunkční kapilární kolona

Extrakce a separace

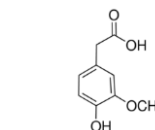
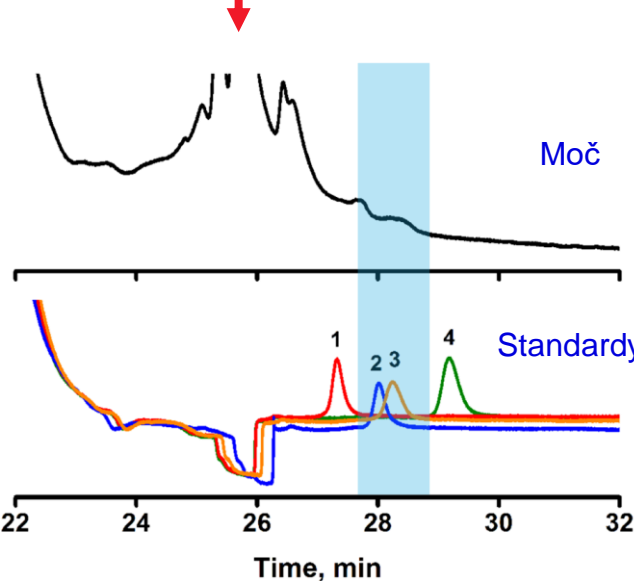
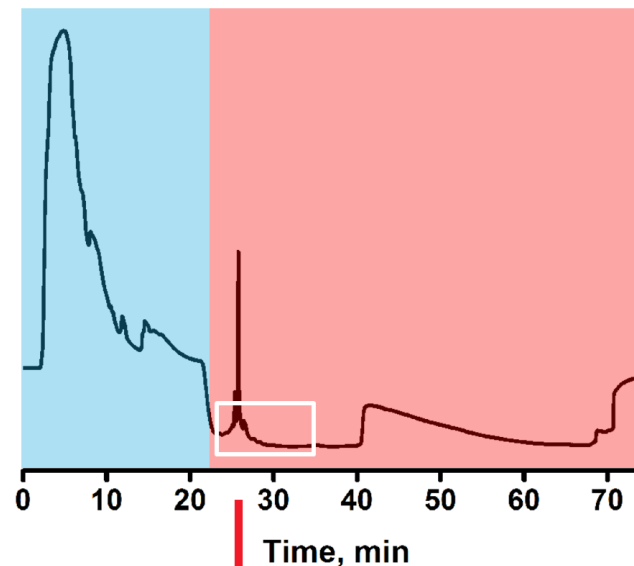
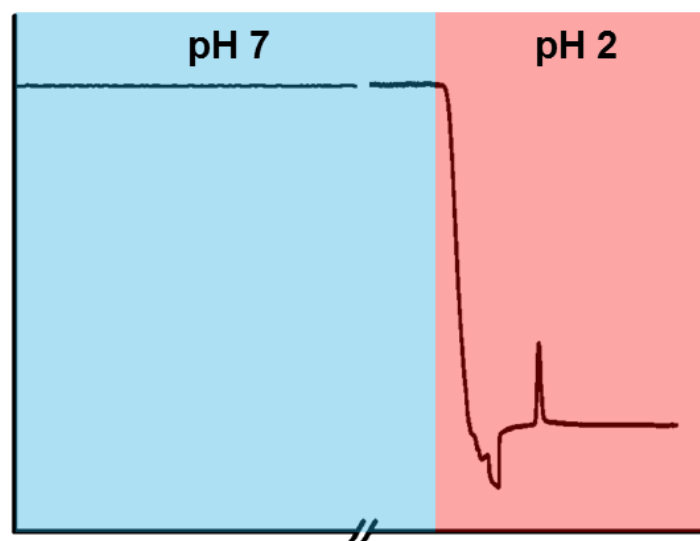


Selektivní záchyt

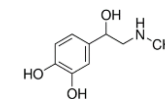
Separace



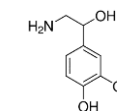
Kyselina vinylphenyl boritá



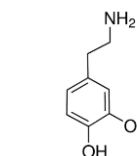
1 - Normetanefrin



2 - Epinefrin



3 - Norepinefrin

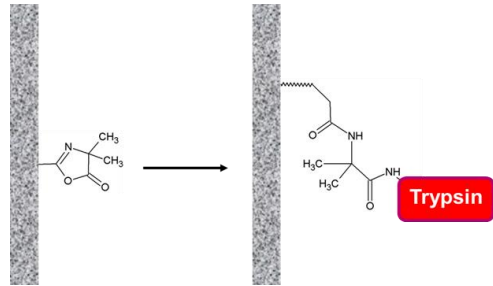


4 - Dopamin

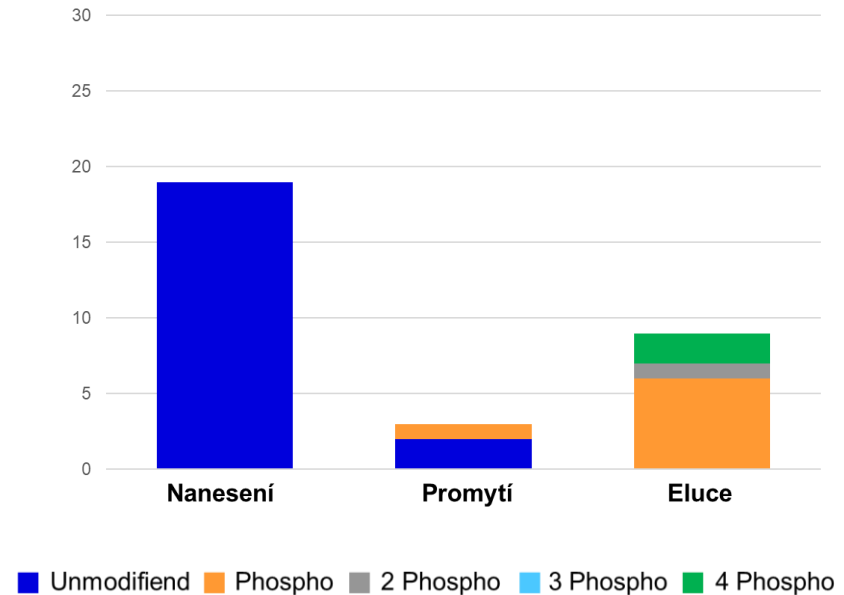
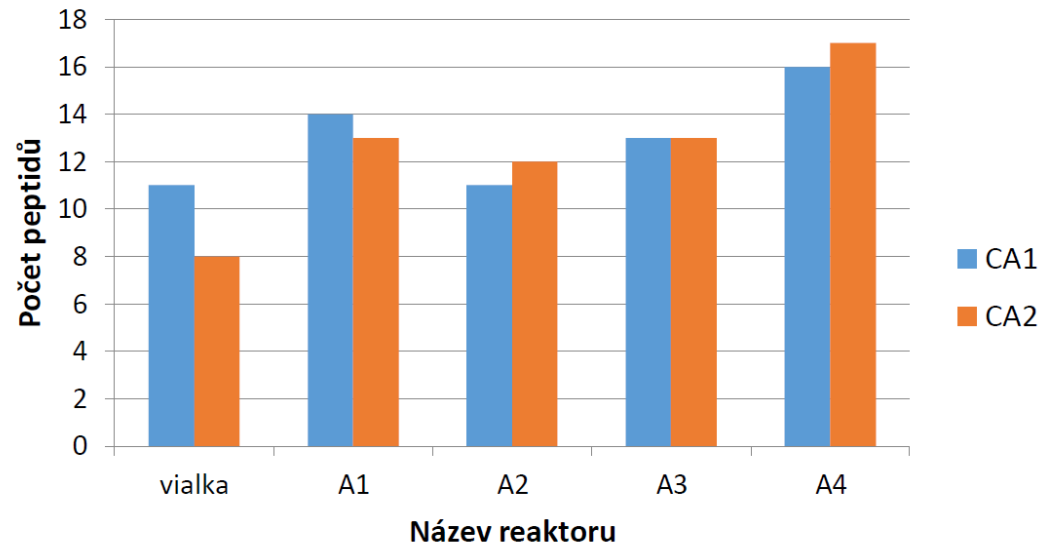
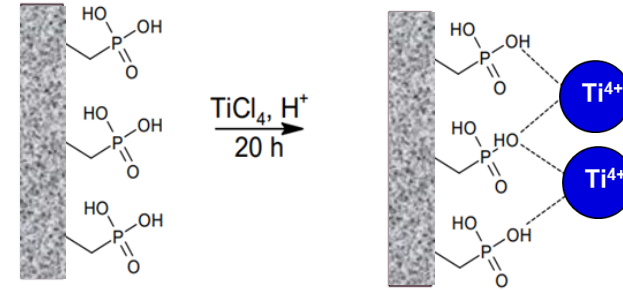


# Příprava vzorku pro proteomickou analýzu

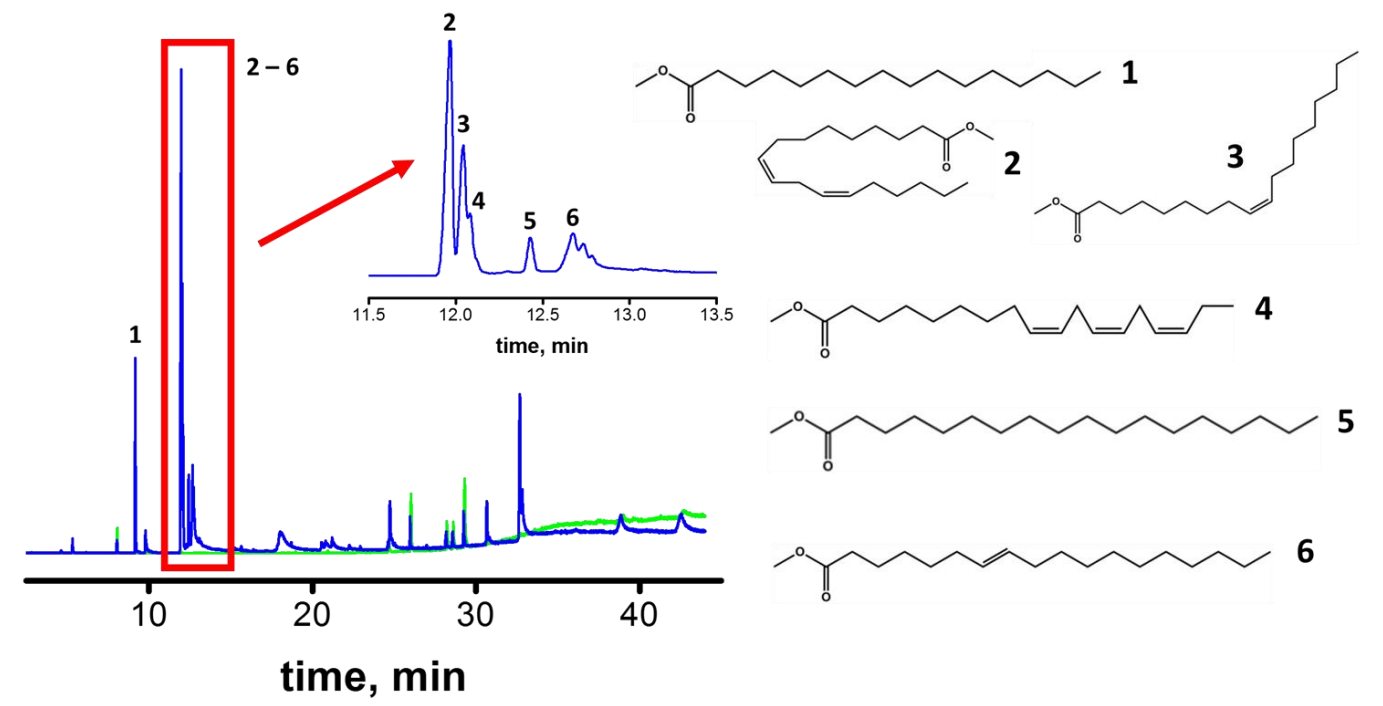
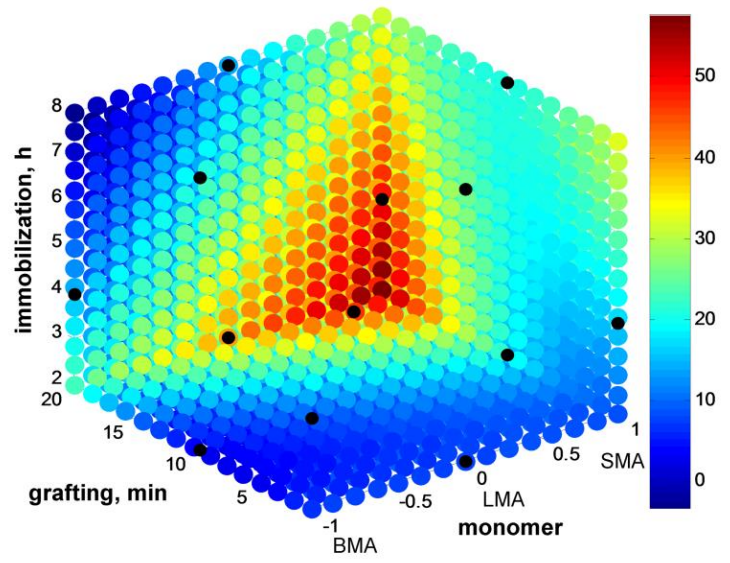
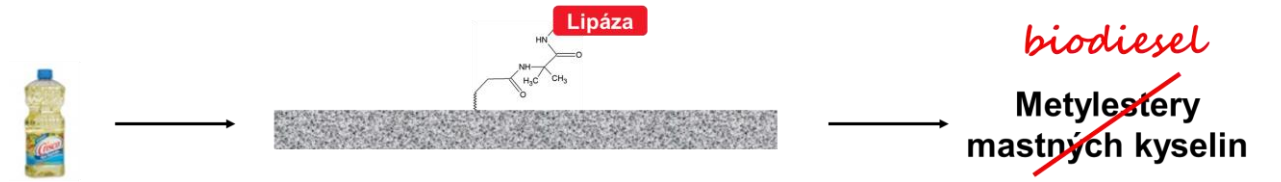
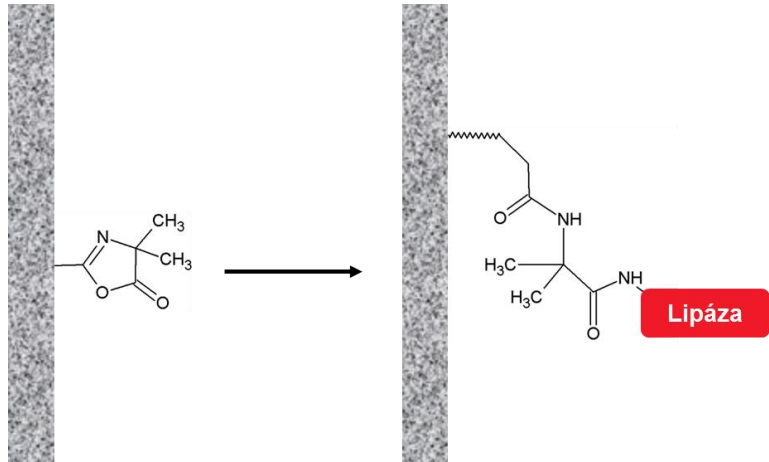
## Proteolýza



## Záchyt fosfopeptidů

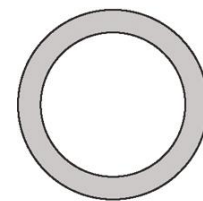
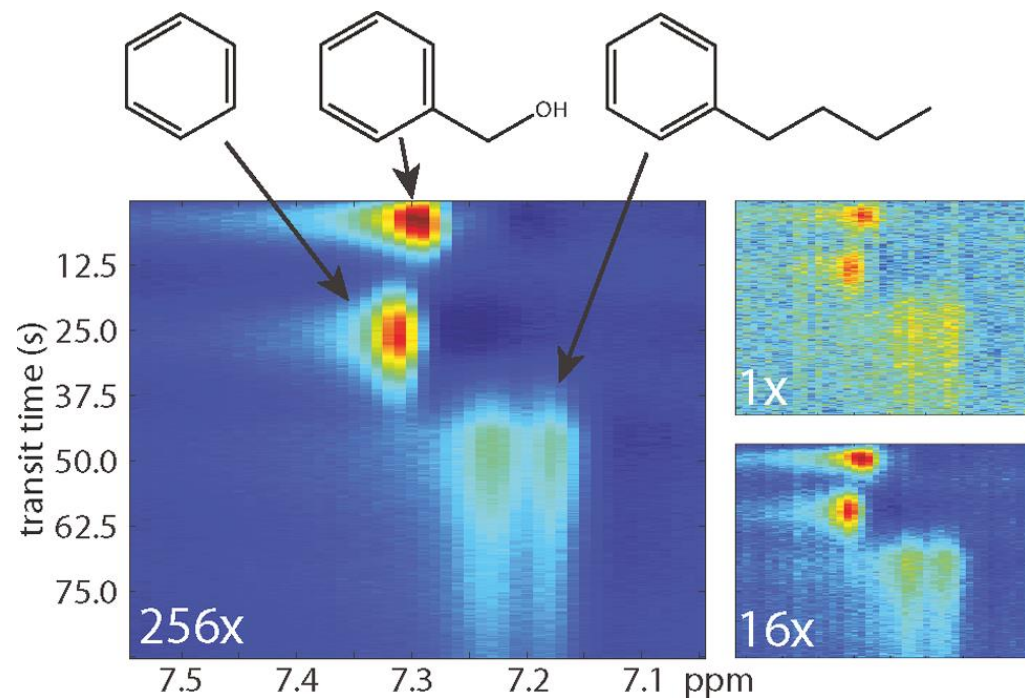
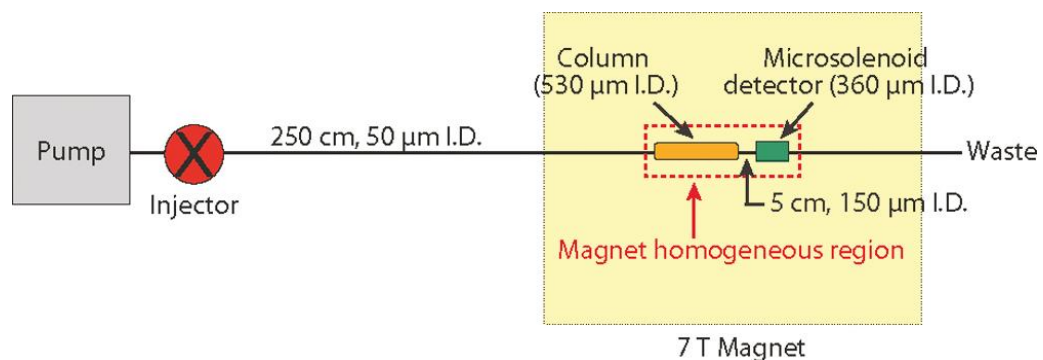


# “Výroba” biodieselu

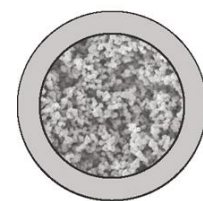


# Spojení s netradiční detekcí

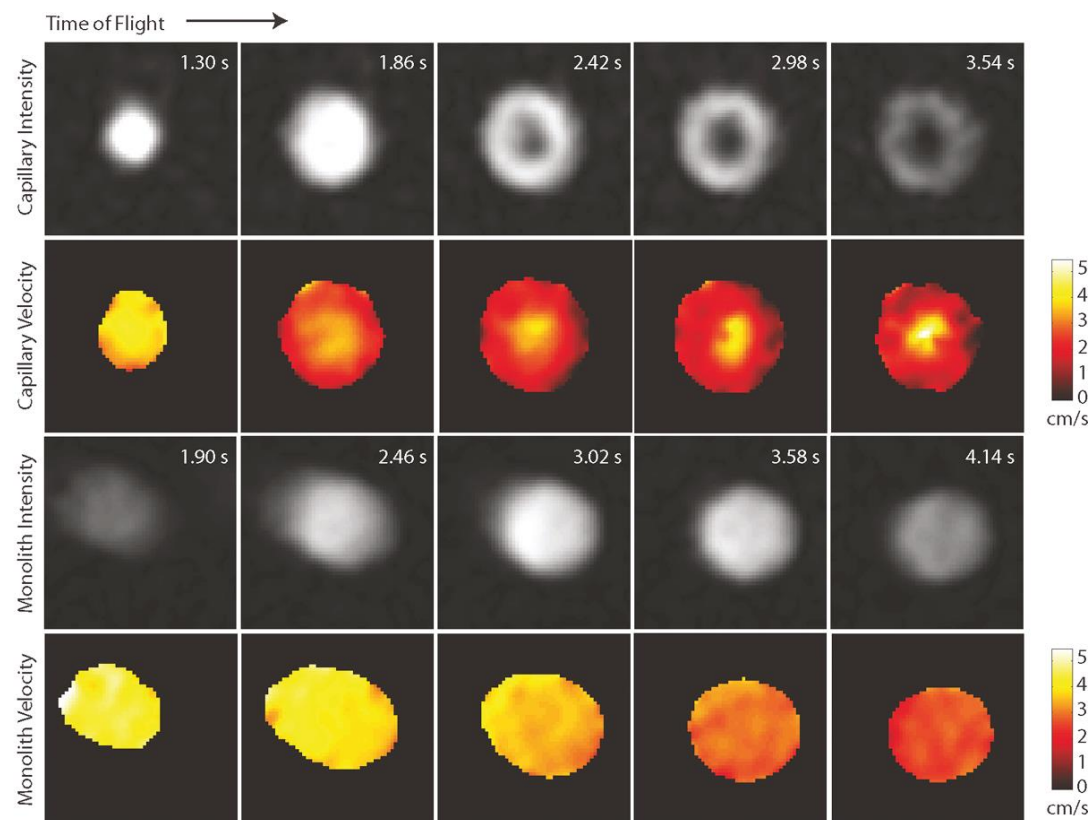
Online LC-NMR



Empty capillary (530 μm I.D.)



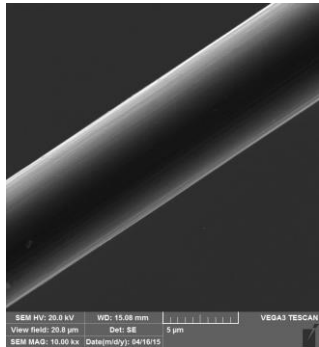
Hypercrosslinked Monolith (530 μm I.D.)



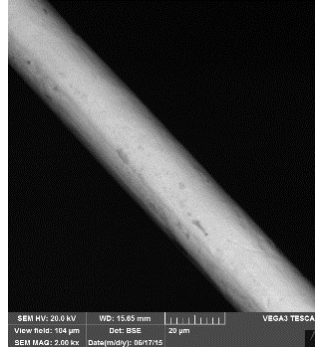
<http://doi.org/10.1021/ac2010108>

# Integrované analytické systémy

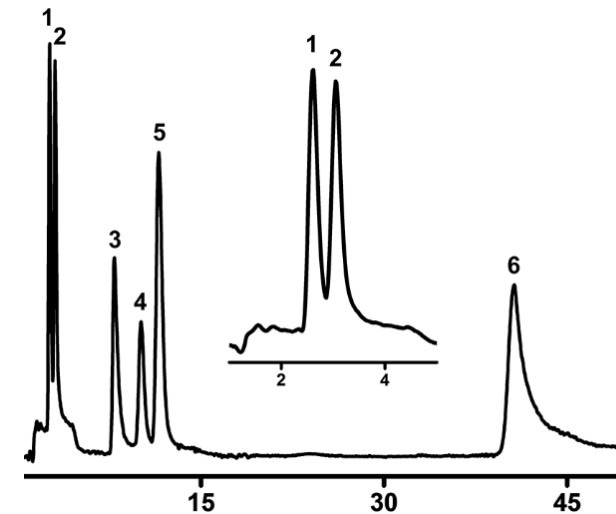
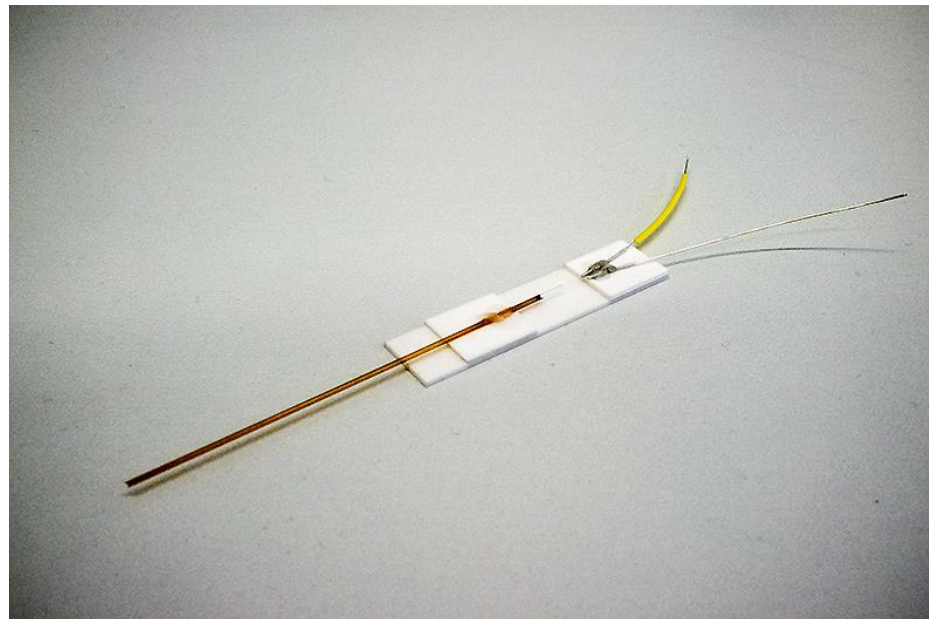
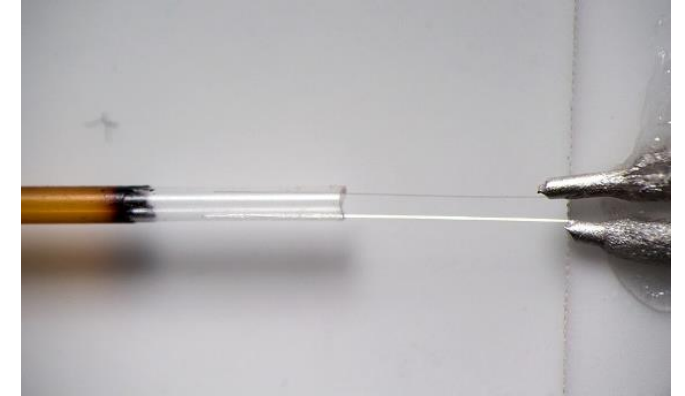
Miniaturizovaná elektrochemická detekce



Uhlíkové vlákno, 7  $\mu\text{m}$

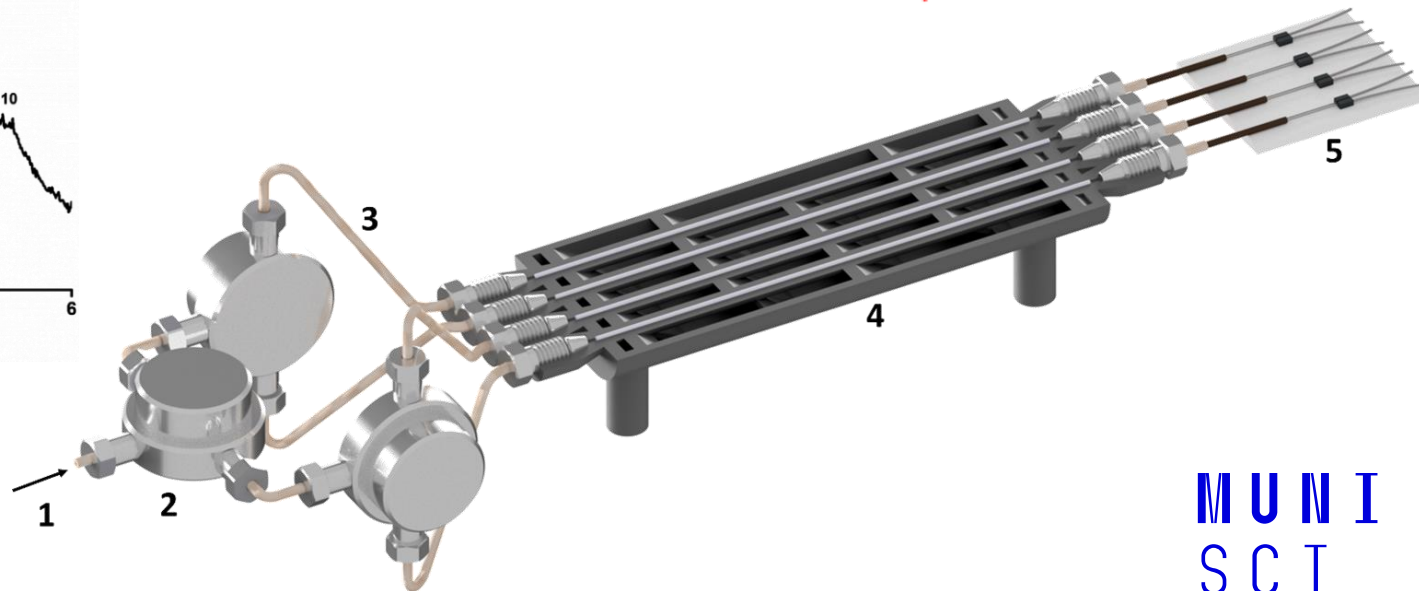
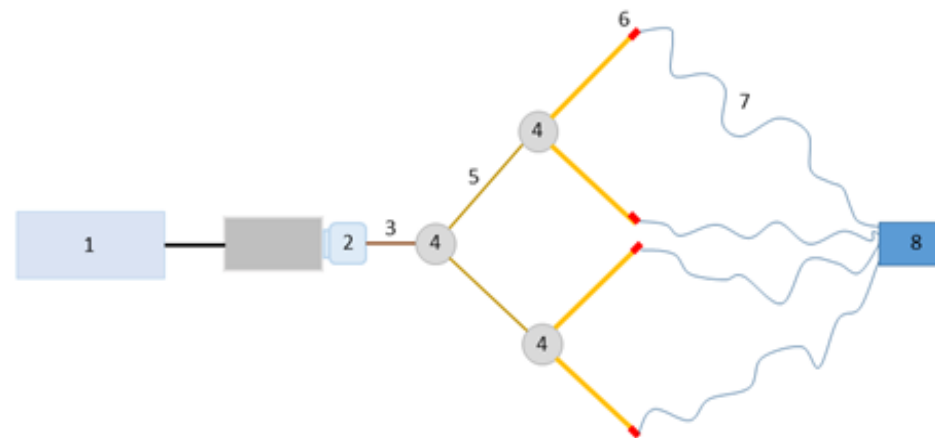
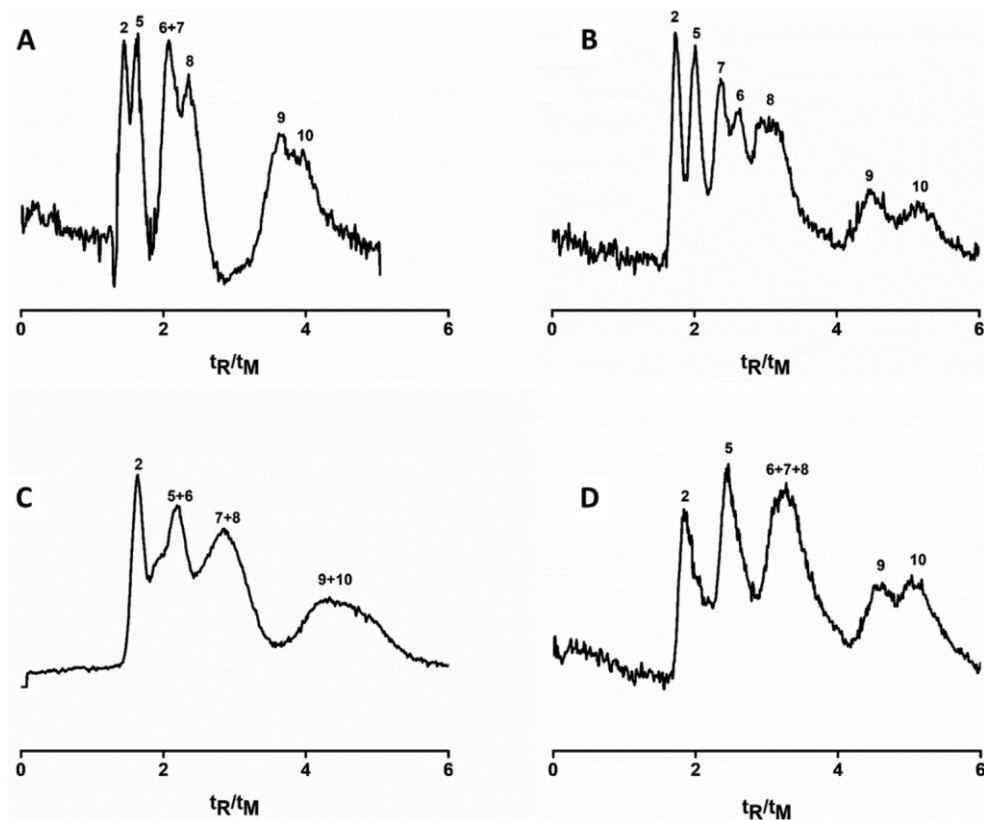


Stříbrný mikrodrát, 25  $\mu\text{m}$



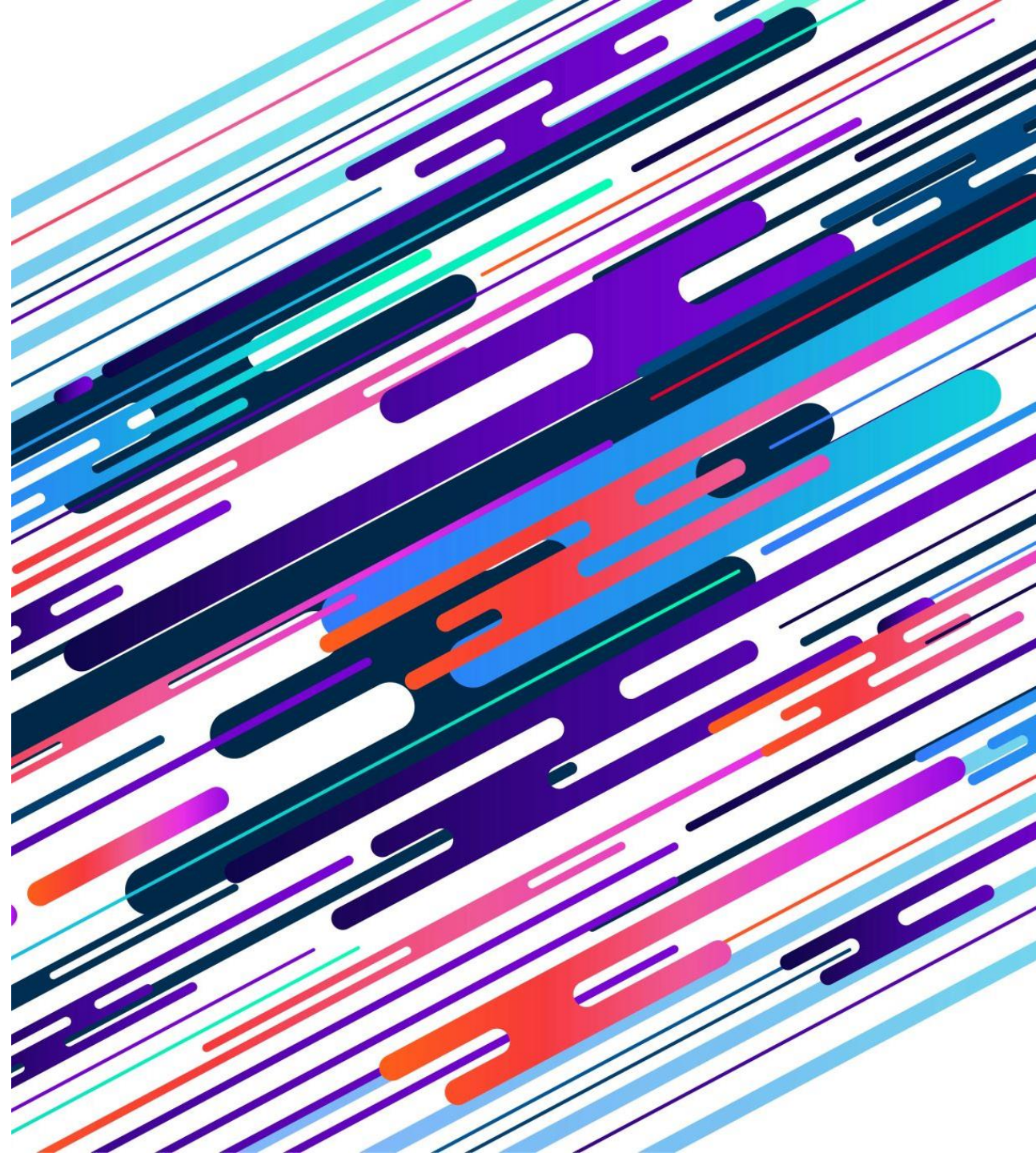
<http://dx.doi.org/10.1016/j.chroma.2017.06.057>

# Integrované analytické systémy

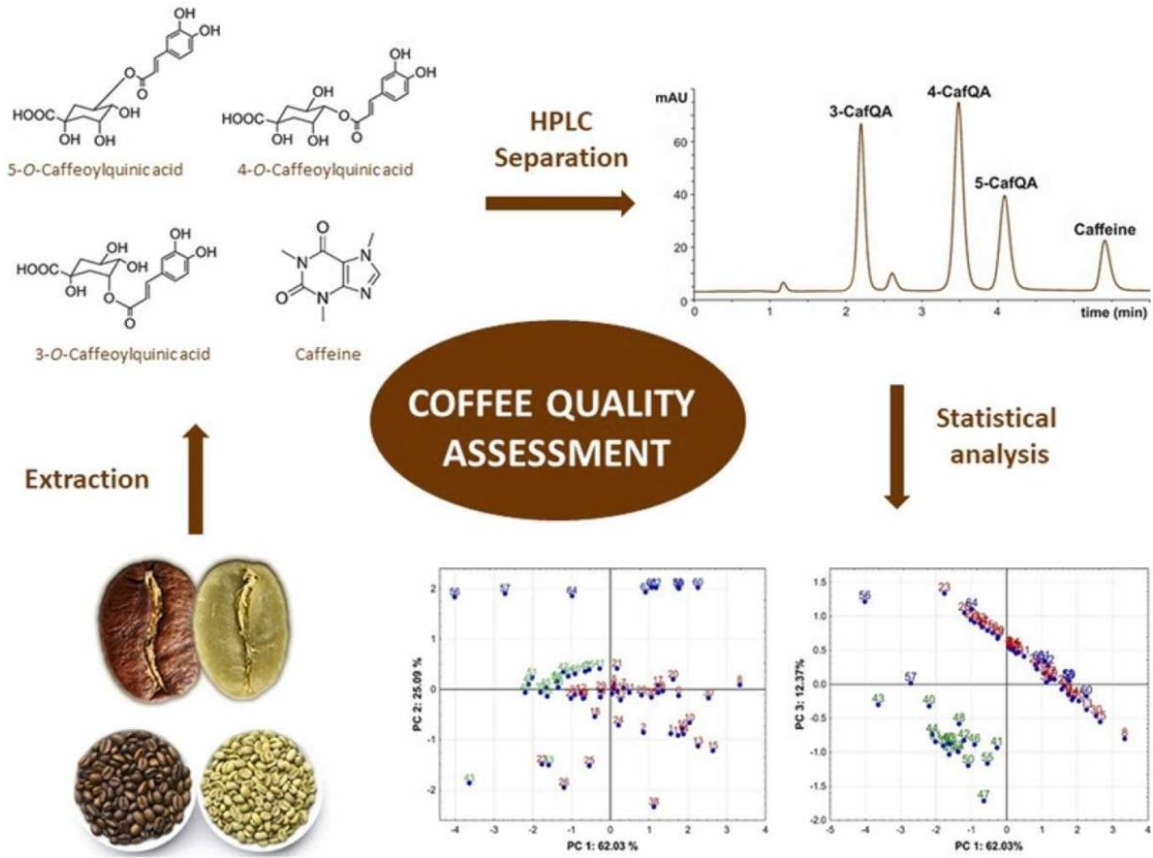


MUNI  
SCI

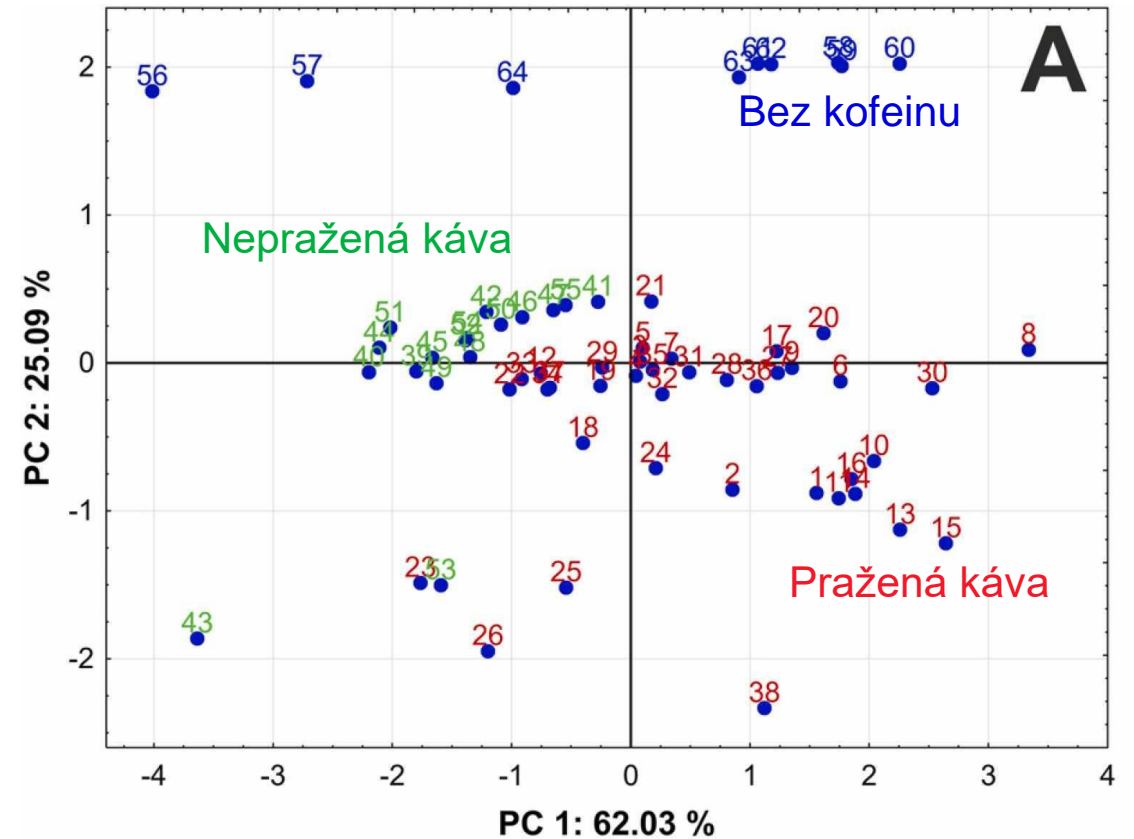
# Využití chromatografie



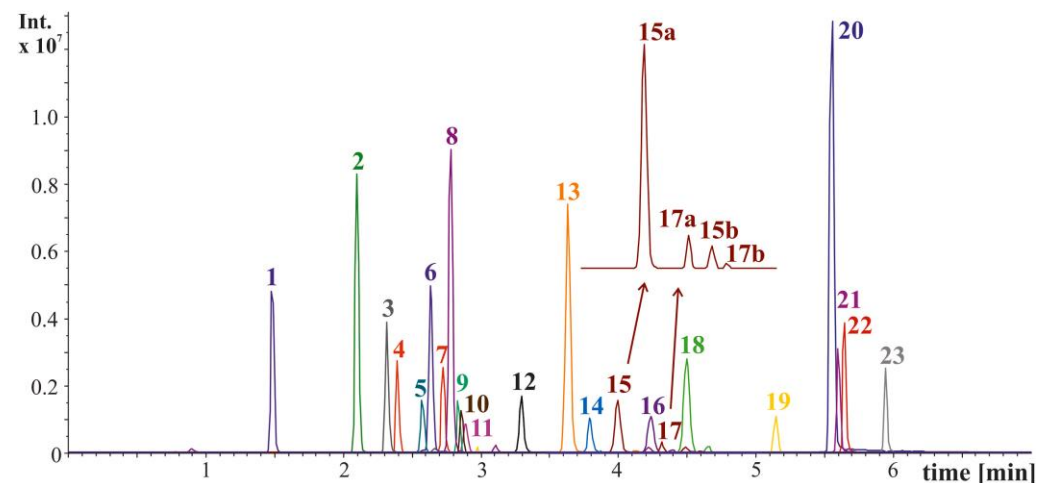
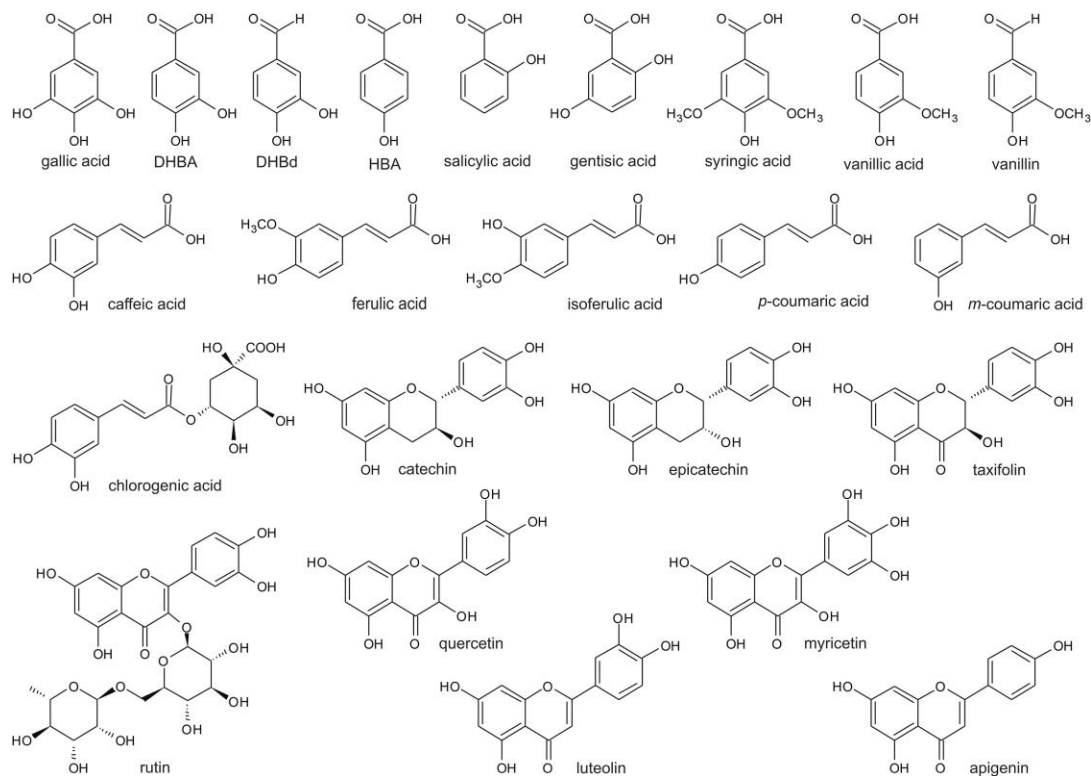
# Analýza kávy



## Analýza kafeinu a izomerů kyseliny chlorogenové



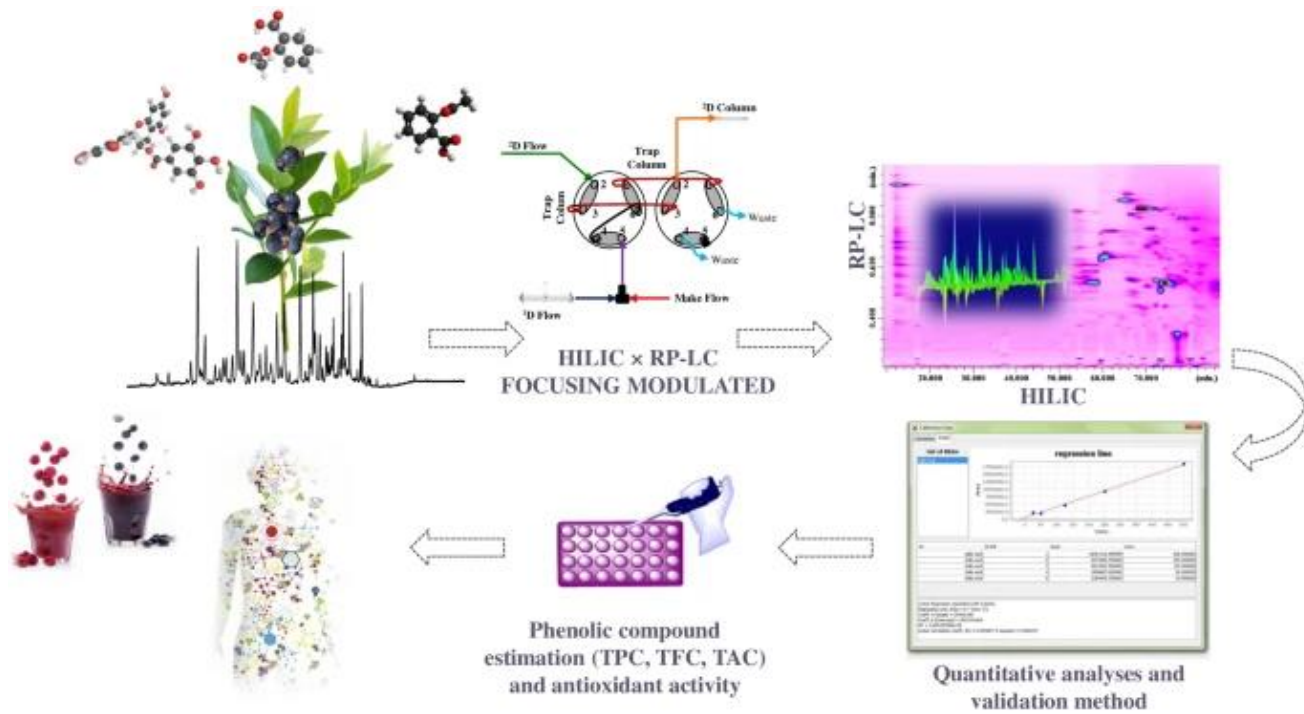
# Kontrola kvality u medovin (?)



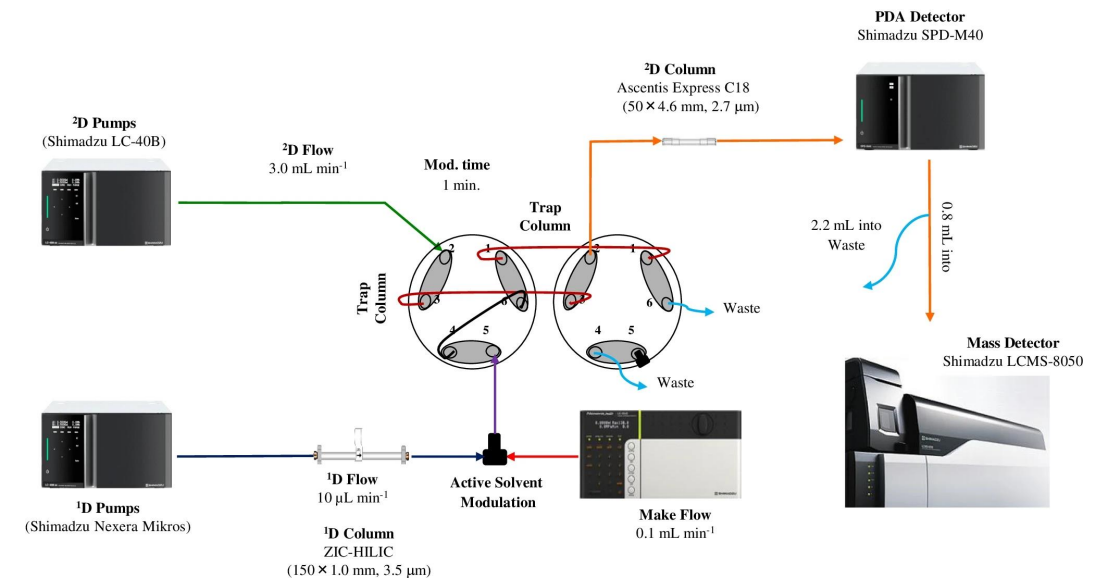
Profil fenolických látek v medovině silně závisí na typu, množství a kvalitě použitého medu. Proto by mohl být použit pro kontrolu kvality jednotlivých vzorků.



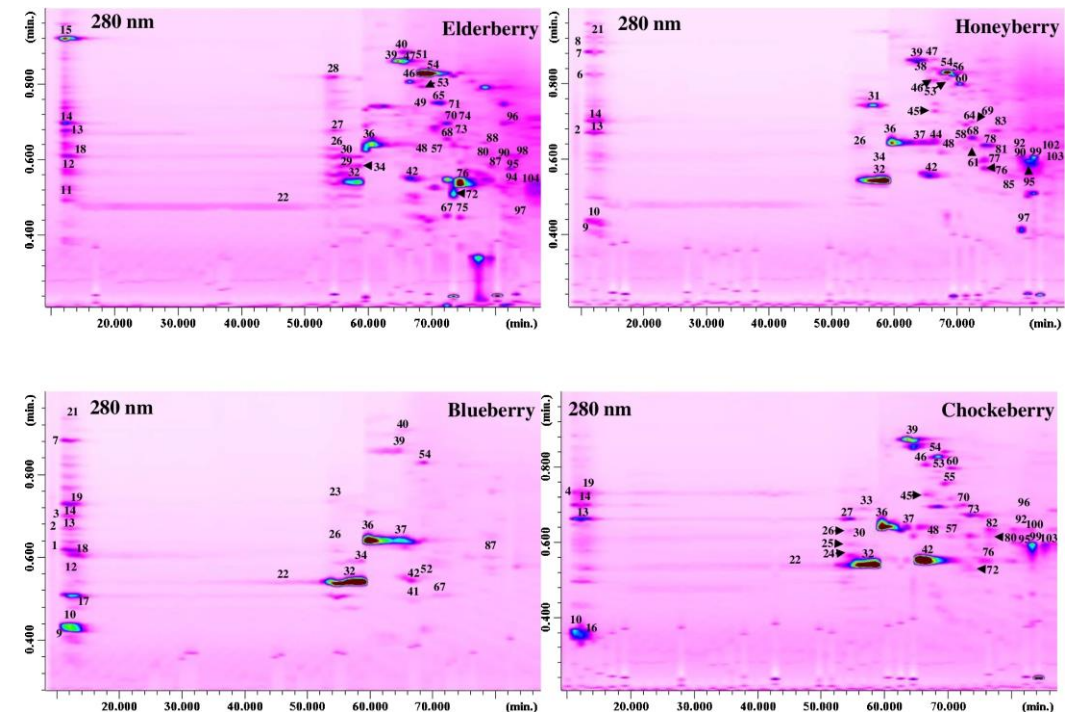
# Pijte černý bez

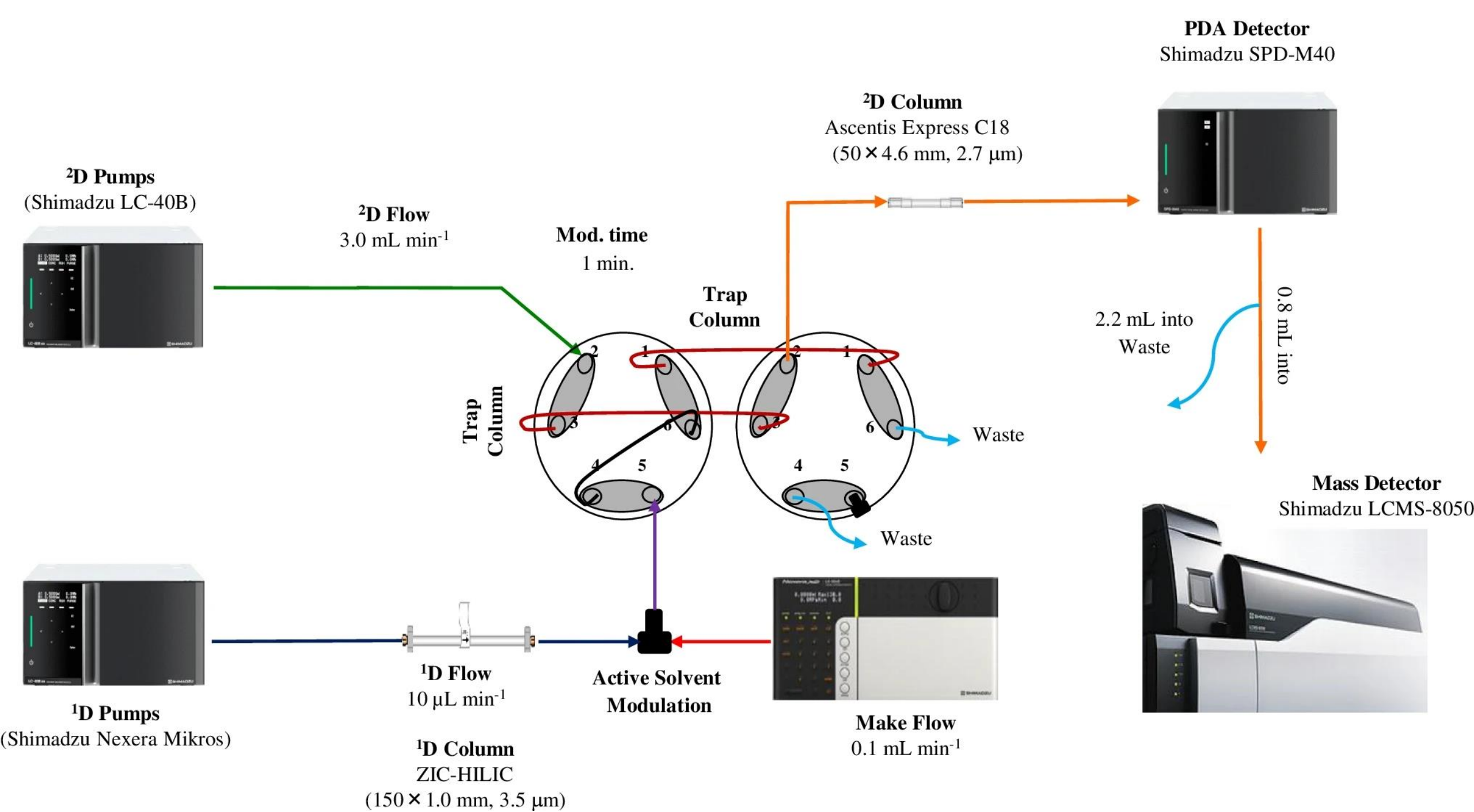


Borůvka (*Vaccinium myrtillus L.*, *Vaccinium corymbosum L.*), černý rybíz (*Ribes nigrum L.*), aronie (*Aronia melanocarpa (Michx.) Elliott*), černý bez (*Sambucus ebulus L.*), zimolez modrý (*Lonicera caerulea L.*) amalina (*Rubus idaeus L.*)

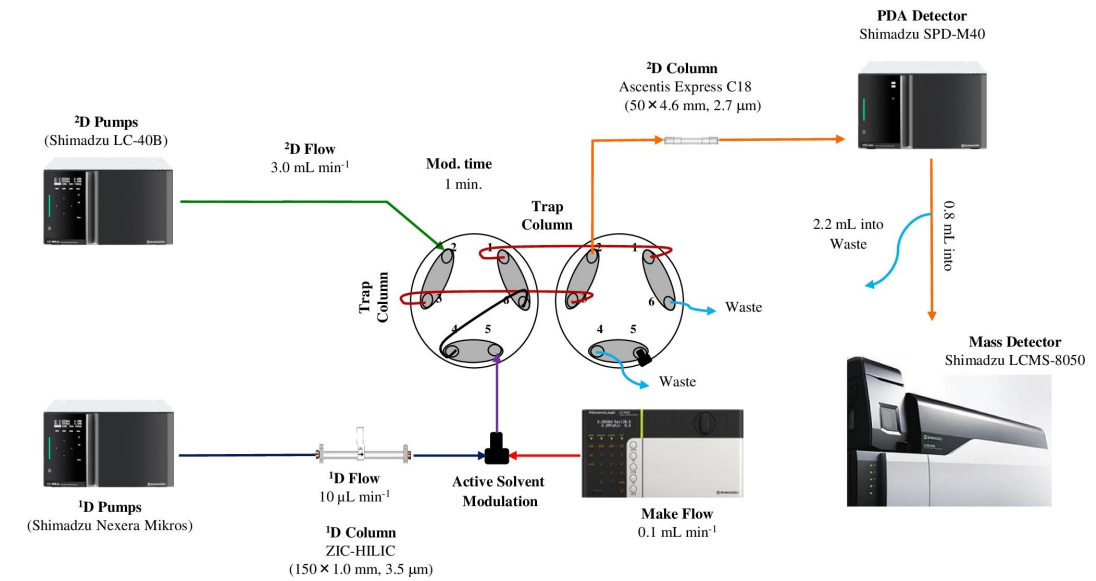
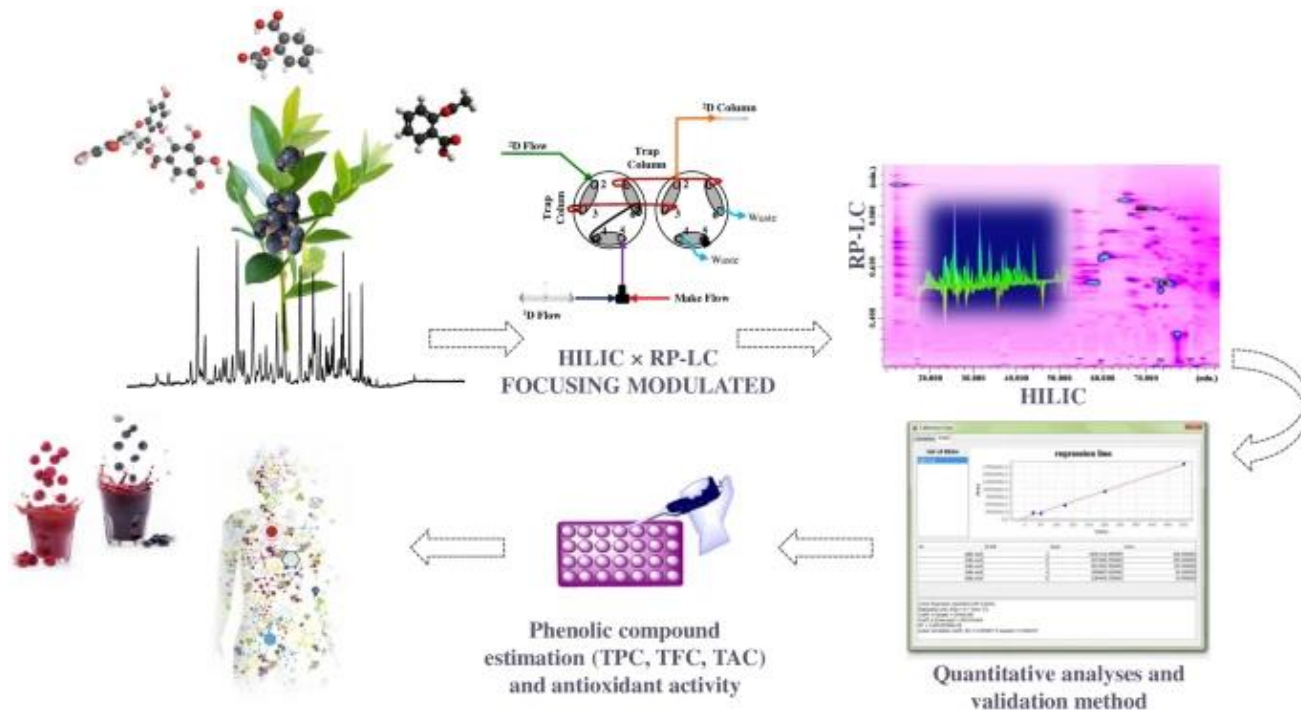


104 různých polyfenolických látek

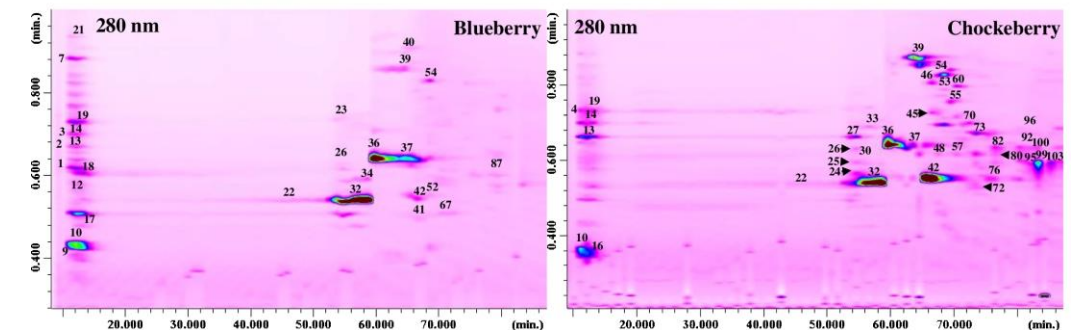
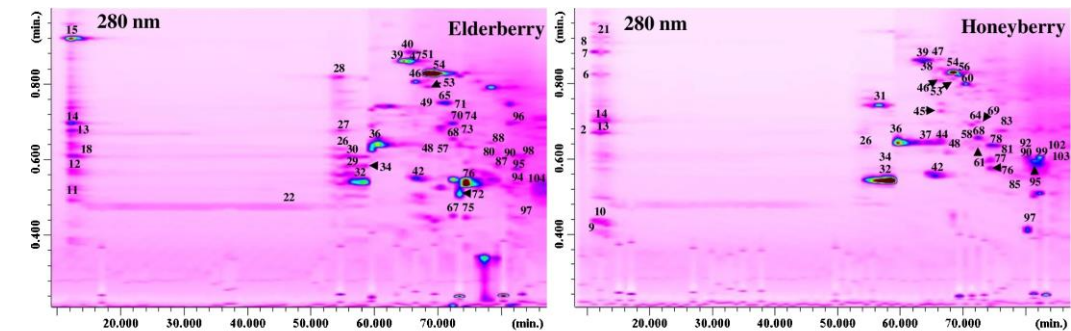




# Pijte černý bez

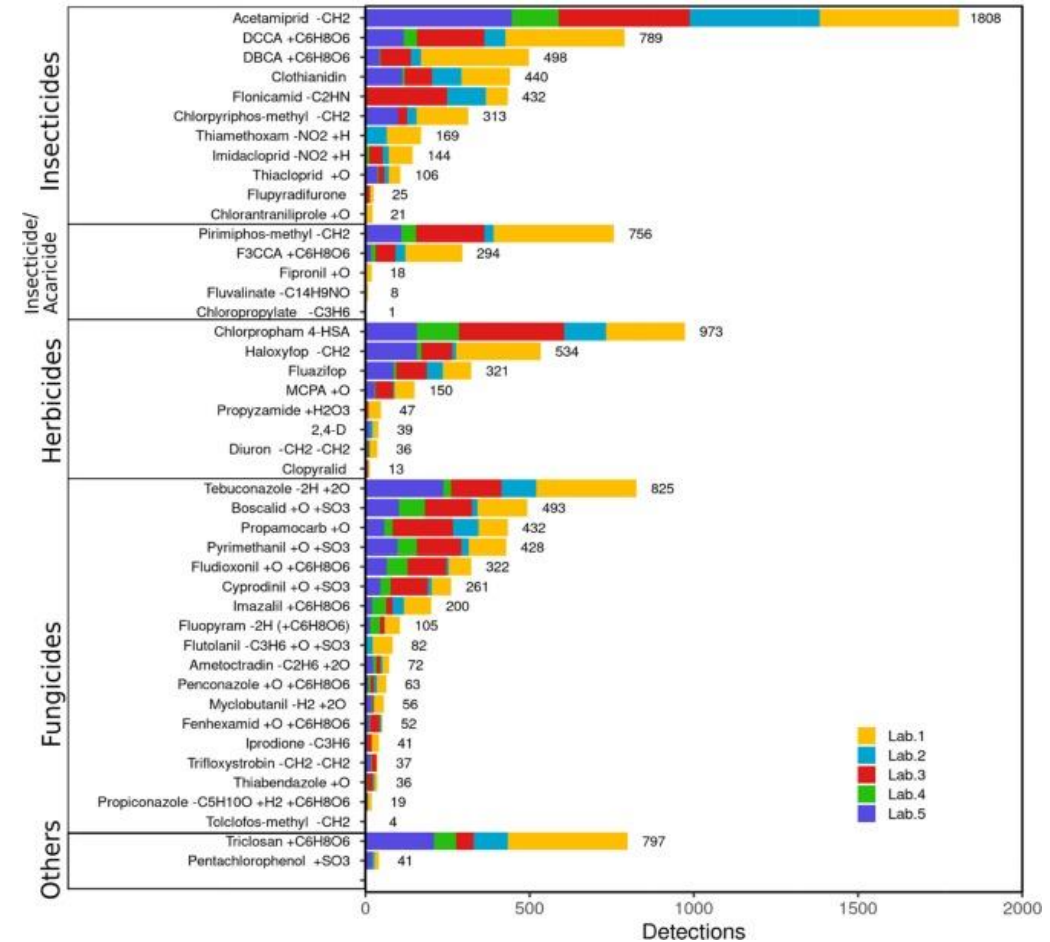
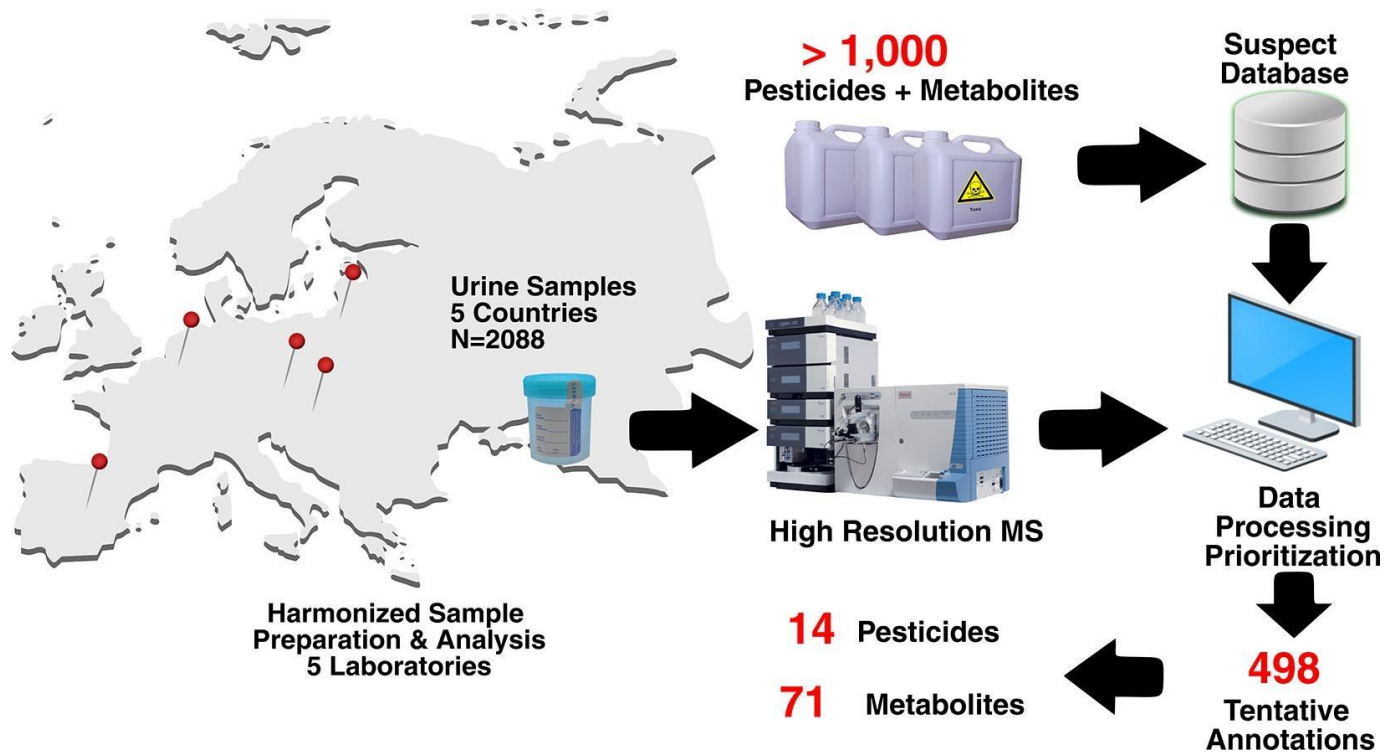


104 různých polyfenolických látek

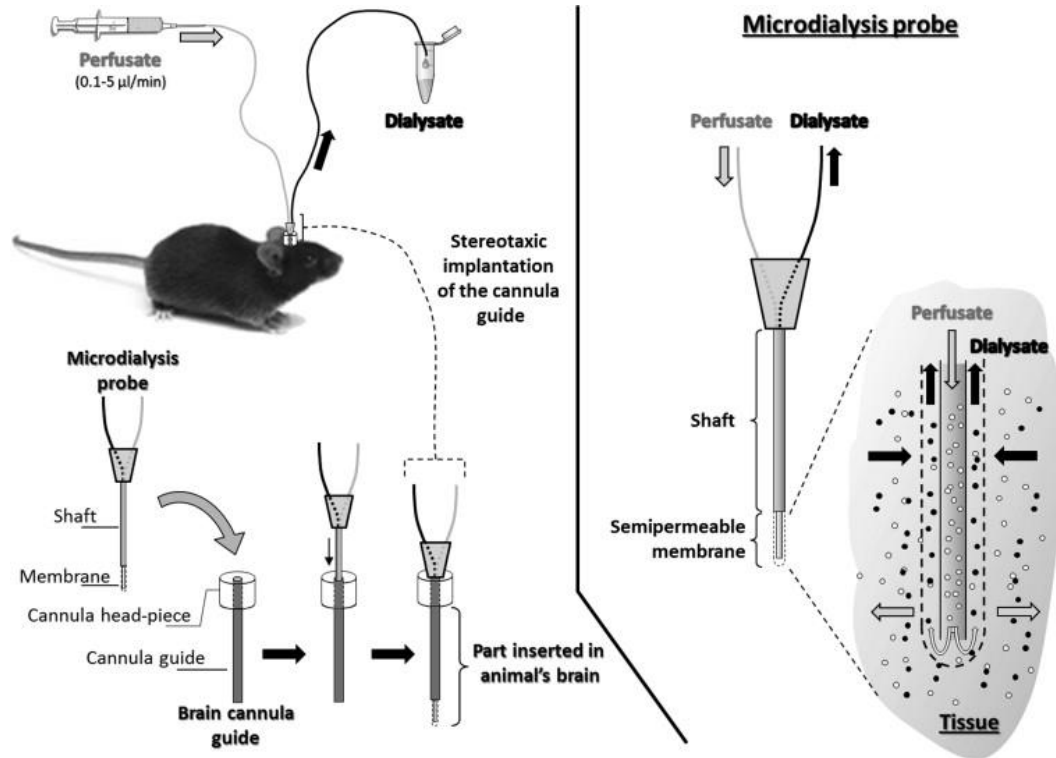


Borůvka (*Vaccinium myrtillus L.*, *Vaccinium corymbosum L.*), černý rybíz (*Ribes nigrum L.*), aronie (*Aronia melanocarpa (Michx.) Elliott*), černý bez (*Sambucus ebulus L.*), zimolez modrý (*Lonicera caerulea L.*) amalina (*Rubus idaeus L.*)

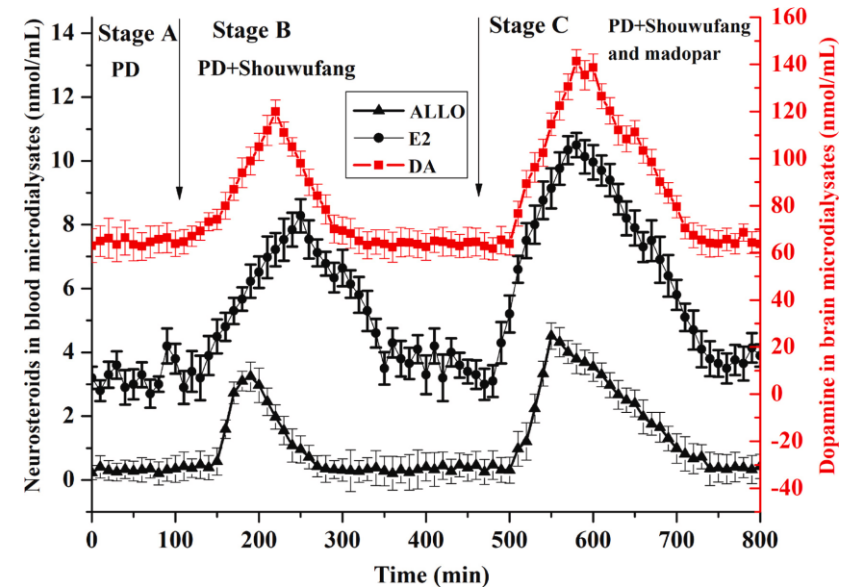
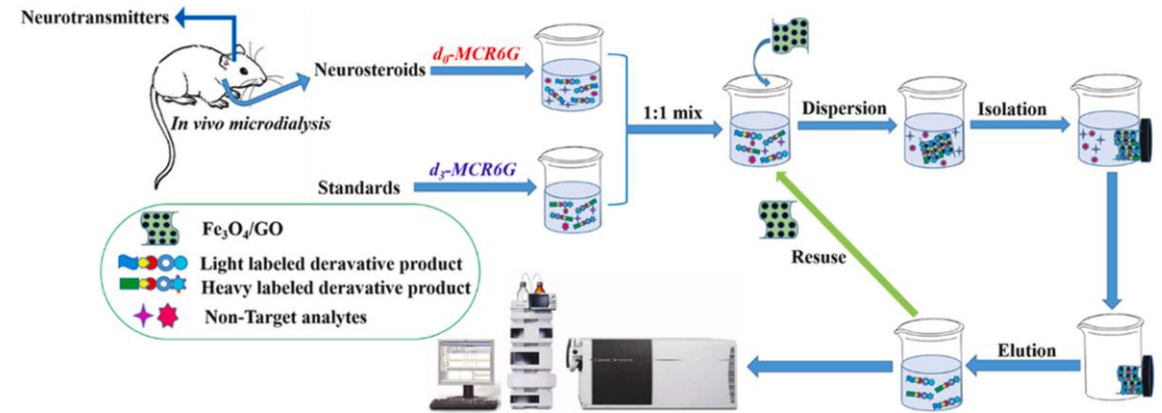
# Pesticidy v moči



# Online analýza nervových přenašečů

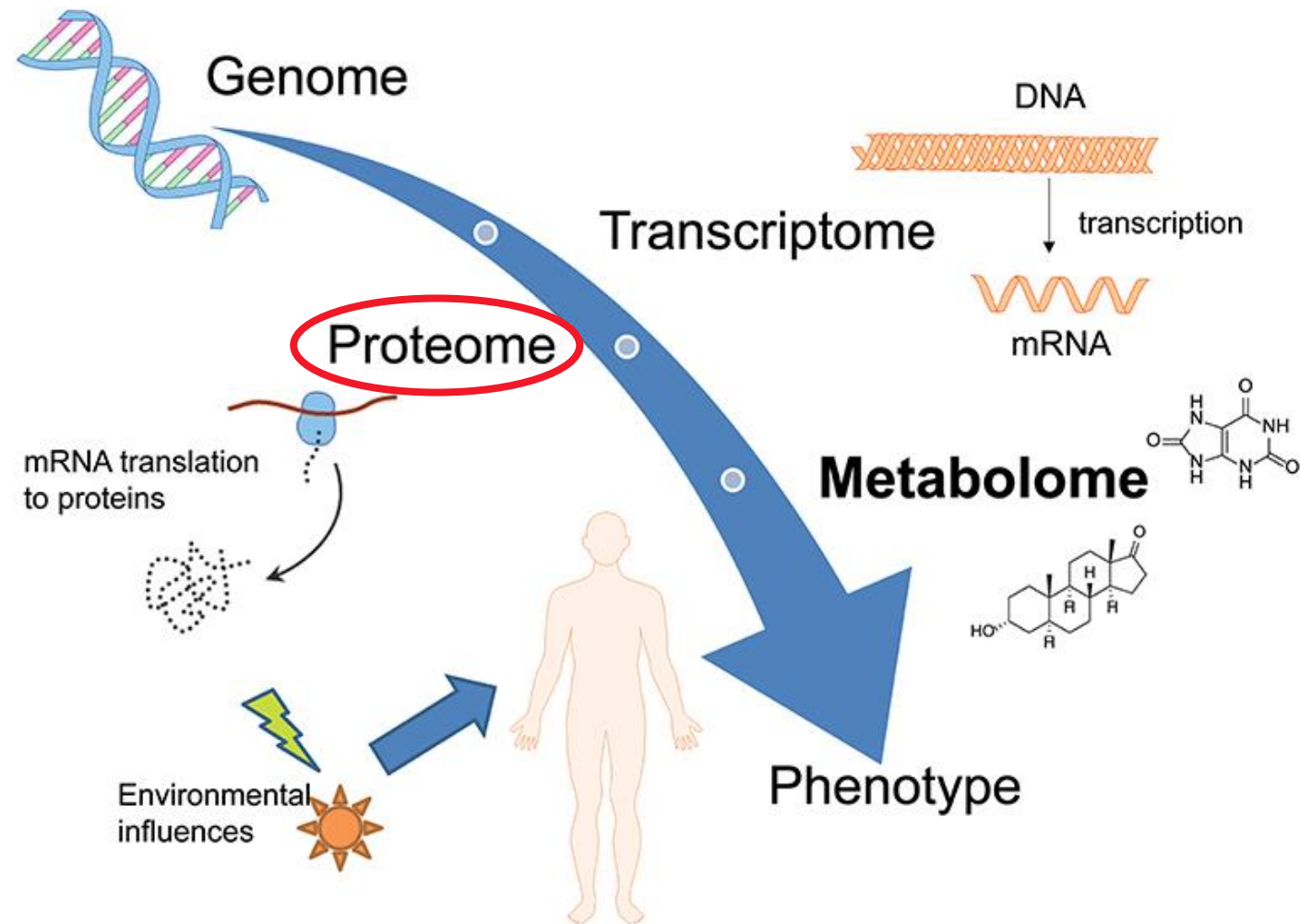


<https://doi.org/10.1016/B978-0-12-415894-8.00024-5>

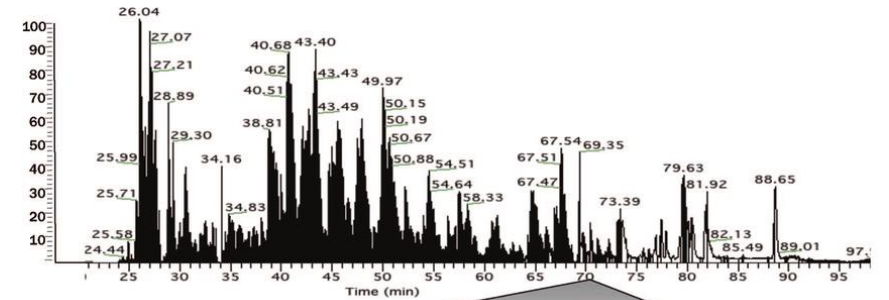
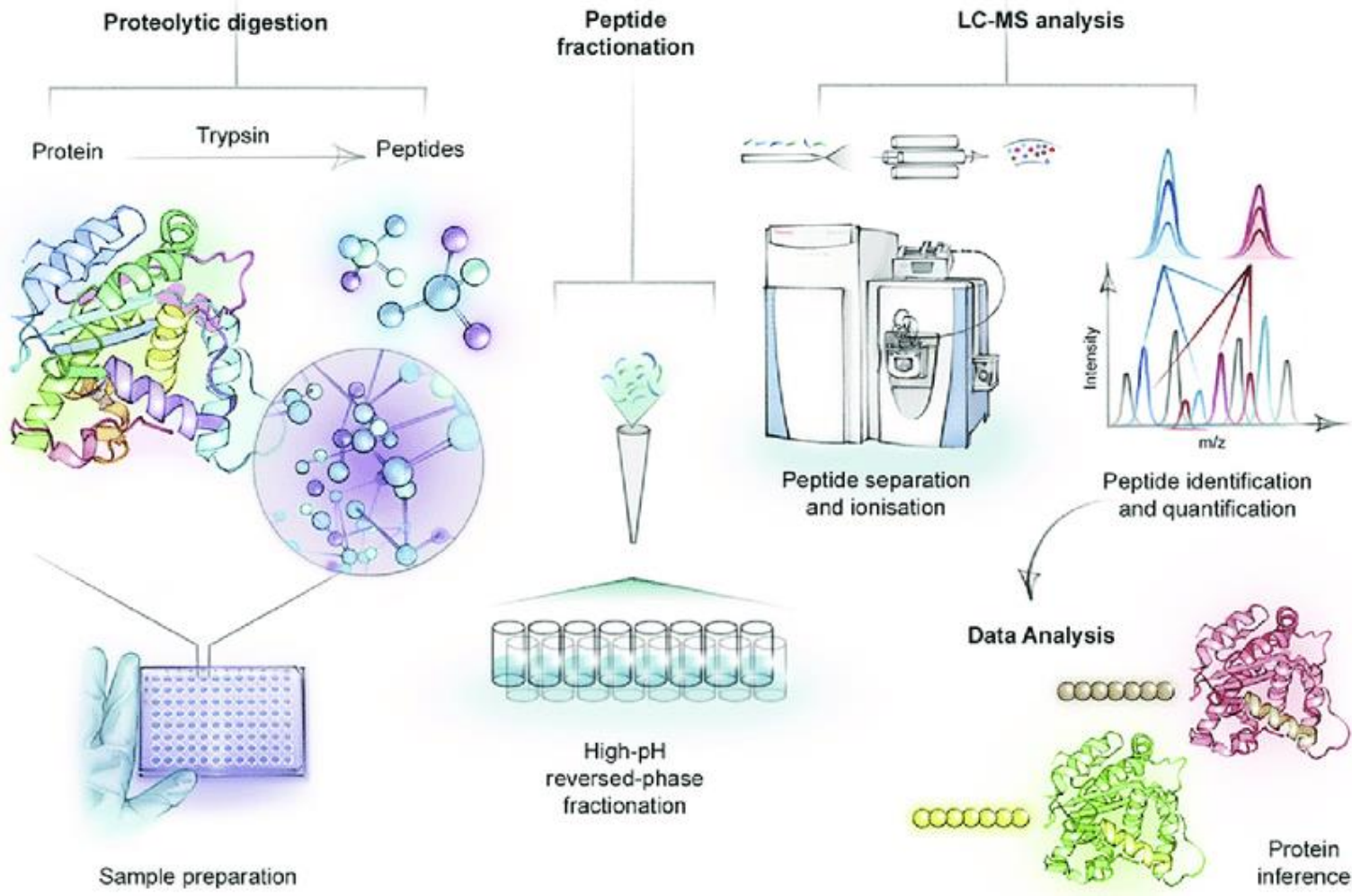


<https://doi.org/10.1016/j.talanta.2019.02.011>

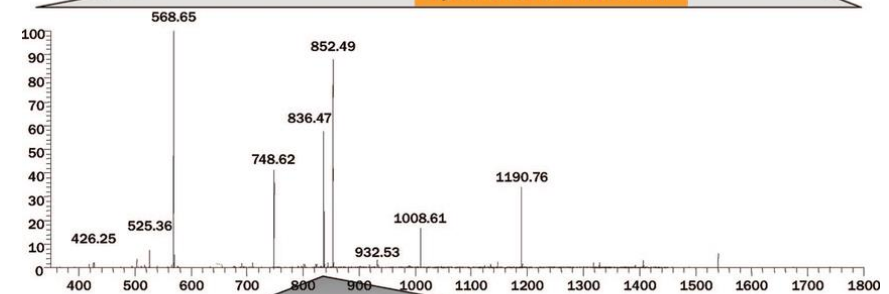
# -omics



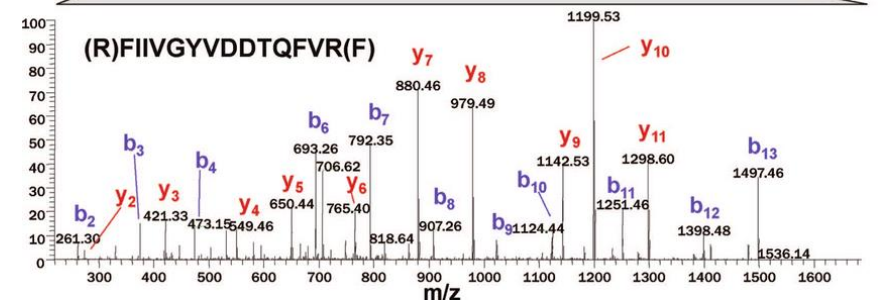
# Proteomická analýza



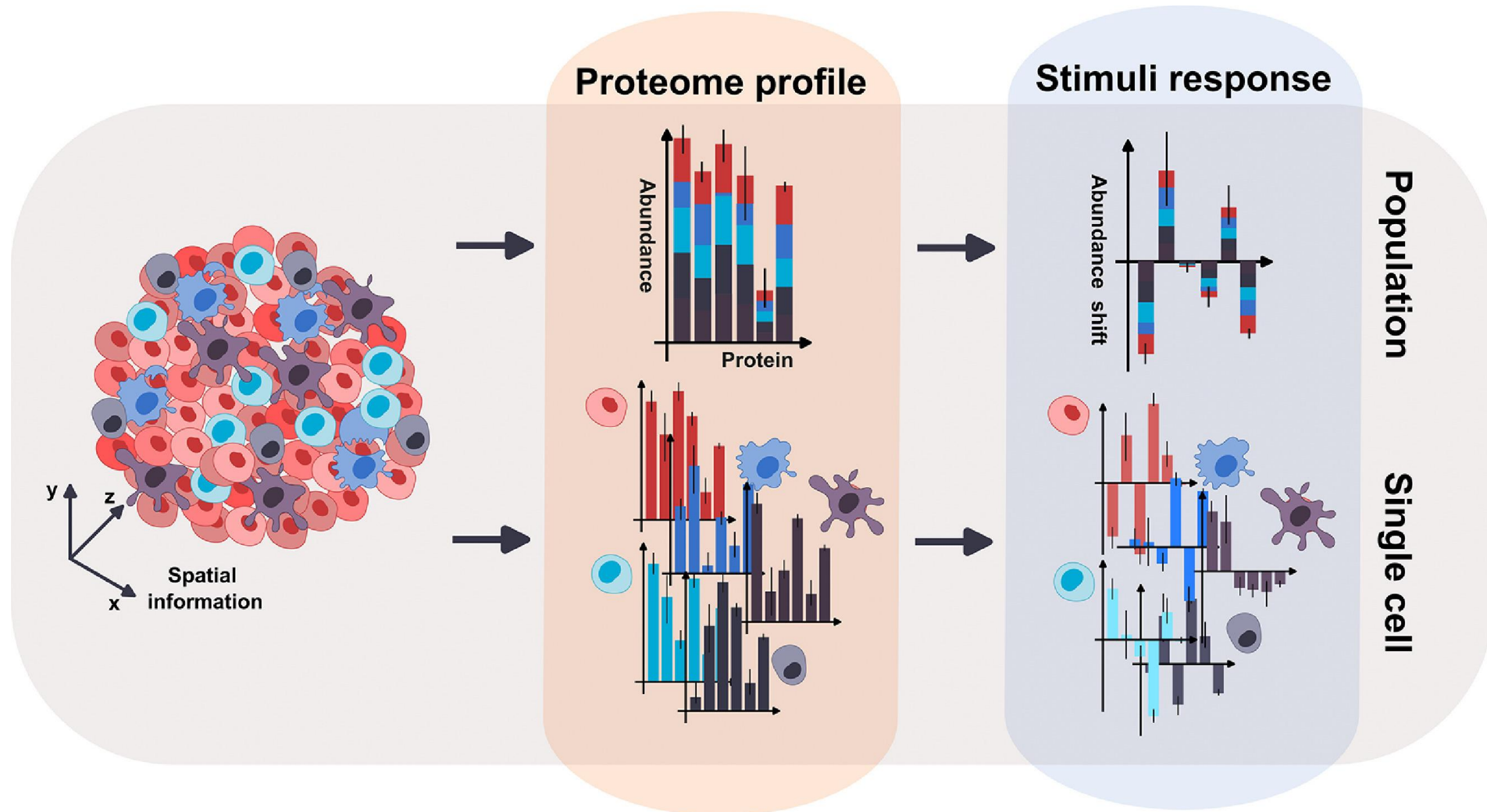
Electrospray Mass Spectrum at 70.55min.



MS/MS of m/z 836.47



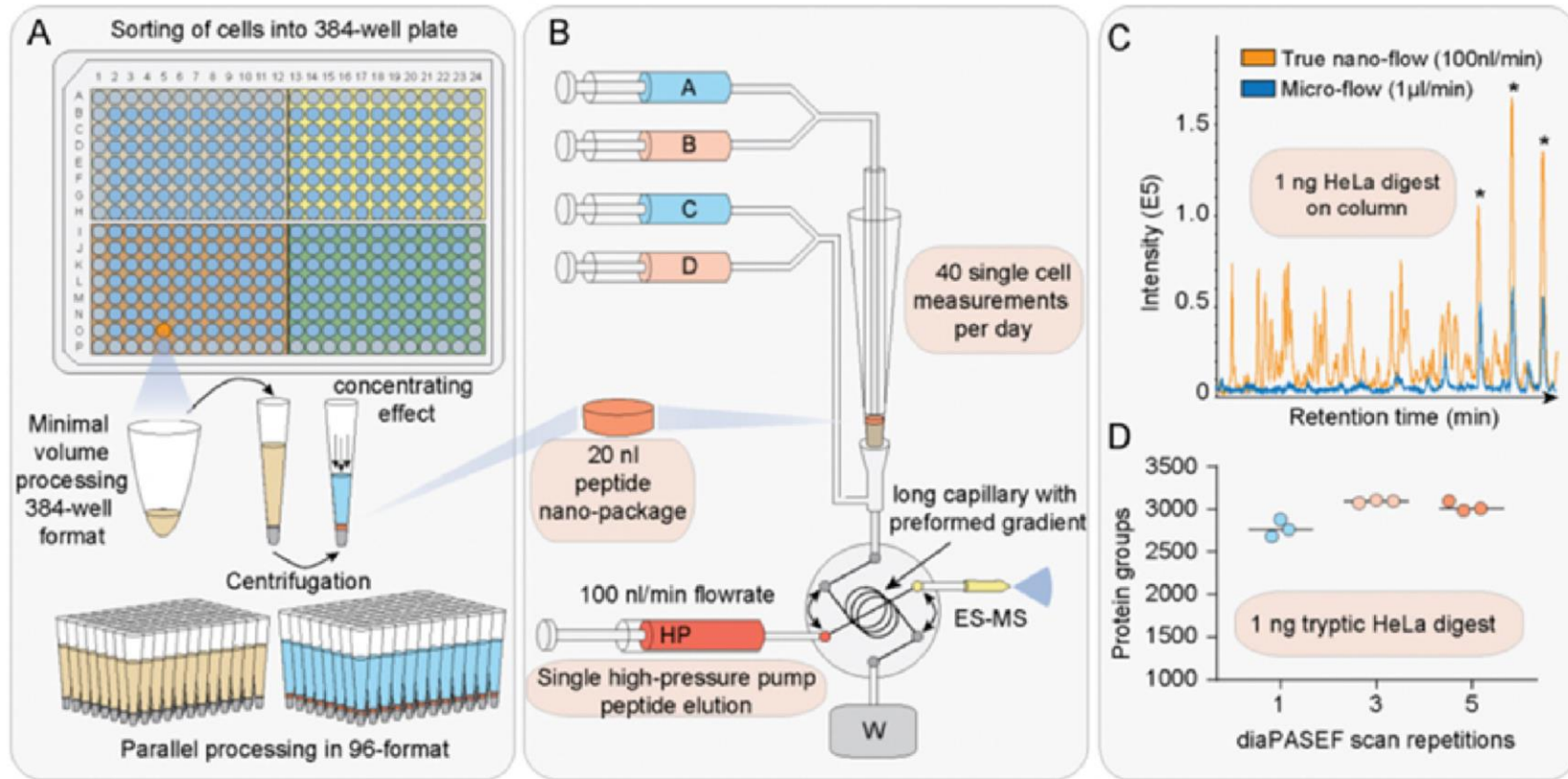
# Analýza jedné buňky (single-cell proteomics)



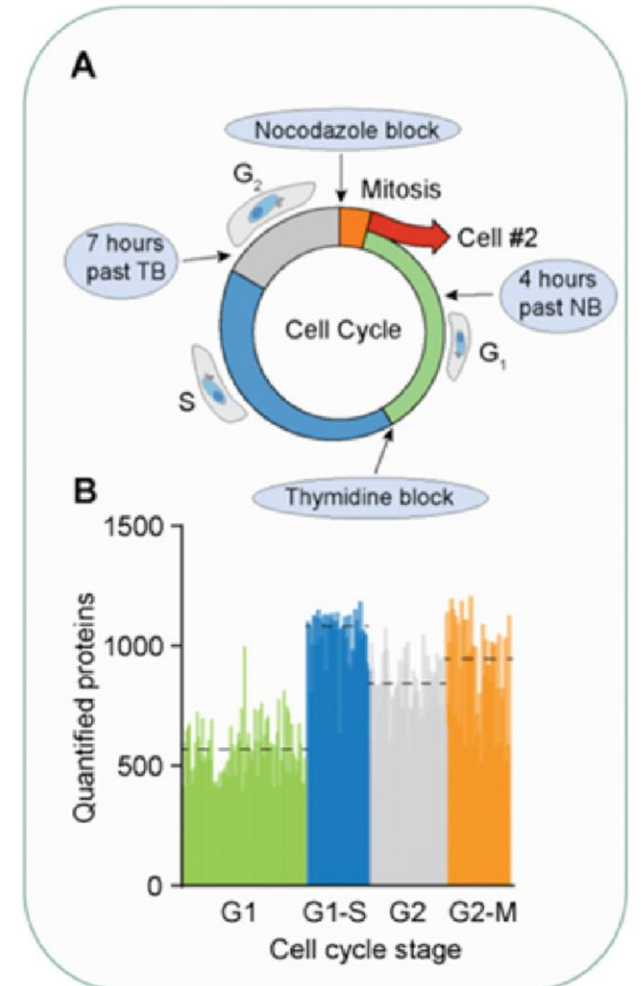


# Single-cell proteomics

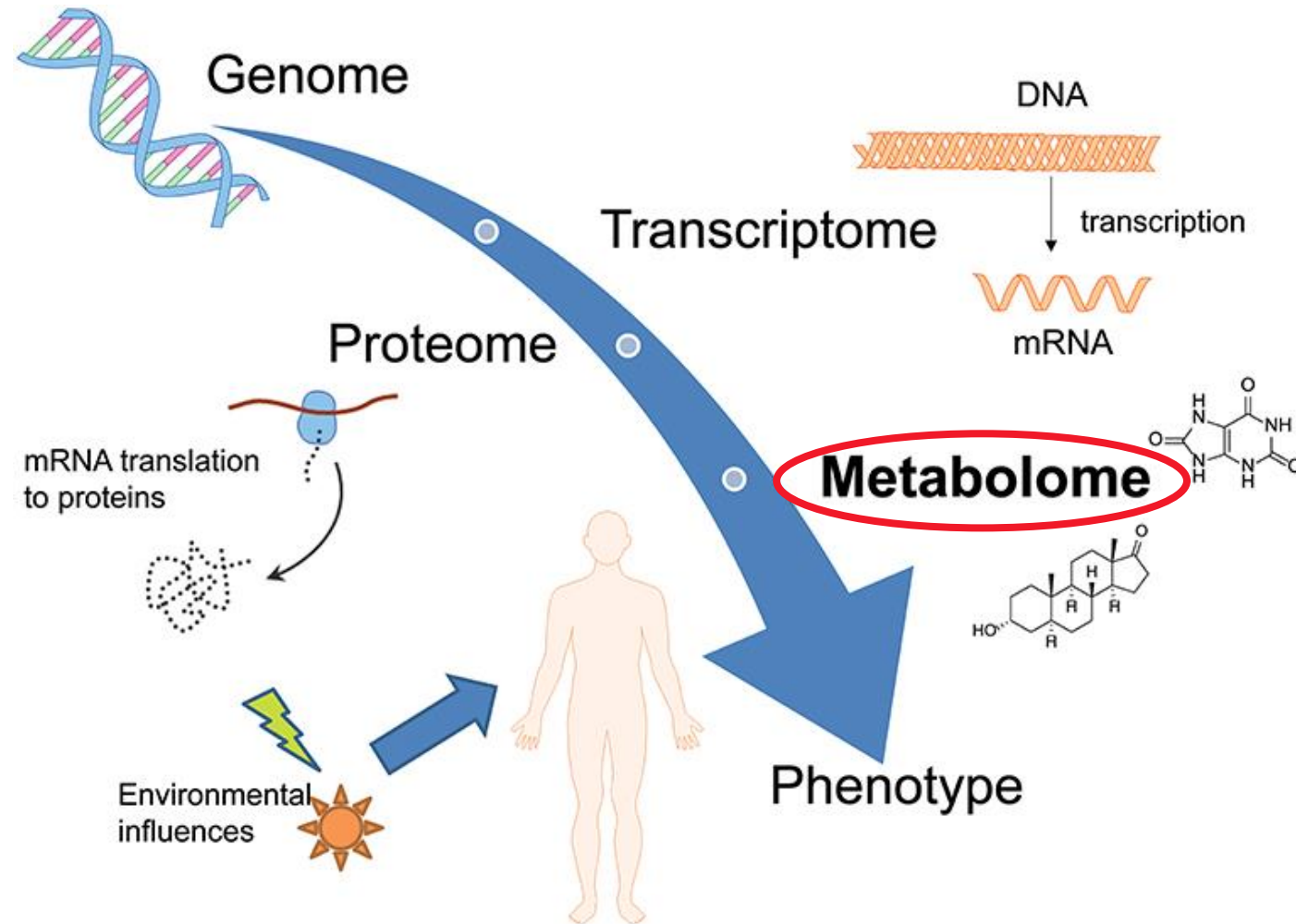
## Workflow using HELA Cells



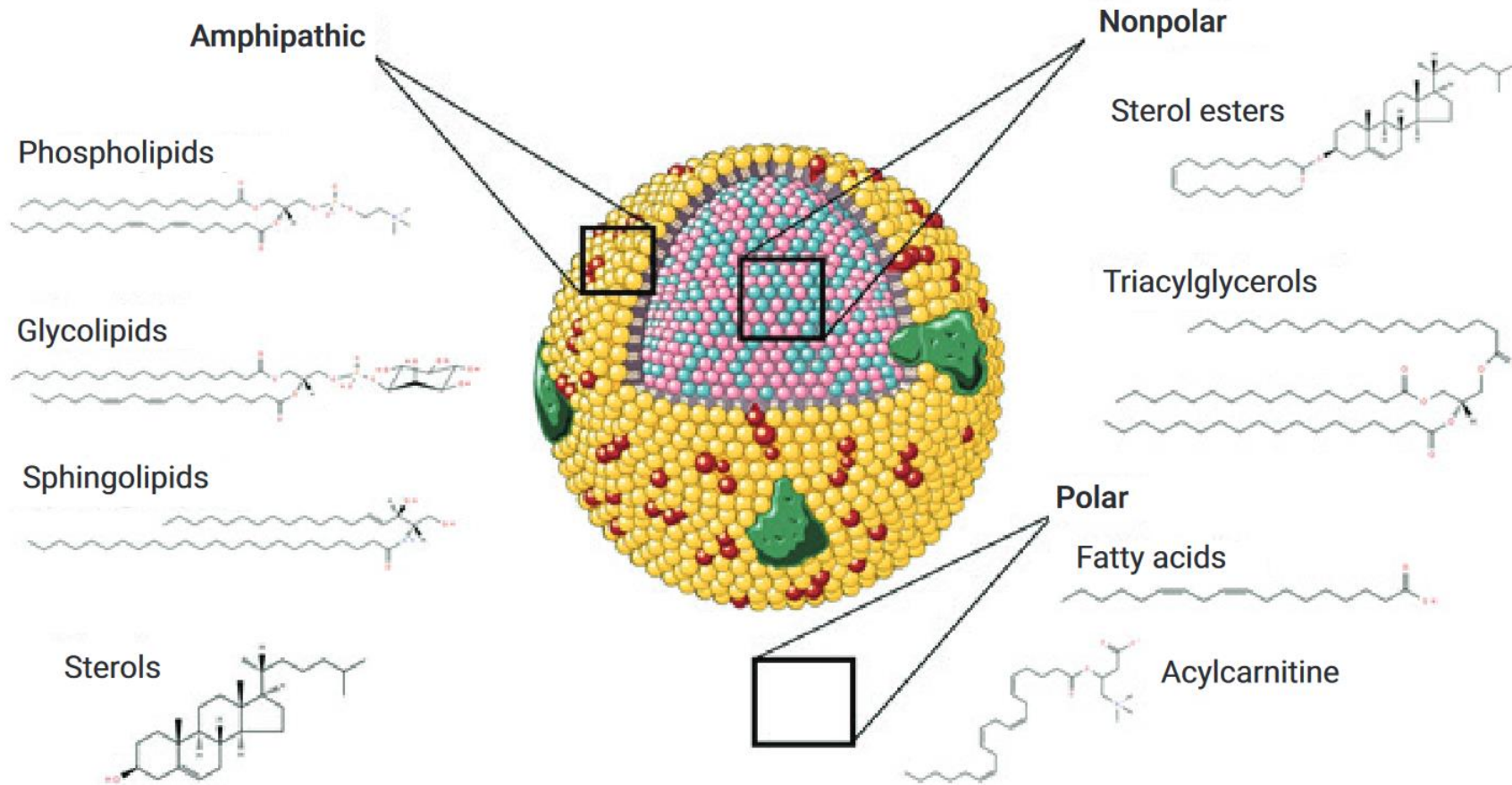
## Single Cell Results



# -omics



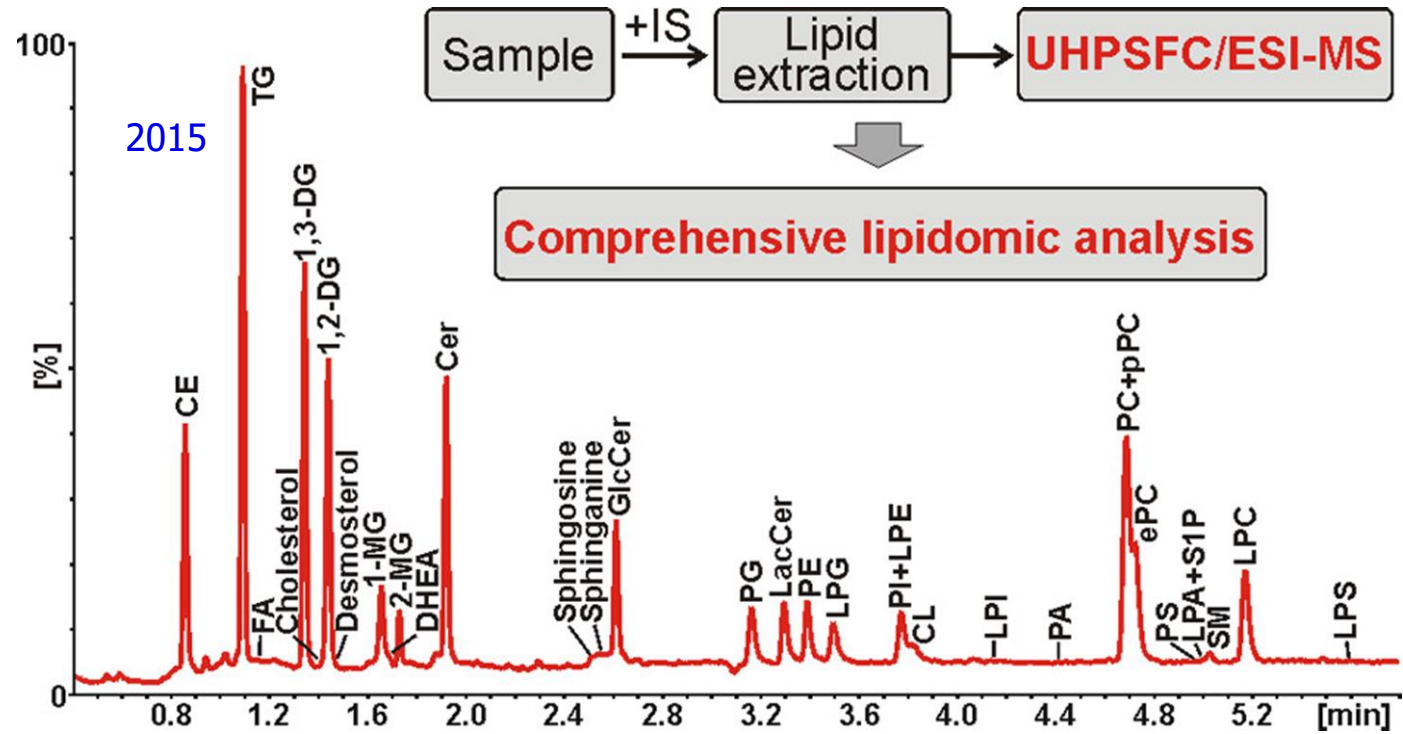
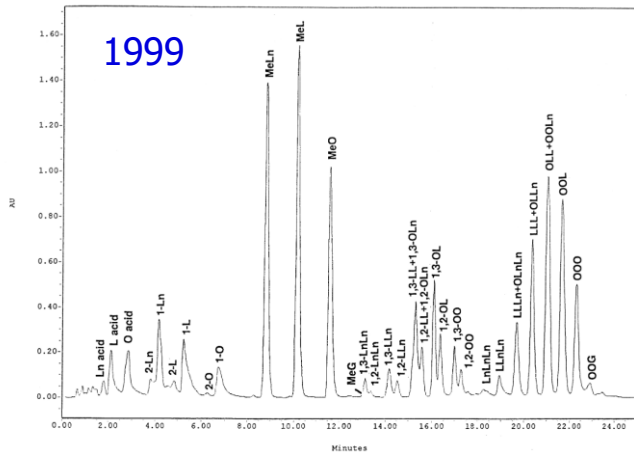
# Lipidomická analýza



# Lipidomická analýza



Michal Holčapek, Univerzita Pardubice

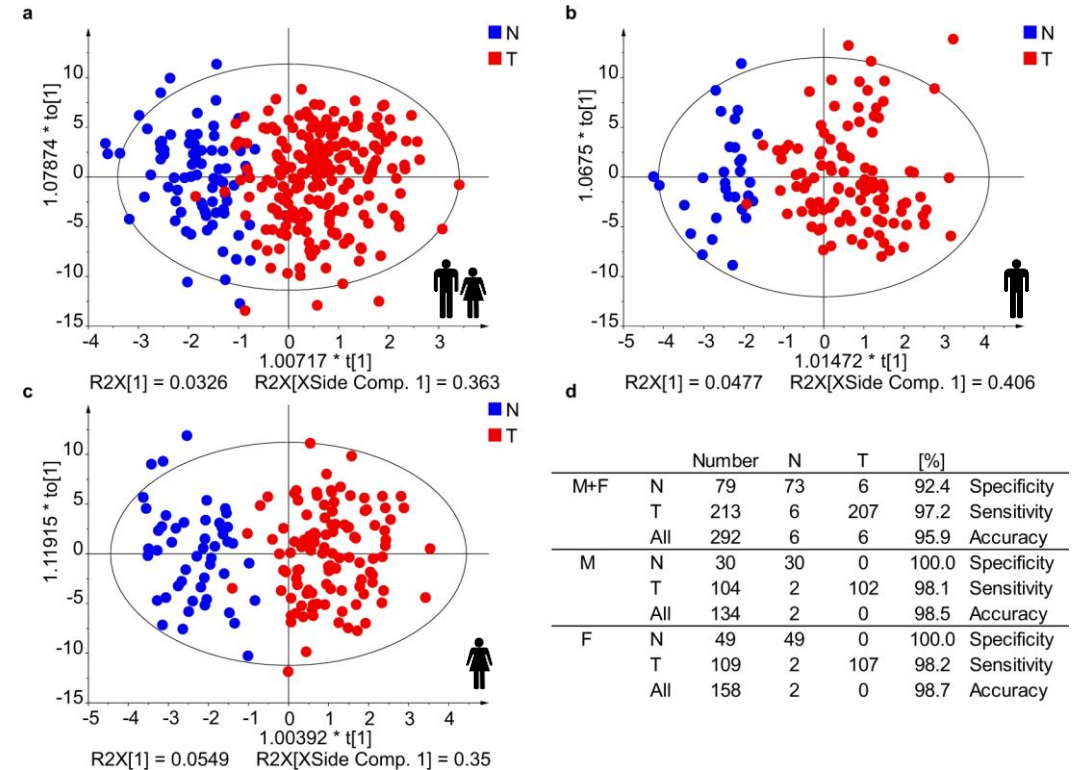
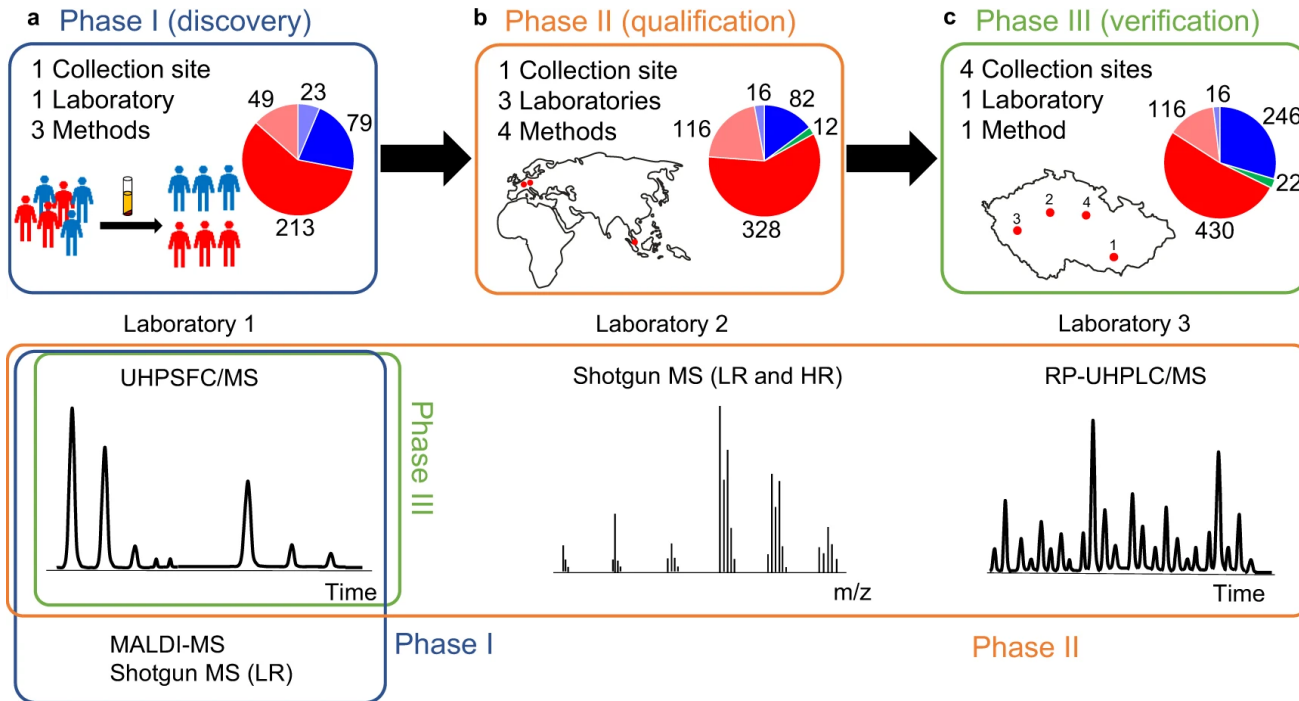


<https://doi.org/10.1021/acs.analchem.5b01054>



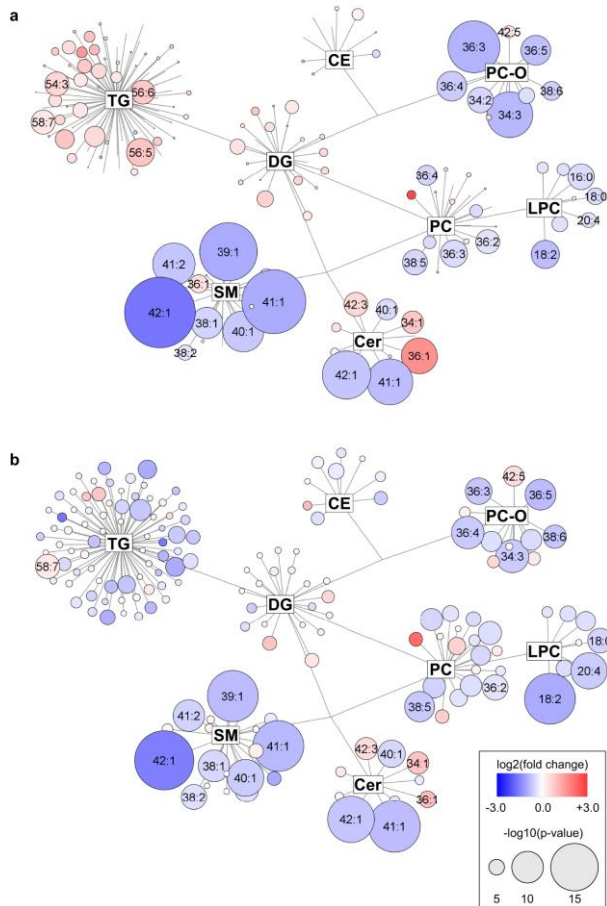
# Včasná diagnostika rakoviny slinivky

zdraví / nemocní



# Včasná diagnostika rakoviny slinivky

## Lipidomické dráhy



# MUNI SCI



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**Sky is the limit**

