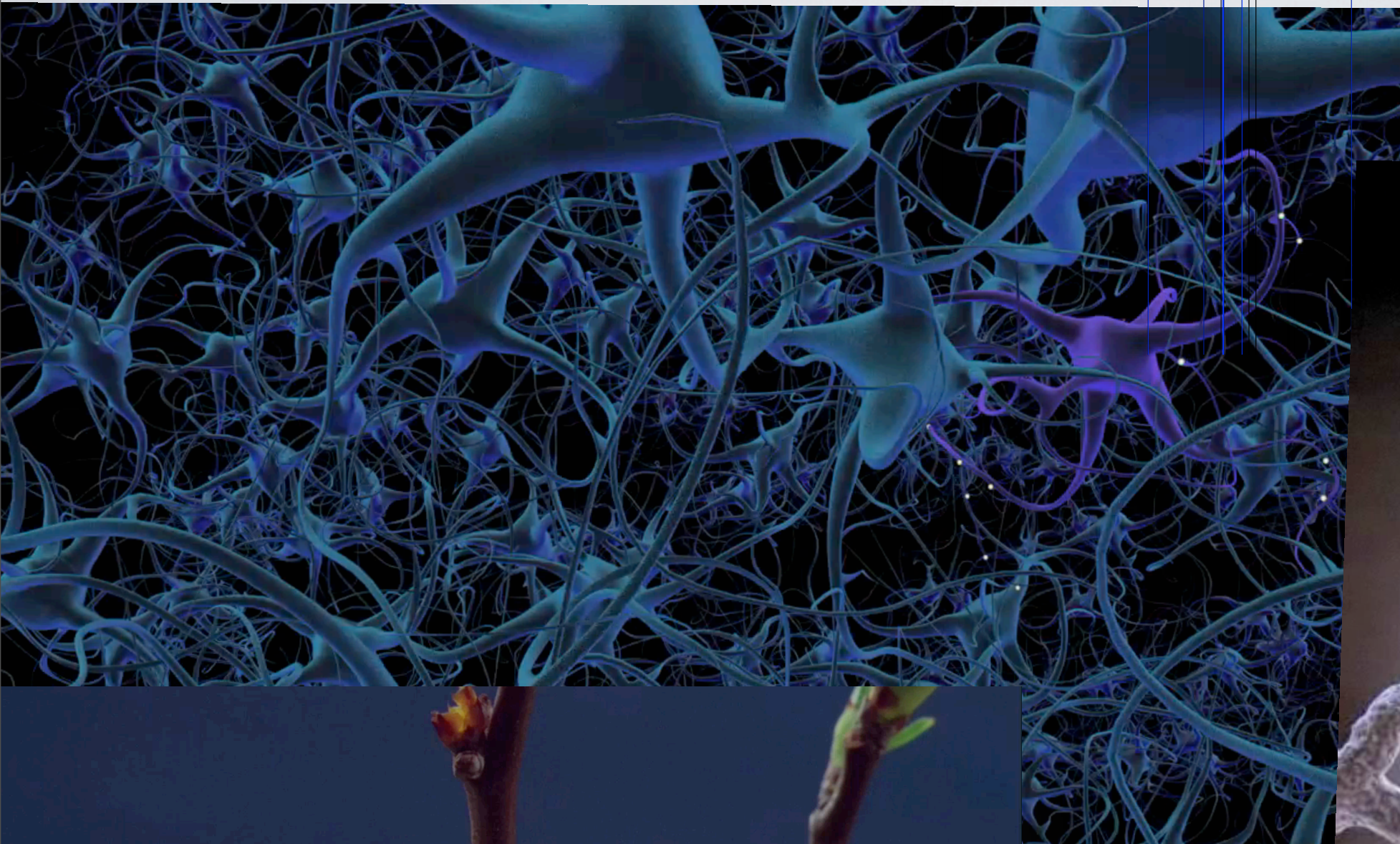
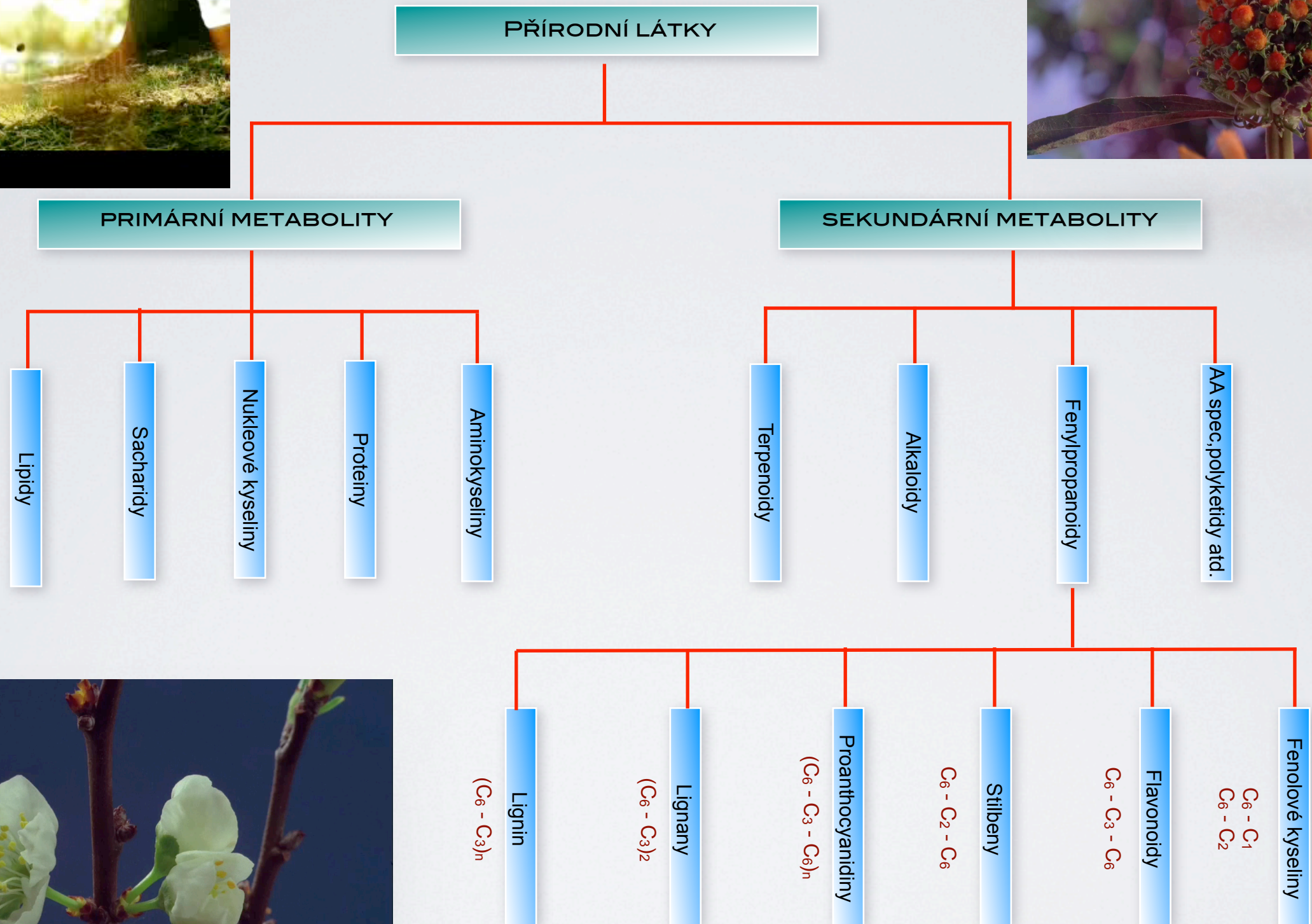
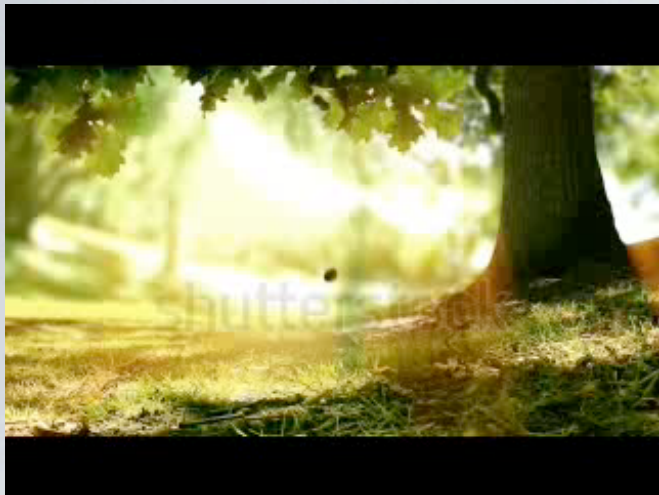
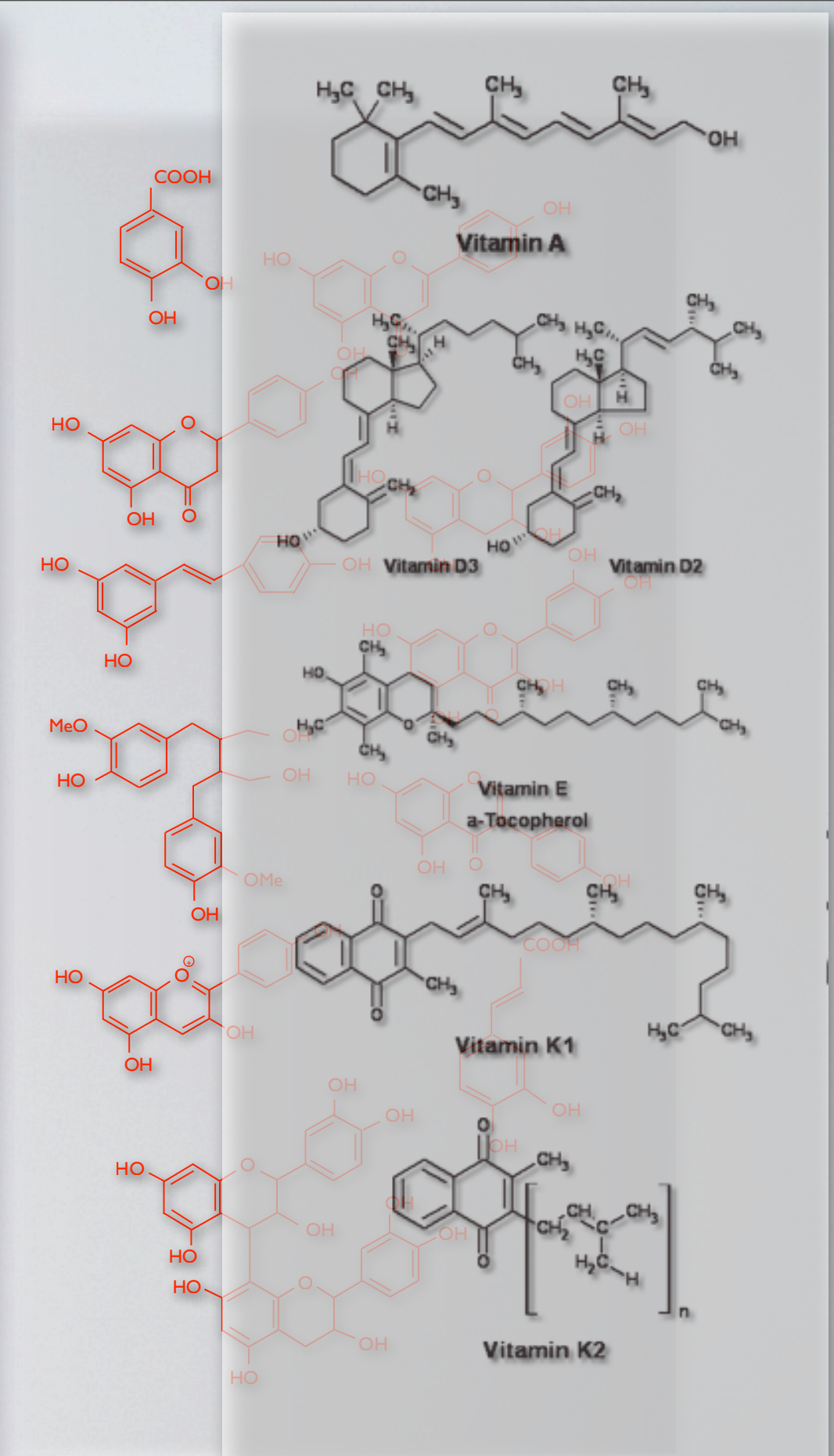
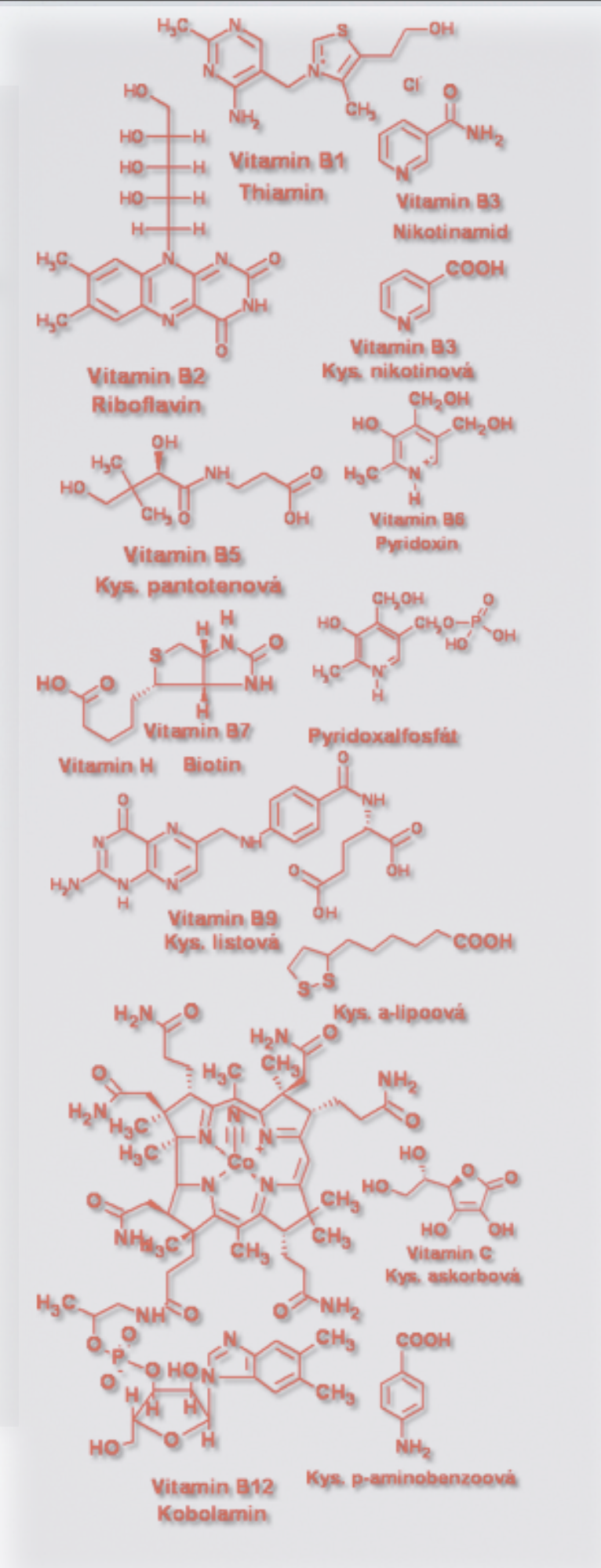
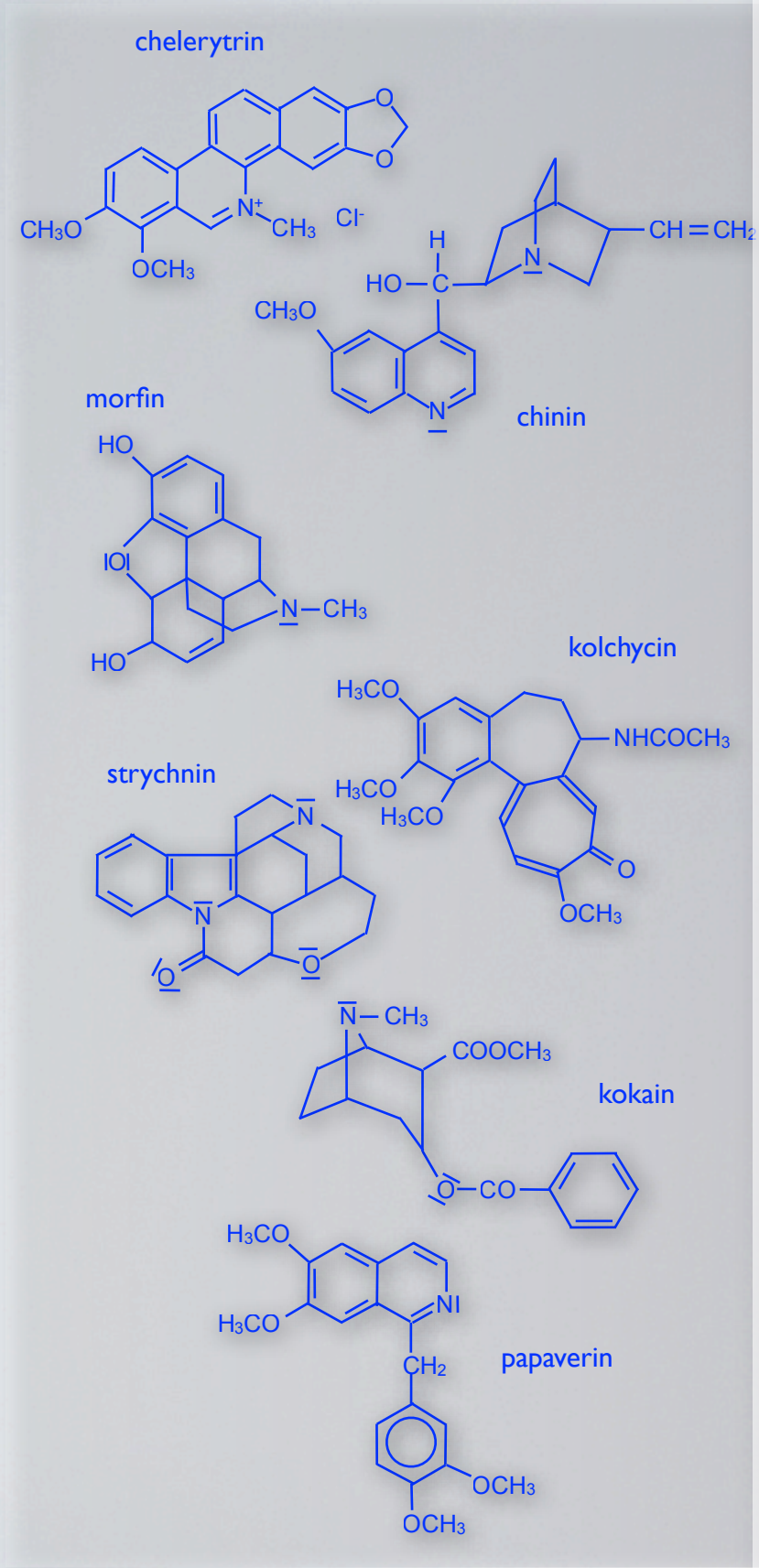


ANALYTICKÉ SYSTÉMY V METABOLOMICE

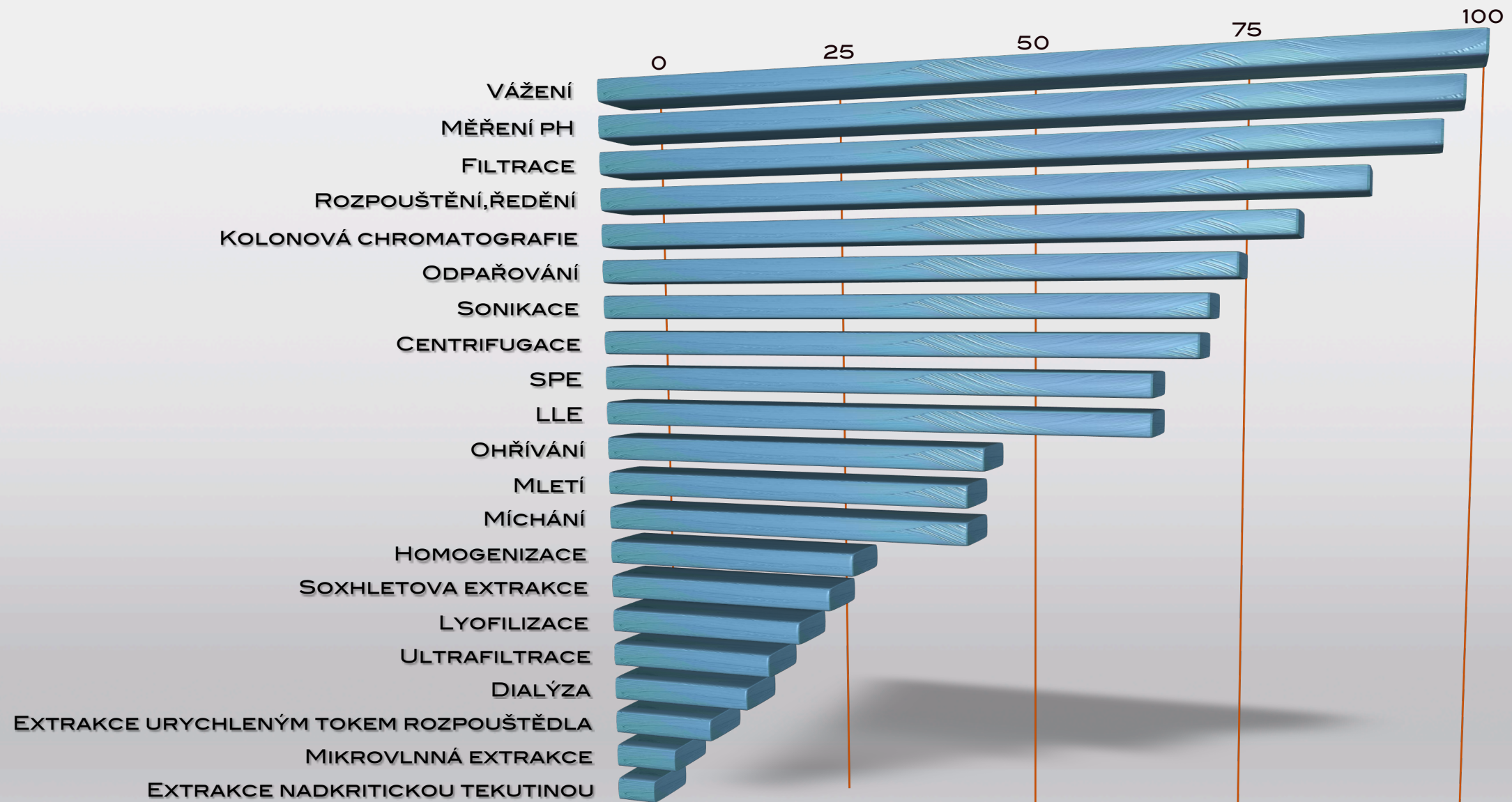
BIOLOGICKY AKTIVNÍ LÁTKY



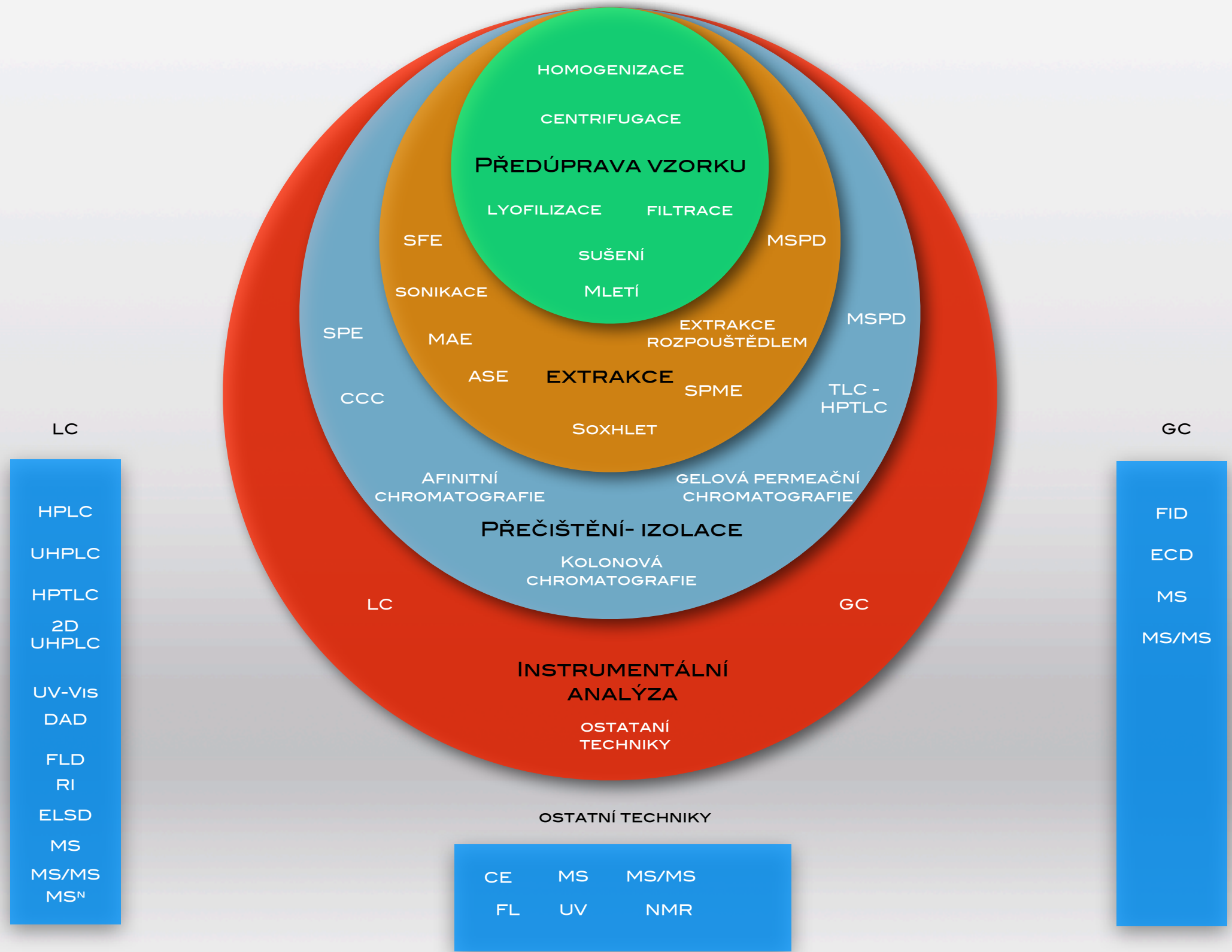




METODY PŘÍPRAVY VZORKŮ



STRATEGIE STANOVENÍ PŘÍRODNÍCH LÁTEK



MODERNÍ EXTRAKČNÍ METODY

SOXHLETOVA MODIFIKOVANÁ
METODA

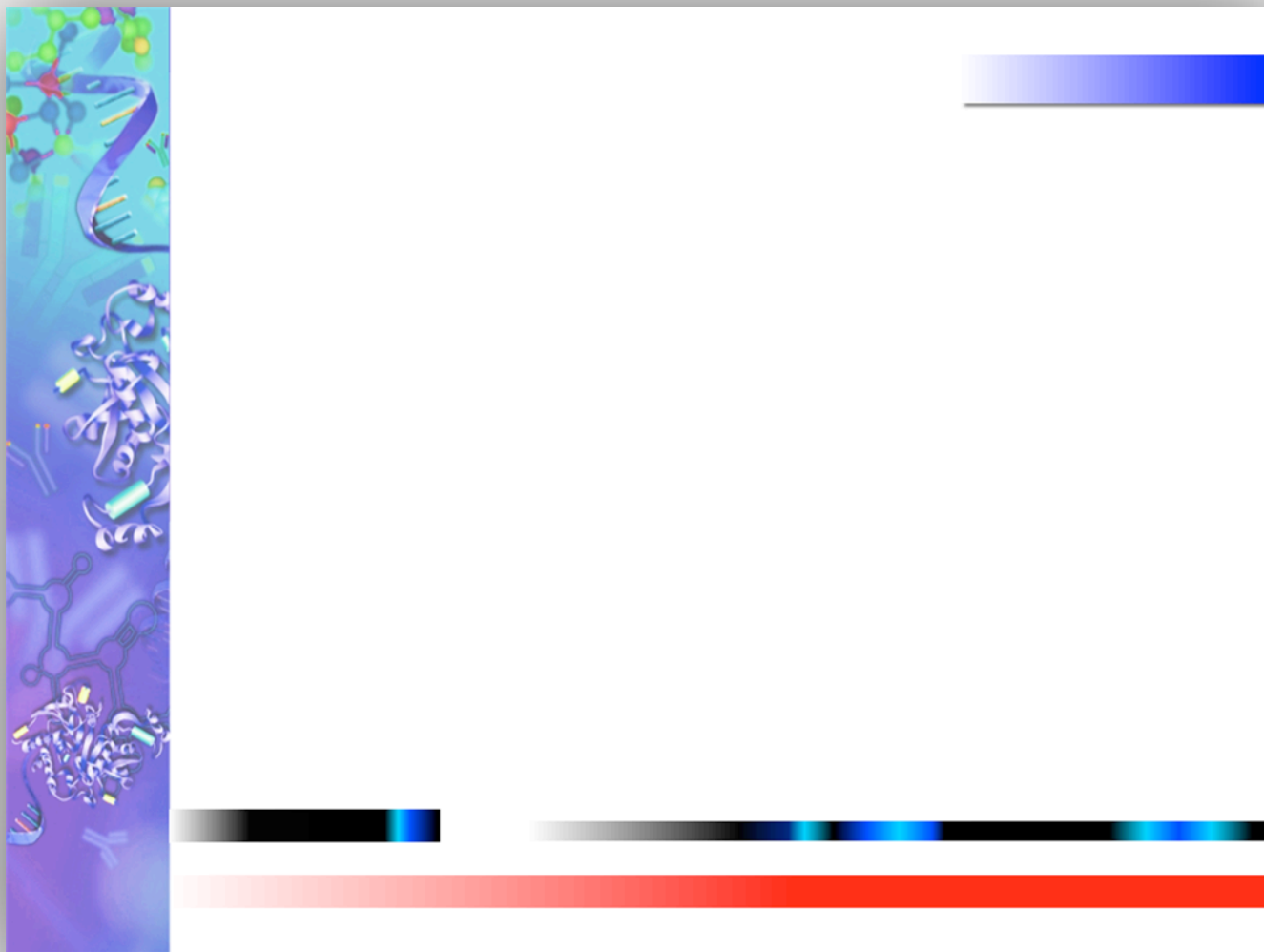
EXTRAKCE STLAČENOU
HORKOU VODOU

EXTRAKCE NADKRITICKOU
TEKUTINOU

TLAKOVÁ EXTRAKCE
ROZPOUŠTĚDLEM

MIKROVLNNÁ EXTRAKCE

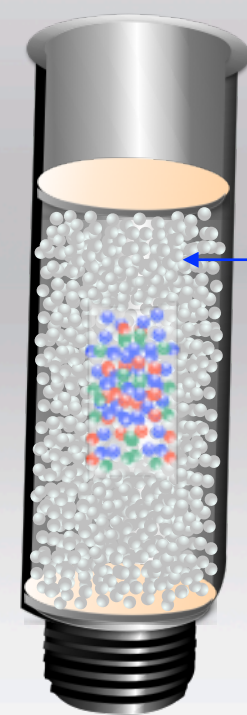
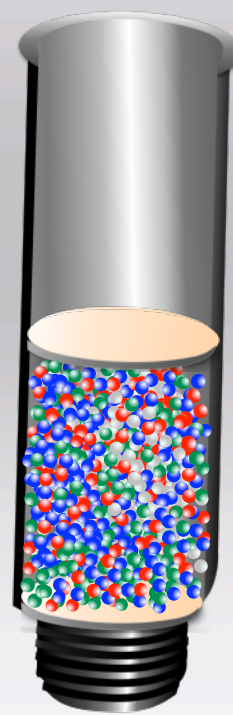
**TLAKOVÁ EXTRAKCE
ROZPOUŠTĚDLEM**



EXTRAKČNÍ CELY



VZOREK+MATRIX



MATRIX

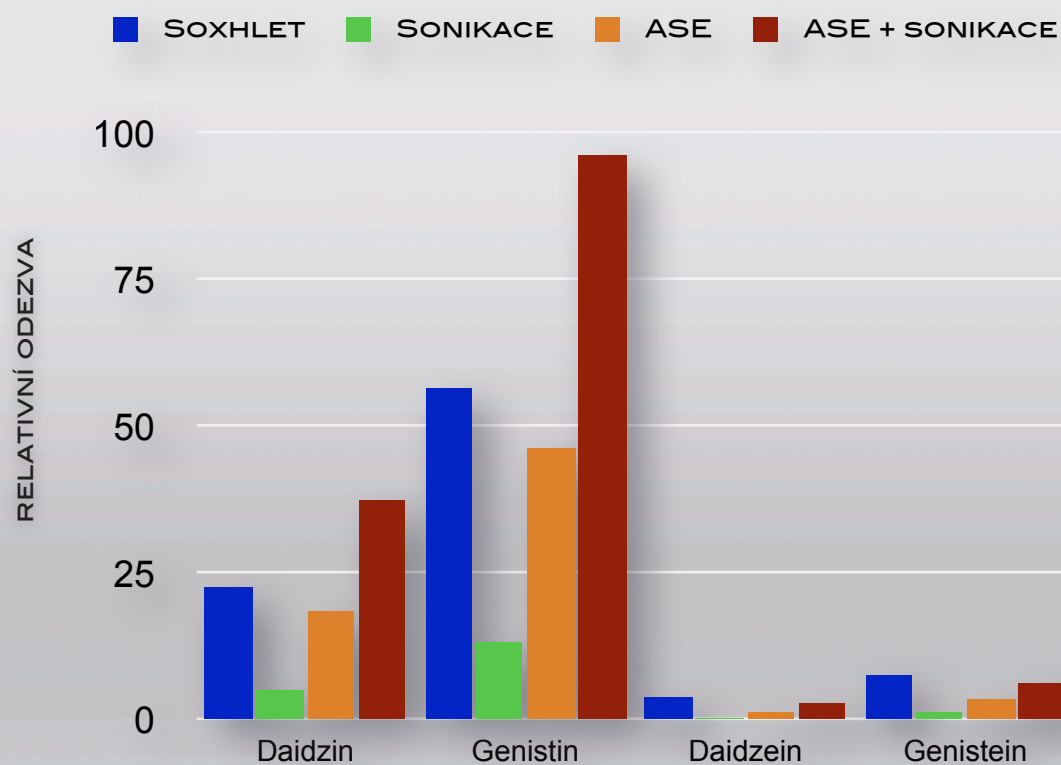
VZOREK
ZABALEN VE
FILTRAČNÍM
PAPÍŘE

TLAKOVÁ EXTRAKCE ROZPOUŠTĚDLEM

EXTRAKCE ISOFLAVONŮ

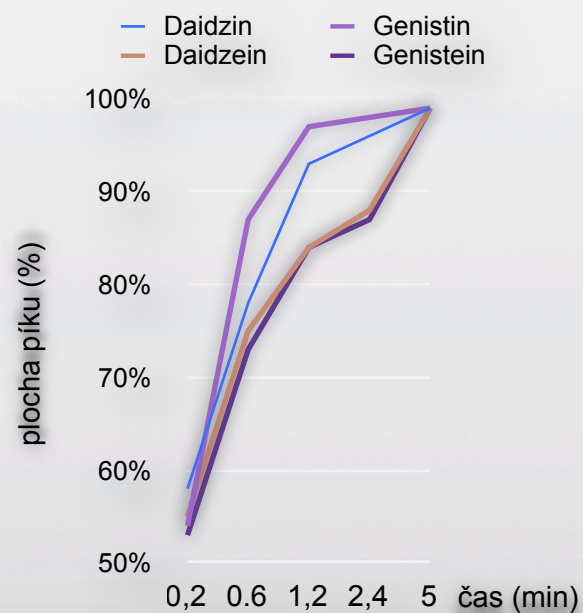
ISOFLAVONY:
TLAK: 15 MPA, TEPLOTA 135°C,
DYNAMICKÁ EXTRAKCE:
POČET CYKLŮ: 3, ČAS: 3X5 MIN,
SOLVENT: MEOH (90% V/V)

SROVNÁNÍ EXTRAČNÍCH TECHNIK

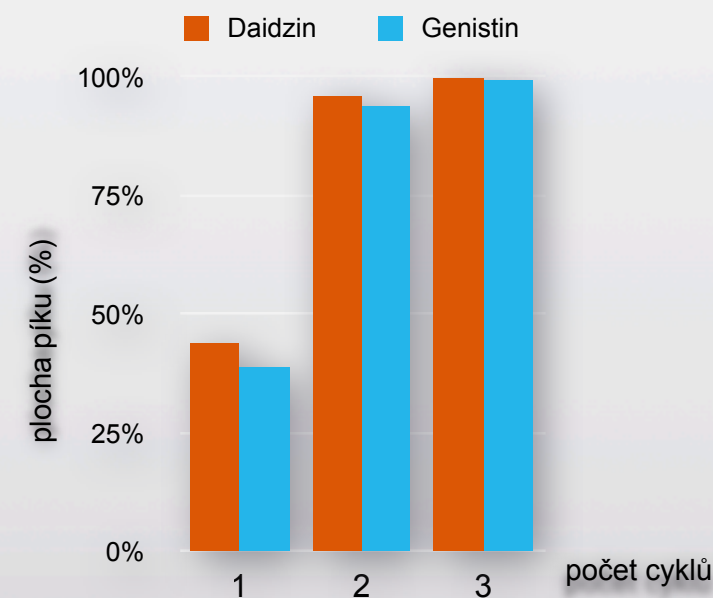


OPTIMALIZACE ASE + SONIKACE

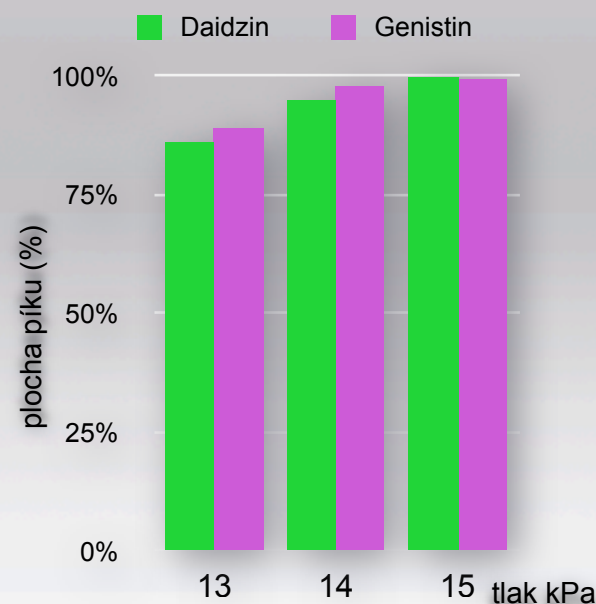
OPTIMALIZACE SONIKACE



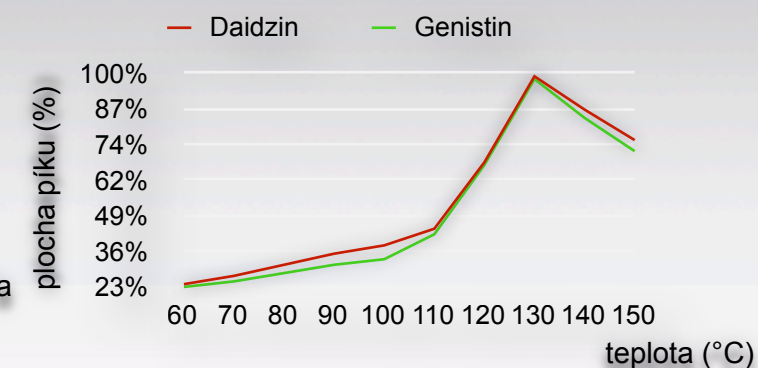
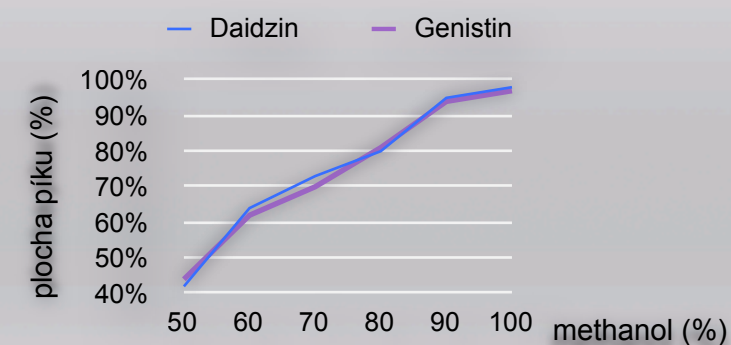
OPTIMALIZACE POČTU CYKLŮ



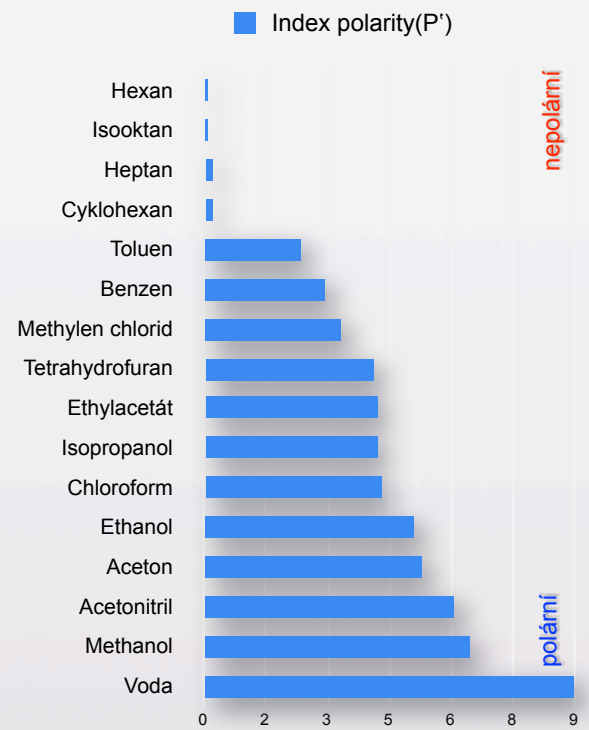
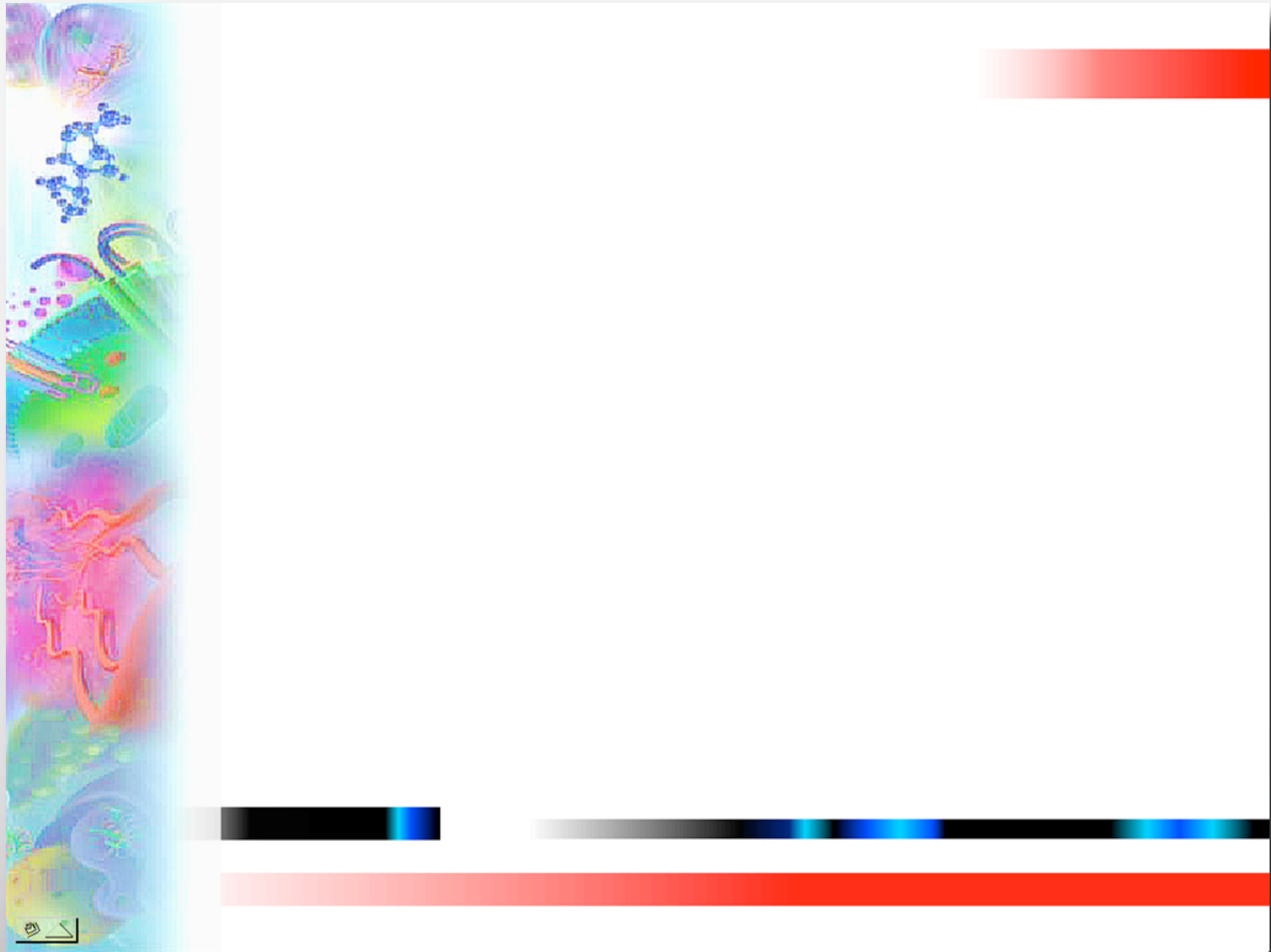
OPTIMALIZACE TLAKU



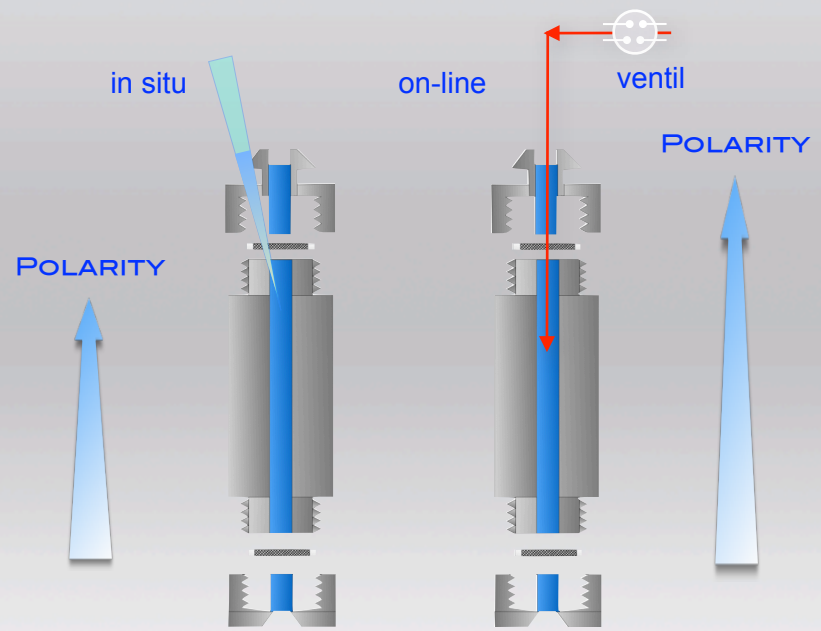
OPTIMALIZACE SOLVENTU A TEPLoty



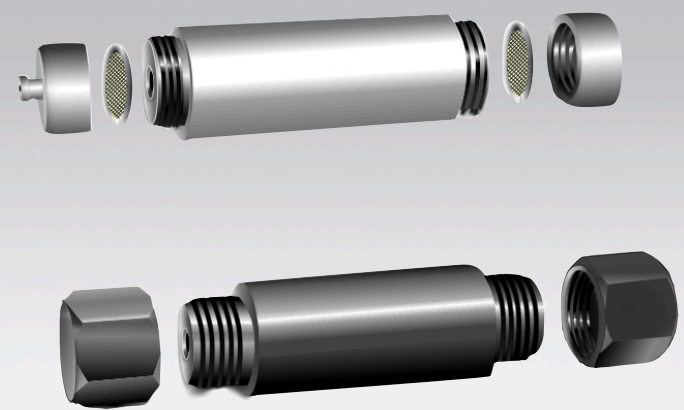
EXTRAKCE NADKRITICKOU TEKUTINOU



EXTRAKCE POLÁRNÍCH LÁTEK



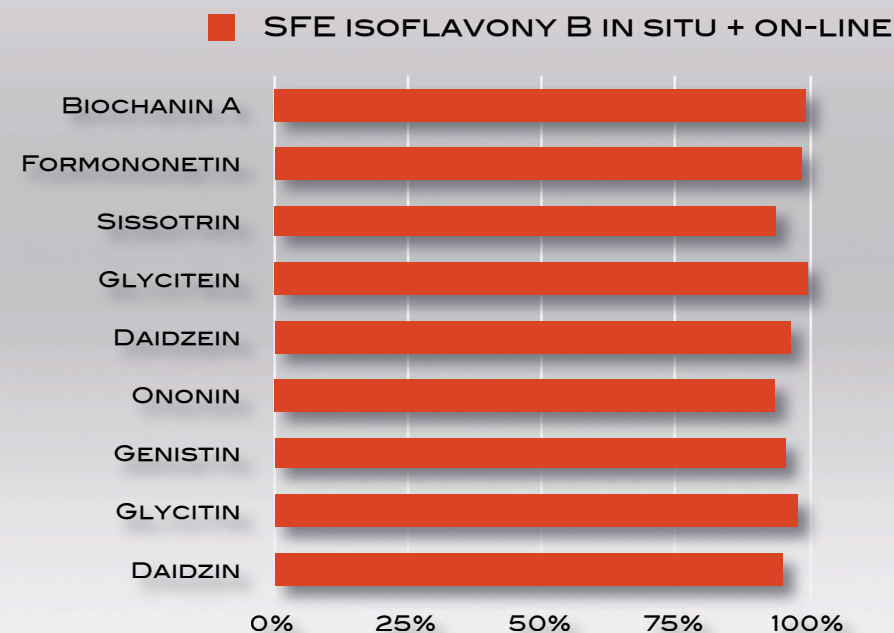
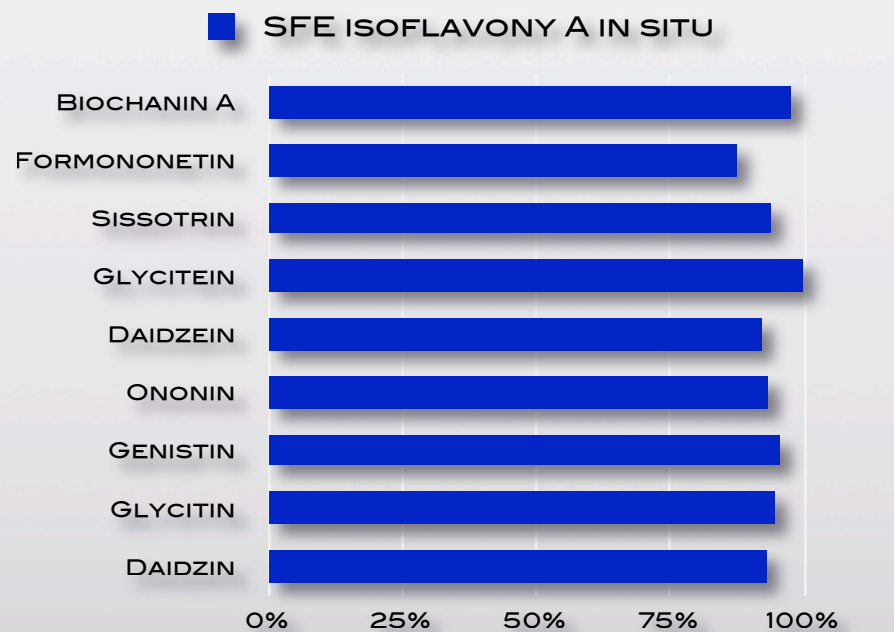
EKTRAKČNÍ CELY



ÚČINNOST EXTRAKCE POLÁRNÍCH ANALYTŮ

EXTRAKCE ISOFLAVONŮ

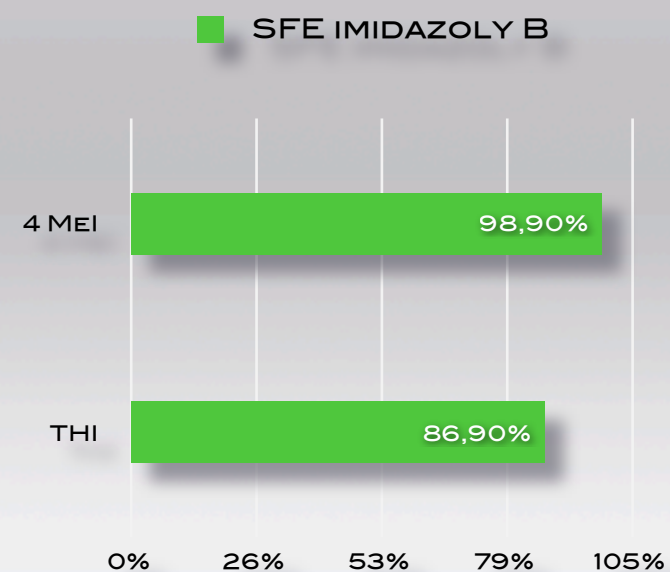
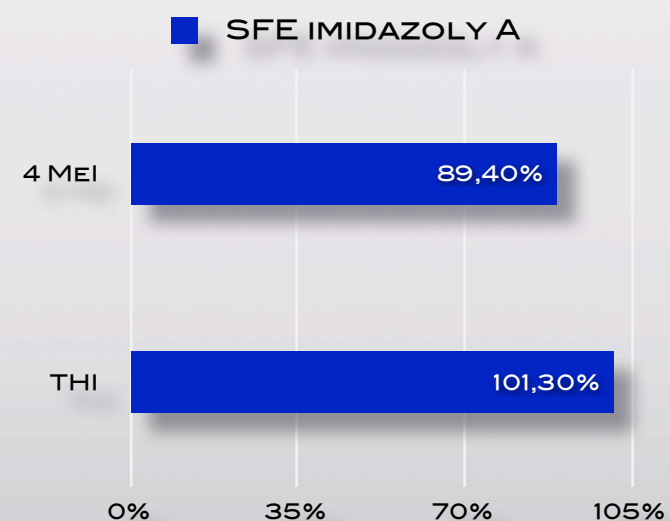
ISOFLAVONY:
TLAK: 30 MPA, TEPLOTA 38°C, ČAS: 30 MIN,
MODIFIKÁTOR: MEOH/H₂O (14:1 v/v):
300 μL IN SITU + 500 μL ON-LINE



EXTRAKCE IMIDAZOLŮ Z KÁVY

IMIDAZOLY A:
TLAK: 40 MPA, TEPLOTA 150°C, ČAS: 45 MIN,
MODIFIKÁTOR: MEOH/H₂O (5:1 v/v):
200 μL IN SITU + 500 μL ON-LINE

IMIDAZOLY B:
TLAK: 40 MPA, TEPLOTA 150°C, ČAS: 45 MIN,
MODIFIKÁTOR: MEOH/H₂O (9:1 v/v):
200 μL IN SITU + 500 μL ON-LINE



ÚČINNOST EXTRAKCE POLÁRNÍCH ANALYTŮ

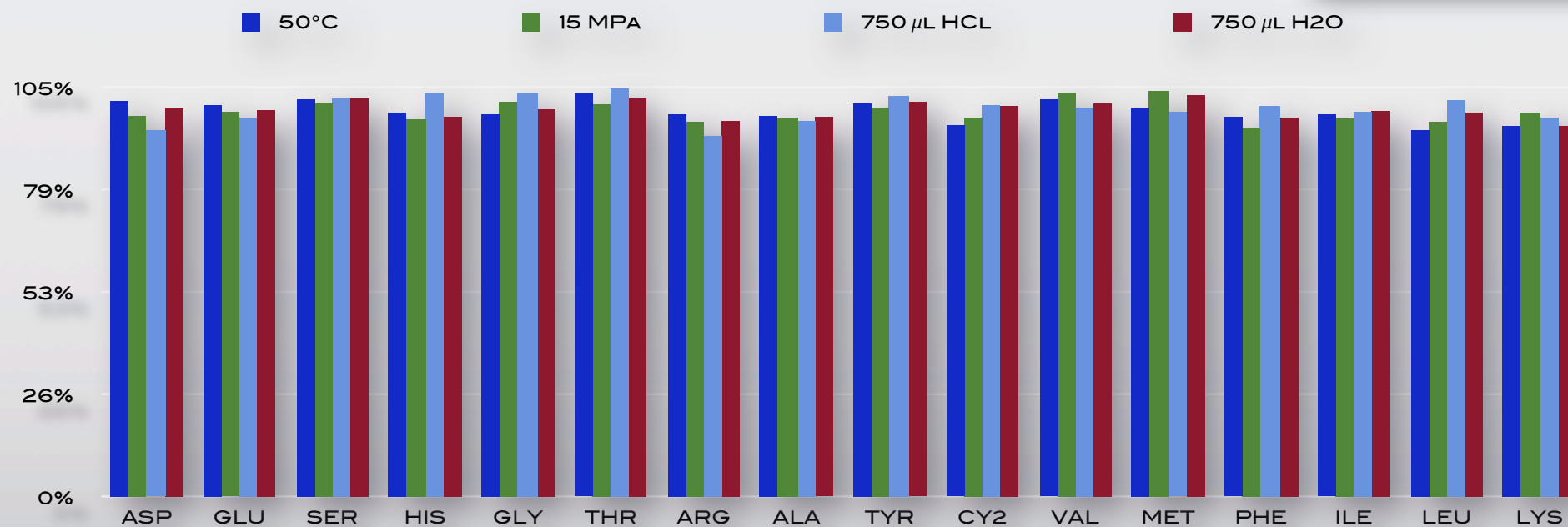
EXTRAKCE VOLNÝCH AMINOKYSELIN Z PŮDNÍCH VZORKŮ

AMINOKYSELINY:

TLAK: 15 MPA, TEPLOTA 50°C, ČAS: 30 MIN,

MODIFIKÁTOR: H₂O: 750 μL ON-LINE

ENTRAINER PRO MODELOVÉ VZORKY: 750 μL HCL IN SITU



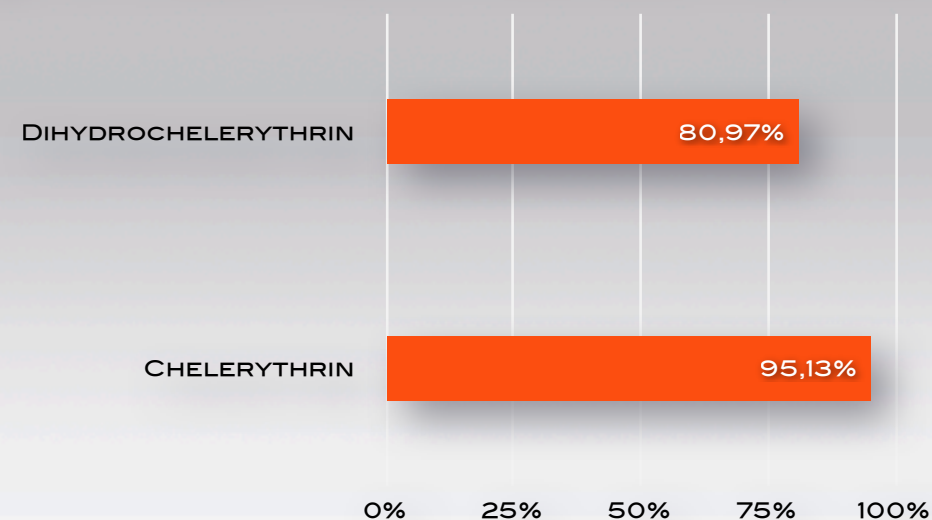
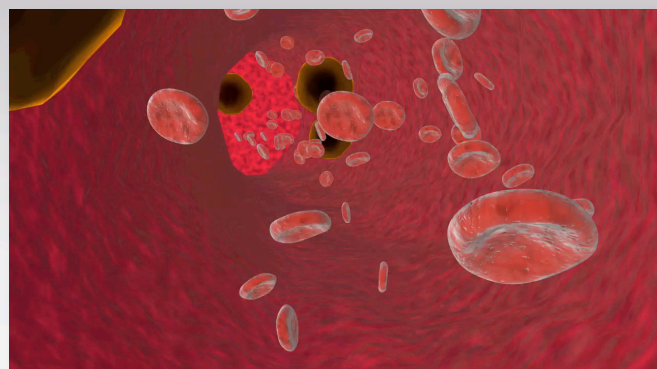
EXTRAKCE ALKALOIDŮ Z KREVNI PLAZMY

CHELERYTHRIN A DIHYDROCHELERYTHRIN:

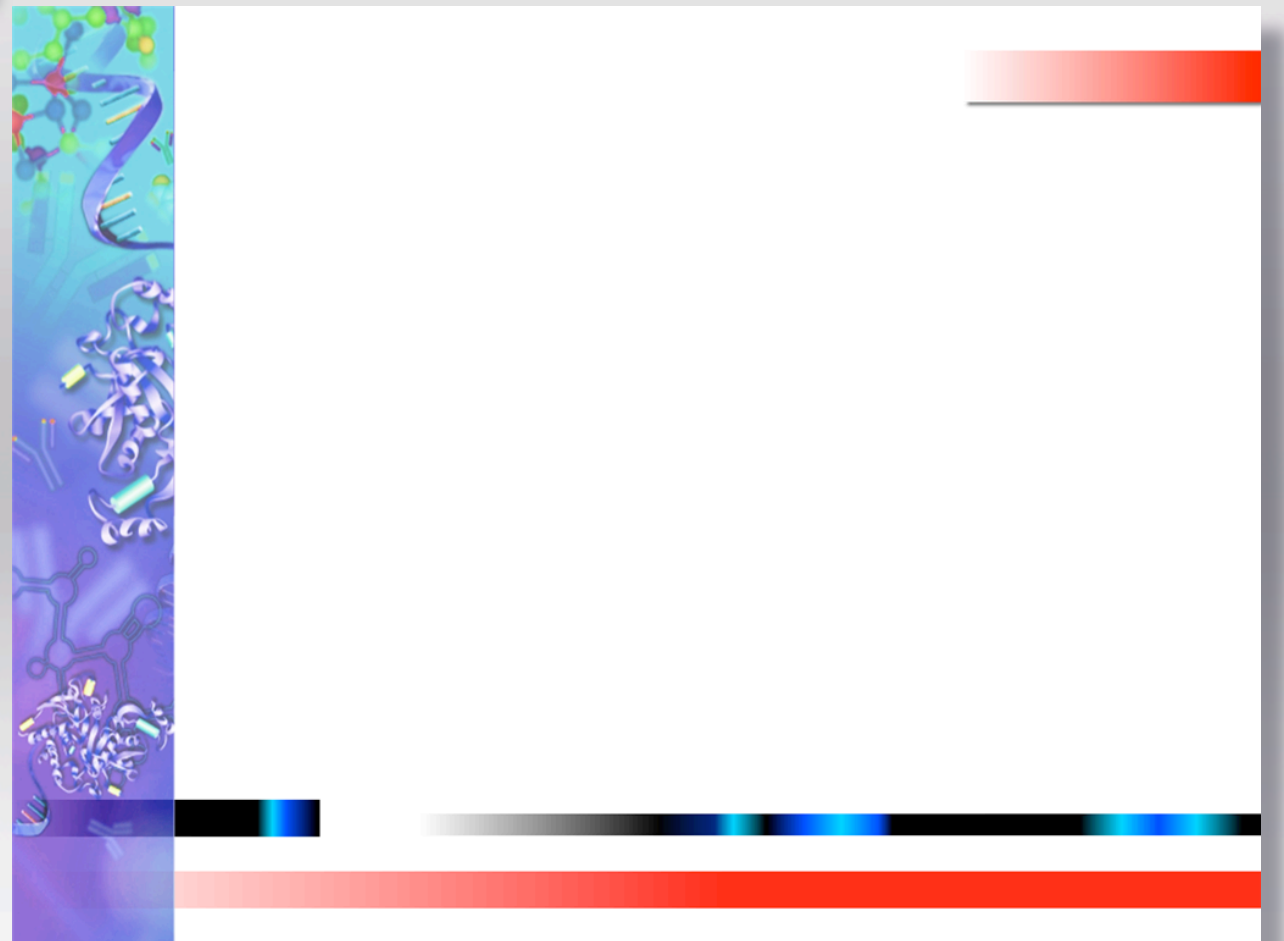
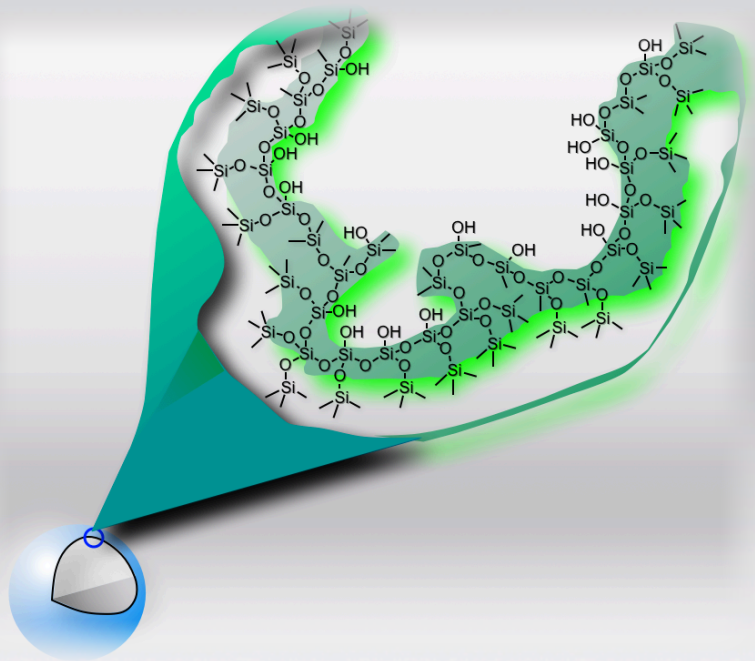
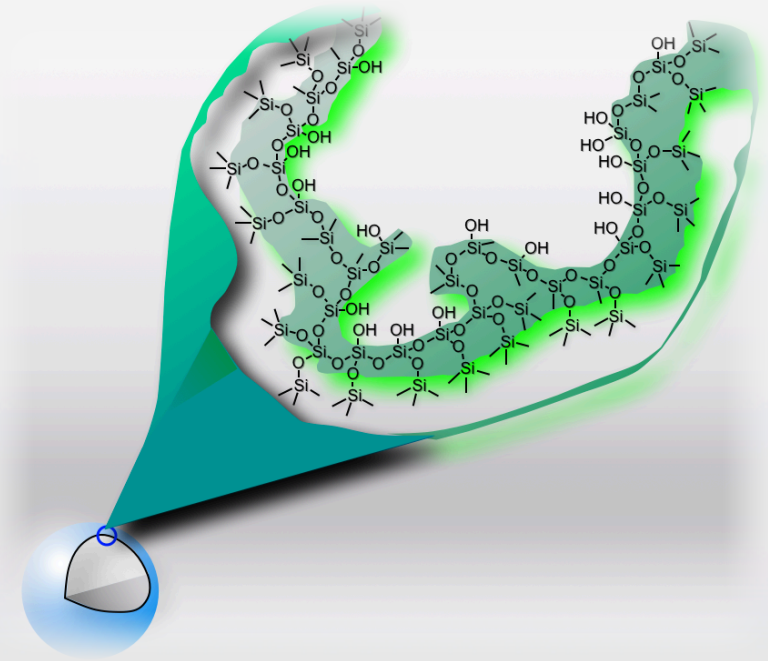
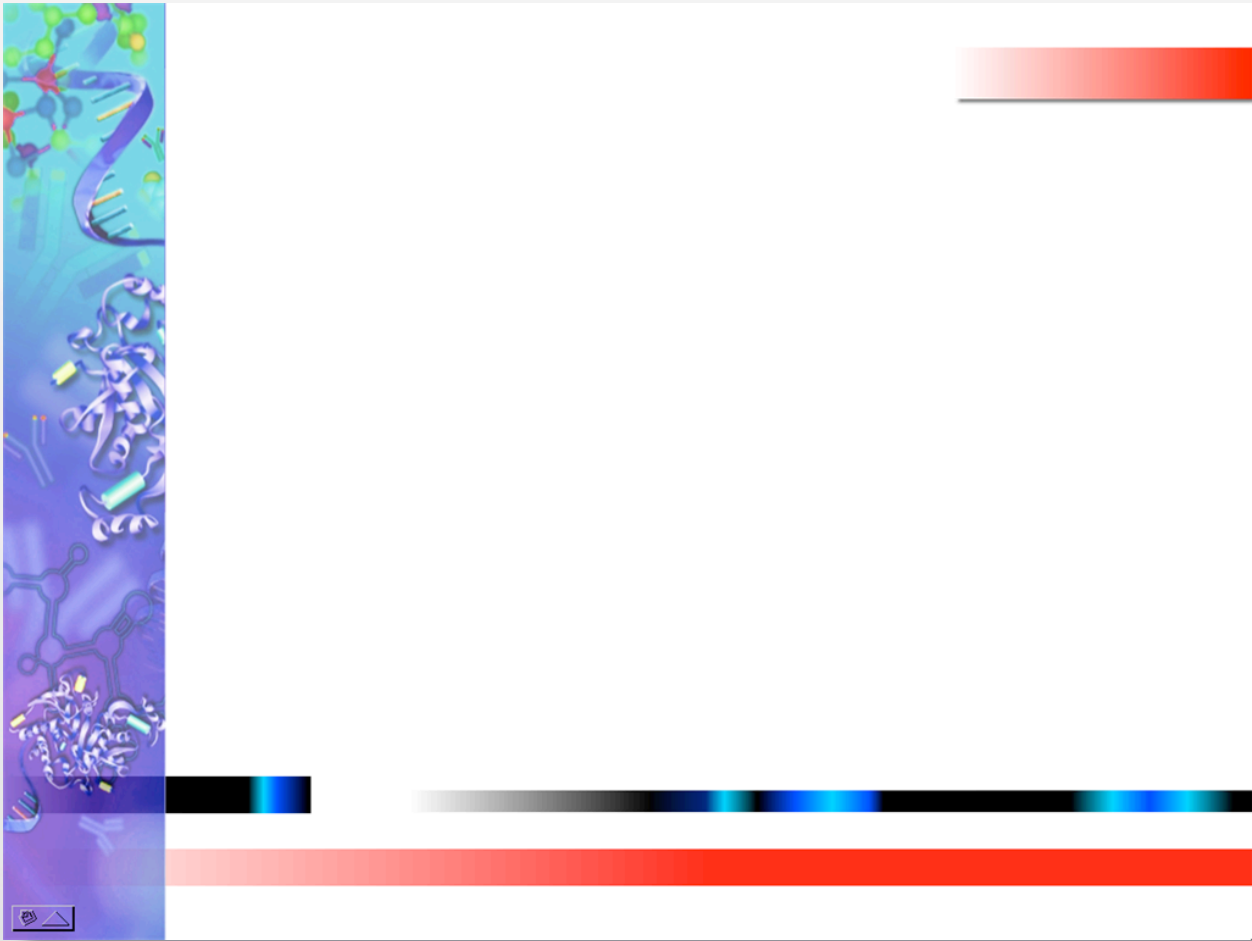
TLAK: 10 MPA, (15 MPA Z KREVNI PLAZMY) TEPLOTA 35°C, ČAS: 20 MIN,

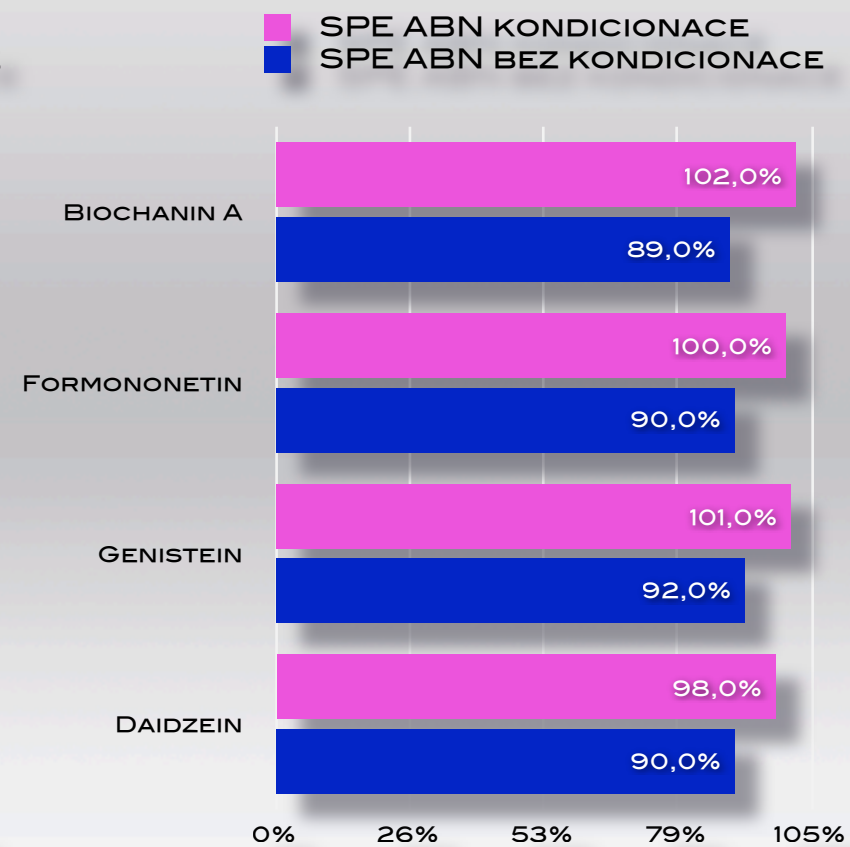
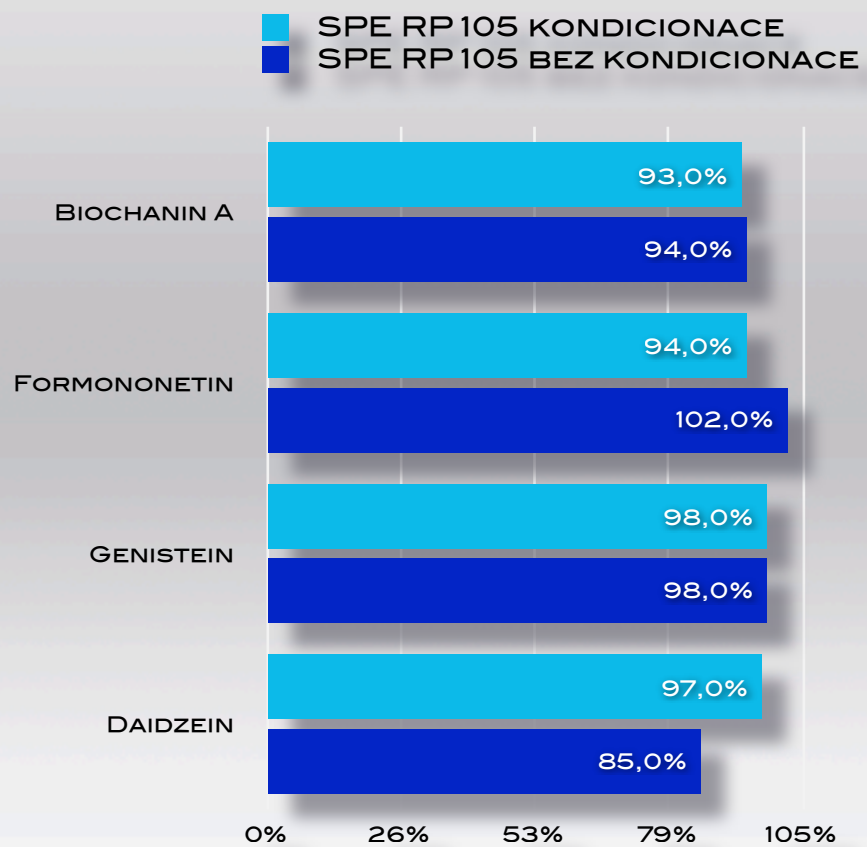
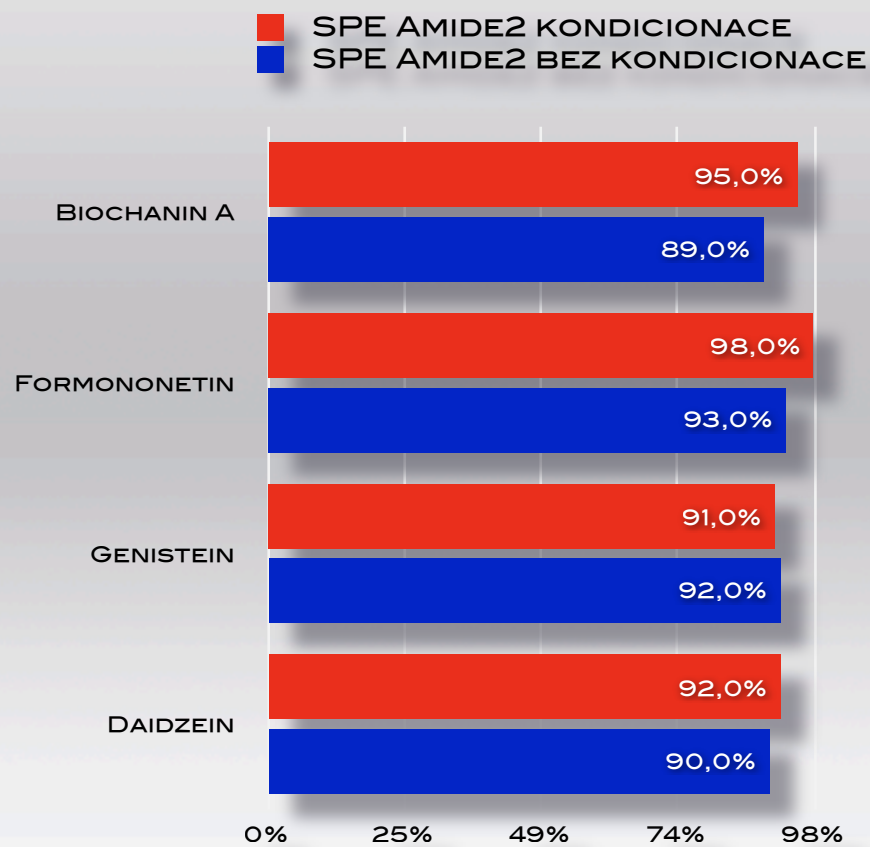
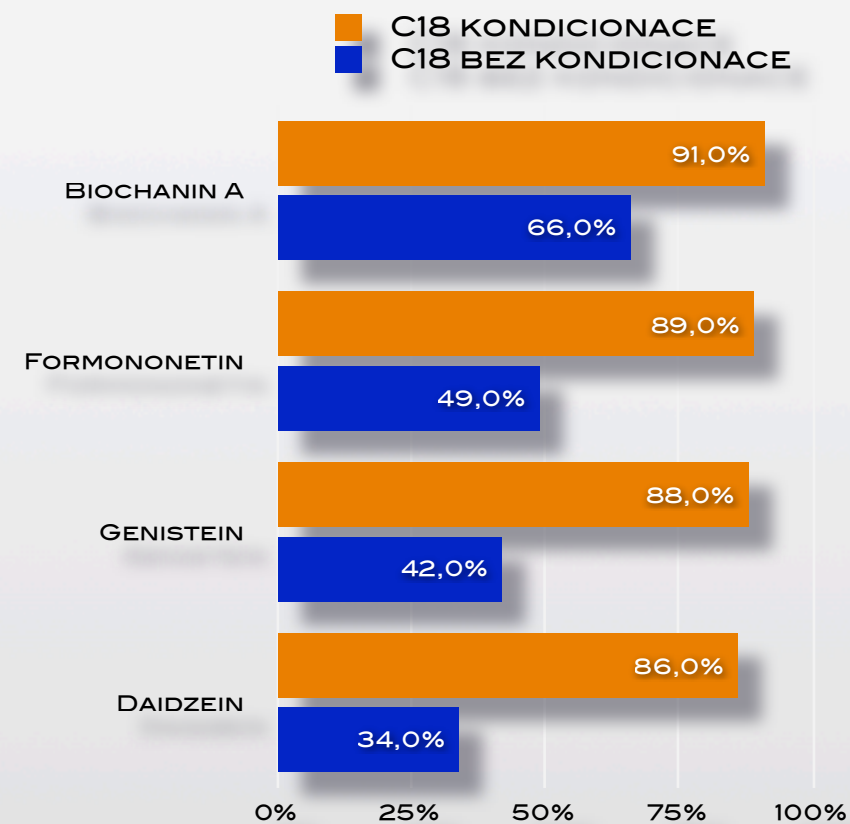
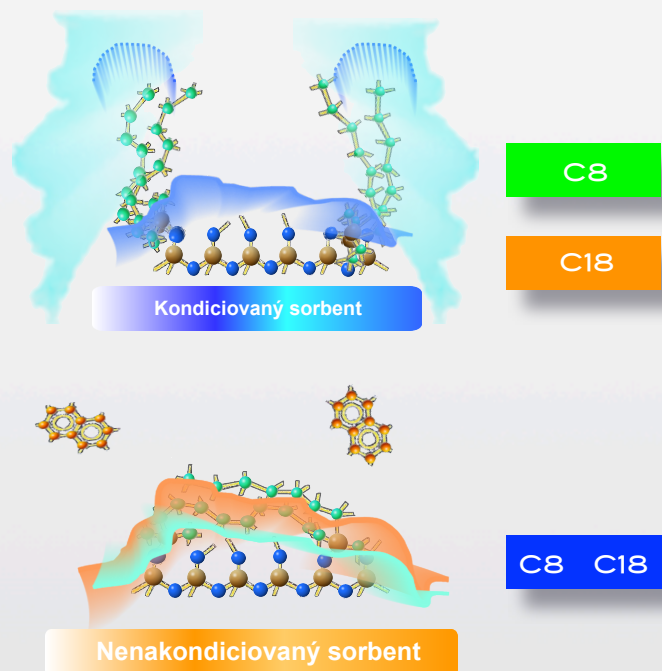
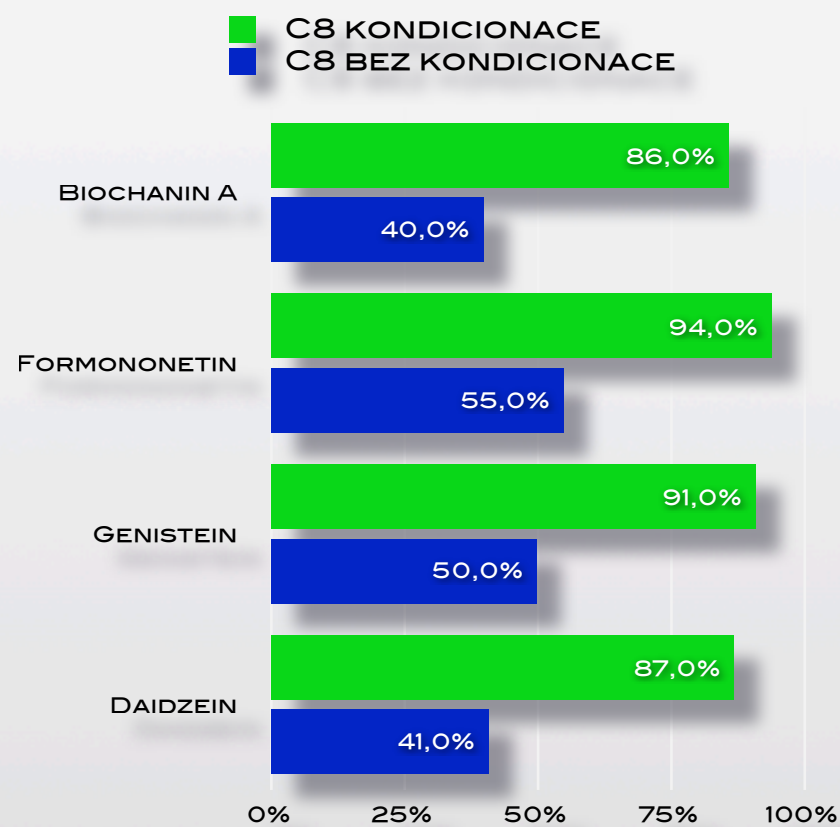
MODIFIKÁTOR: MEOH/H₂O: 1:1 (V/V) 500 μL IN SITU + 500 μL ON-LINE

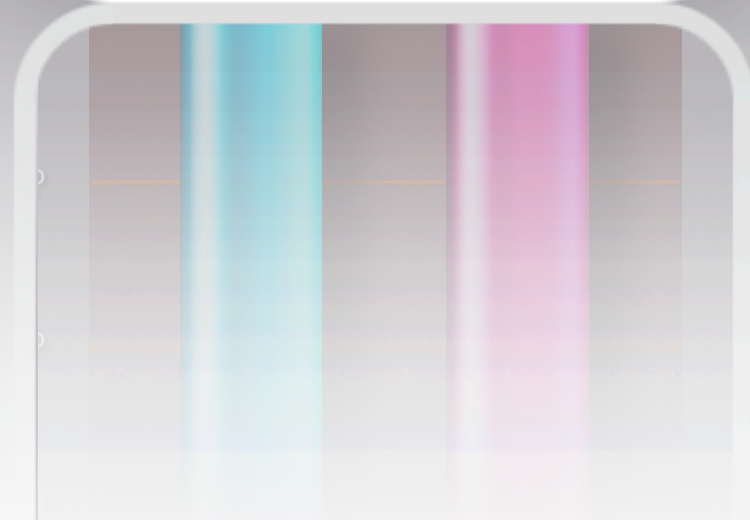
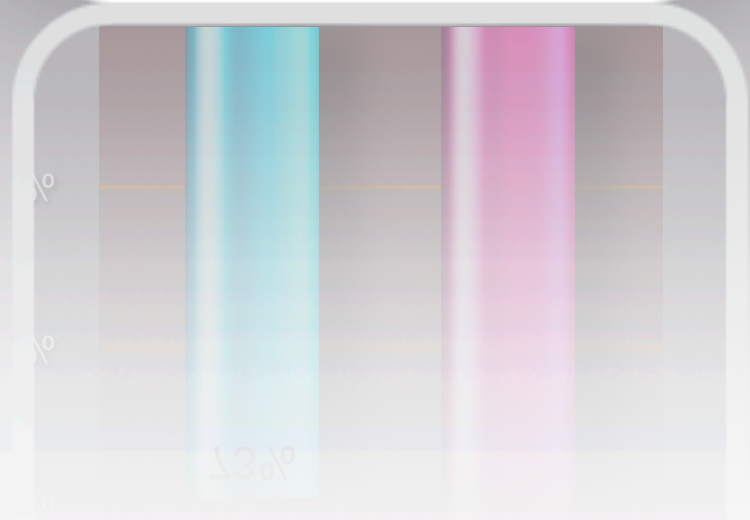
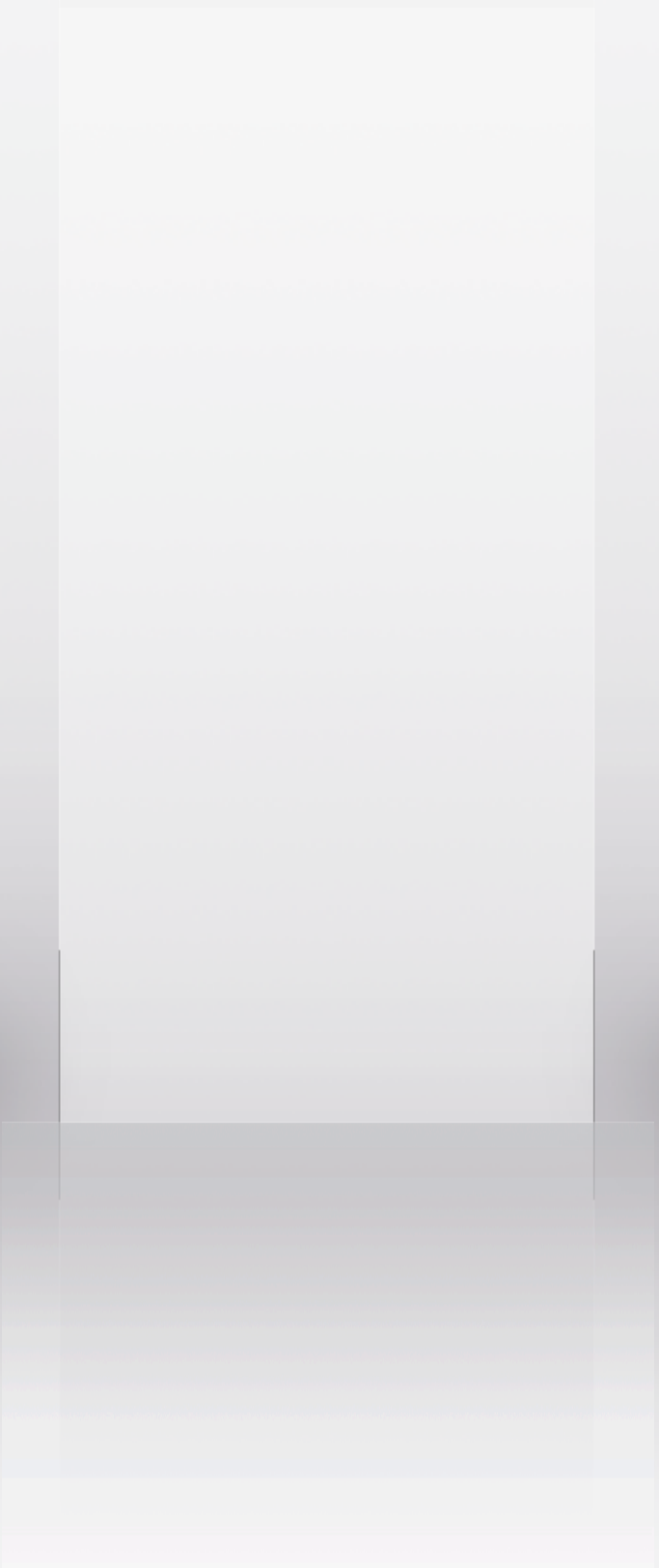
SFE CHELERYTHRIN, DIHYDROCHELERYTHRIN (KREVNI PLAZMA)



EXTRAKCE PEVNOU FÁZÍ



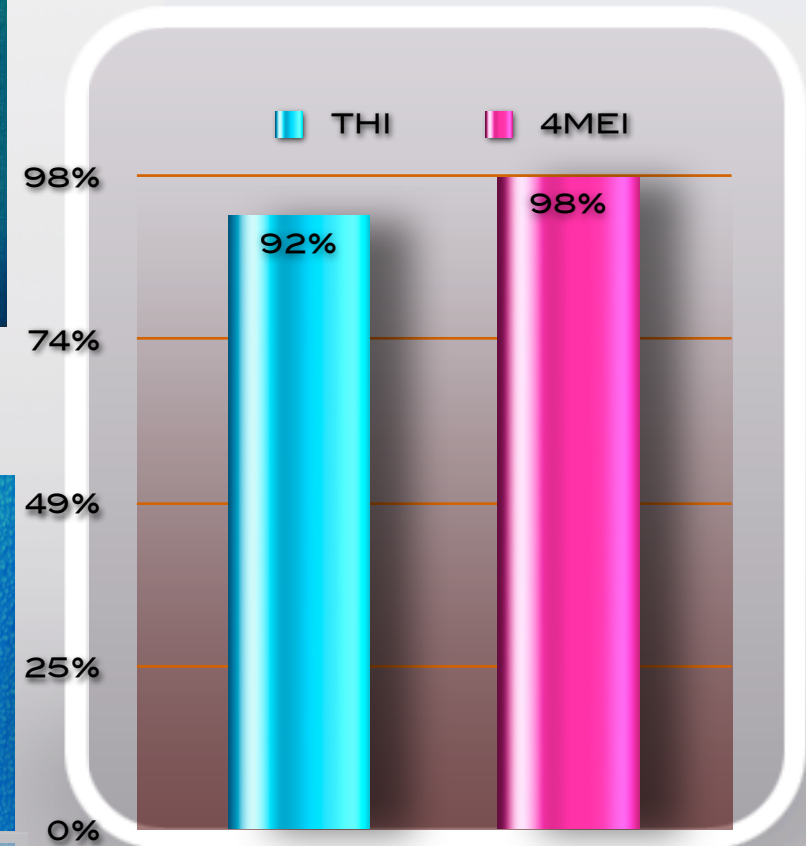
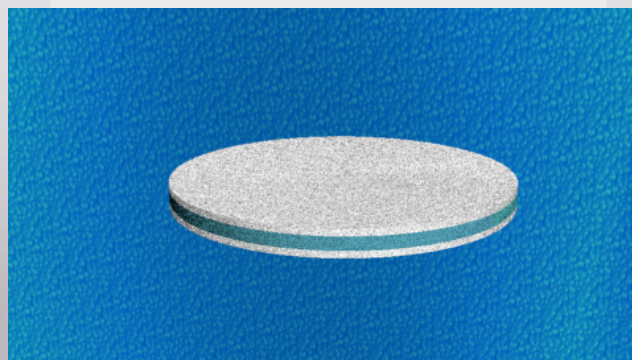
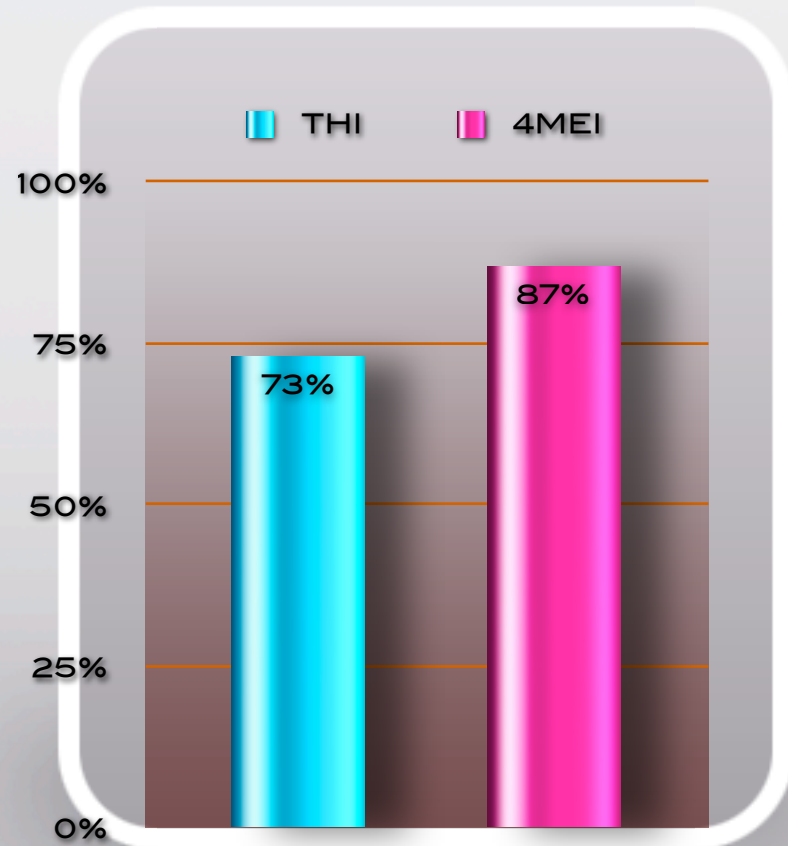




SROVNÁNÍ SPE SCX A SPE SPEC DISC SCX SEPARACE NEUROTOXINŮ

SPE SCX
 KONDICIONACE: 1000 μ L METOH
 1000 μ L H₂O
 VZOREK: 3000 μ L
 PROMYTÍ: 1000 μ L METOH
 ELUCE: 3000 μ L METOH+5M HCL

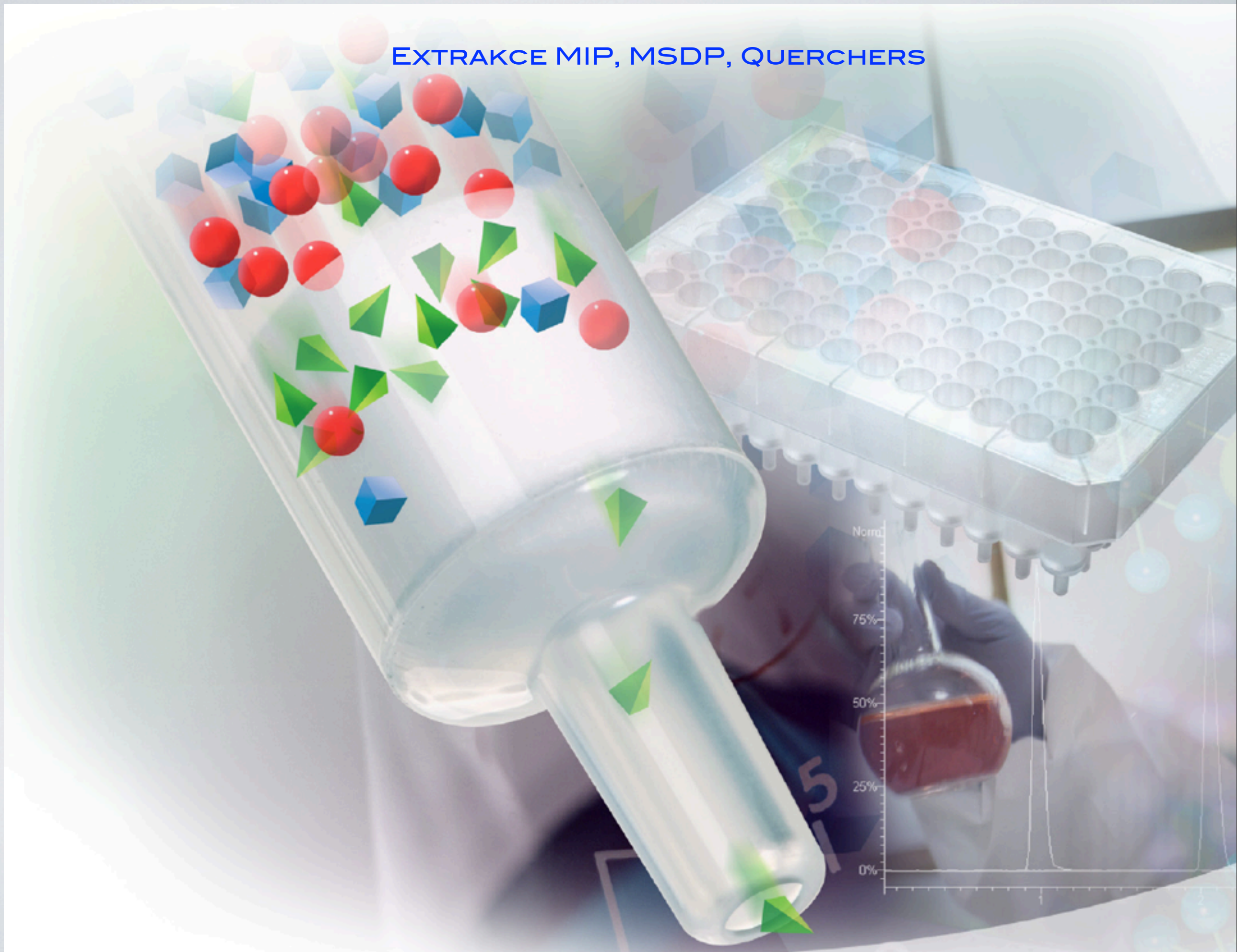
SPE SCX DISC
 KONDICIONACE: 100 μ L METOH
 100 μ L H₂O
 VZOREK: 300 μ L
 PROMYTÍ: 100 μ L METOH
 ELUCE: 100 μ L METOH+5M HCL



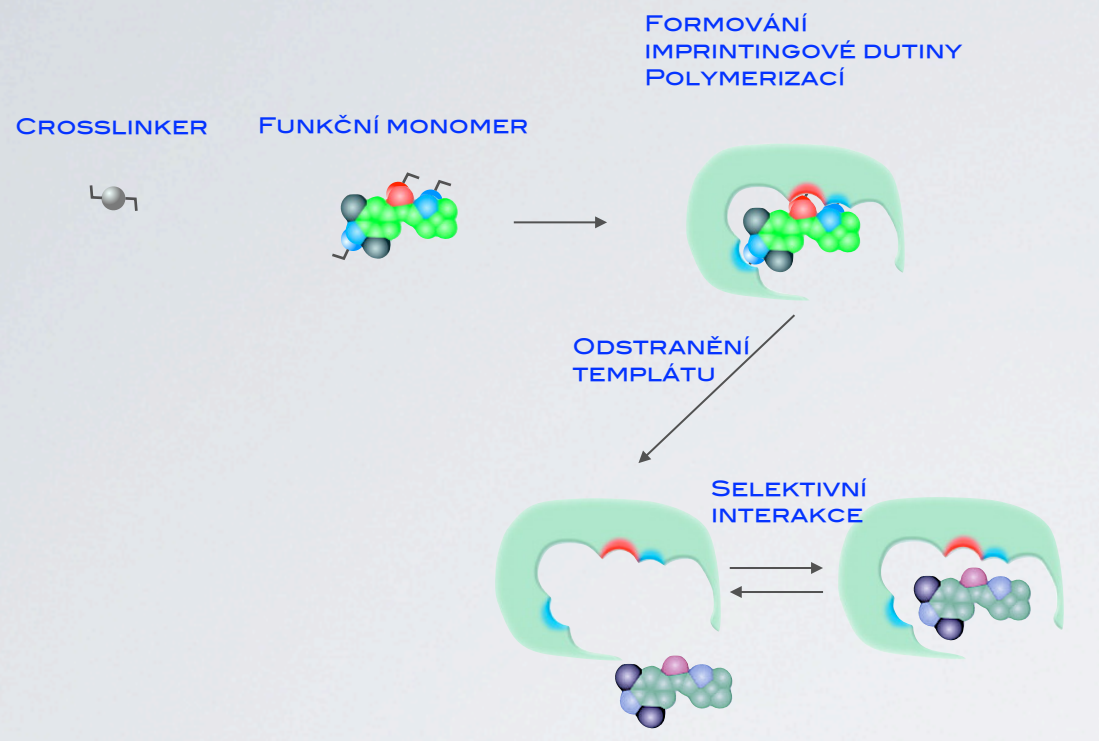
SPE SCX

SPE SCX DISC

EXTRAKCE MIP, MSDP, QUERCHERS



EXTRAKCE MIP, MSPD, QUERCHERS

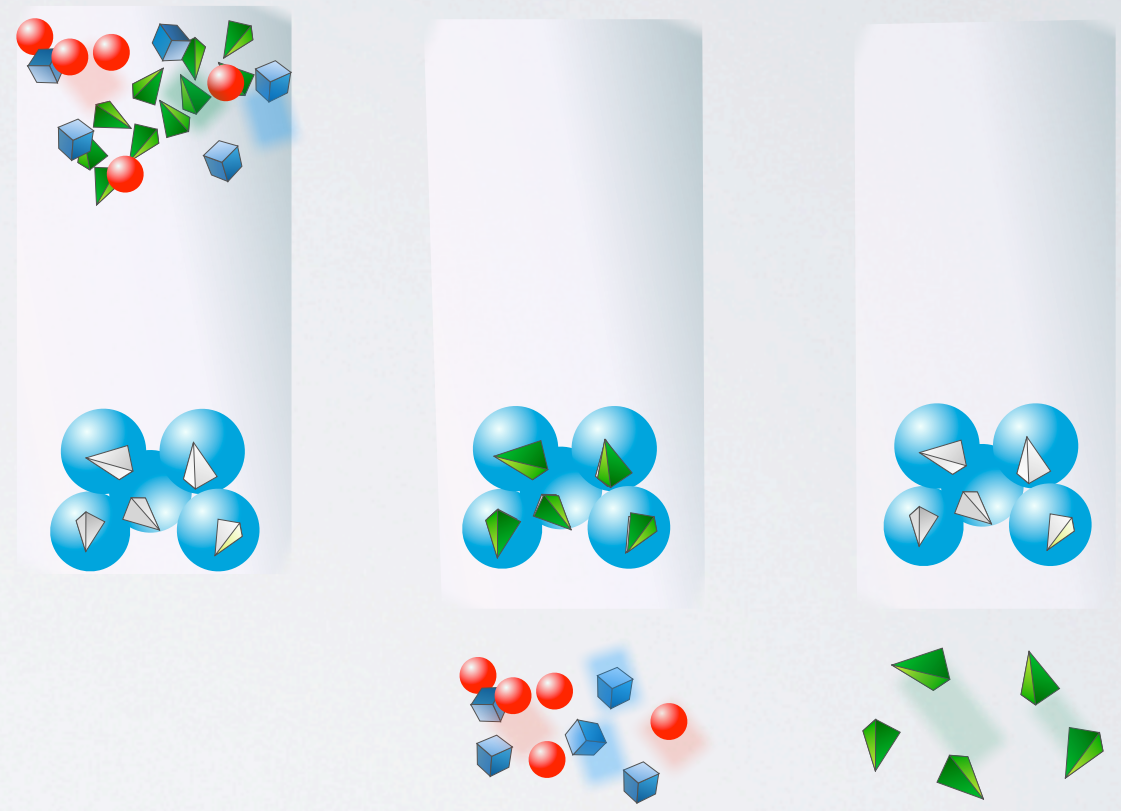


EXTRAKCE MIP POLYMERY (MOLECULAR IMPRINTED POLYMERS)

NANESENÍ VZORKU

PROMYTÍ

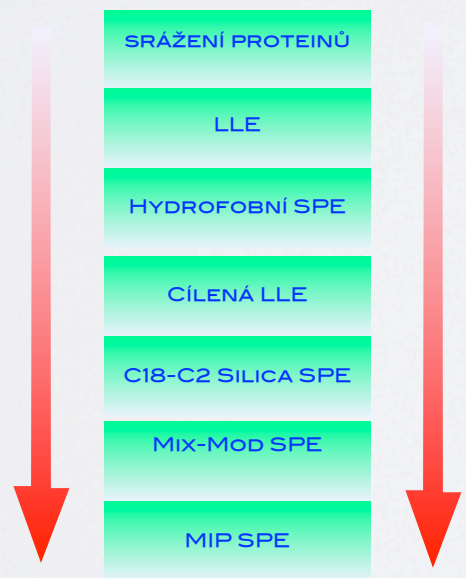
ELUCE



RELATIVNÍ SELEKTIVITA PREP TECHNIK

NESELEKTIVNÍ

SUROVÝ EXTRAKT

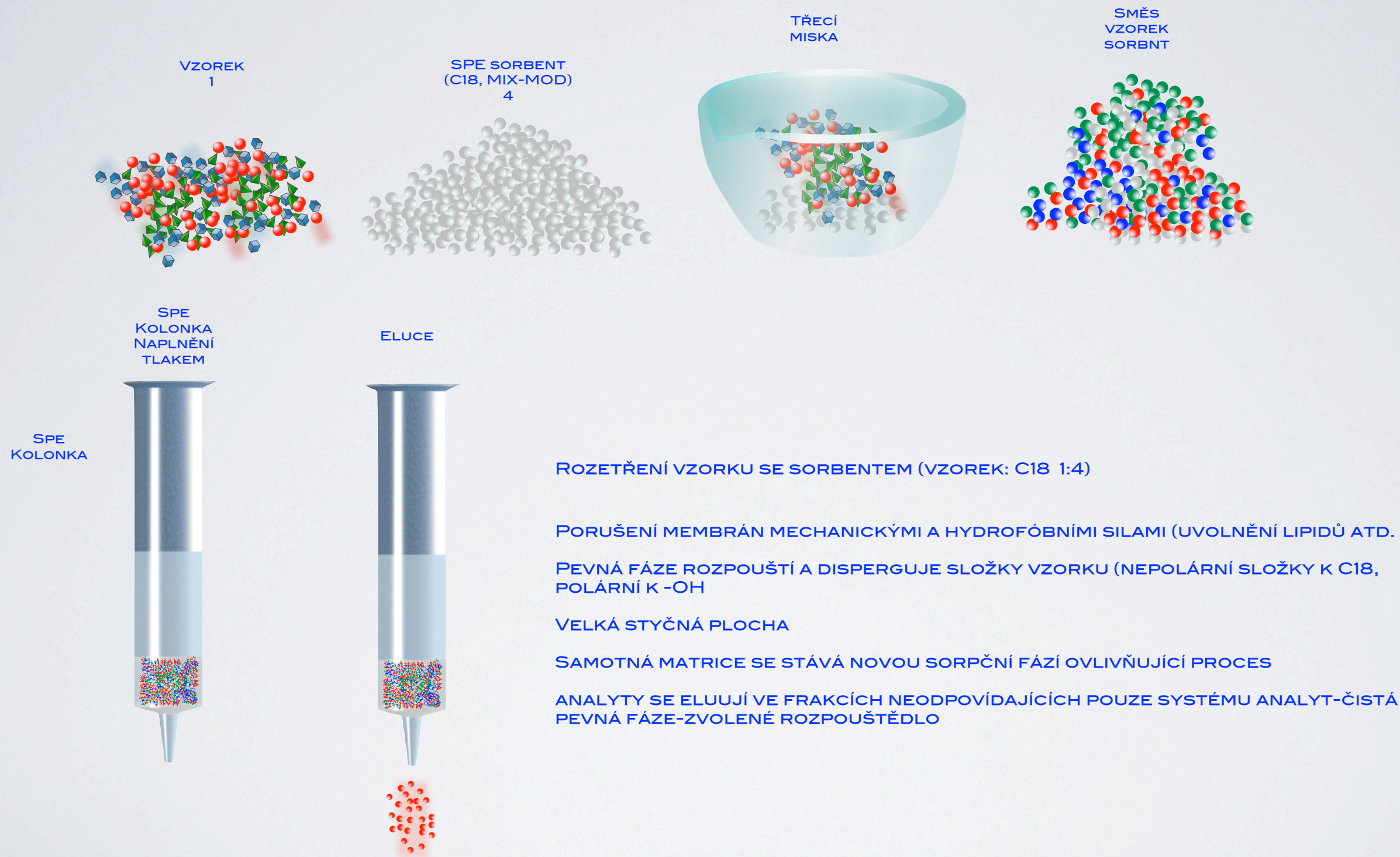


VYSOCE SELEKTIVNÍ

PŘEČIŠTĚNÝ EXTRAKT (NÍZKÝ LOD, LOQ)

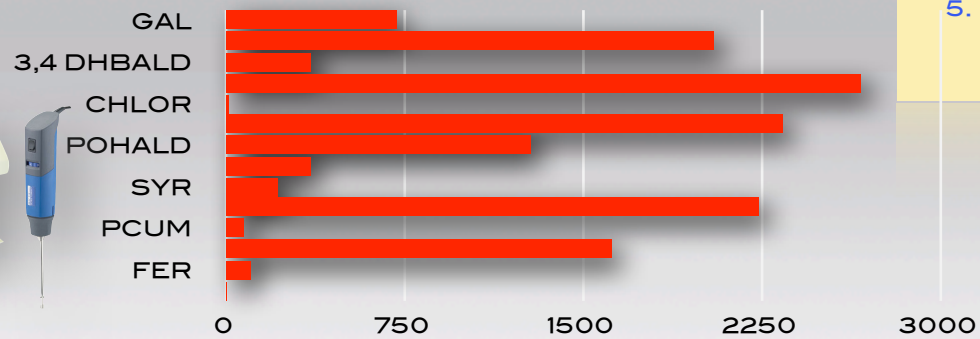
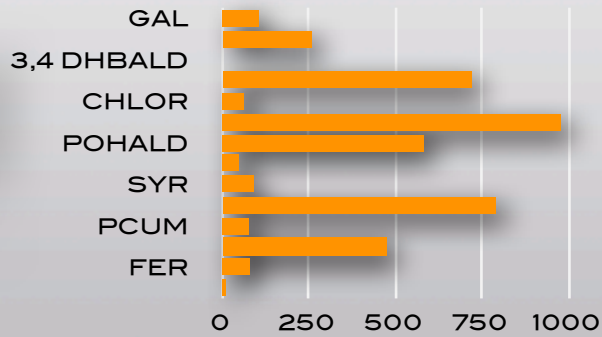
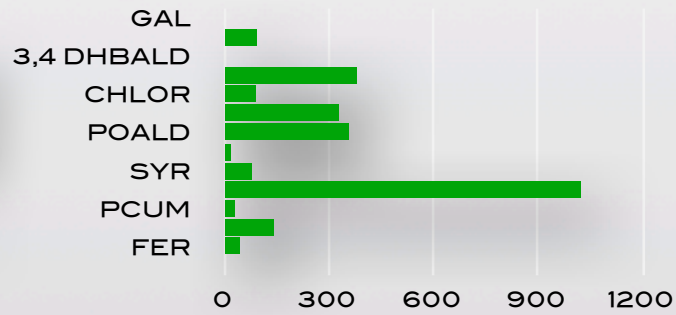
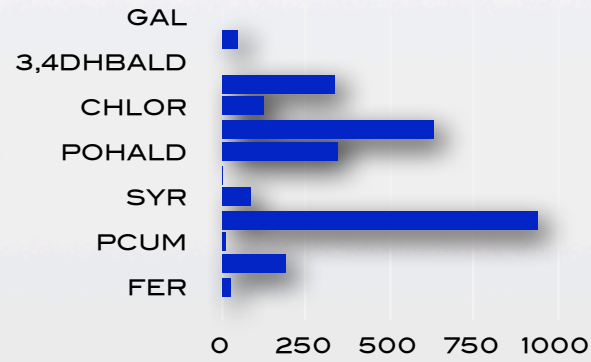
EXTRAKCE MIP, MSDP, QUERCHERS

MSDP - MATRIX SOLID PHASE DISPERSION

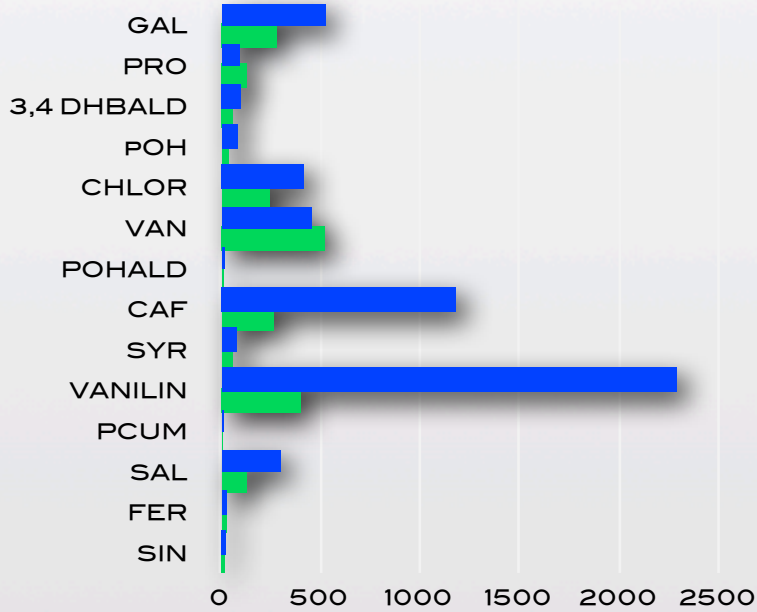


EXTRAKČNÍ KOMBINOVANÉ METODY UAPLE μ SPE: FRAKCE ŘASY CYSTOSEIRA ABIES

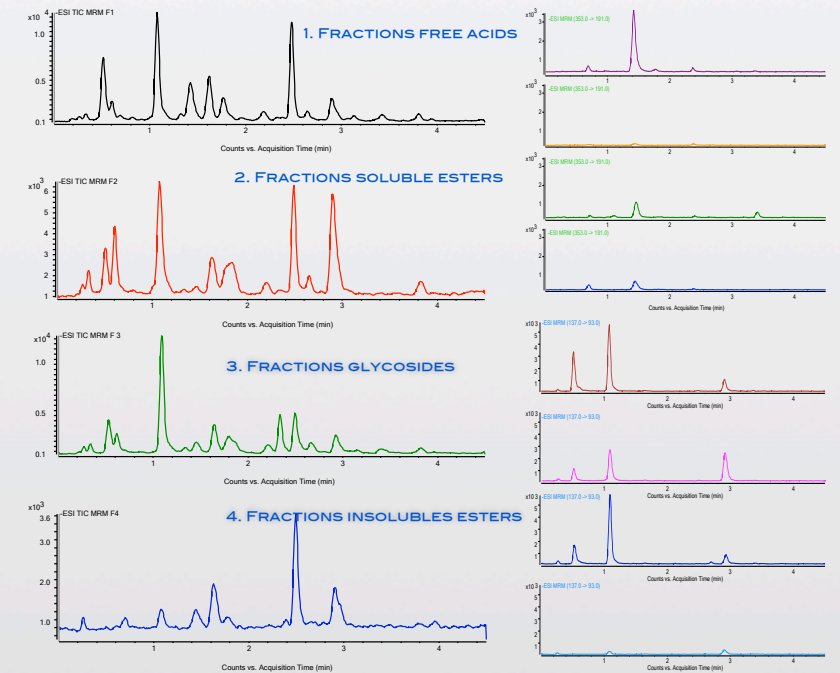
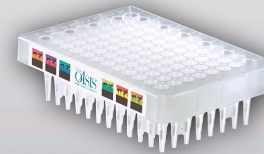
SROVNÁNÍ EXTRAKČNÍCH TECHNIK



μ ELUTE MCX VERSUS MCX 3CC (UAPLE EXTRAKCE)



■ CYSTOS μ ELUTE MCX ■ CYSTOS MCX 3CC

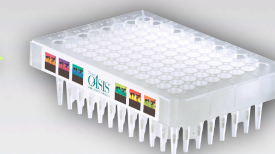


μ ELUTION PLATE:

1. CONDITION: 50 μ L MEOH
2. CONDITION: 50 μ L WATER
3. LOAD SAMPLE: 500 μ L
4. WASH: 20 μ L 5% MEOH WITH 2% ACETIC ACID
5. ELUTION: 4x20 μ L (5%, 10%, 15%, 20% MEOH (V/V) WITH 2% NH₄OH)

SPE:

1. CONDITION: 500 μ L MEOH
2. CONDITION: 500 μ L WATER
3. LOAD SAMPLE: 1000 μ L
4. WASH: 500 μ L 5% MEOH WITH 2% ACETIC ACID
5. ELUTION: 4x1000 μ L (5%, 10%, 15%, 20% MEOH (V/V) WITH 2% NH₄OH)



UA

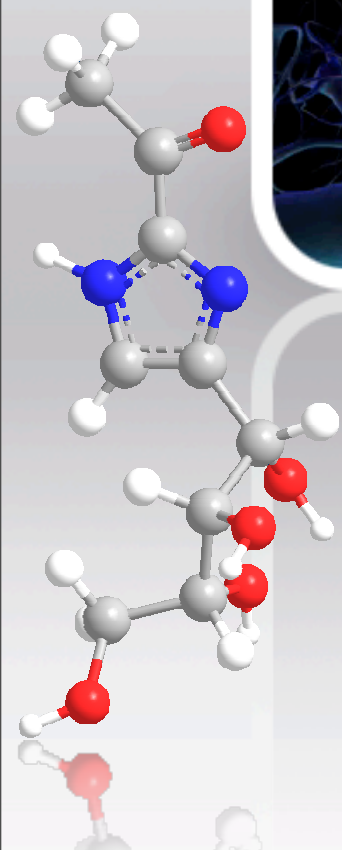
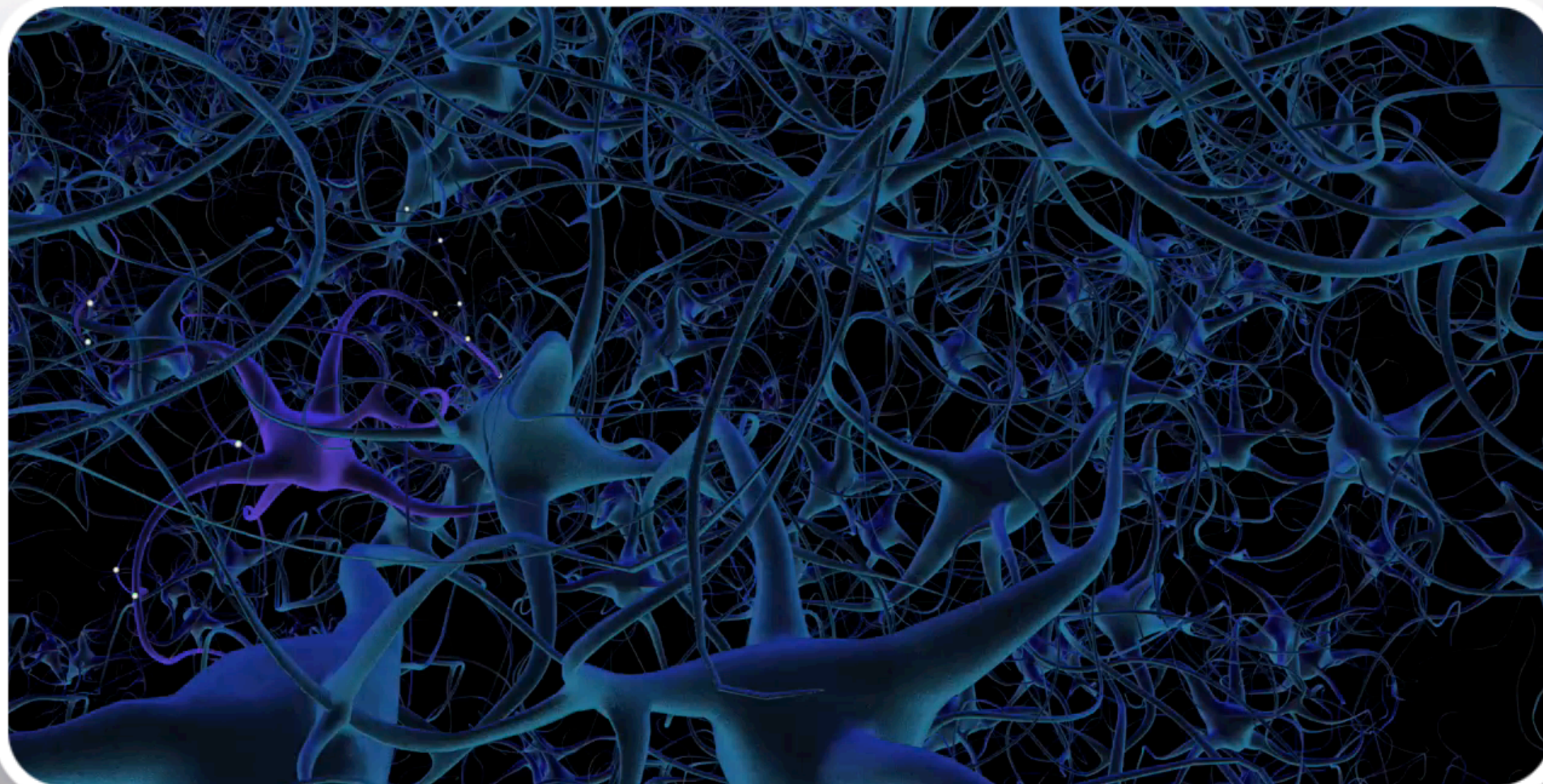
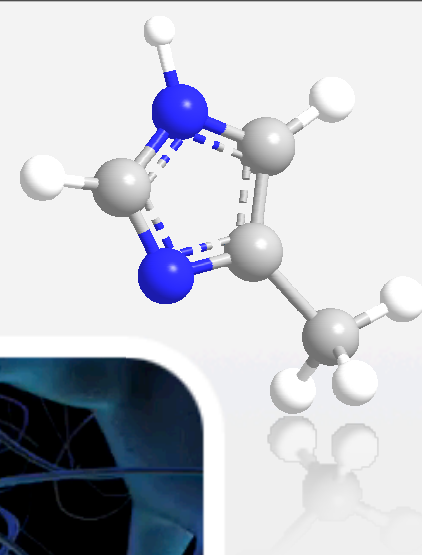
PLE

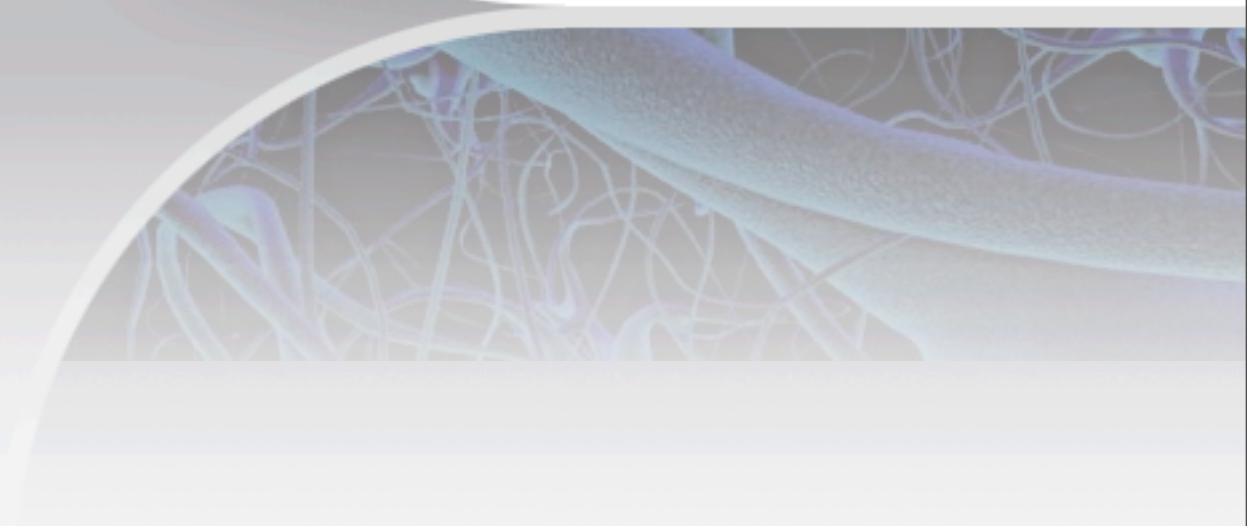
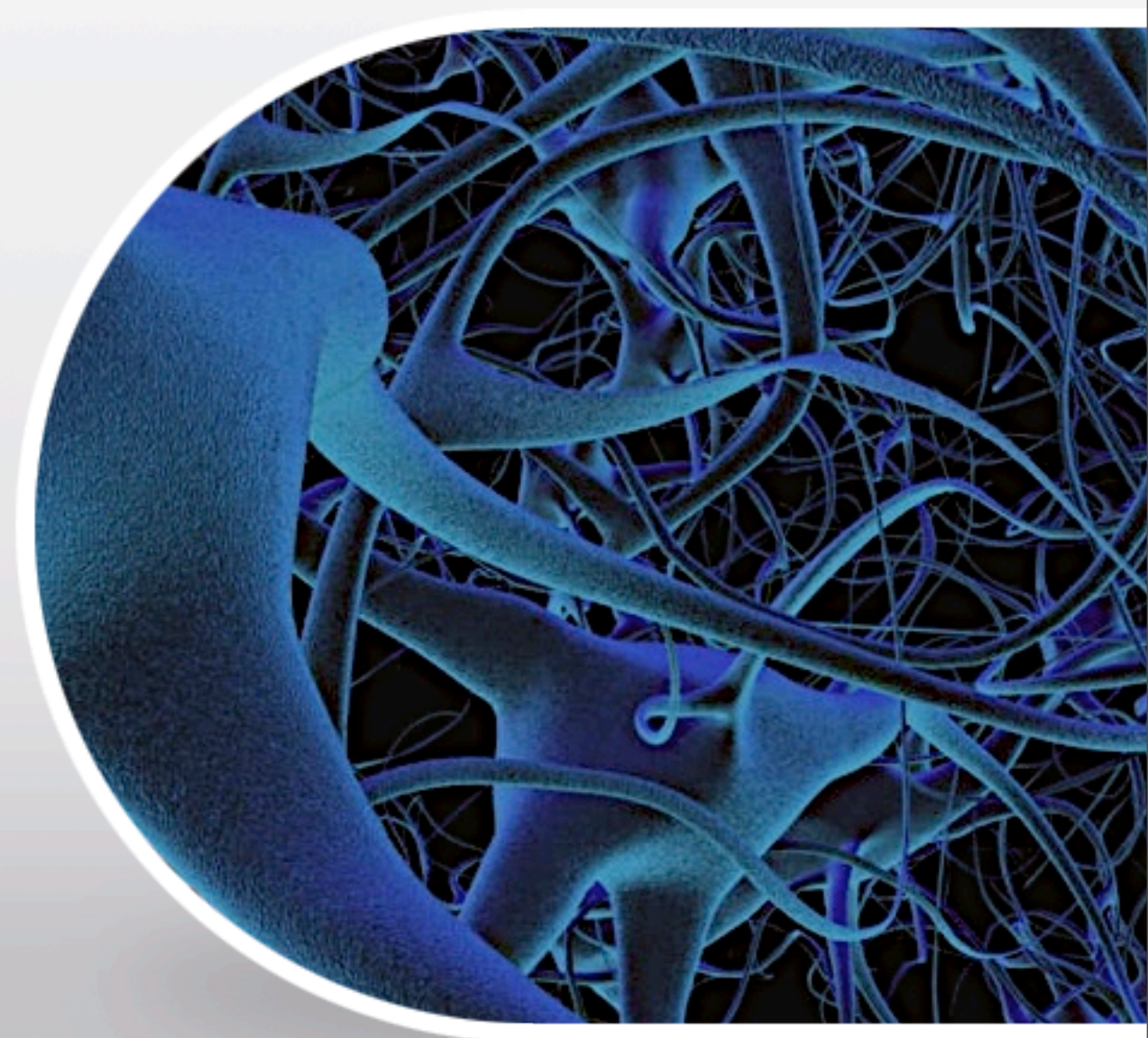
μ SPE

MS/MS

CHROMATOGRAFIE

CHROMATOGRAFIE NEUROTOXINŮ

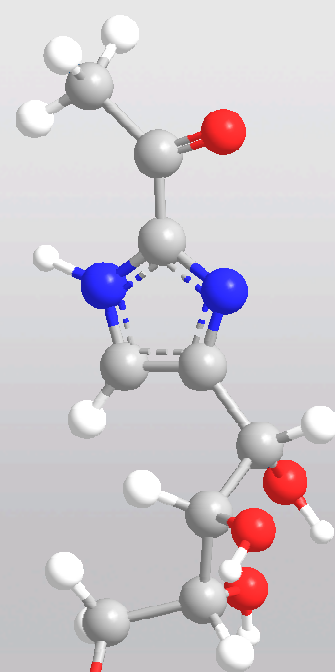
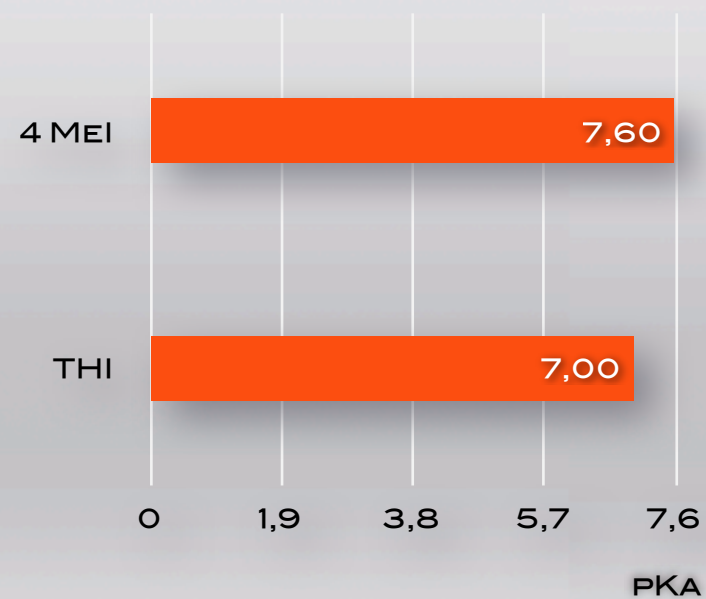




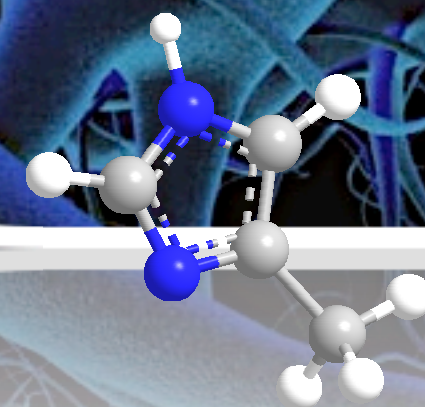
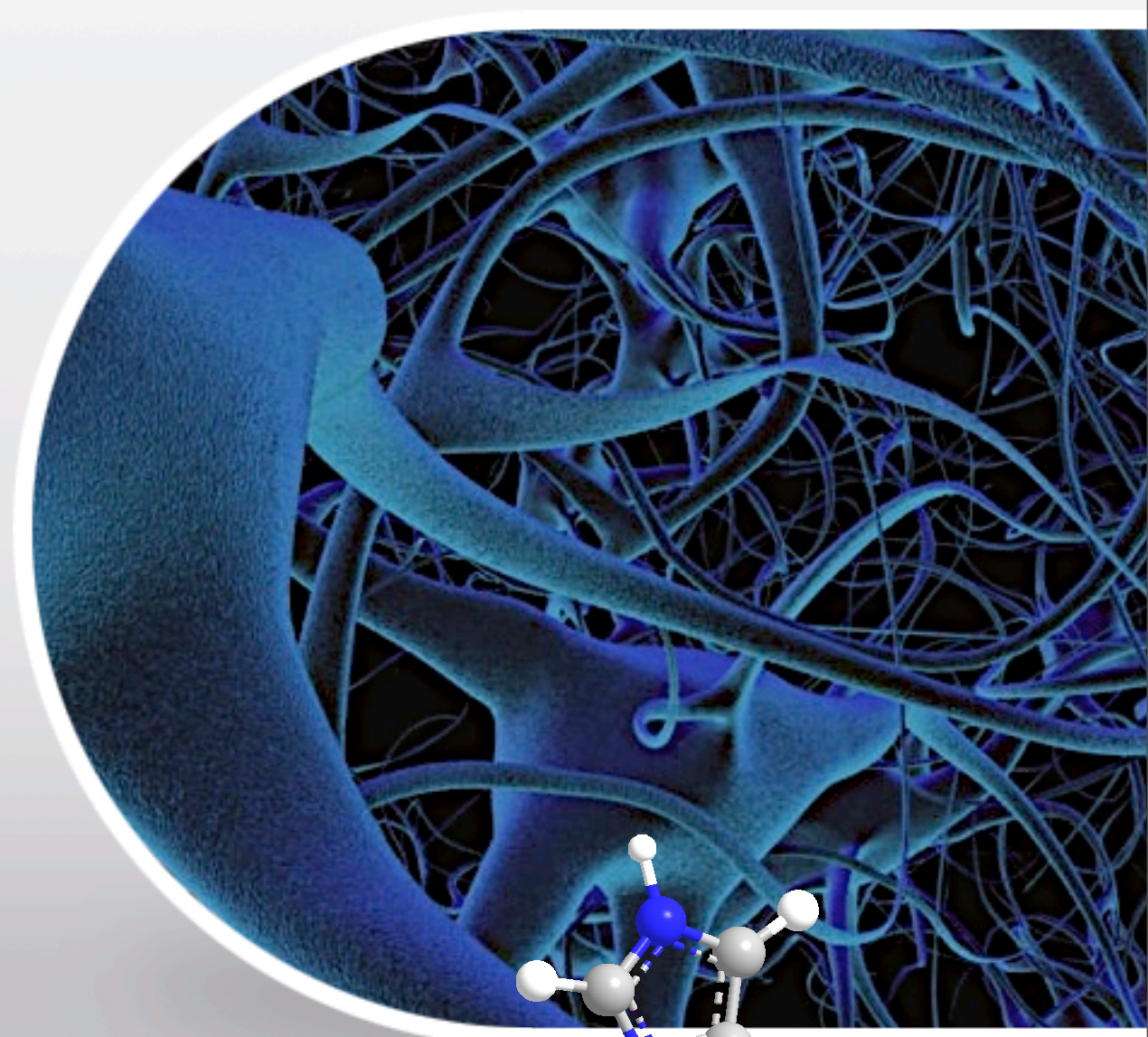
STRATEGIE SEPARACE IMIDAZOLŮ

- VLASTNOSTI IMIDAZOLŮ
- IMIDAZOLY:
VYSOCE POLÁRNÍ ANALYTY
SILNĚ BAZICKÉ

■ PKA THI, 4 MEI



2-ACETYL-4(5)-(1,2,3,4-TETRAHYDROXYBUTYL)-IMIDAZOL



4(5)-METHYLIMIDAZOL

OPTIMALIZACE CHROMATOGRAFICKÝCH PODMÍNEK

VÝBĚR CHROMATOGRAFICKÉ KOLONY:

JEDNODUCHÝ ENDCAPING
 DVOJTÝ ENDCAPING
 TROJTÝ ENDCAPING
 ENDCAPING S VLOŽENOU POLÁRNÍ SKUPINOU

VOLBA MOBILNÍ FÁZE:

VODNÁ FÁZE: H₂O, KYS. OCTOVÁ, MRAVENČÍ, MRAVENČAN (SODNÝ, DRASELNÝ, AMONNÝ), OCTAN (SODNÝ, DRASELNÝ, AMONNÝ), AMONIAK

ORGANICKÁ FÁZE: METHANOL, ACETONITRIL

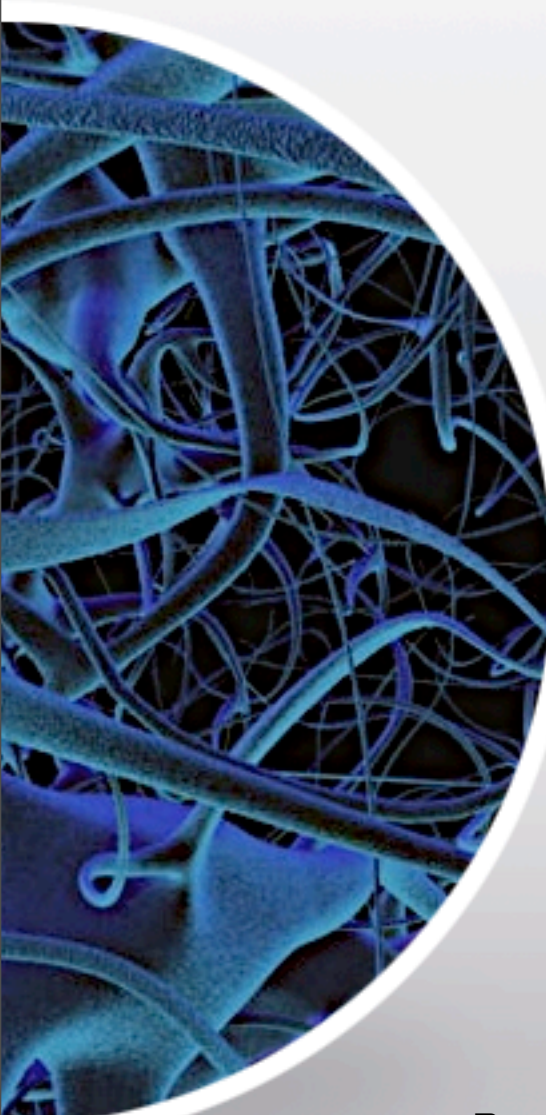
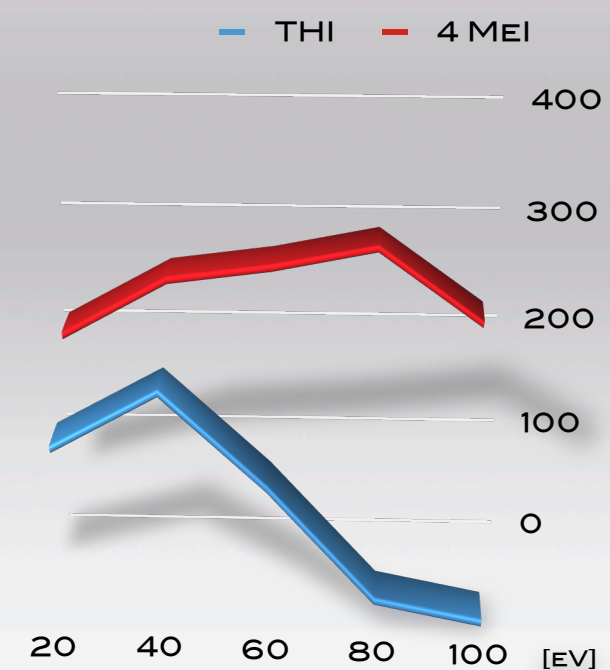
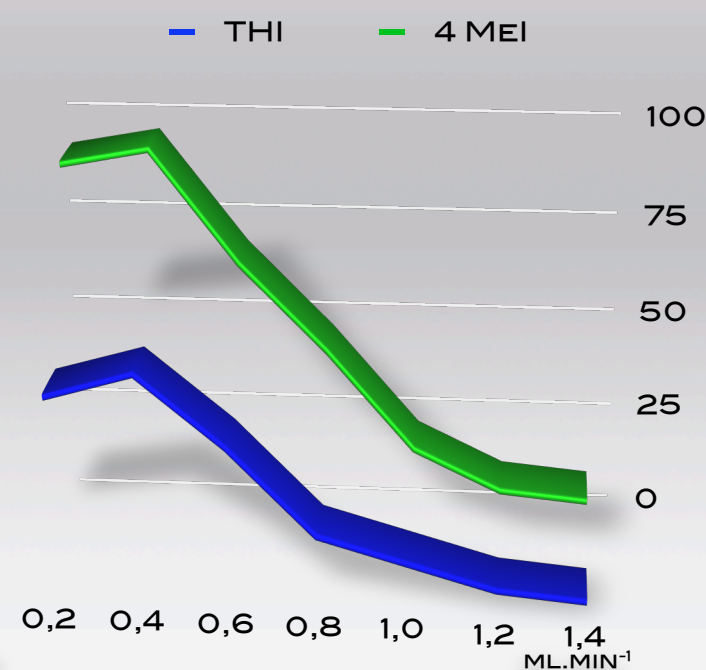
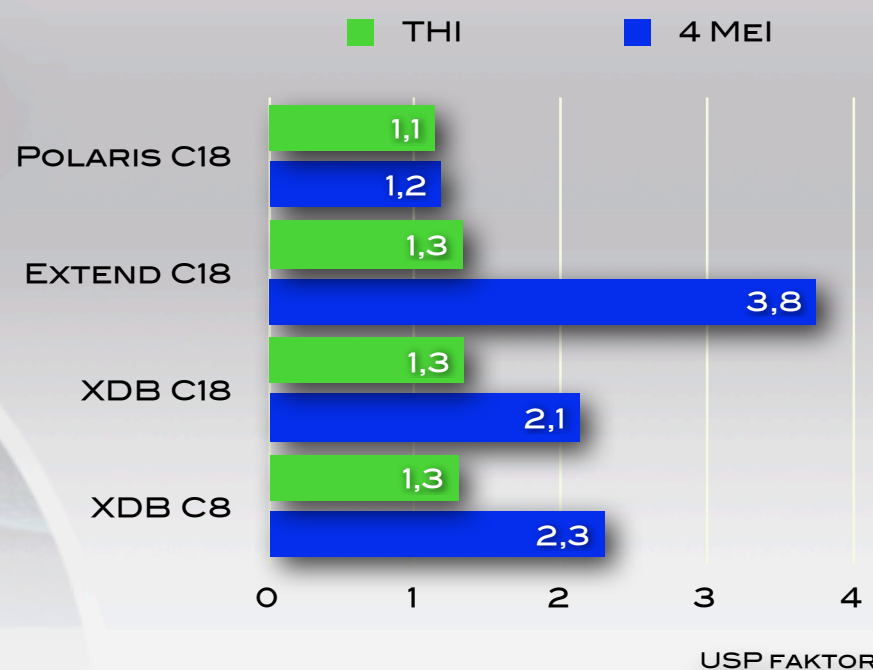
HMOTNOSTNÍ DETEKCE

PARAMETRY LC A MS

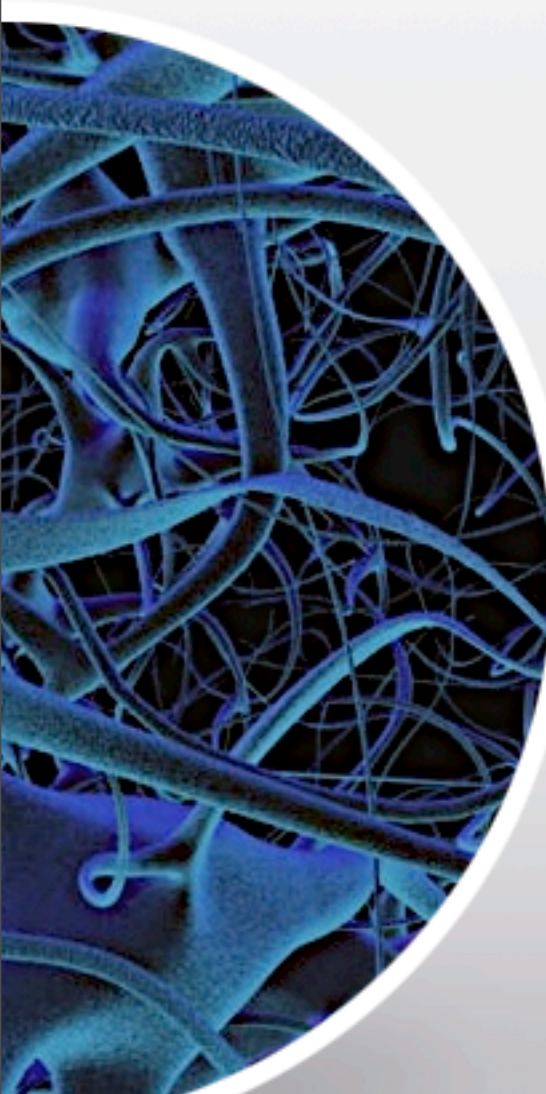
SYMETRIE PÍKŮ

VLIV PRŮTOKOVÉ RYCHLOSTI NA RELATIVNÍ CITLIVOSTI MS SIGNÁLU

VLIV FRAGMENTAČNÍHO NAPĚTÍ NA CITLIVOSTI MS SIGNÁLU



OPTIMALIZACE CHROMATOGRAFICKÝCH PODMÍNEK



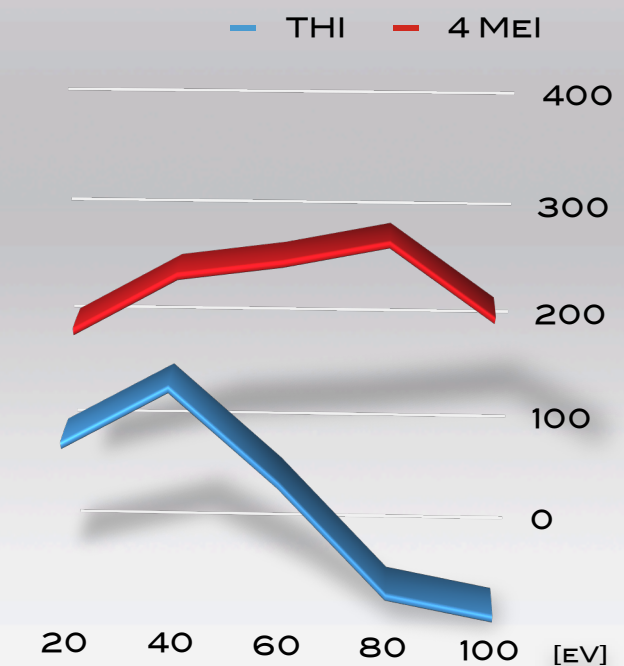
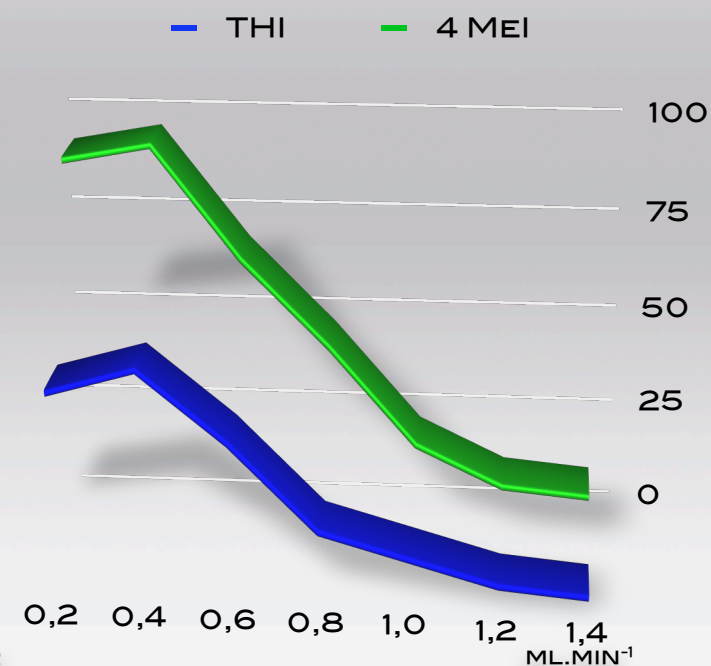
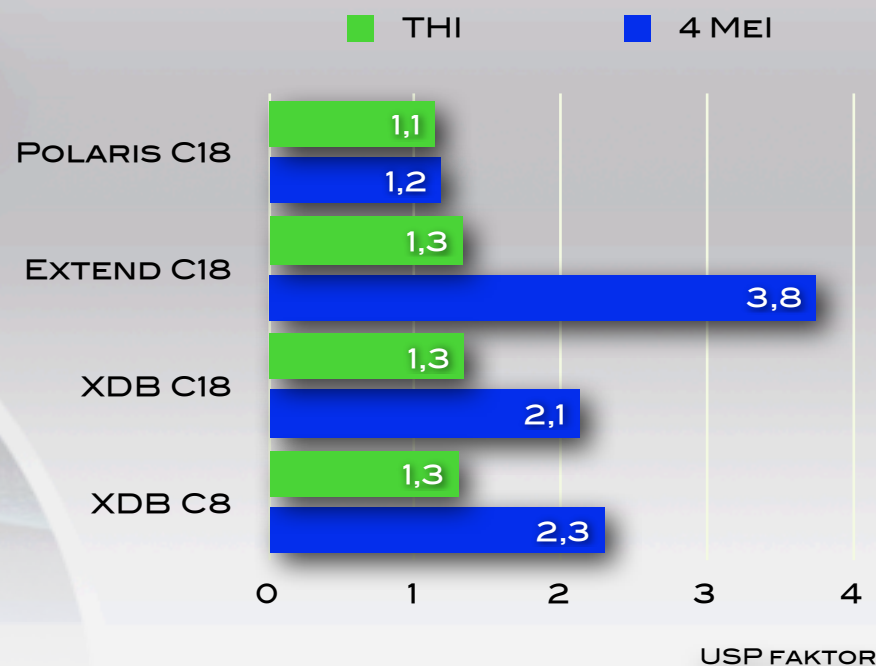
- VÝBĚR CHROMATOGRAFICKÉ KOLONY:
 - JEDNODUCHÝ ENDCAPING
 - DVOJTÝ ENDCAPING
 - TROJTÝ ENDCAPING
 - ENDCAPING S VLOŽENOU POLÁRNÍ SKUPINOU

- VOLBA MOBILNÍ FÁZE:
 - VODNÁ FÁZE: H₂O, KYS. OCTOVÁ, MRAVENČÍ, MRAVENČAN (SODNÝ, DRASELNÝ, AMONNÝ), OCTAN (SODNÝ, DRASELNÝ, AMONNÝ), AMONIAK
 - ORGANICKÁ FÁZE: METHANOL, ACETONITRIL

- HMOTNOSTNÍ DETEKCE

- PARAMETRY LC A MS

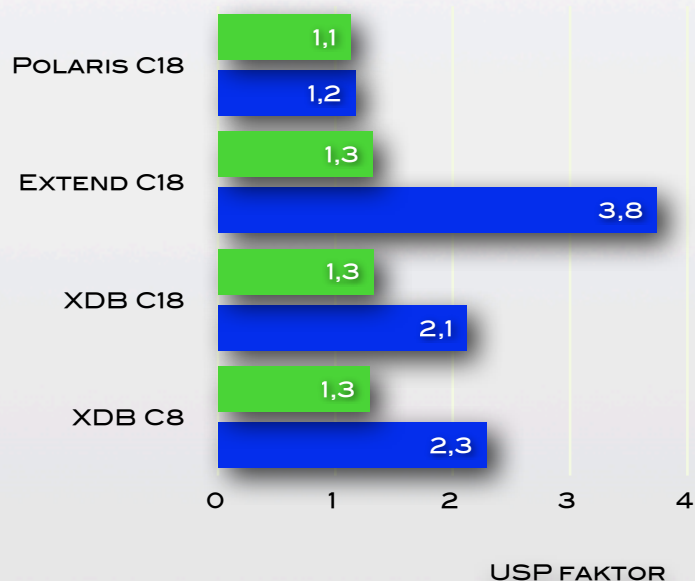
- SYMETRIE PÍKŮ
 - VLIV PRŮTOKOVÉ RYCHLOSTI NA RELATIVNÍ CITLIVOSTI MS SIGNÁLU
 - VLIV FRAGMENTAČNÍHO NAPĚTÍ NA CITLIVOSTI MS SIGNÁLU



CHROMATOGRAFIE IMIDAZOLŮ TEST REVERZNÍCH FÁZÍ

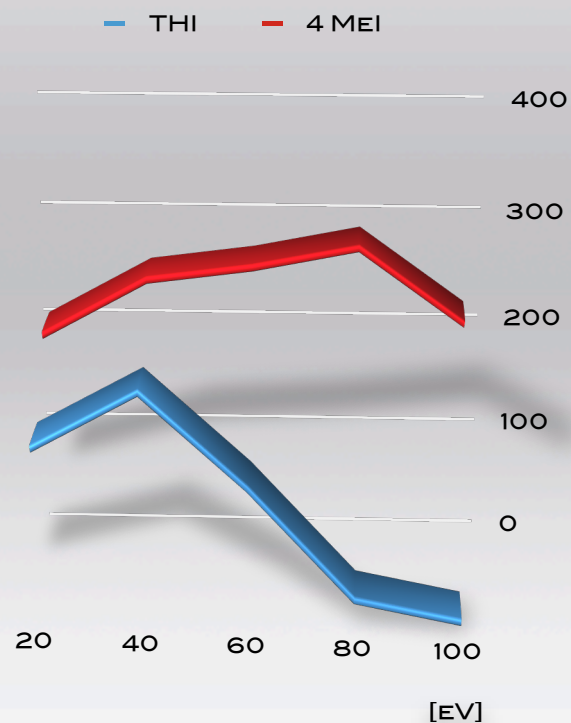
● SYMETRIE PÍKŮ

■ THI ■ 4 MEI



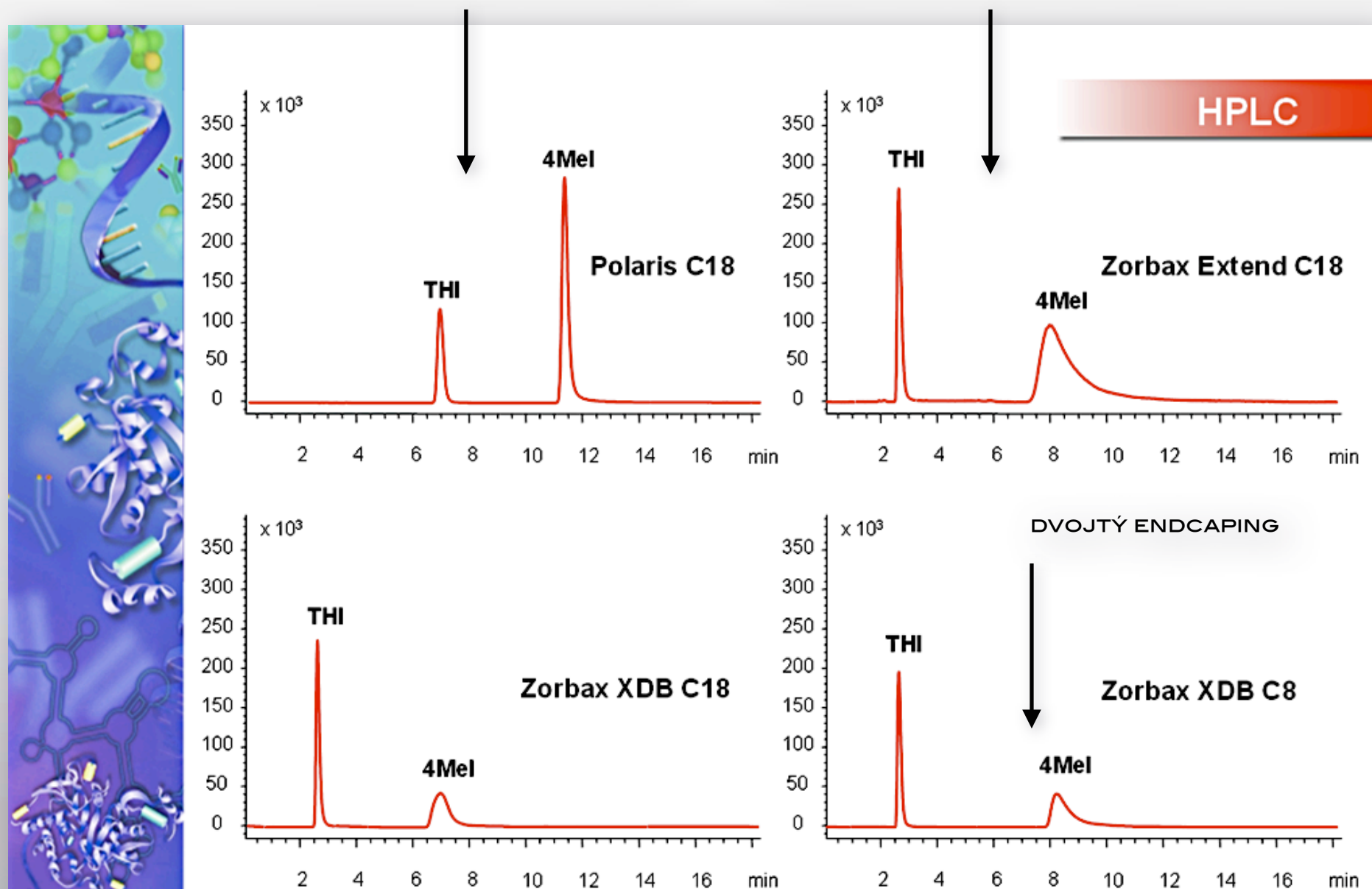
● HMOTNOSTNÍ DETEKCE

VLIV FRAGMENTAČNÍHO NAPĚTÍ NA CITLIVOSTI MS SIGNÁLU



ENDCAPPING S VLOŽENOU POLÁRNÍ SKUPINOU

ENDCAPPING POMOCÍ PROPYLÉNOVÝCH MŮSTKŮ

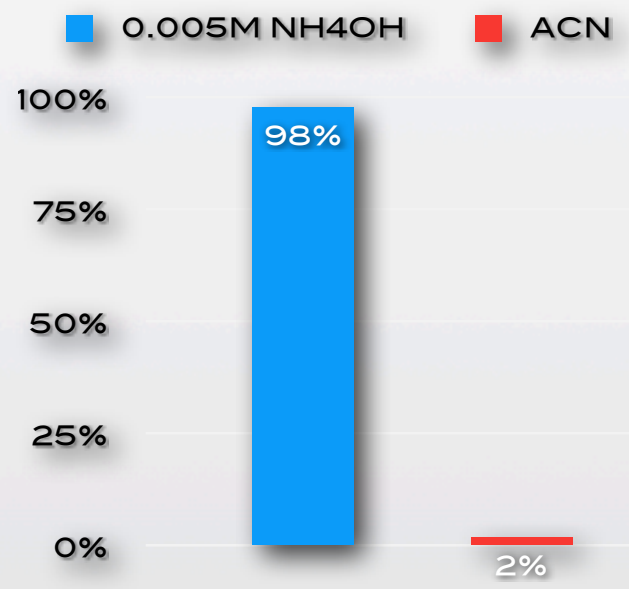


Porovnání stacionárních fází
Chromatografie polárních látek

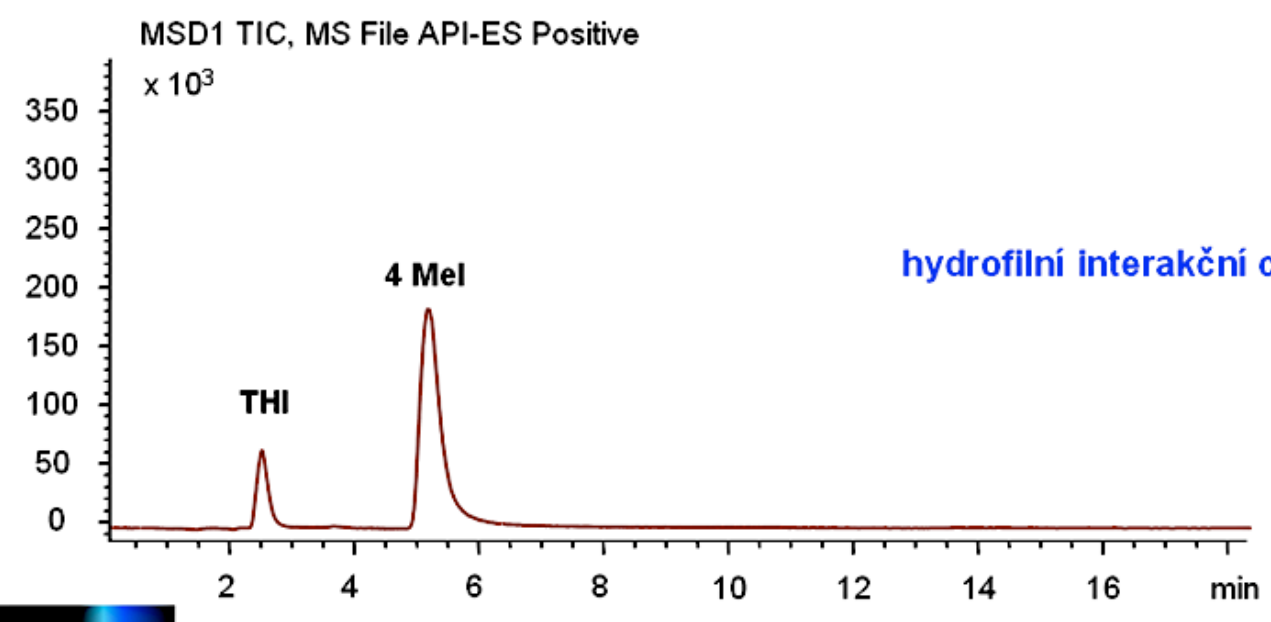
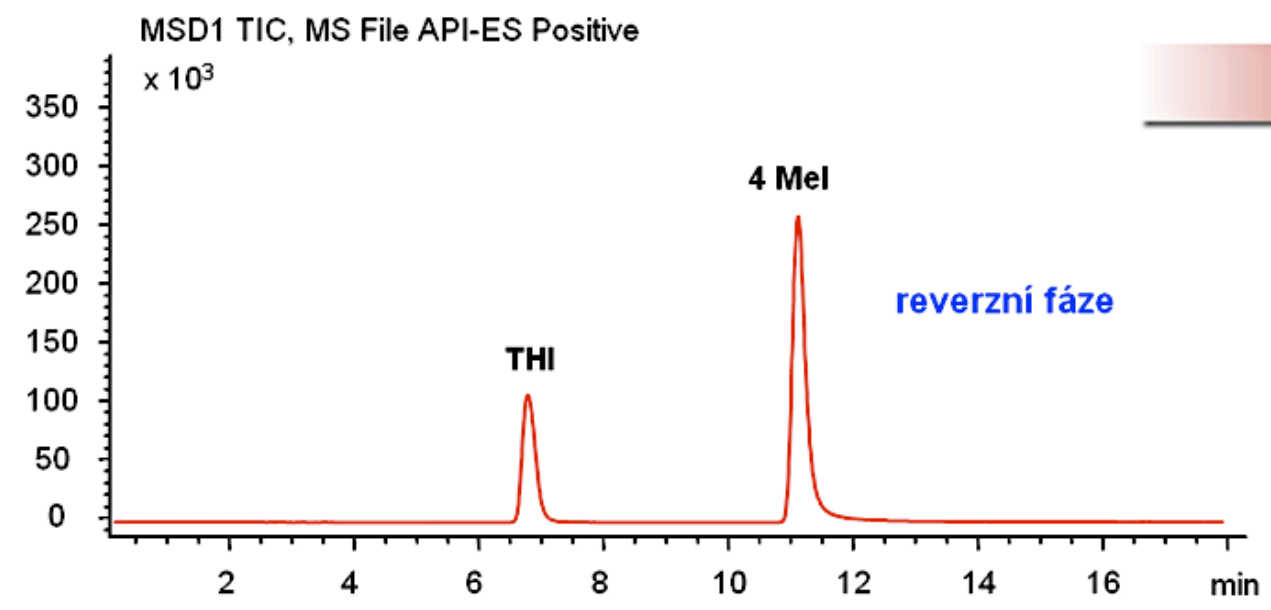
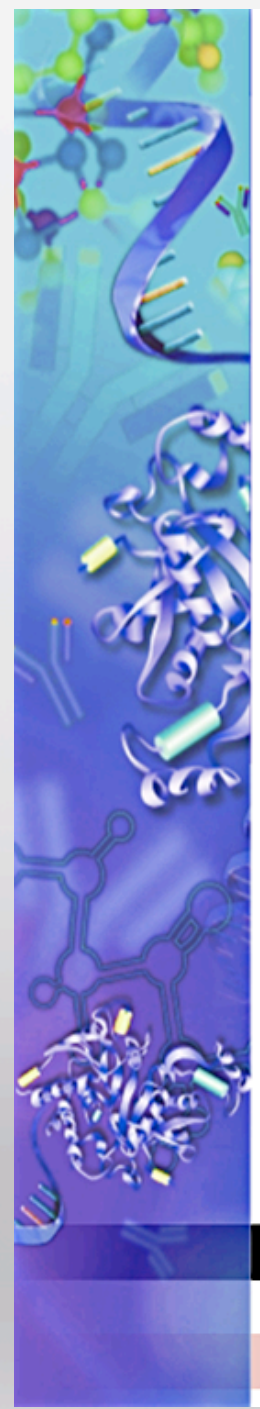
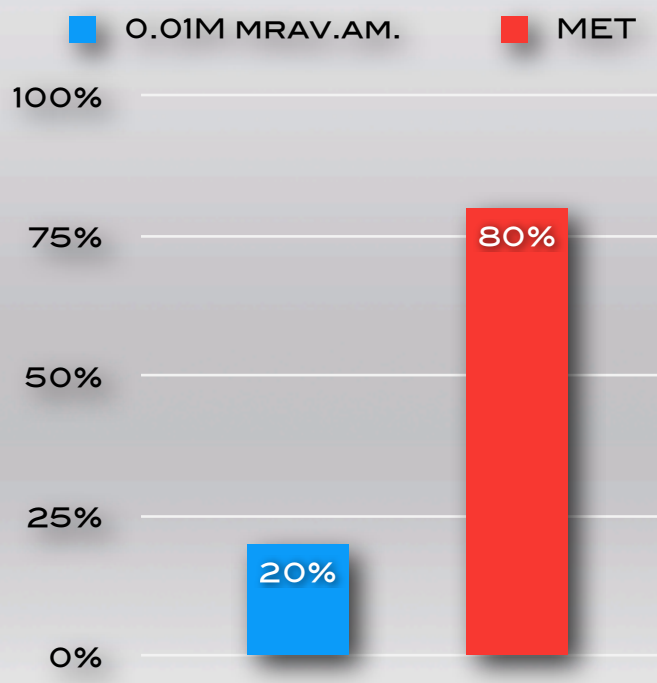
Chromatografie polárních látek
Porovnání stacionárních fází

HYDROFILNÍ INTERAKČNÍ CHROMATOGRRAFIE

REVERZNÍ FÁZE



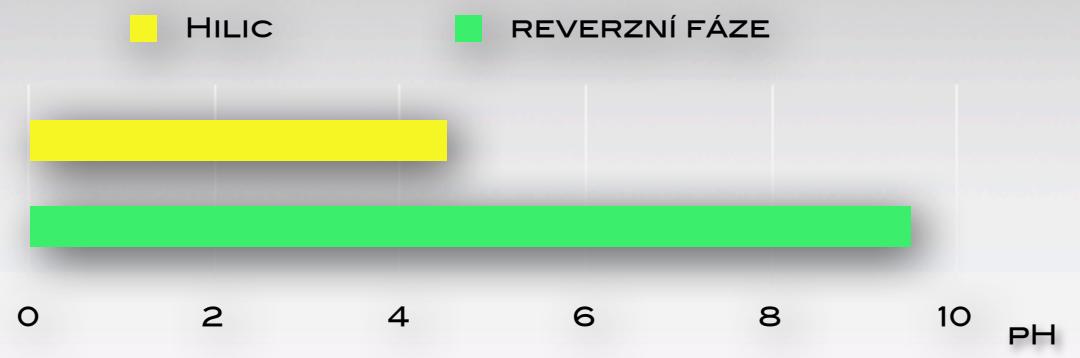
HILIC



Srovnání reverzní fáze – hydrofilní chromatografie

Chromatografie polárních látek

LOD REVERZNÍ FÁZE 3 PG THI 1PG 4 MEI
 LOD HILIC 2 PG THI 1.5 PG 4 MEI



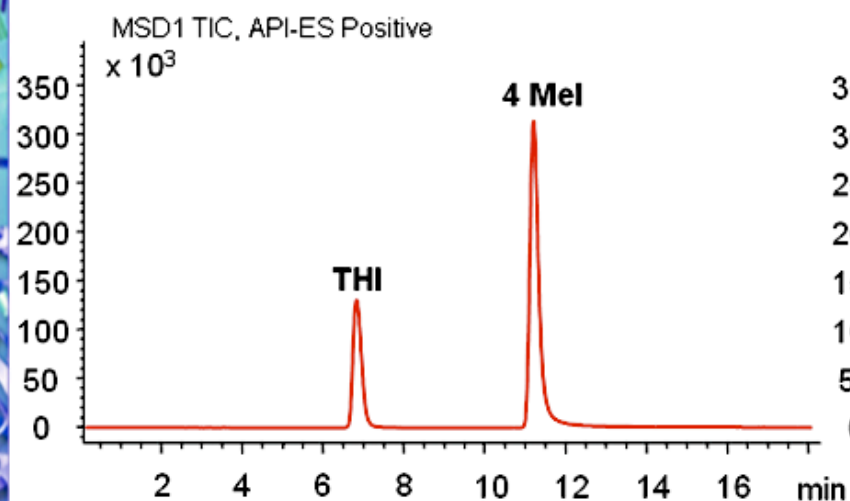
CHROMATOGRÁFIE IMIDAZOLŮ REÁLNÉ VZORKY

HPLC

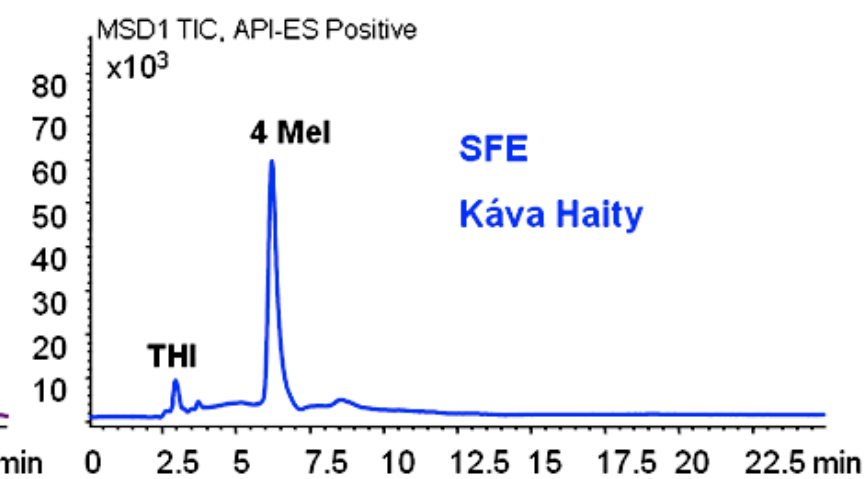
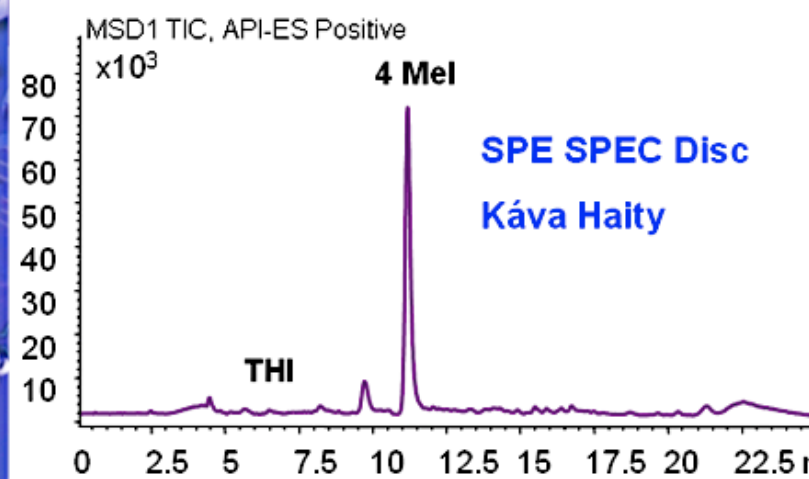
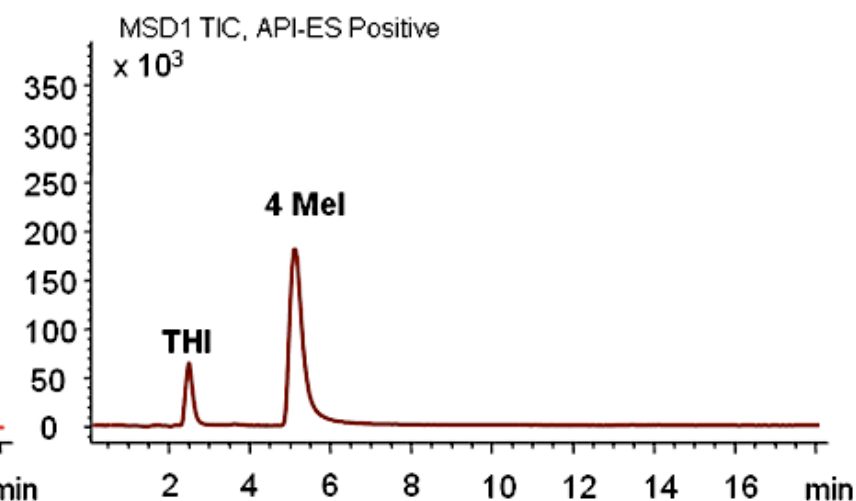
KÁVA	THI $\mu\text{G}\cdot\text{G}^{-1}$	4 MEI
MARILA	0.004	1.05
JIHLAVANKA	0.003	0.72
BRAZIL	0.052	0.60
MEXICO	0.033	0.39
HAITY	0.006	1.38
VITMELTA	0.069	2.05

ČERNÉ PIVO	NG.ML ⁻¹	
SAMSON	9.40	3.69
STAROPRAMEN	7.47	10.51
KRUŠOVICE	3.45	3.81
BERNARD	12.28	12.70
GUINES	3.24	4.05
VELKOPOP.KOZEL	13.19	1.58
STAROBRNO	8.93	28.03

Reverzní fáze s vloženou polární skupinou



Hydrofilní interakční chromatografie



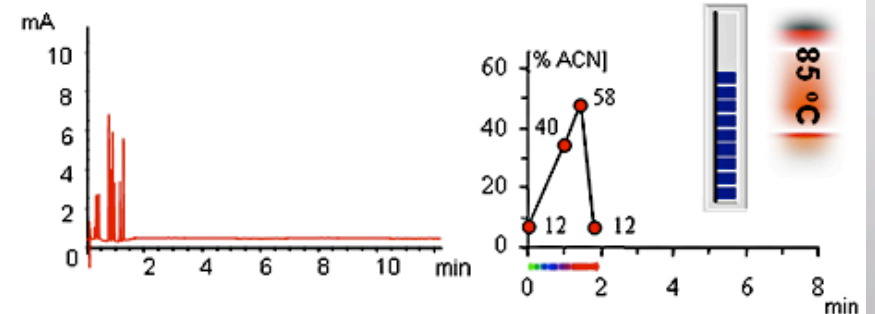
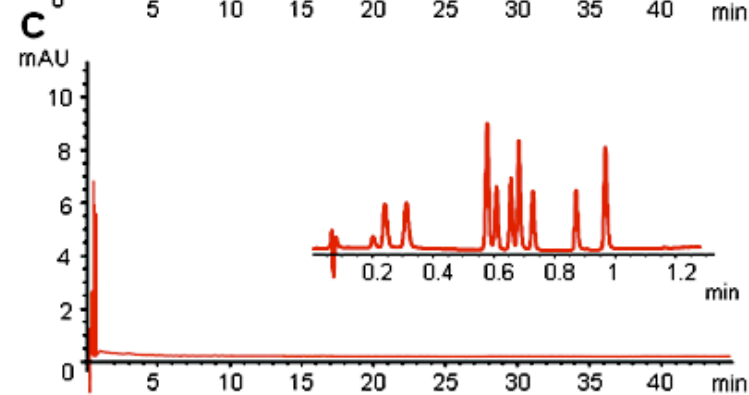
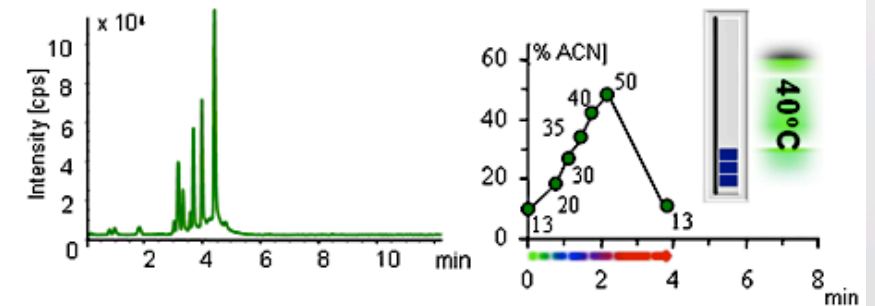
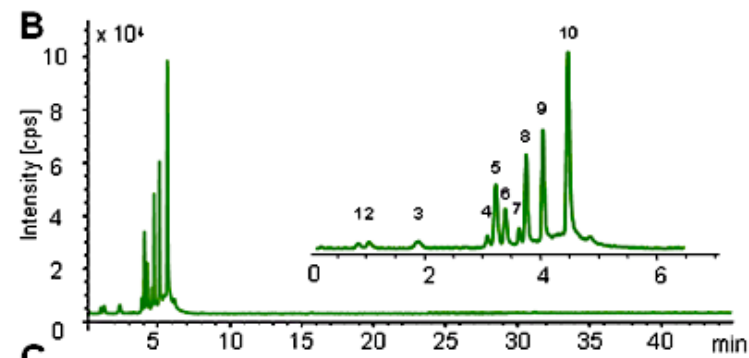
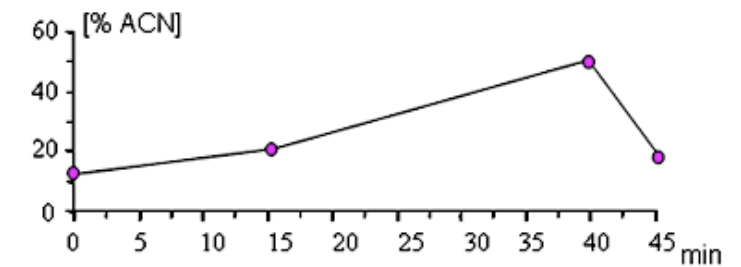
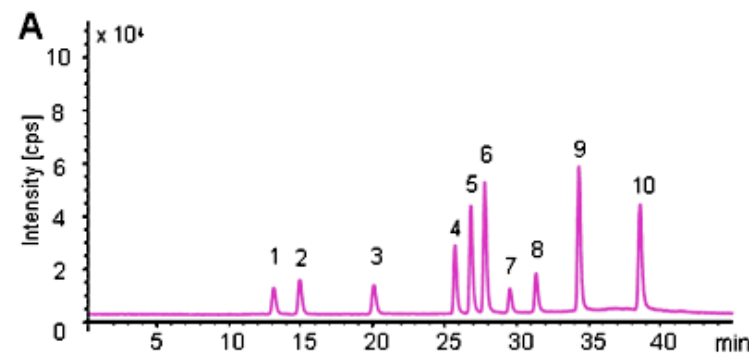
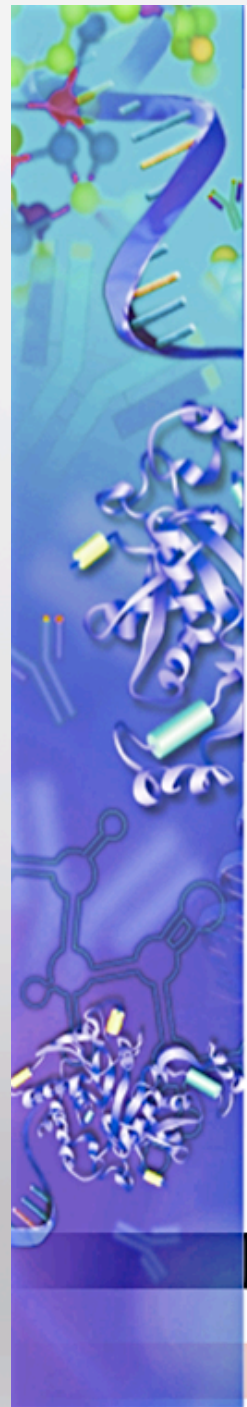
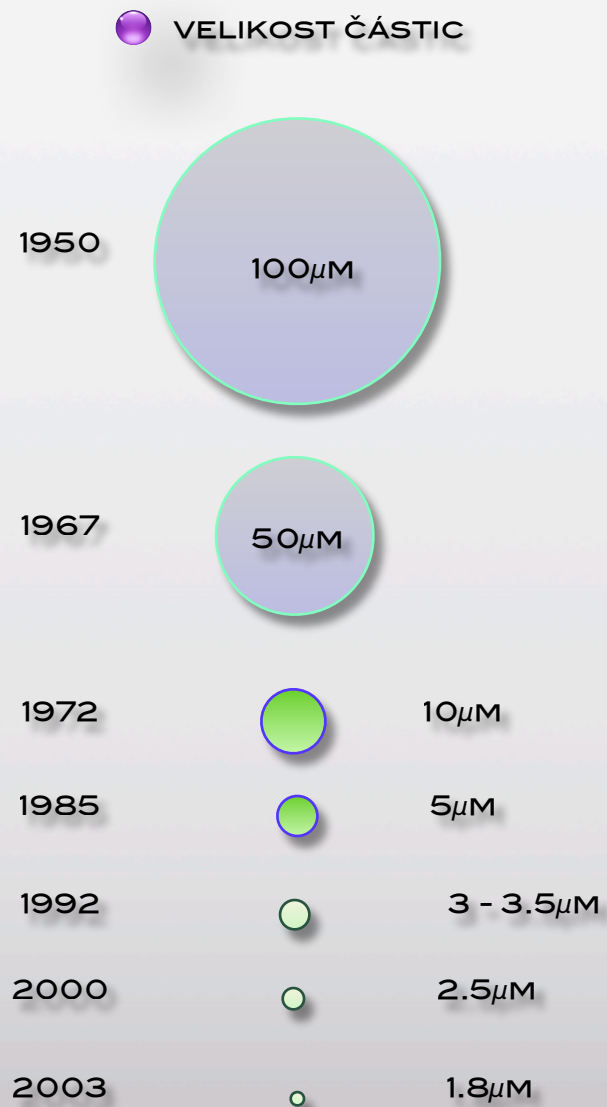
Srovnání reverzní fáze – hydrofilní chromatografie

Chromatografie polárních látek

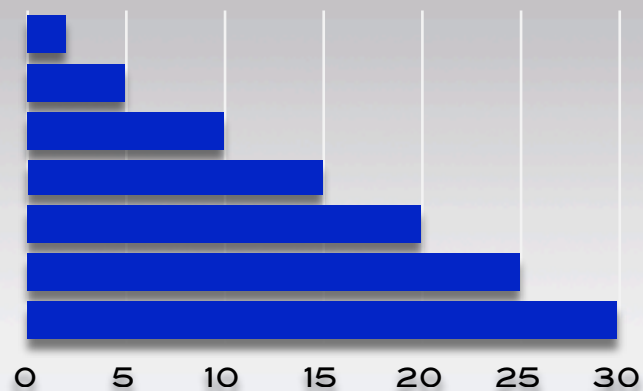
● LIMIT TOXICITY: 10 MG THI 200 MG 4 MEI VZTAŽENO NA KG CLASS III. CAMEL ●

ULTRA RYCHLÁ CHROMATOGRRAFIE

ULTRA RYCHLÁ CHROMATOGRRAFIE



■ DÉLKA KOLONY (CM)



Vývoj gradientu

Ultra rychlá chromatografie

● PRŮTOK

● A. 150X2.1; 3 μm ; PRŮTOK 0.2 ML.MIN⁻¹

1.DAIDZIN
2.GLYCITIN
3.GENISTIN
4.ONONIN
5.DAIDZEIN

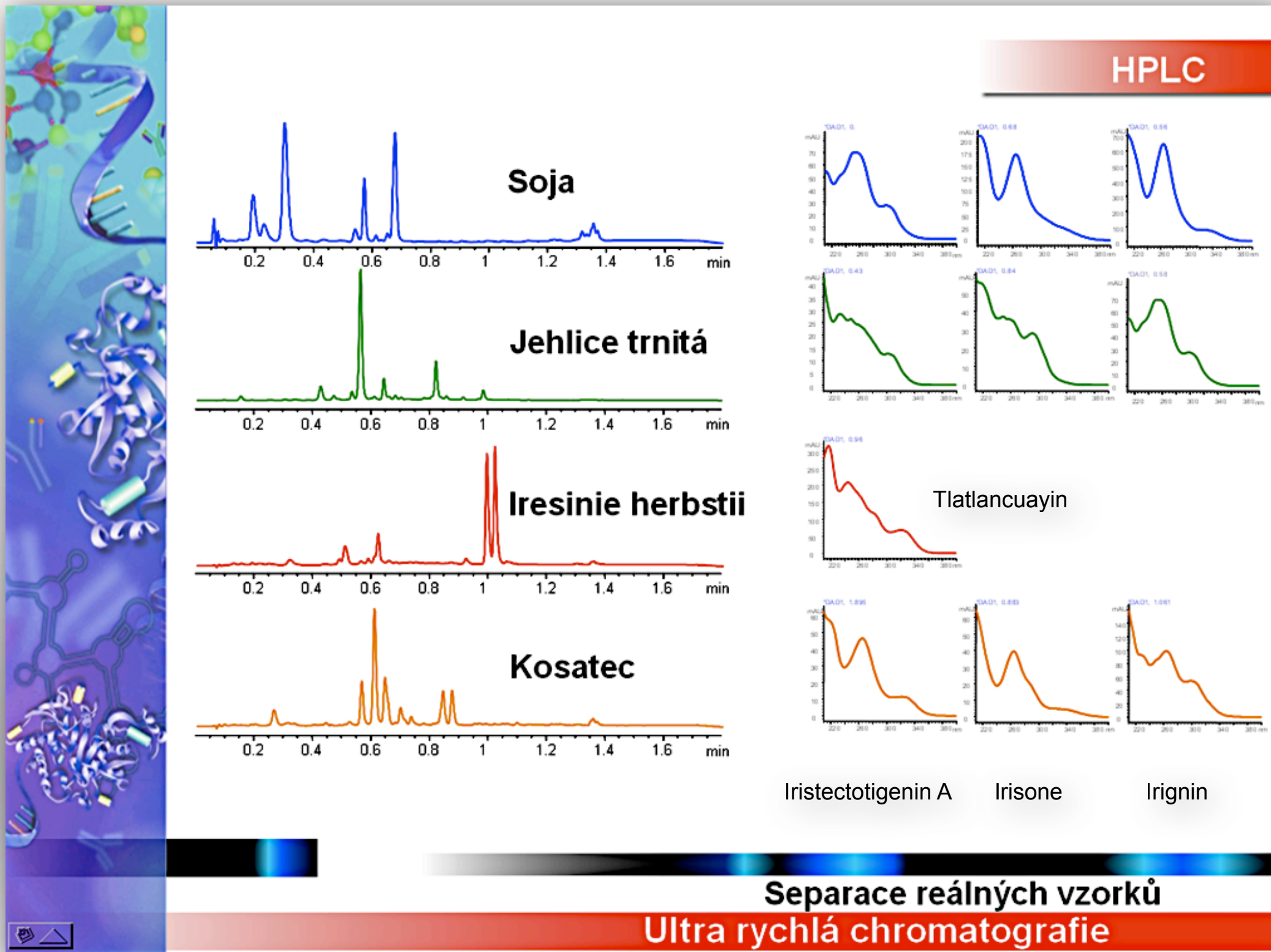
6.GLYCITEIN
7.SISSOTRIN
8.GENISTEIN
9.FORMONONETIN
10.BIOCHANINA

● TEPLOTA

● B. 20X2.1; 3 μm ; PRŮTOK 0.68 ML.MIN⁻¹

● C. 30X2.1; 1.8 μm ; PRŮTOK 1.3 ML.MIN⁻¹

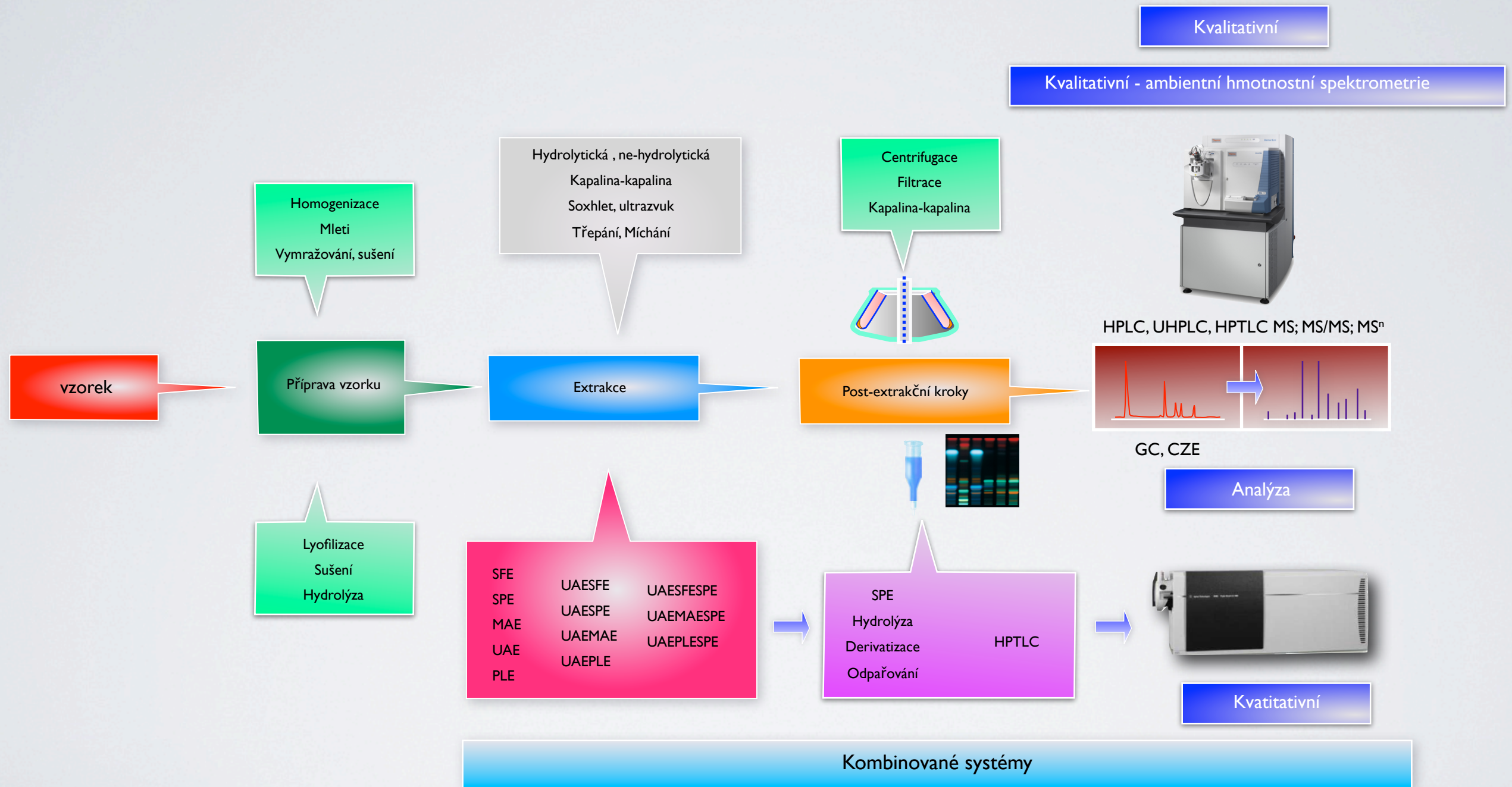
ULTRA RYCHLÁ CHROMATOGRRAFIE - SEPARACE IZOFLAVONŮ Z REÁLNÝCH VZORKŮ



● 30X2.1;1.8 μ M ; PRŮTOK 1.3 ML.MIN⁻¹, TEPLOTA 85°C

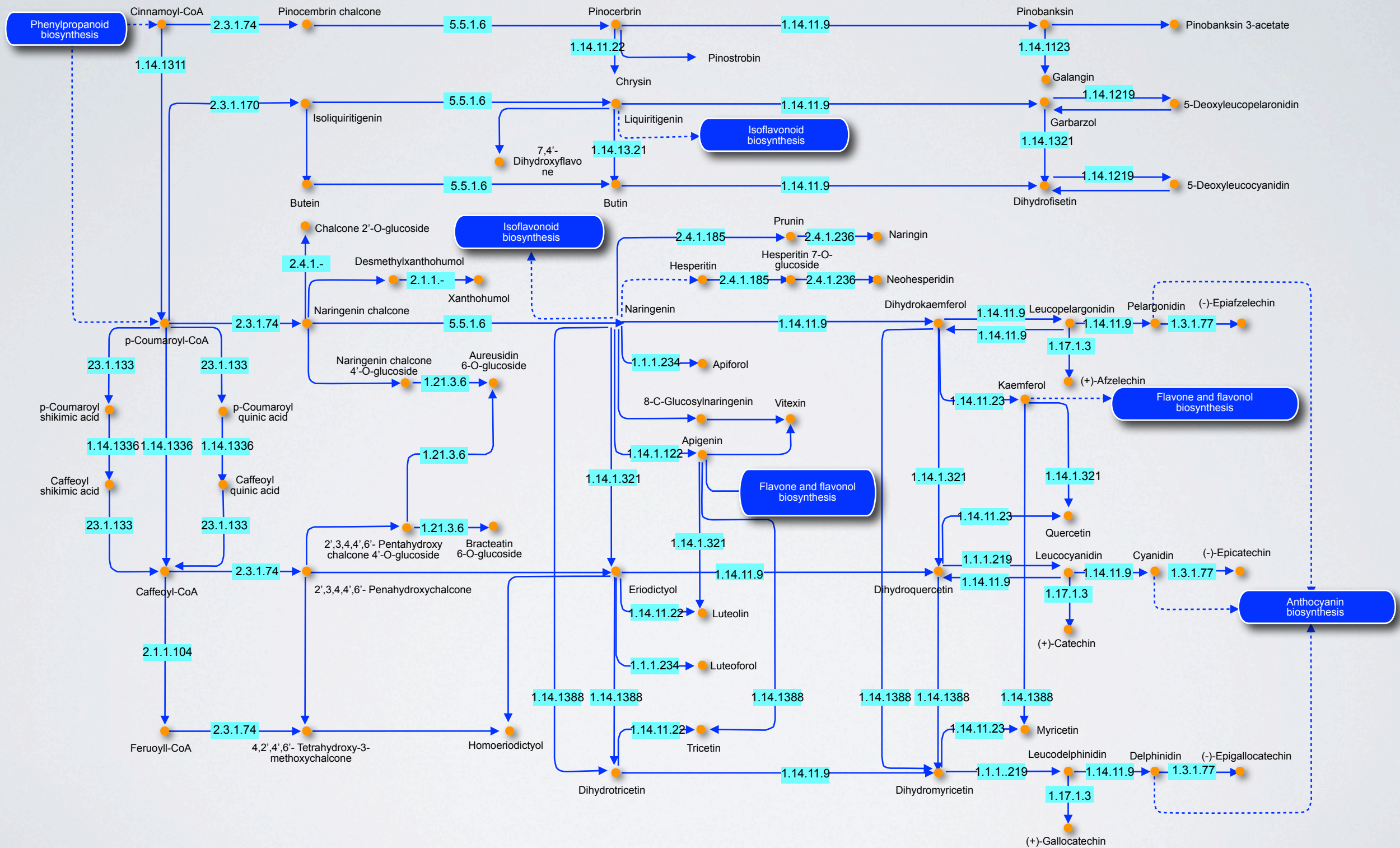
KOMBINOVANÉ SYSTÉMY

STRATEGIE EXTRAKCE PRO KOMBINOVANÉ SYSTÉMY



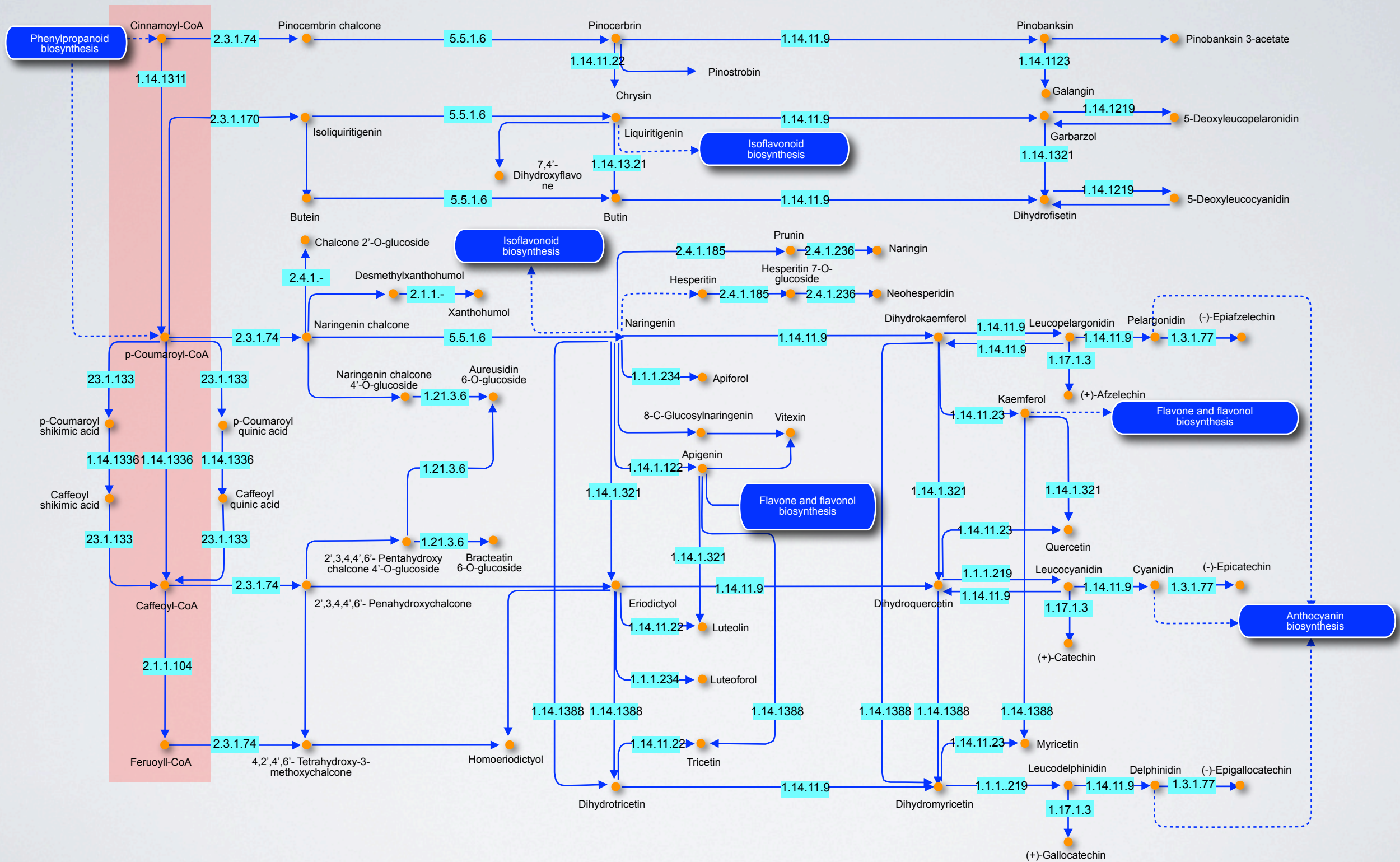
STUDIUM ROSTLINNÝCH METABOLITŮ

BIOSYNTÉZA FLAVONOIDŮ



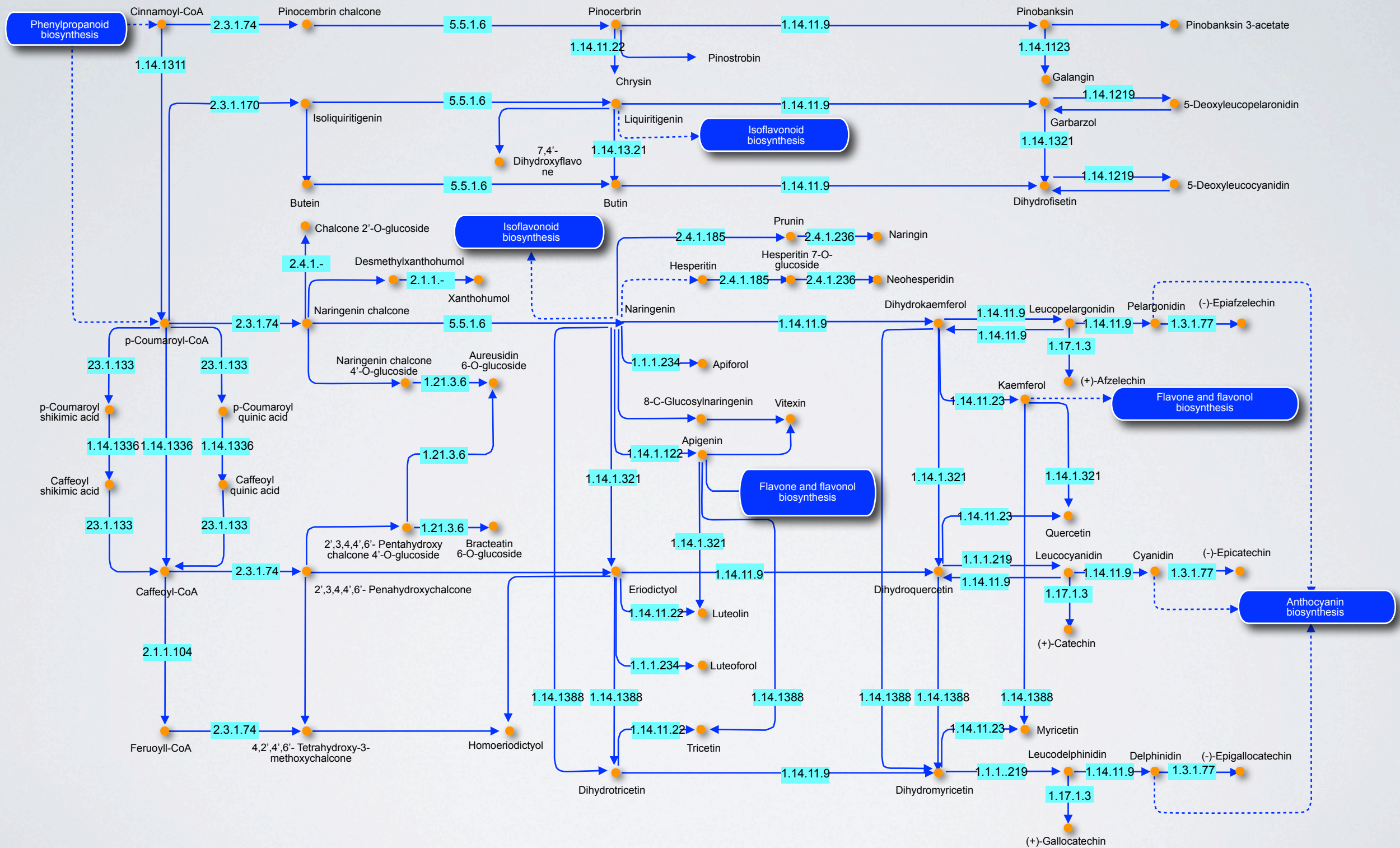
STUDIUM ROSTLINNÝCH METABOLITŮ

BIOSYNTÉZA FLAVONOIDŮ



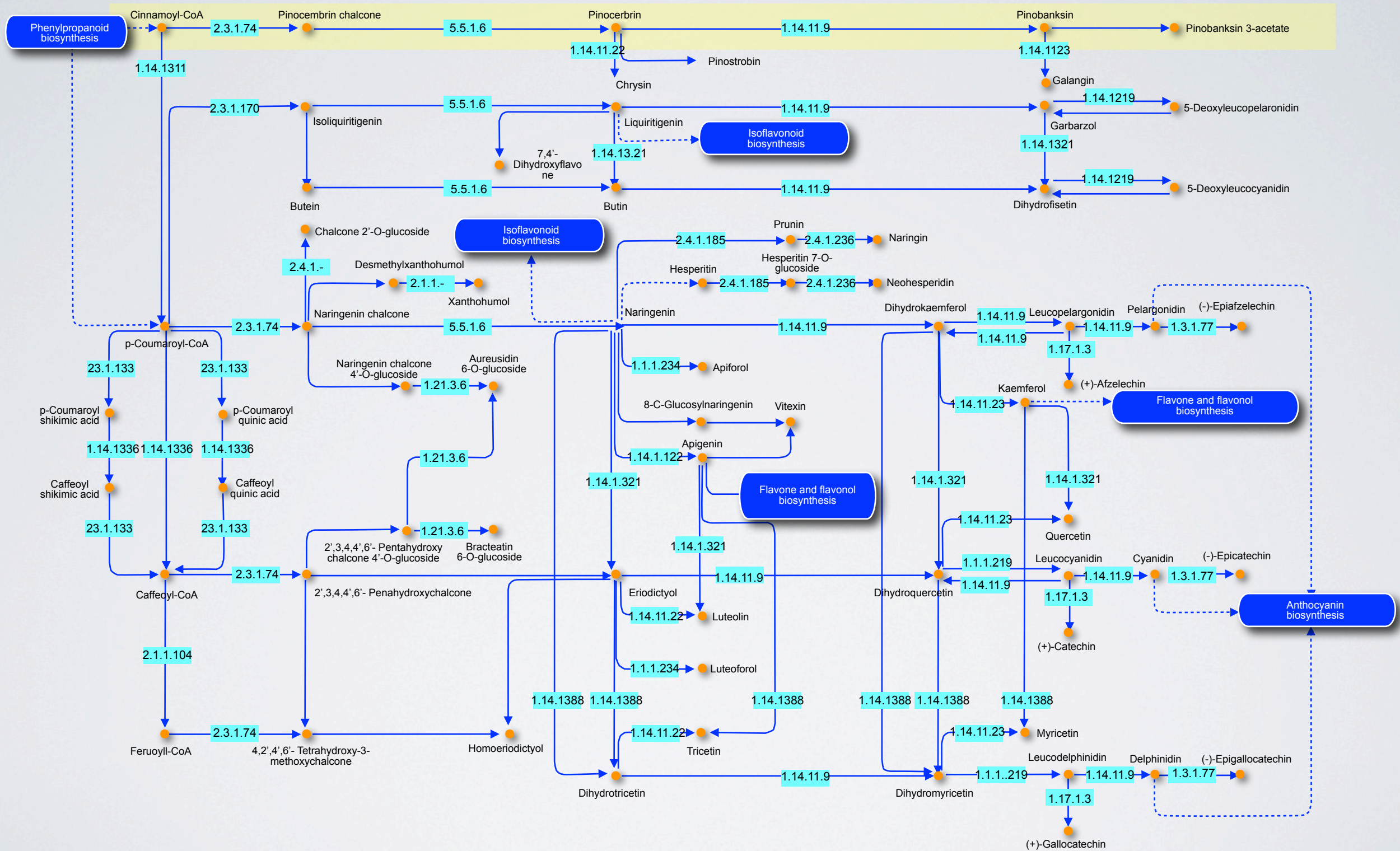
STUDIUM ROSTLINNÝCH METABOLITŮ

BIOSYNTÉZA FLAVONOIDŮ



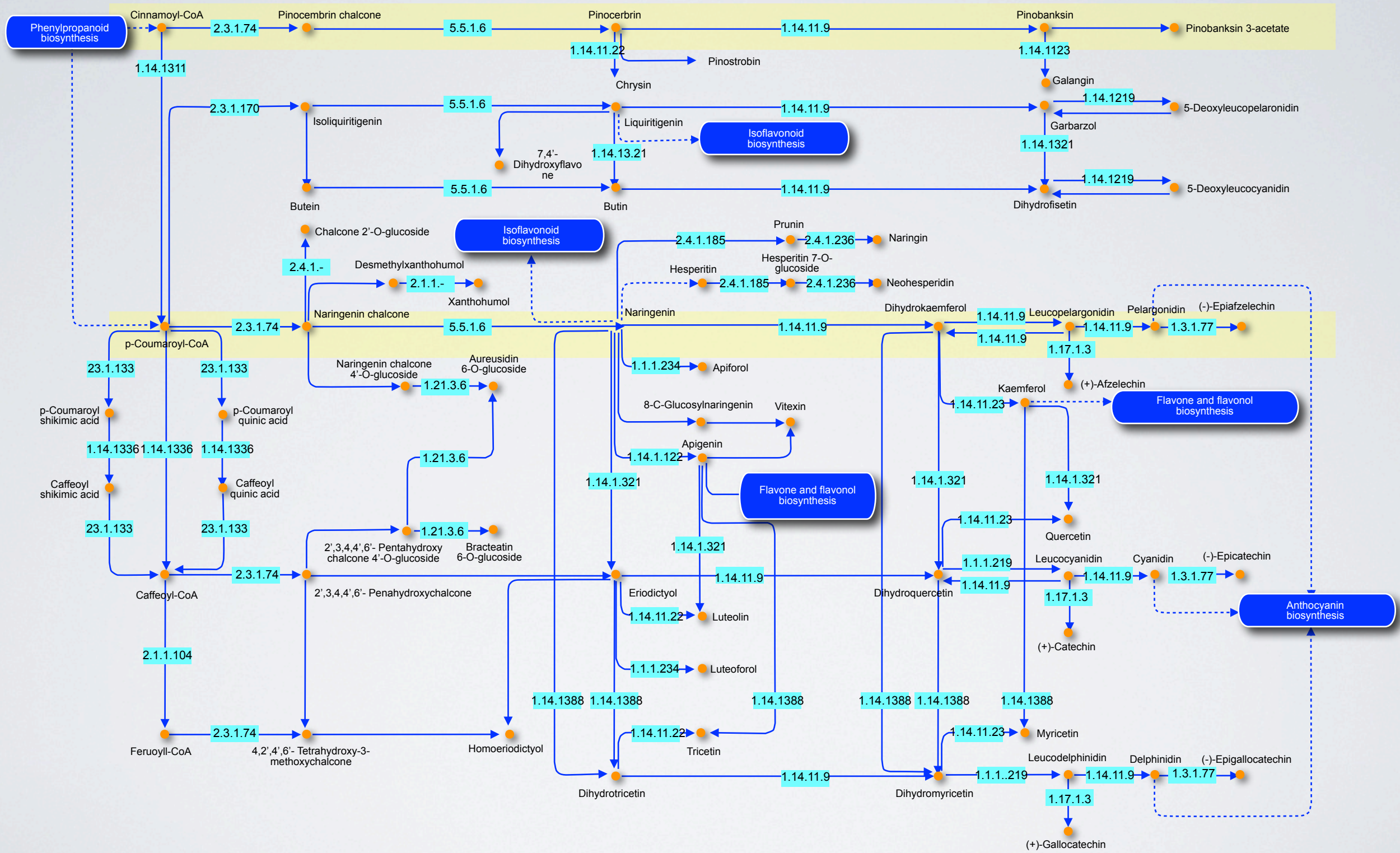
STUDIUM ROSTLINNÝCH METABOLITŮ

BIOSYNTÉZA FLAVONOIDŮ



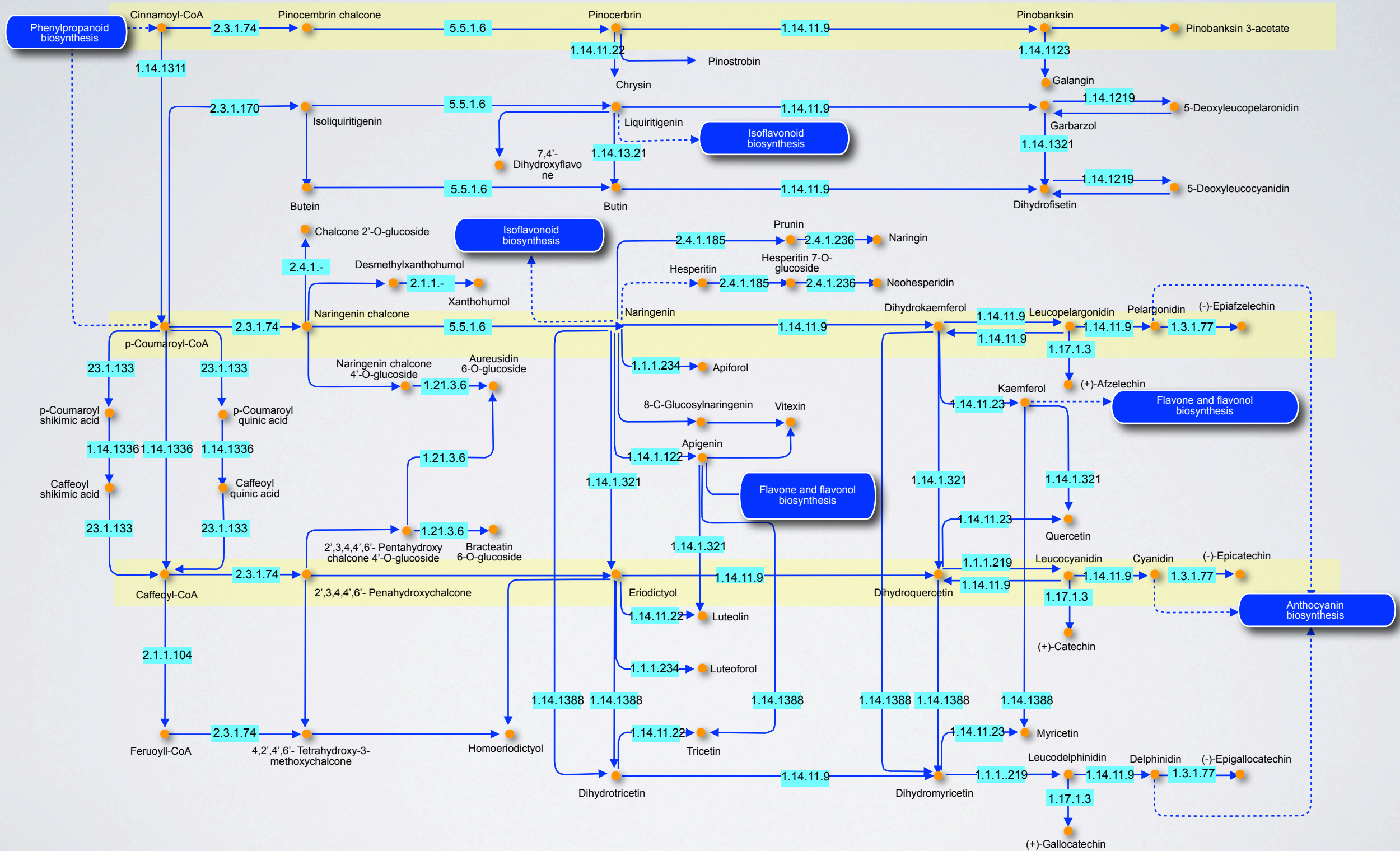
STUDIUM ROSTLINNÝCH METABOLITŮ

BIOSYNTÉZA FLAVONOIDŮ



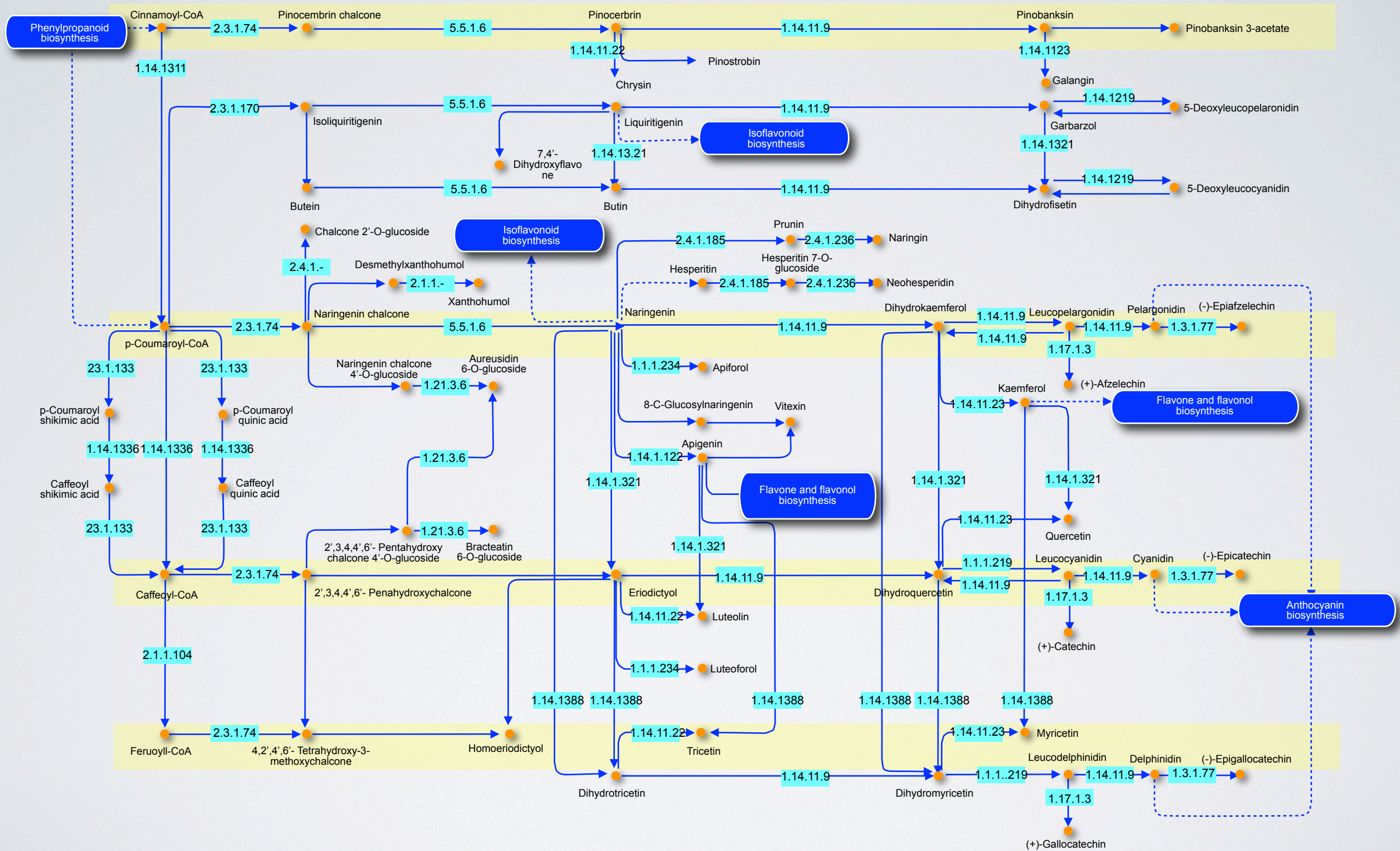
STUDIUM ROSTLINNÝCH METABOLITŮ

BIOSYNTÉZA FLAVONOIDŮ



STUDIUM ROSTLINNÝCH METABOLITŮ

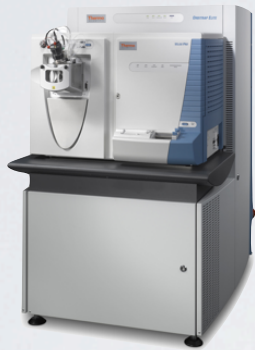
BIOSYNTÉZA FLAVONOIDŮ



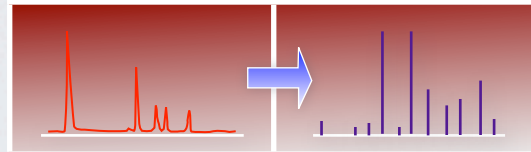
METABOLICKÉ STUDIE POMOCÍ HNOTNOSTNÍ SPEKTROMETRIE

Kvalitativní - přesná hmota

Kvalitativní - ambientní hmotnostní spektrometrie



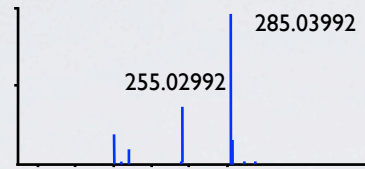
HPLC, UHPLC, HPTLC MS; MS/MS; MSⁿ



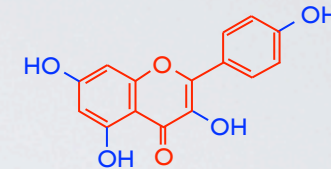
Analýza



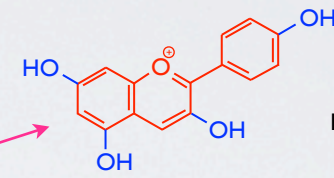
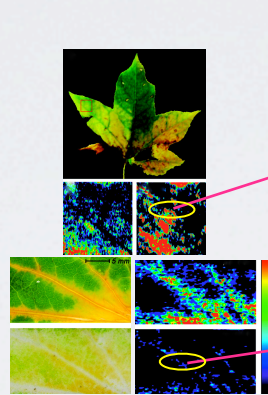
Kvantitativní



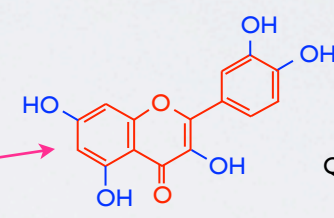
285.03992 C₁₅H₉O₆
255.02992 C₁₄H₇O₅



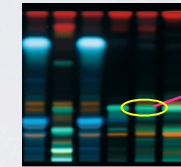
Kaemferol



Pelargonin



Quercetin



Proanthocyanidin dimer B1

Ambientní hmotnostní spektrometrie

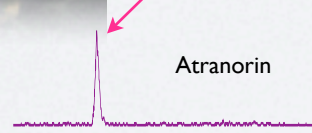
HPTLC MS s ambientní technikami

Lišejníkové metabolity



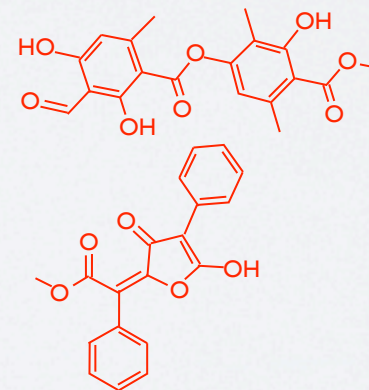
100 attomol

Atranorin



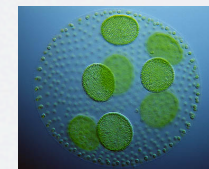
10 attomol

Vulpinic acid



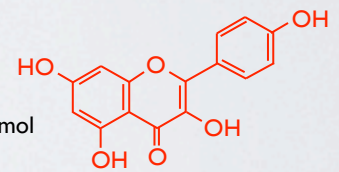
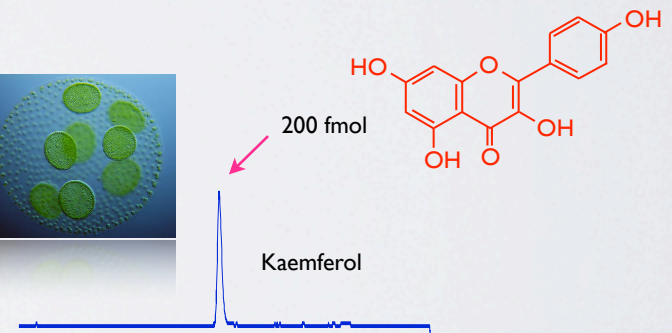
MS/MS (QQQ) s Jet Stream Technologies

Flavonoidy v řasách



200 fmol

Kaemferol



METABOLICKÉ STUDIE POMOCÍ HNOTNOSTNÍ SPEKTROMETRIE

AMBIENTNÍ TECHNIKY

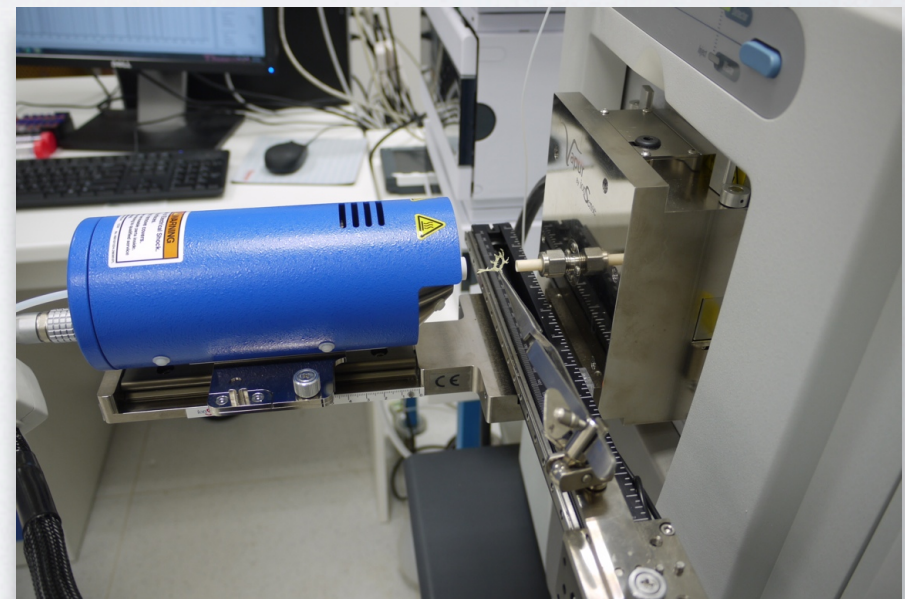
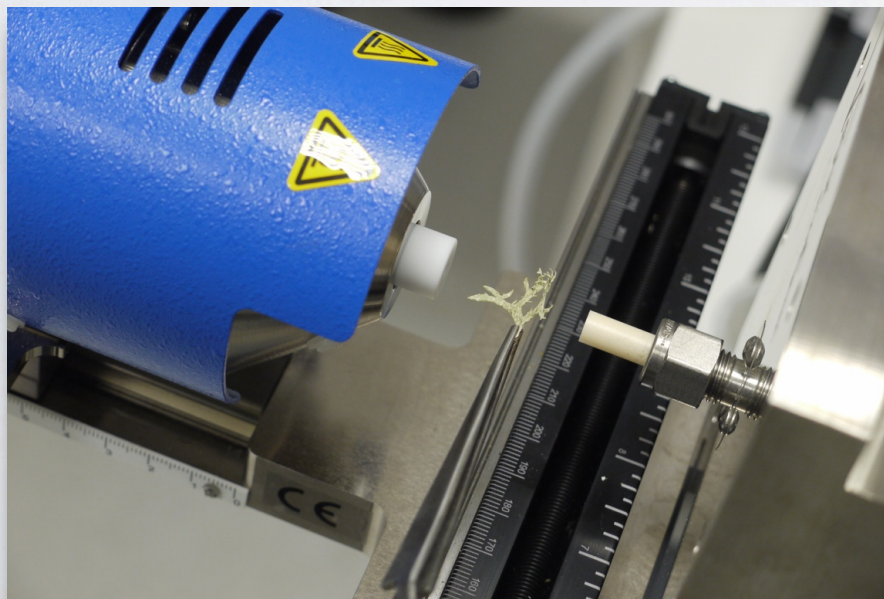
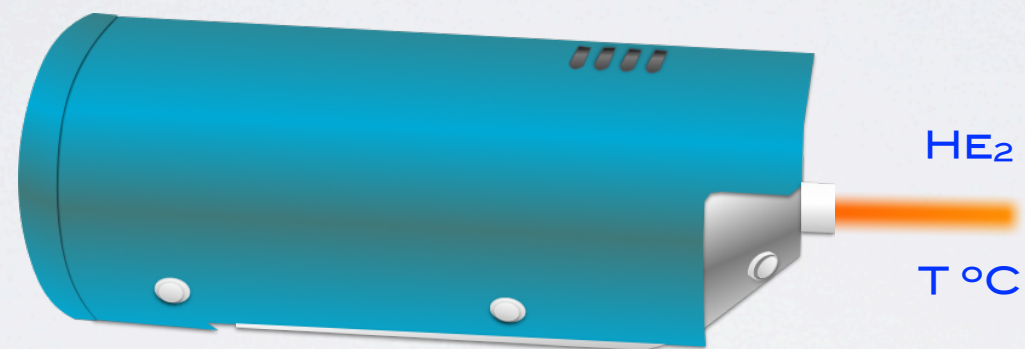
DART - DIRECT ANALYSIS IN REAL TIME

PŘÍMÁ ANALÝZA V REÁLNÉM ČASE

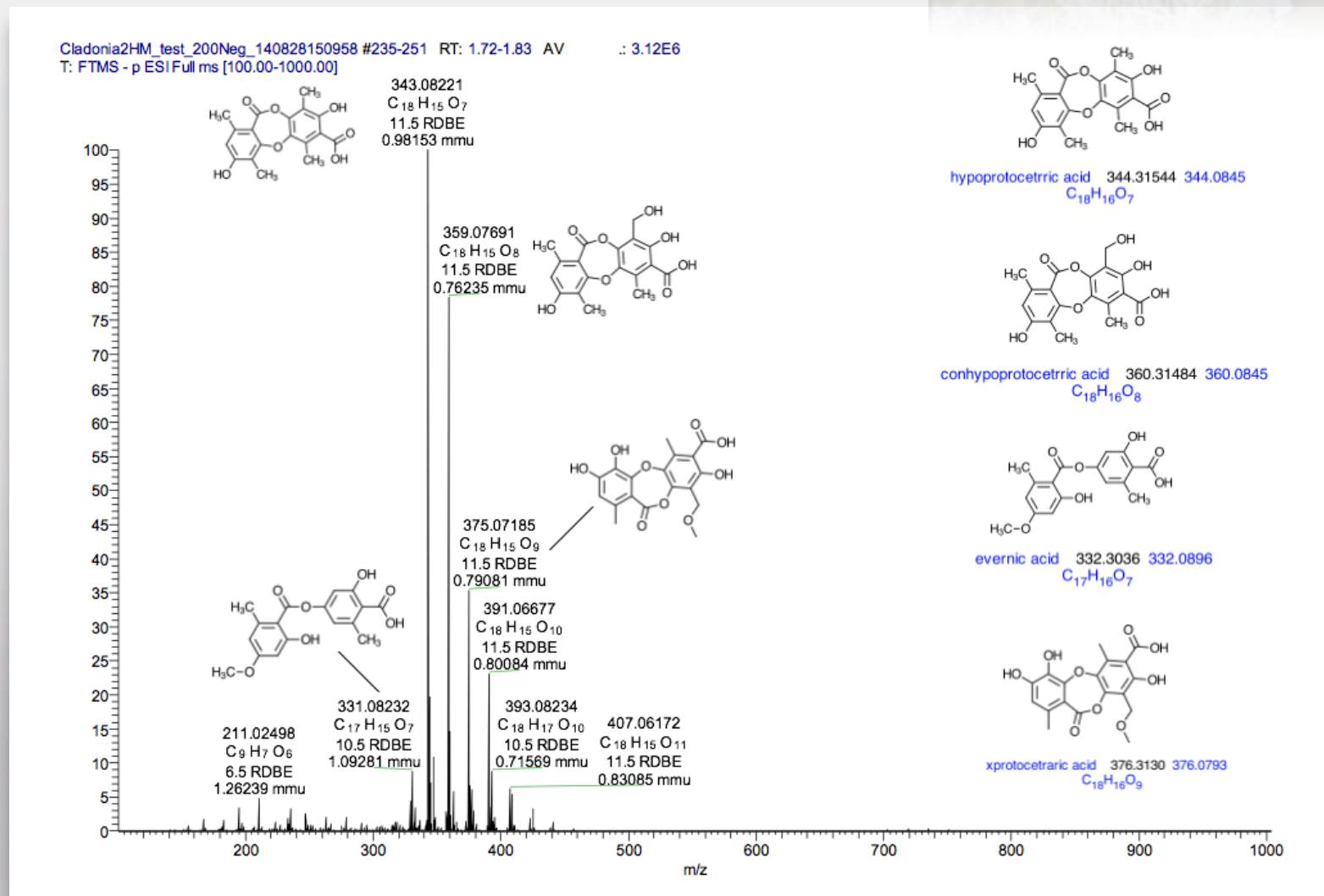
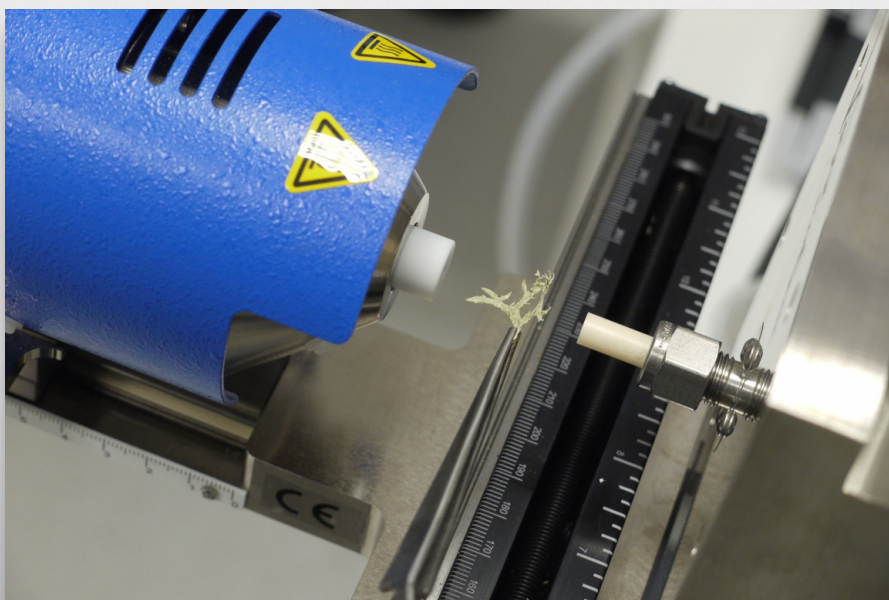
IONIZACE ANALYTU NASTÁVÁ PO KOLIZI MOLEKUL S METASTABILNÍMI MOLEKULAMI HELIA NEBO DUSÍKU V EXCITOVANÉM STAVU

ODPADÁ ÚPRAVA VZORKU

ODPADÁ CHROMATOGRRAFIE

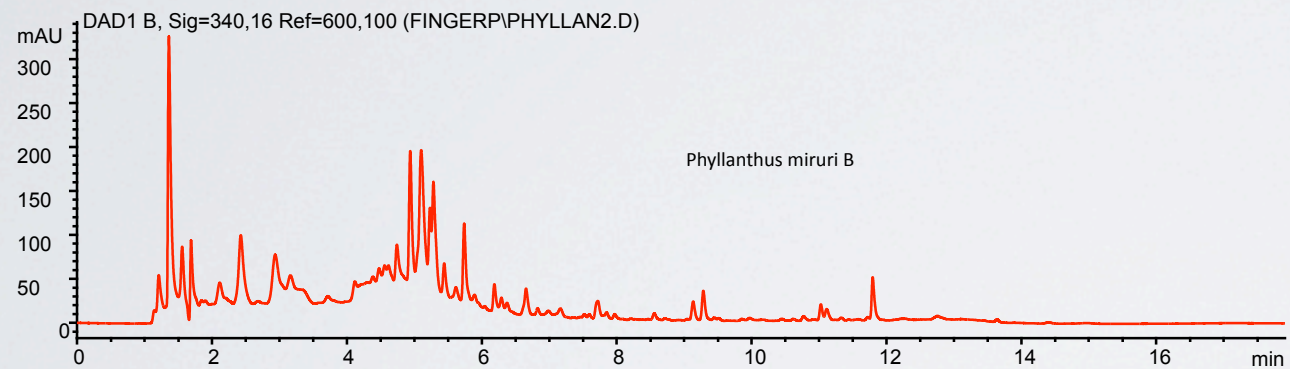
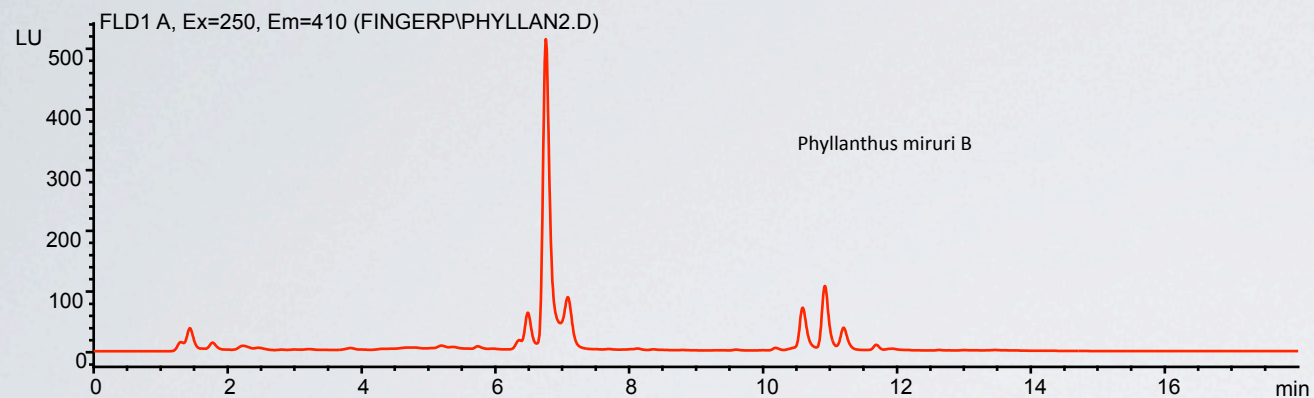


DART - DIRECT ANALYSIS IN REAL TIME MASSFINGERPRINT CLADONIA CONVOLUTA



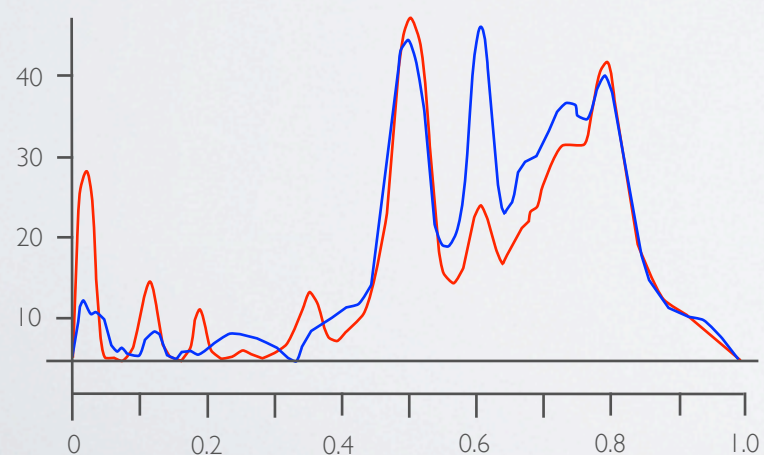
METABOLICKÉ STUDIE POMOCÍ HPTLC S HMOTNOSTNÍ SPEKTROMETRIÍ

FINGERPRINTY



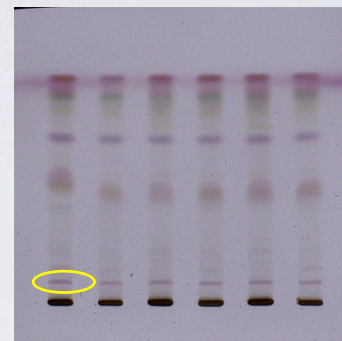
DENSITOGRAM

PHYLLANTHUS MIRURI

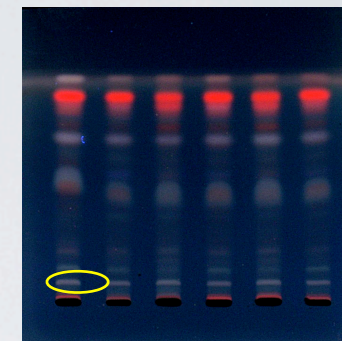


HPTLC PHYLLANTHUS MIRURI

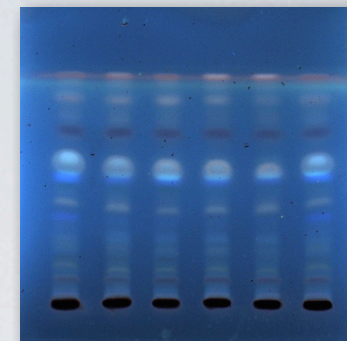
SYSTEM 1 UV VIS



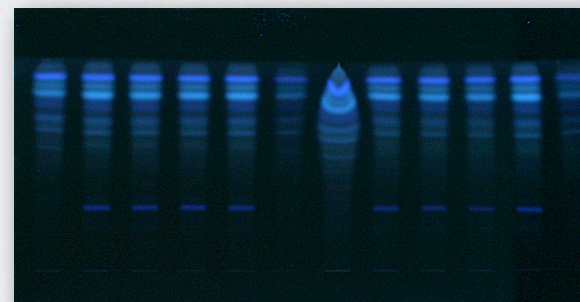
SYSTEM 1 336NM DERIVATIZACE



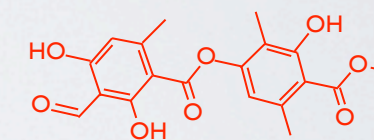
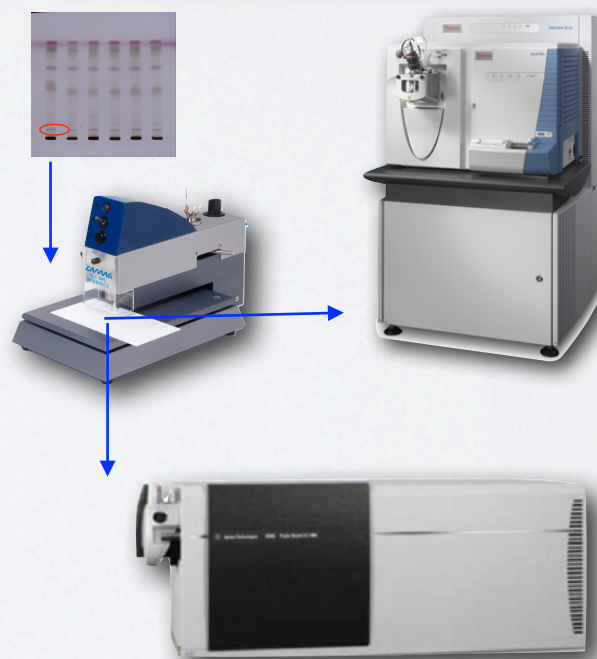
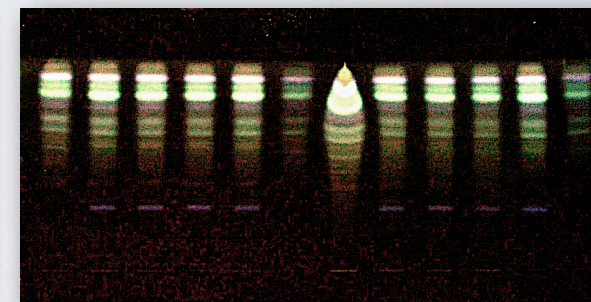
SYSTEM 1 254NM DERIVATIZACE



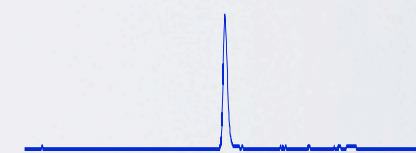
SYSTEM 3 336NM DERIVATIZACE

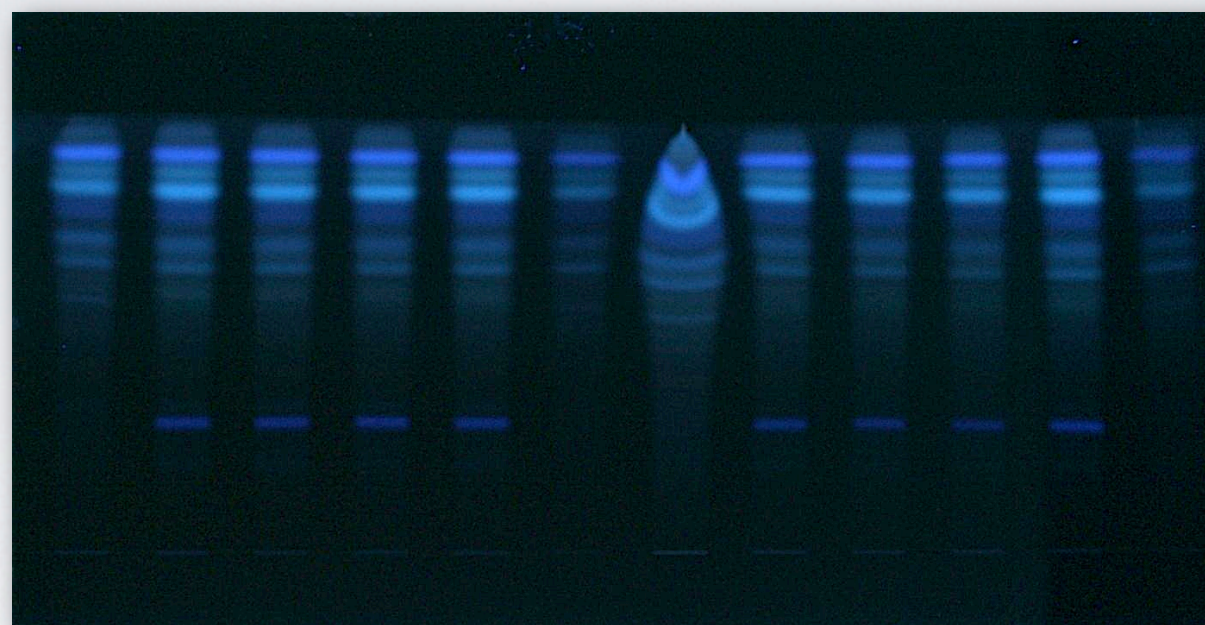


SYSTEM 4 336NM DERIVATIZACE



Atranorin





DĚKUJI

