

# Co je nového ve spotřebním materiálu a v automatizaci

**Martina Riesová**

*VIZE 2022, 22.11.2022 Hotel Hermitage Praha & stream*

Waters  
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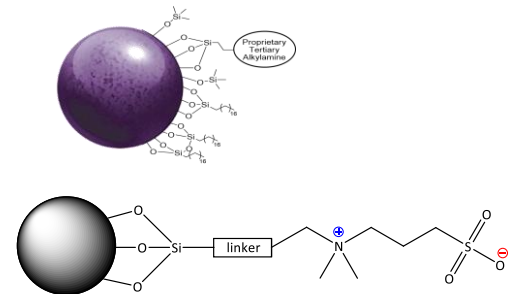
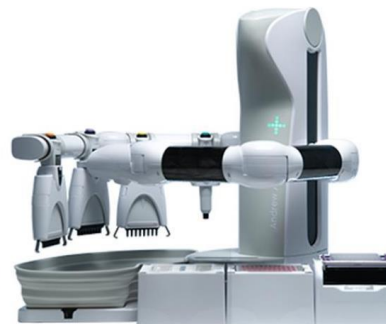
PREMIER Otto Andrew

Martina Riesová  
VIZE 2020 24.11.2020

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Kolony pro polární analyty

Martina Riesová  
VIZE 2021, 23.11.2021 Hotel Hermitage Praha & stream

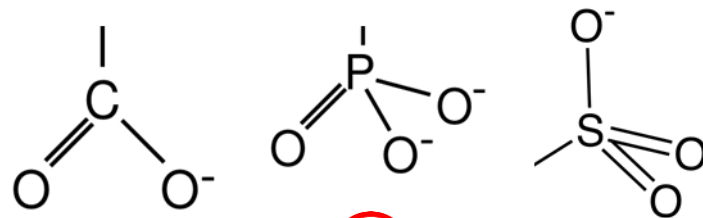


# MaxPeak HPS technologie

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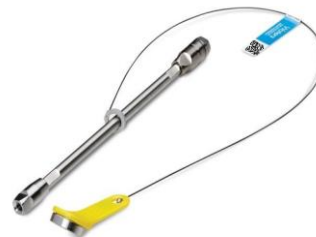
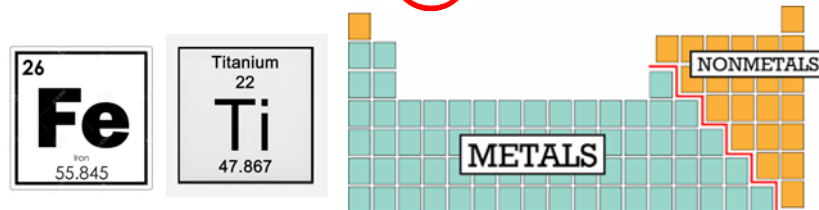
**MAXPEAK™**  
HIGH PERFORMANCE SURFACES

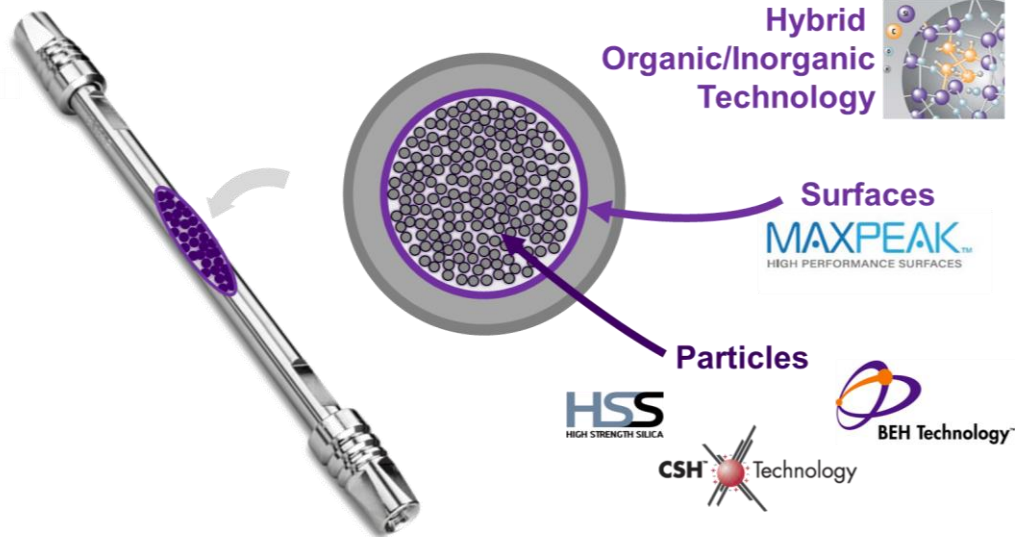
- Zamezení nspecifických interakcí lewisových bází s kovovým povrchem



## Dosavadní možnosti:

- Jiný materiál (PEEK, Titan..)
- Pasivace
- Odstínění interakcí – aditiva do MF
- Potažení (vzorkem)



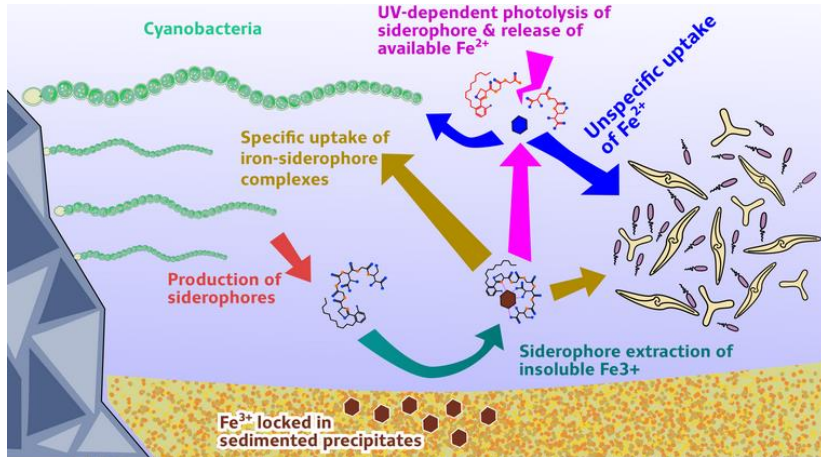


## Co můžeme z MaxPeak HPS vytěžit?

- Věrohodnost výsledků již od prvního nástřiku
- Bez pasivace/ aditiv

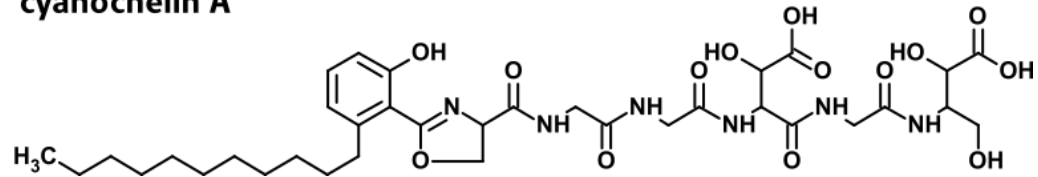
## Siderofory

- Látky schopné reverzibilně vázat železo přes koordinační vazbu
- Syntetizovány mikroorganismy za účelem vychytávání železa z prostředí



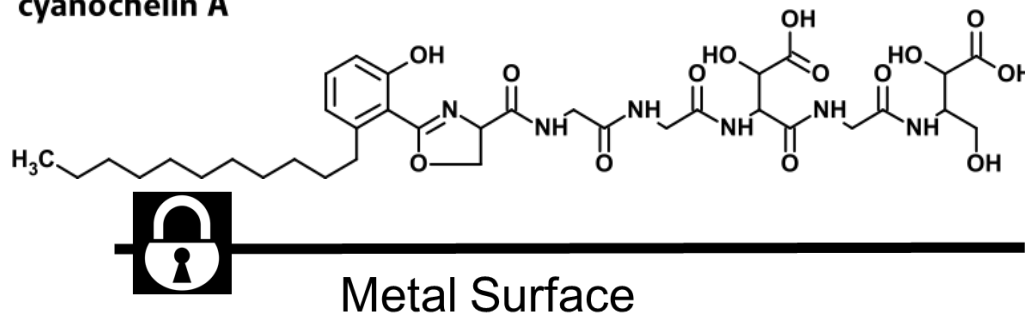
<https://www.alga.cz/c-43-skupina-pavla-hrouzka.html>

### cyanochelin A



Metal Surface

cyanochelin A



## ■ Pasivace

“práce na celý den”

## ■ Běžná C18

- ACN (0.1% FA)/H<sub>2</sub>O (0.1% FA)
- TFA
- Výsledky nebyly vždy opakovatelné, více nástřiků

## ■ Pasivace ?

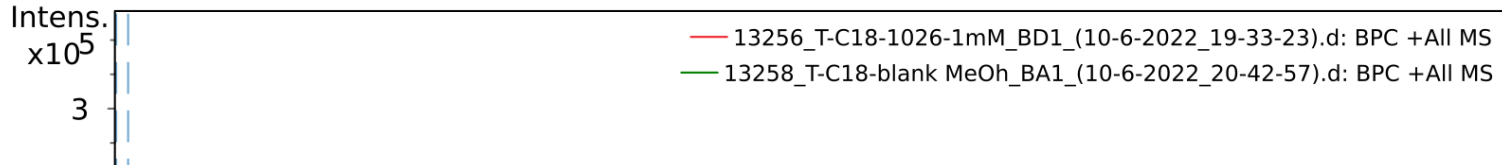
“zapojit a jedem”

## ■ XBridge Premier BEH C18

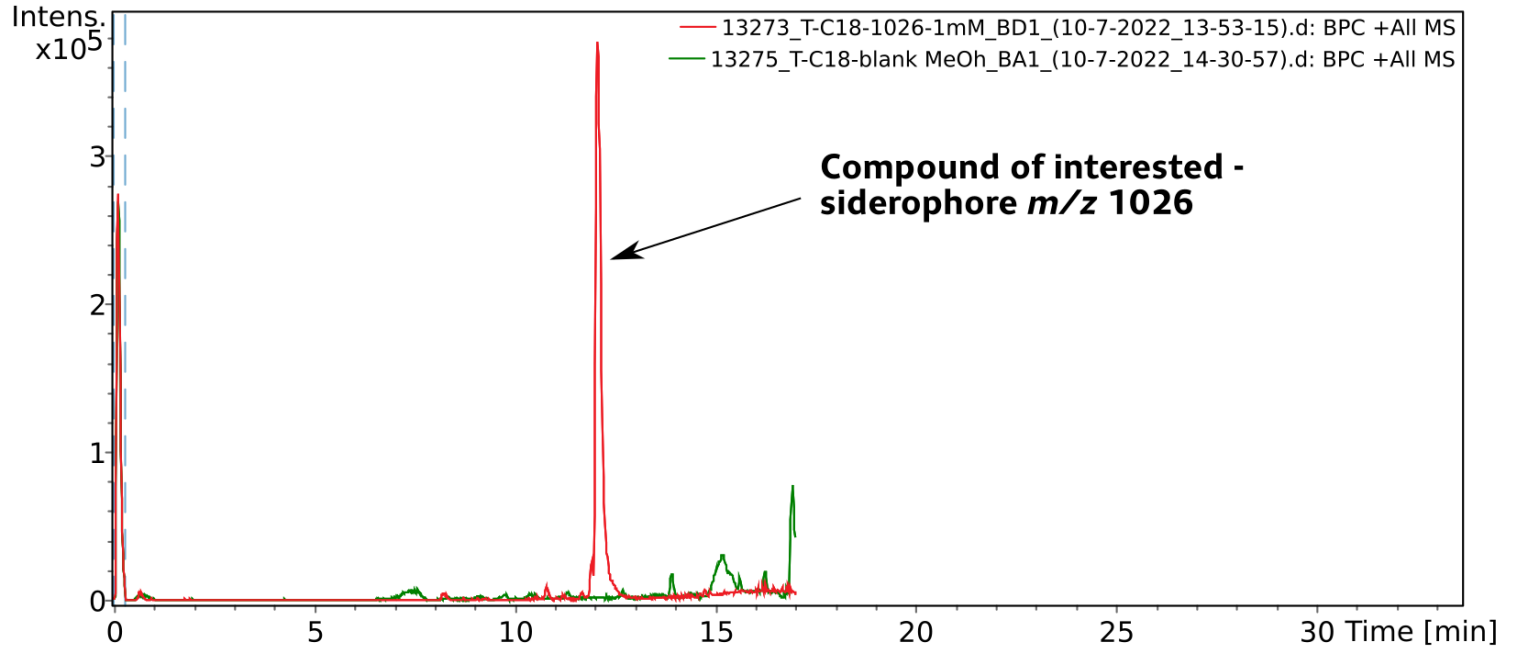
- ACN (0.1% FA)/H<sub>2</sub>O (0.1% FA)
- Bez TFA
- Opakovatelné od prvního nástřiku

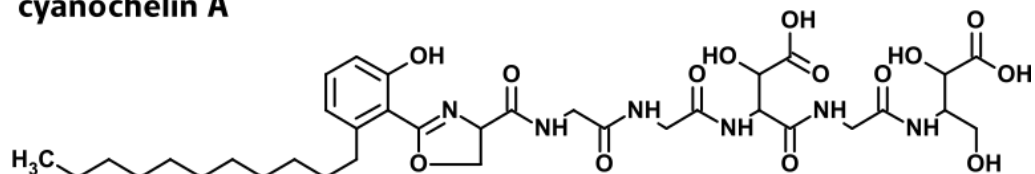
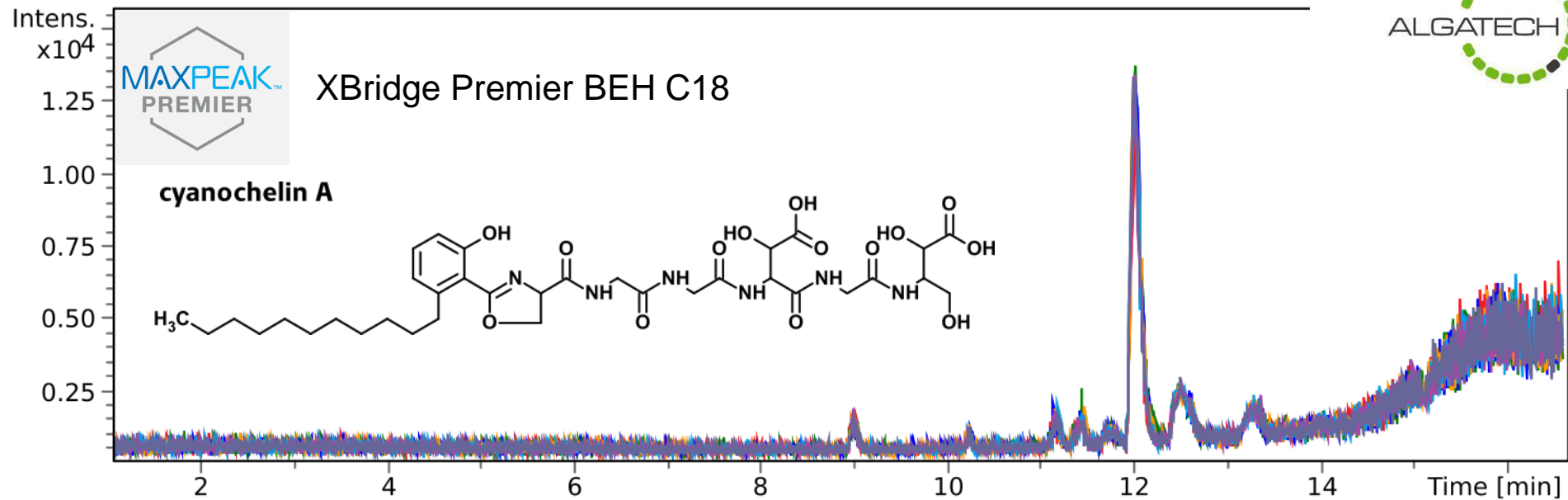


Regular C-18 column



XBridge PRM BEHC18  
2.5µm 2.1x50mm FIT





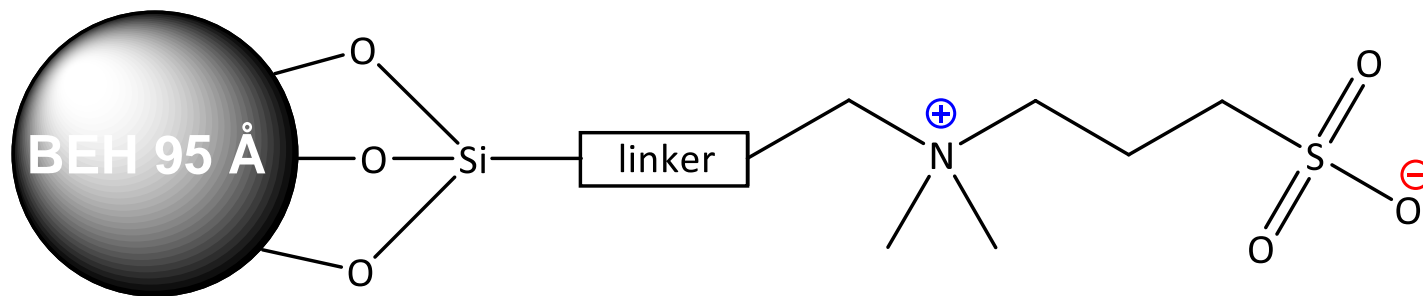
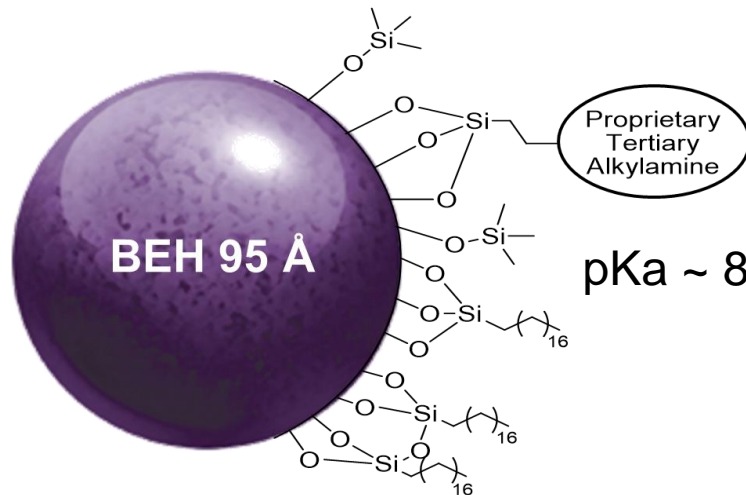
- 13471\_T-C18-1026-10uM-inj1ul\_BC5\_(10-17-2022\_16-04-14).d: BPC +All MS
- 13476\_T-C18-1026-10uM-inj1ul\_BC5\_(10-17-2022\_17-38-06).d: BPC +All MS
- 13477\_T-C18-1026-10uM-inj1ul\_BC5\_(10-17-2022\_17-56-51).d: BPC +All MS
- 13478\_T-C18-1026-10uM-inj1ul\_BC5\_(10-17-2022\_18-15-34).d: BPC +All MS
- 13482\_T-C18-1026-10uM-inj1ul\_BC5\_(10-17-2022\_19-30-32).d: BPC +All MS
- 13483\_T-C18-1026-10uM-inj1ul\_BC5\_(10-17-2022\_19-49-15).d: BPC +All MS
- 13484\_T-C18-1026-10uM-inj1ul\_BC5\_(10-17-2022\_20-08-00).d: BPC +All MS



# “Nové” sorbenty – C18 AX a Z-HILIC

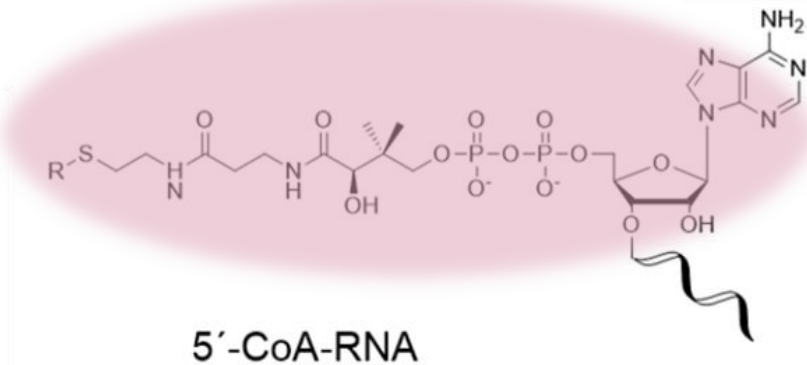
## ▪ Čekali na:

- BEH částice
- Spolehlivé navázání ligandů
- MaxPeak HPS (Premier) technologii

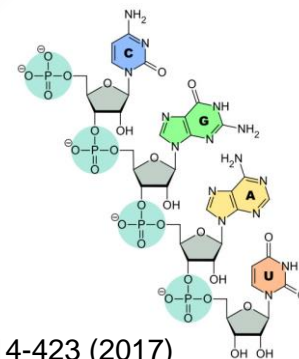
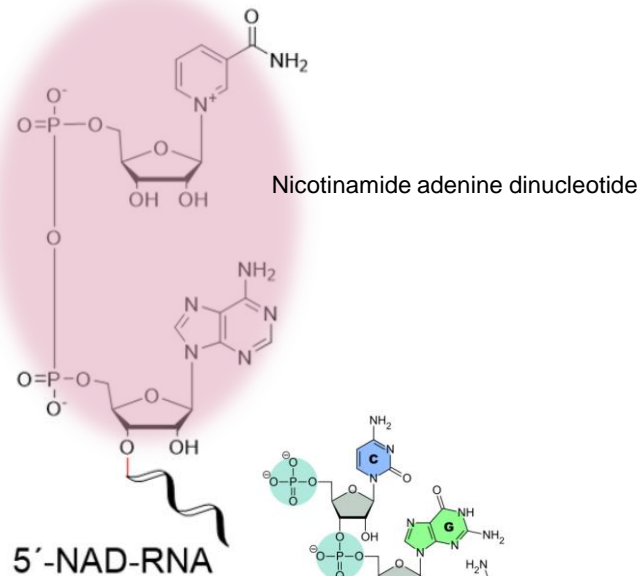


# RNA čepičky

- Modifikace na 5' konci RNA řetězce
- Stabilita RNA, obrana před exonukleázami, její aktivita

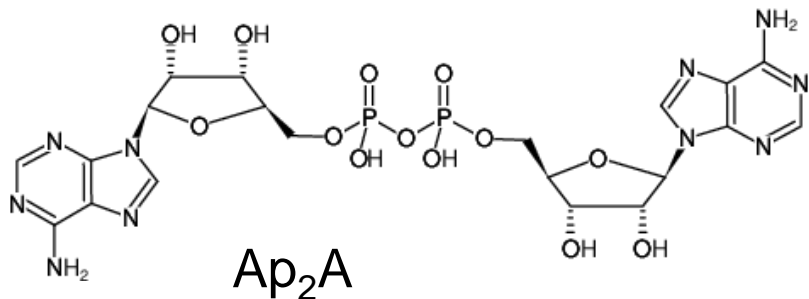


- Nově objevené čepičky – Alarmony  
(dinukleosid polyfosfáty)

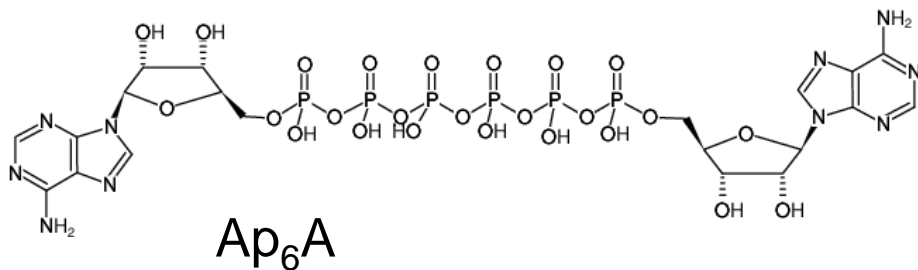
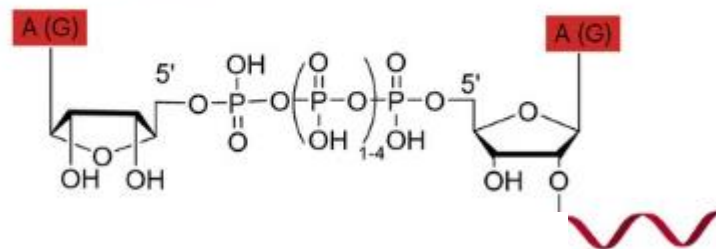


Chem. Listy 111, 414-423 (2017)

# Alarmony dinukleosidpolyfosfáty $Np_nN$



## $Np_nN$ -capped RNA



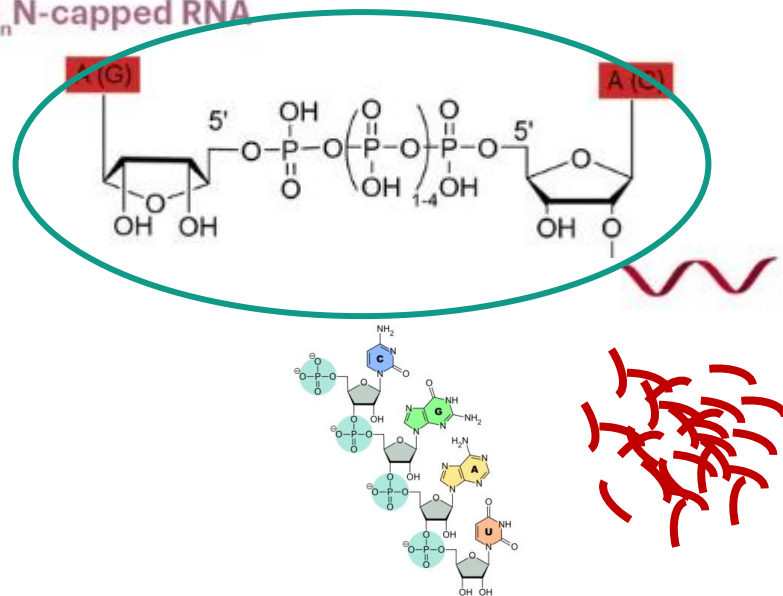
## V buňkách: LC-UV

- IP činidla TEA ve vysokých koncentracích (>100mM)
- C18

## RNA čepičky: LC MS/MS

- Nemůžu použít běžná IP činidla v dostatečné konc.
- C18 nefunguje
- $Np_nN$  vs nukleosidmonofosfáty

$Np_nN$ -capped RNA



<https://cahova.group.uochb.cz/en>

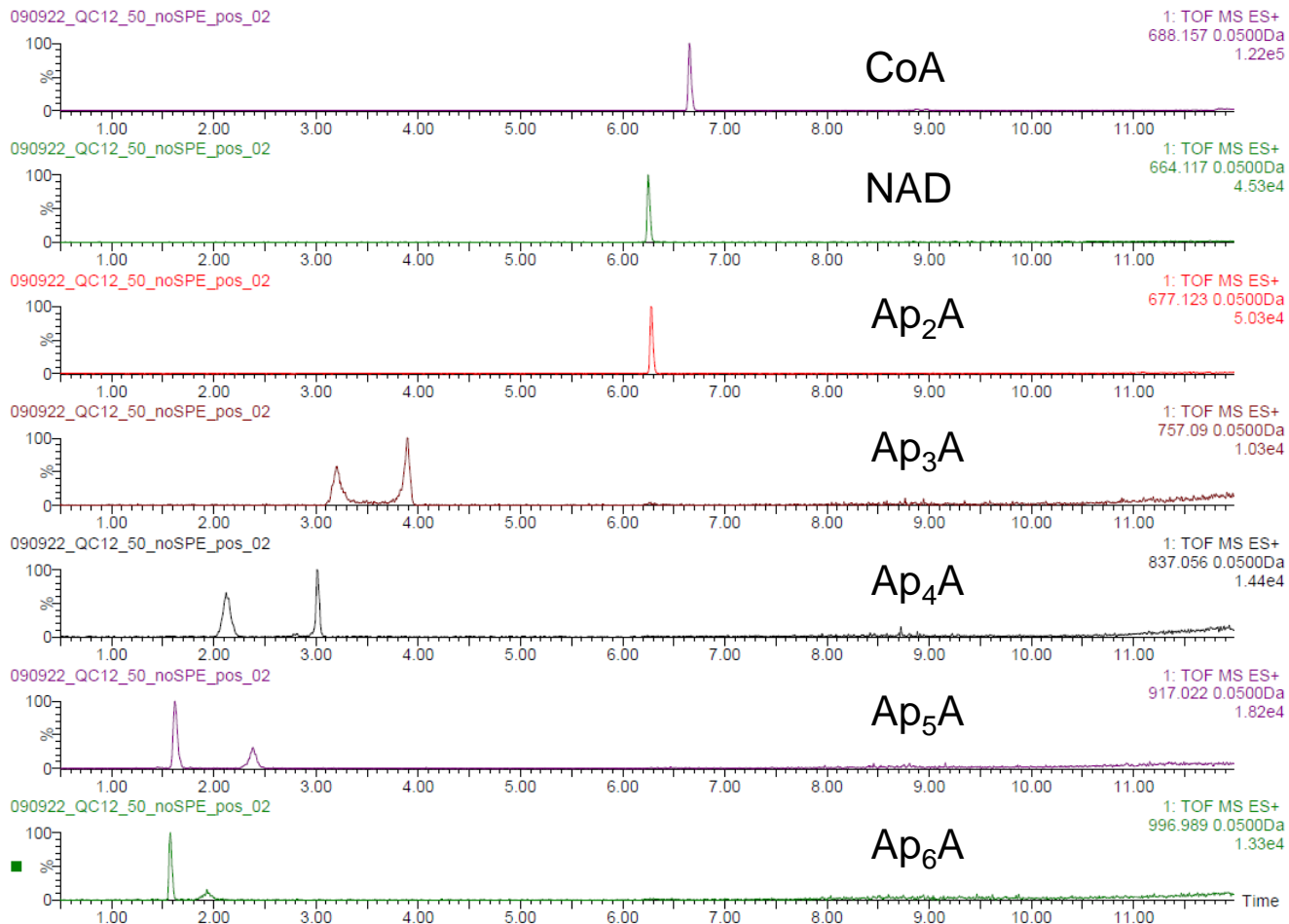
# RNA čepičky LC MS/MS

## Kolona C18 (USP L1)

- 100% vodná fáze
- zvýšená retence polárních látek
- drobný kladný povrchový náboj

## MF

- 20mM  $\text{NH}_4\text{HCO}_3$ , pH 8 / ACN



# RNA čepičky LC MS/MS

## Atlantis Premier C18 AX

(USP L78)

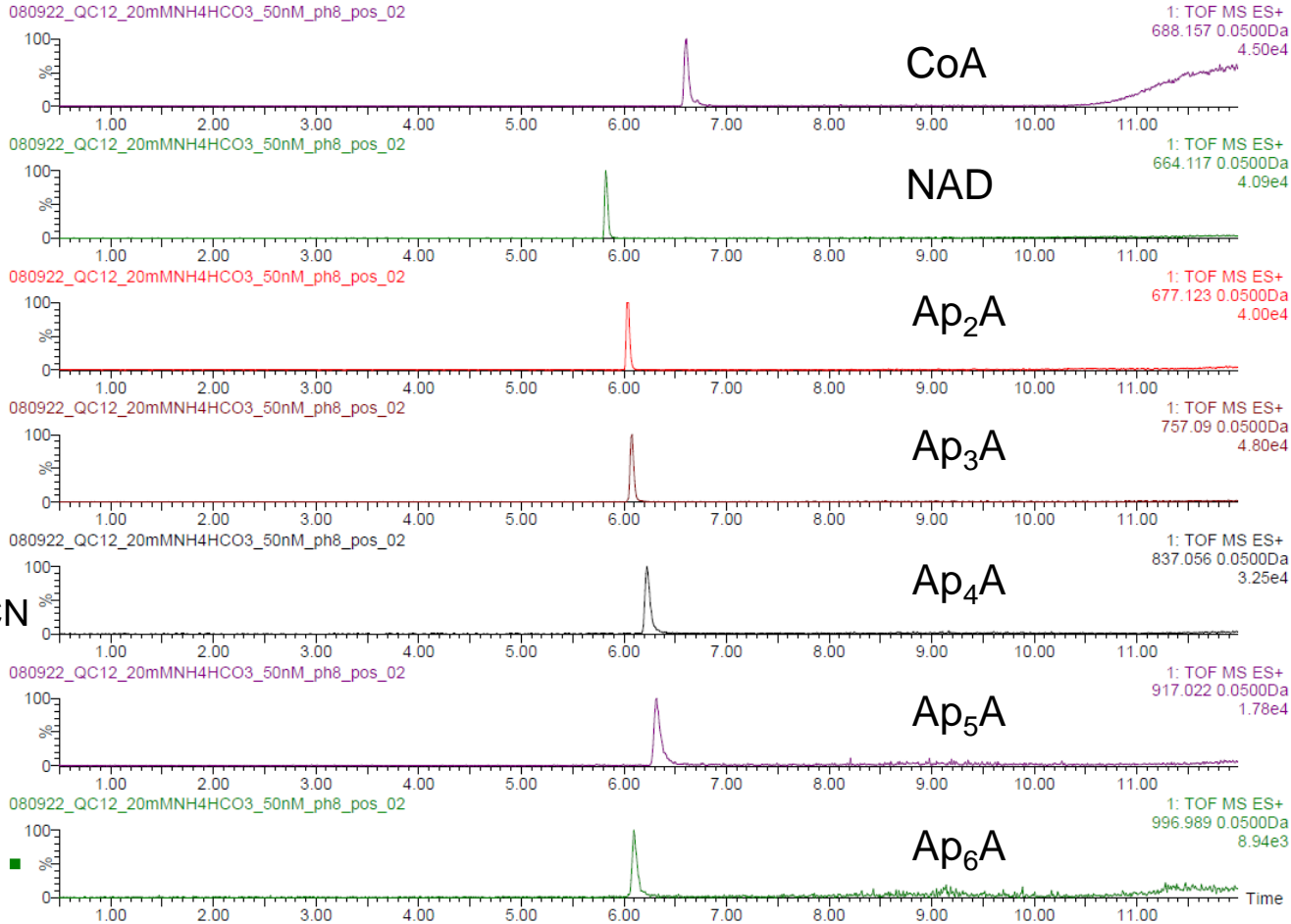
- 100% vodná fáze
- **Mixed-mode**
- Anion Exchange

## MF

- 20mM NH<sub>4</sub>HCO<sub>3</sub>, pH 8 / ACN

## Retence

## Selektivita



# RNA čepičky LC MS/MS

## Atlantis Premier Z-HILIC

- sulfobetain

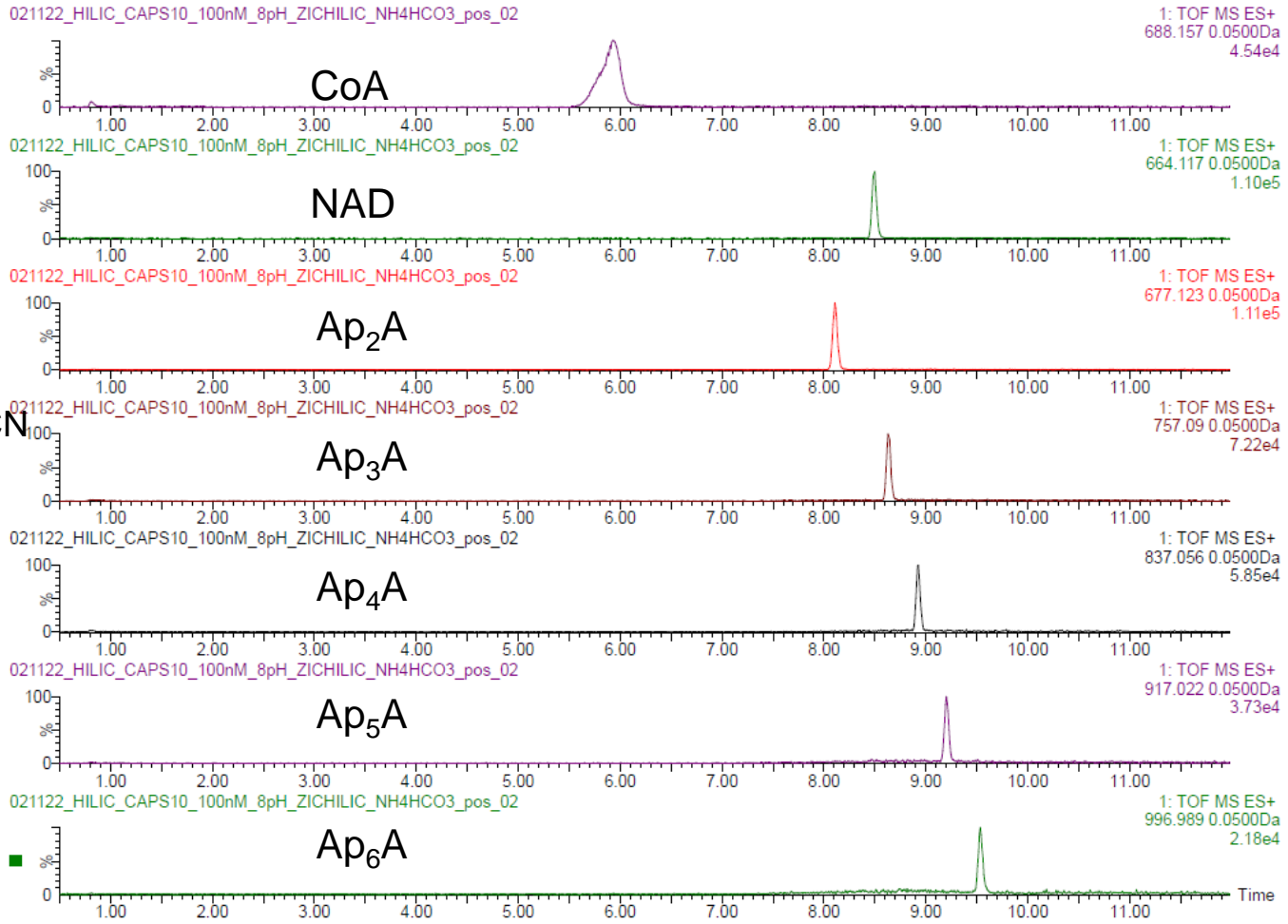
## MF

- 20mM NH<sub>4</sub>HCO<sub>3</sub>, pH 8 / ACN

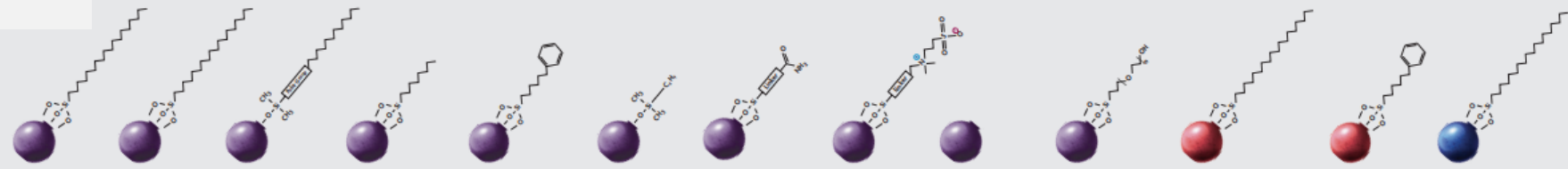
Retence  
Selektivita



? AMP, GMP...

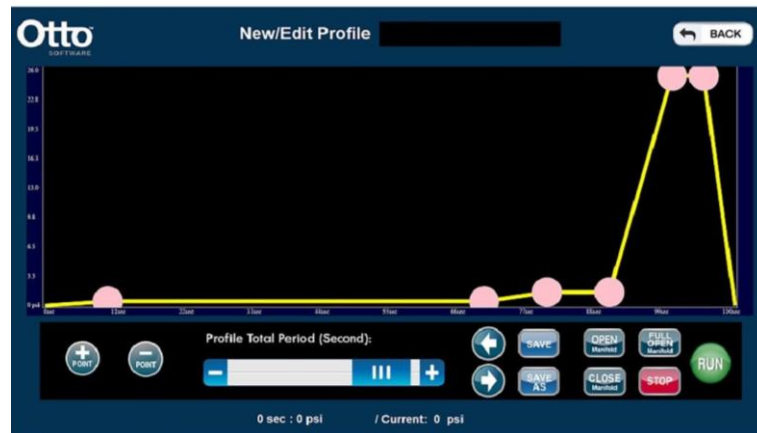






	C <sub>18</sub>	C <sub>18</sub> AX	Shield RP18	C <sub>8</sub>	Phenyl	C <sub>4</sub>	Amide	Z-HILIC	HILIC	BEH-PEO	CSH C <sub>18</sub>	CSH Phenyl-Hexyl	HSS T3
Ligand density	3.1 μmol/m <sup>2</sup>	1.6 μmol/m <sup>2</sup>	3.3 μmol/m <sup>2</sup>	3.2 μmol/m <sup>2</sup>	3.0 μmol/m <sup>2</sup>	2.4 μmol/m <sup>2</sup>	7.5 μmol/m <sup>2</sup>	3.0 μmol/m <sup>2</sup>	n/a	1.5 μmol/m <sup>2</sup>	2.3 μmol/m <sup>2</sup>	2.3 μmol/m <sup>2</sup>	1.6 μmol/m <sup>2</sup>
Pore diameter	130 Å, 300 Å	95 Å	130 Å	130 Å	130 Å	300 Å	130 Å	95 Å	130 Å	250 Å	130 Å	130 Å	100 Å
Carbon load	18%	17%	17%	13%	15%	8%	12%	17%	unbonded	12%	15%	14%	11%
Endcap style	proprietary	proprietary	TMS	proprietary	proprietary	none	none	none	none	none	proprietary	proprietary	proprietary
pH range	1-12	2-10	2-11	1-12	1-12	2-10	2-11	2-10	1-9	2.5-8	1-11	1-11	2-8
Low pH temp. limit	80 °C	60 °C	50 °C	60 °C	80 °C	80 °C	90 °C	60 °C	45 °C	60 °C	80 °C	80 °C	45 °C
High pH temp. limit	60 °C	60 °C	45 °C	60 °C	60 °C	50 °C	90 °C	60 °C	45 °C	60 °C	45 °C	45 °C	45 °C
Surface area	185 m <sup>2</sup> /g	270 m <sup>2</sup> /g	185 m <sup>2</sup> /g	185 m <sup>2</sup> /g	185 m <sup>2</sup> /g	90 m <sup>2</sup> /g	185 m <sup>2</sup> /g	270 m <sup>2</sup> /g	185 m <sup>2</sup> /g	174 m <sup>2</sup> /g	185 m <sup>2</sup> /g	185 m <sup>2</sup> /g	230 m <sup>2</sup> /g
USP classification	L1	L78	L1	L7	L11	L26	L68	L122	L3	L33	L1	L11	L1

# Jak se má Ottík ?

A screenshot of the Otto software interface in 'Edit Method' mode. The screen displays a list of 12 method steps. On the left side, there are several control buttons: 'OPEN Manifold', 'CLOSE Manifold', 'ADD PROFILE', 'PAUSE', 'COPY STEP', 'DELETE STEP', 'MOVE STEP UP', 'MOVE STEP DOWN', 'FULL OPEN Manifold', 'RUN', and 'STOP'. The method steps are as follows:

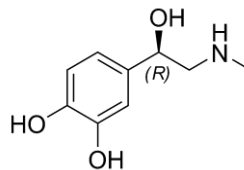
- 1 Y Open manifold
- 2 Y Pause and Waiting for User
- 3 Y Close manifold
- 4 Y Operating profile : 3CC\_LOAD\_CARTRIDGE.prf
- 5 Y Open manifold
- 6 Y Pause and Waiting for User
- 7 Y Pause and Waiting for User
- 8 Y Close manifold
- 9 Y Operating profile : 3CC\_WASH\_CARTRIDGE.prf
- 10 Y Open manifold
- 11 Y Pause and Waiting for User
- 12 Y Pause and Waiting for User

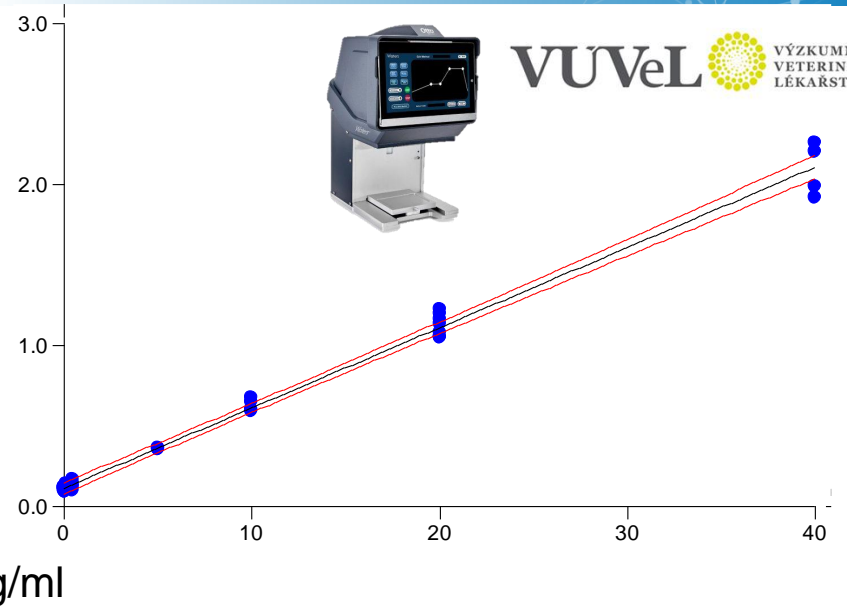
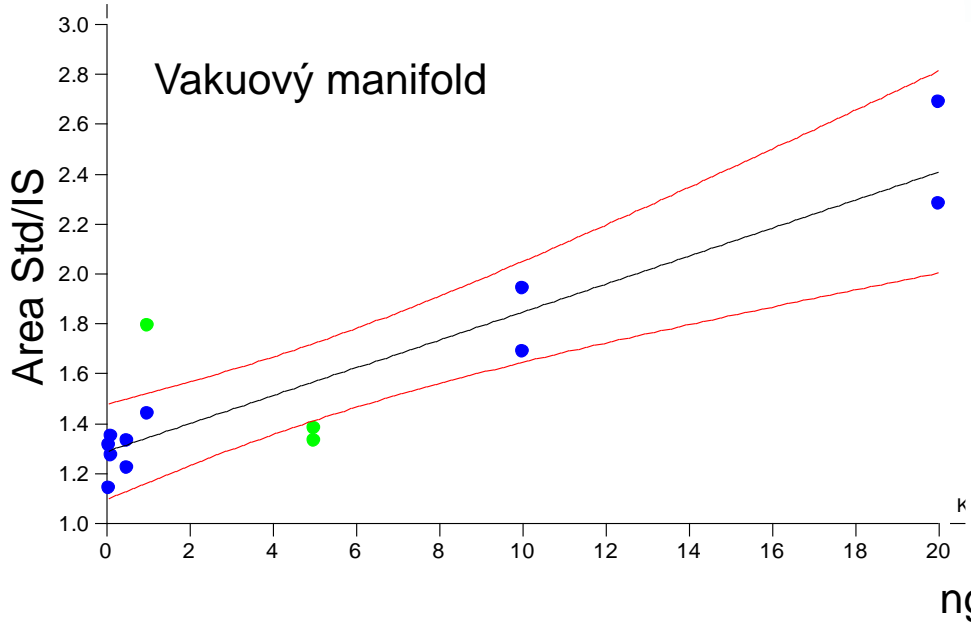
At the bottom, there are fields for 'Sample Name' (water) and 'Method Folder' (3CC\_CARTRIDGE), along with 'SAVE' and 'SAVE AS' buttons. A 'BACK' button is in the top right corner.

# Jak se má Ottík ?

## Klinická studie – Adrenalin (Epinephrin) v lidské plazmě

- GLP
- Guideline pro validace bioanalytických metod podle CHMP (*Committee for Medicinal Products for Human Use*)
- “ultra” stopová analýza (**0,05 – 20 ng/ml**)
- Výrazné zlepšení parametrů kalibrační závislosti a citlivosti metody





$$Y = A + B \cdot X$$

Parametr	Odhad	RSD%
A	0.1379	<b>60.7</b>
B	0.04984	<b>51.2</b>
r	<b>0.9659</b>	

Parametr	Odhad	RSD%
A	0.11278	<b>11.2</b>
B	0.04984	<b>1.4</b>
r	<b>0.9959</b>	

## Extraction



- Plně automatizovaná SPE
- Kompatibilní s:
  - **platičky, mikroplatičky**
  - **1cc, 3cc a 6cc kolonkami**
- Kontrola tlakového profilu (vakua) přes OneLab+
- “Gripper” pro plnou automatizaci



# Poděkování

❖ Mgr. Tomáš Galica, Ph.D.



❖ Mgr. Anton Škríba, Ph.D.



❖ Ing. Kamil Šťastný, Ph.D.



## Děkuji Vám za pozornost

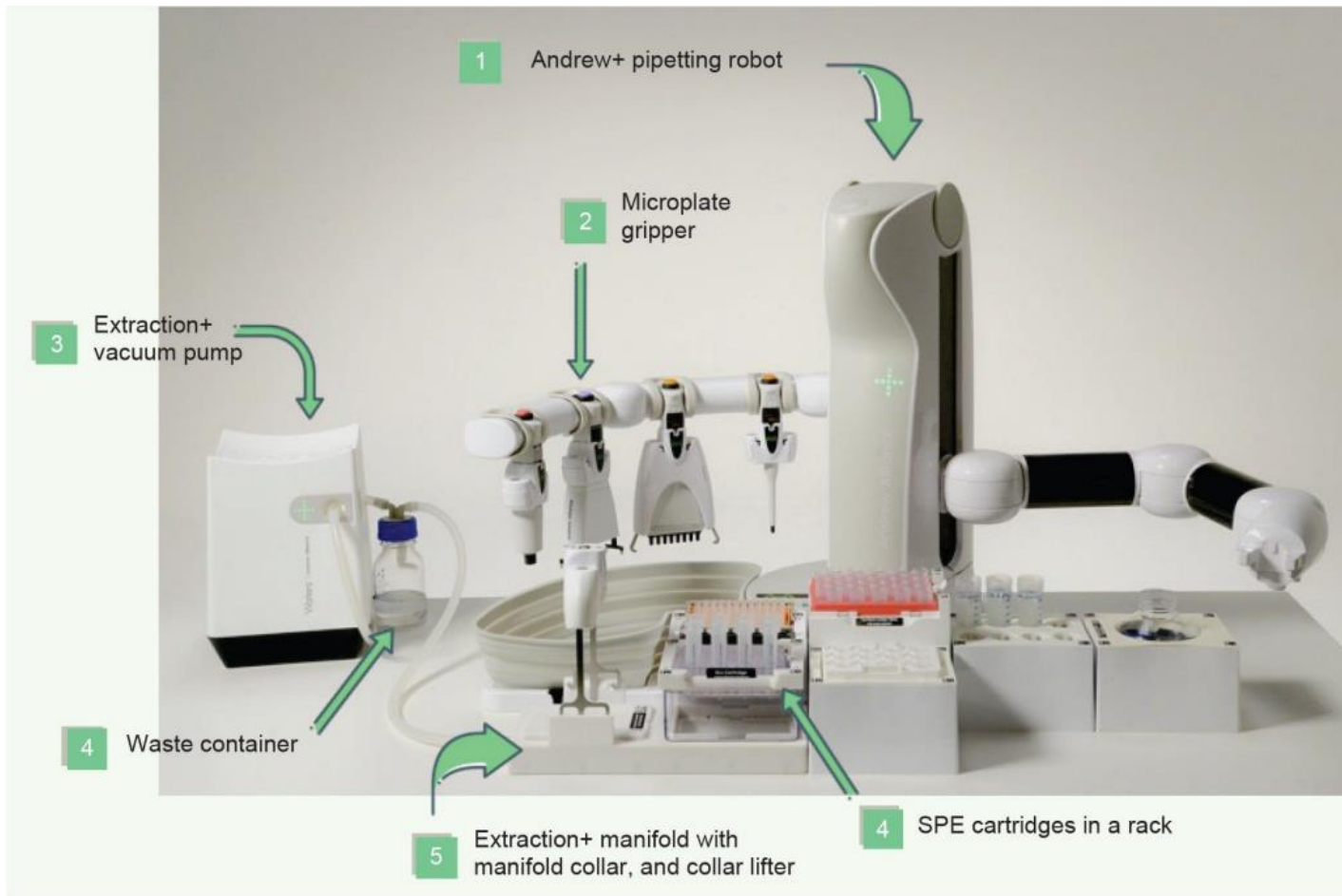




Waters

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# Extraction

