

**Urychlete vývoj HPLC metody o 30%**

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*Sr Chemistry Account Manager, ČR*



- Řešení problémů
- Podnětné diskuze, dobré nápady
- Pocit, že jsem někomu pomohla
- Výhledy, Volejbal, Vlaky, Veverky, piVo



- Věci, co se vlečou a nemusely by
- Něco není včas
- Rybí polévku, málo čtu



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## Přehled toho, co budeme dnes probírat

Method development **goals**

Focus on a particularly sticky **problem**

Discuss a **simple solution** to mitigate risk

Present a few **examples**

Invite you to **learn more**

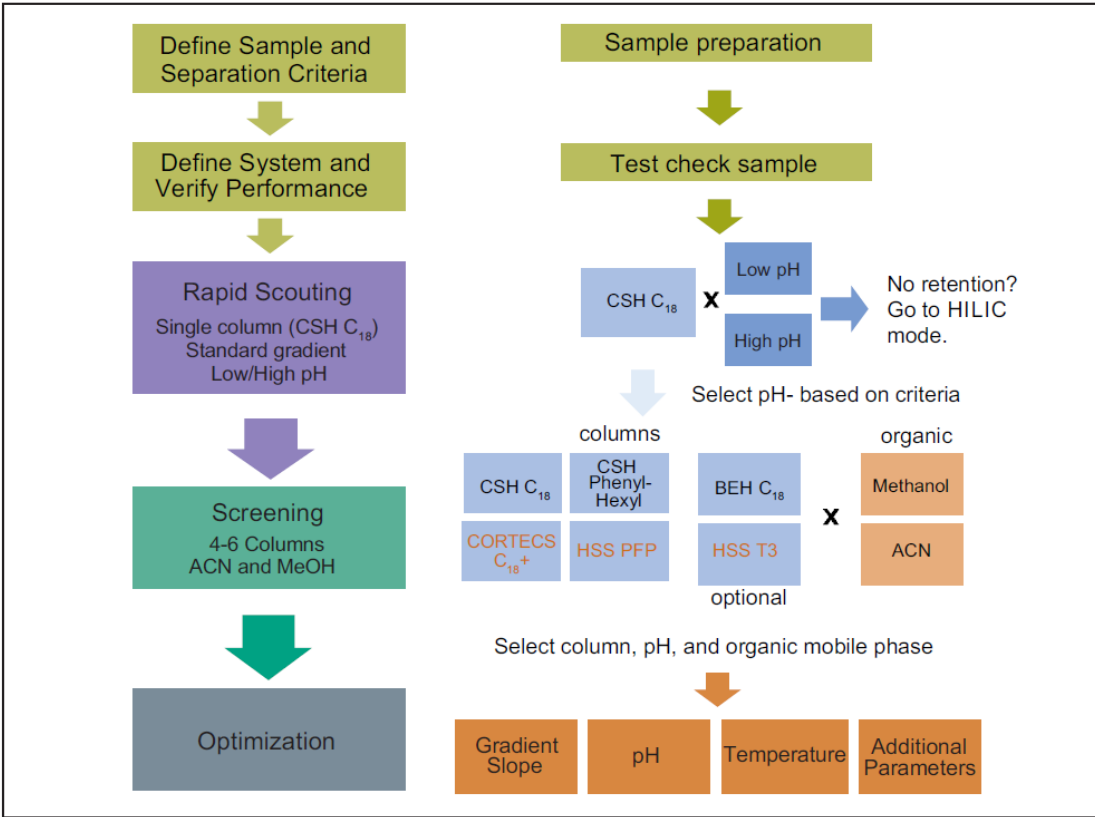
Je možné, aby vývoj metody přinesl tohle všechno?

Peak Shape  
Ruggedness  
Performance  
Sensitivity  
Reproducibility  
Robustness  
Sustainability  
Simplicity  
Speed  
Suitability  
Resolution

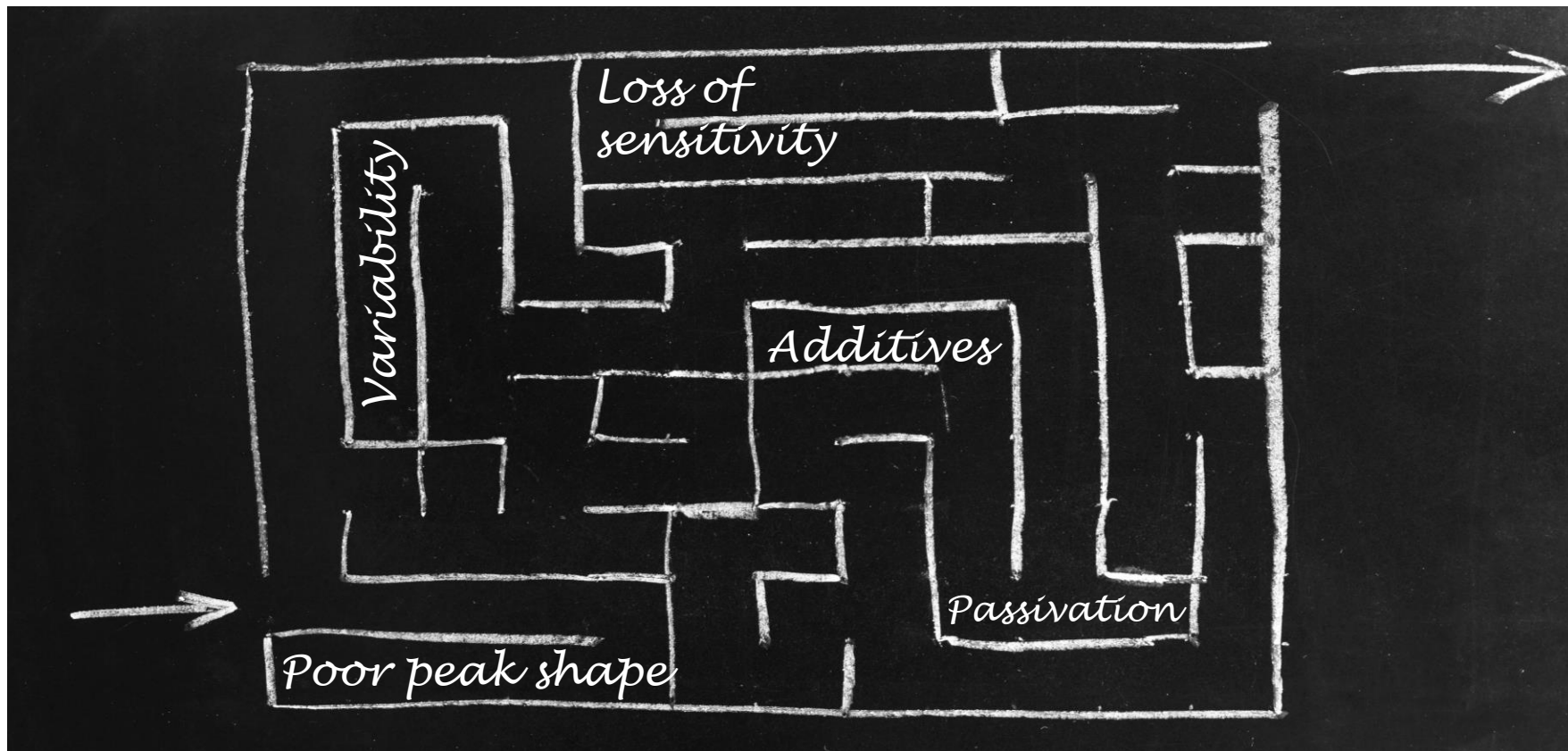


Joy  
Achieve  
Impress the Team  
Confidence  
Make an Impression  
Make a Difference  
Peace of Mind  
Satisfaction  
Inspire

# Systematický přístup pokryje všechny aspekty. Je to pravda?

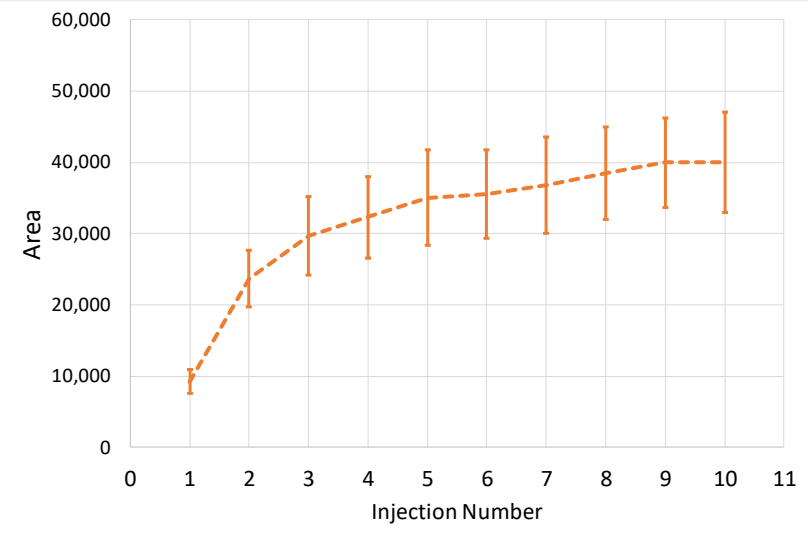


Ne vždy. Vývoj metody není vždy přímočarý

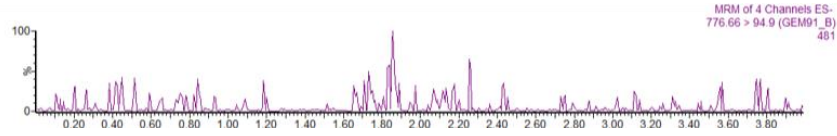


Dokonce i systematický přístup může poskytnout matoucí výsledky

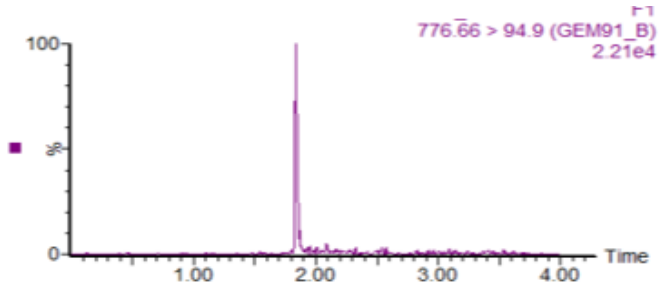
# Variability



# Missing Peaks

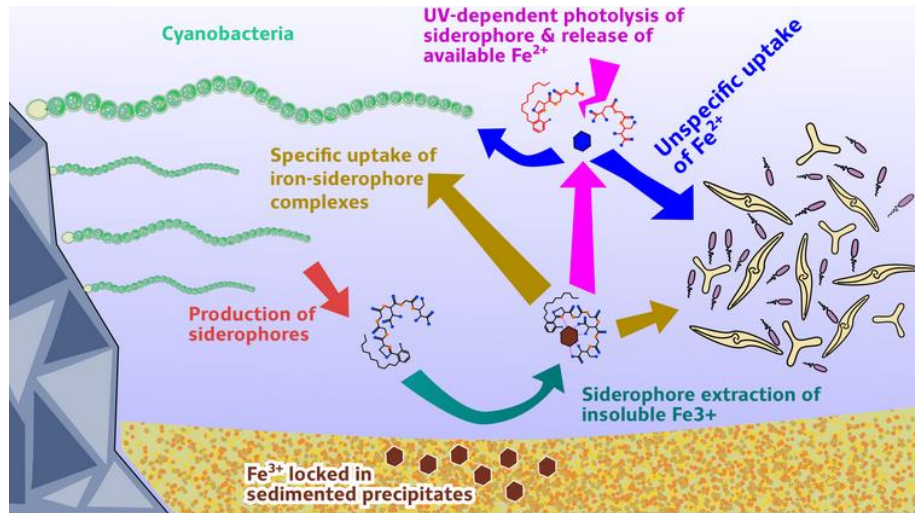
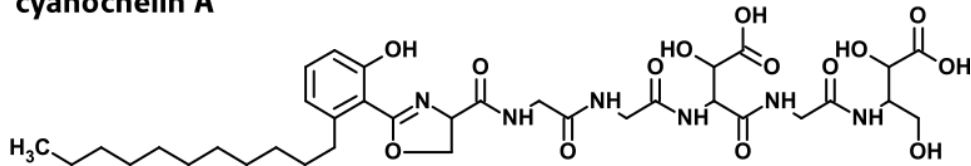


(It should look like this!)



# Případ chybějícího píku

## cyanochelin A

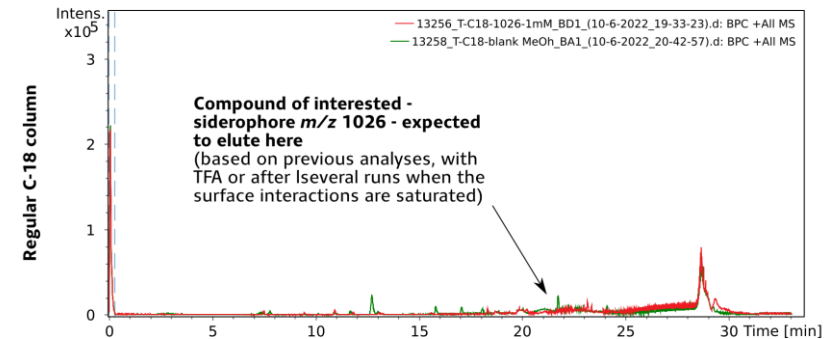
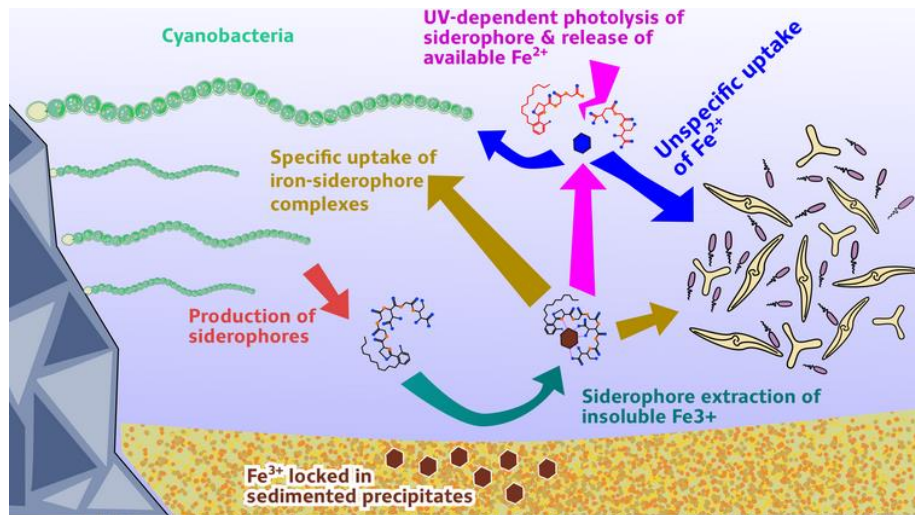
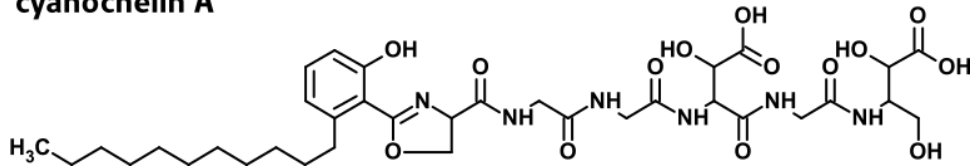


<https://www.alga.cz/c-43-skupina-pavla-hrouzka.html>



# Případ chybějícího píku

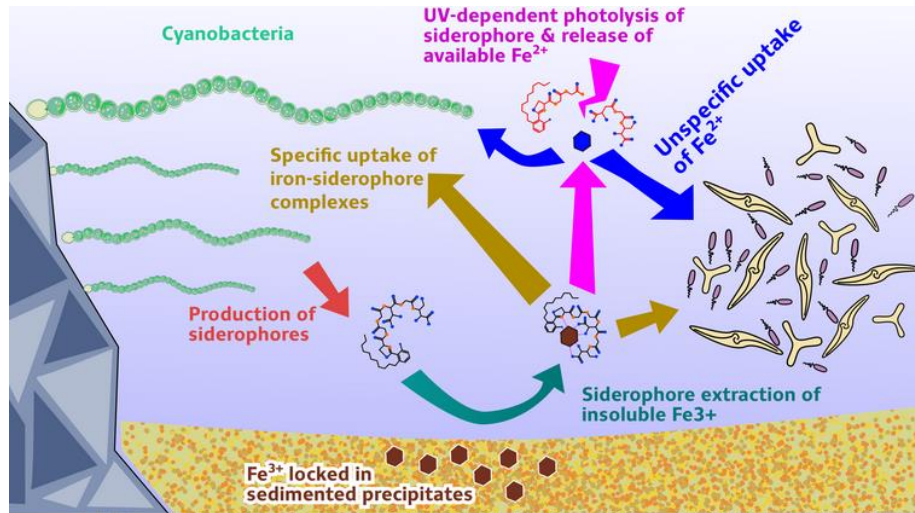
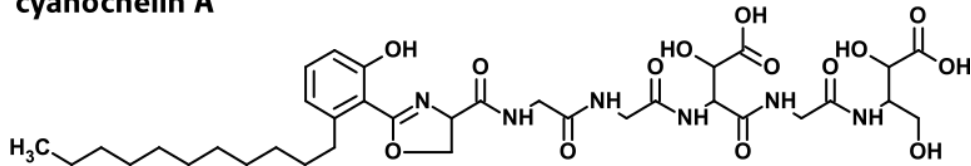
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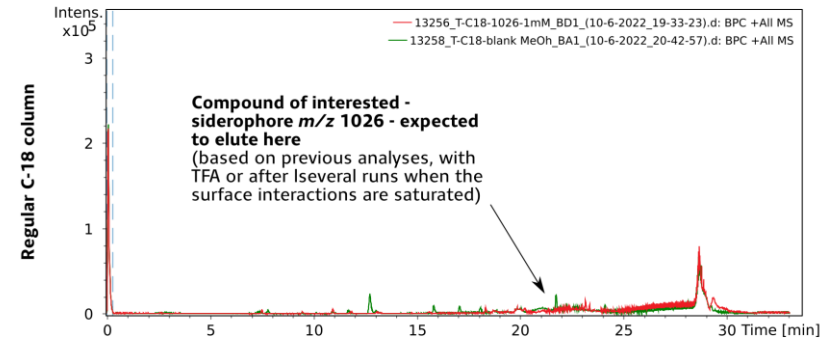
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# Případ chybějícího píku

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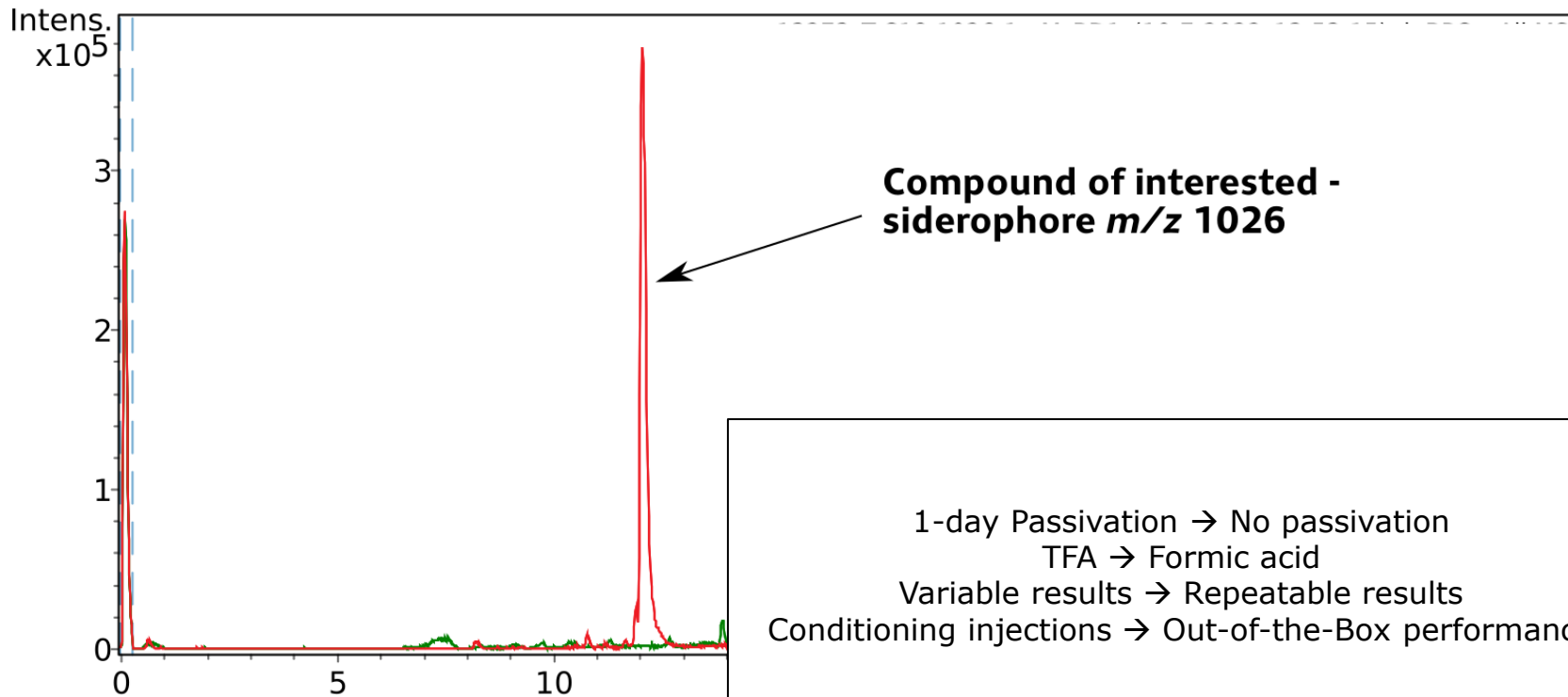
1-day Passivation  
TFA additive  
Variable results  
Conditioning injections

# Alternativní přístup poskytl mnohem jednodušší metodu



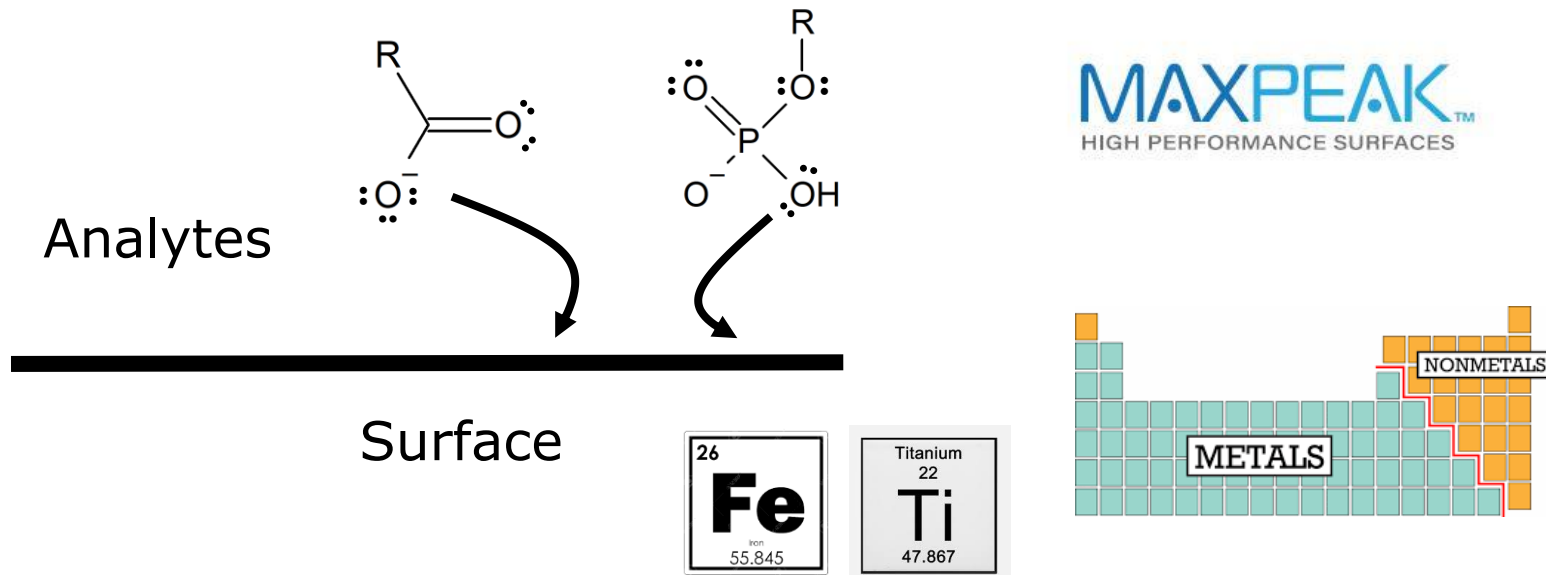
Waters™

XBridge PRM BEHC18  
2.5µm 2.1x50mm FIT



1-day Passivation → No passivation  
TFA → Formic acid  
Variable results → Repeatable results  
Conditioning injections → Out-of-the-Box performance

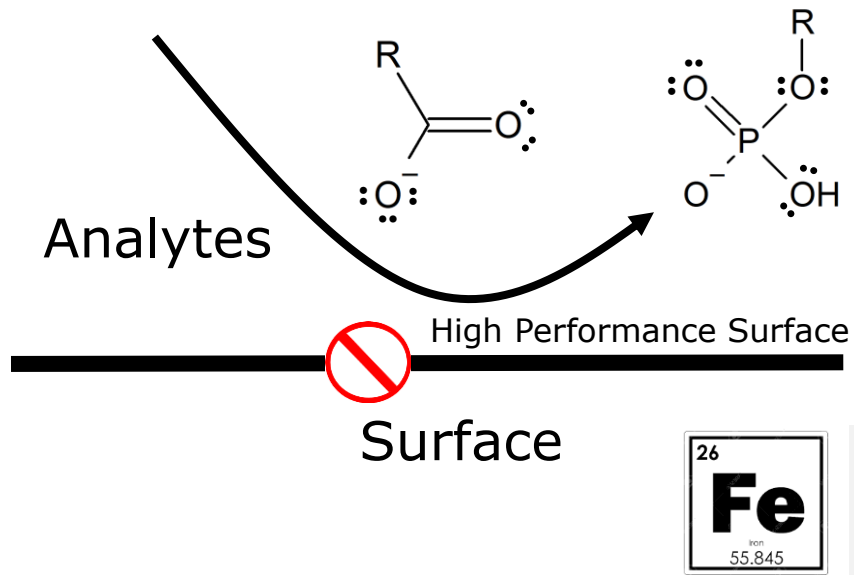
Některé analyty mohou interagovat s povrchy, s nimiž přicházejí do styku



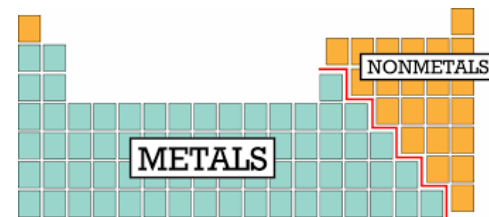
„Non-specific binding“ je jakákoliv nechtěná nebo nezamýšlená interakce  
Hydrofóbní a iontové interakce  
Zmírnění NSB pomocí kontroly prostředí a omezení styku s povrchem

# Vyřešte problém s MaxPeak™ High Performance Surfaces

Waters™



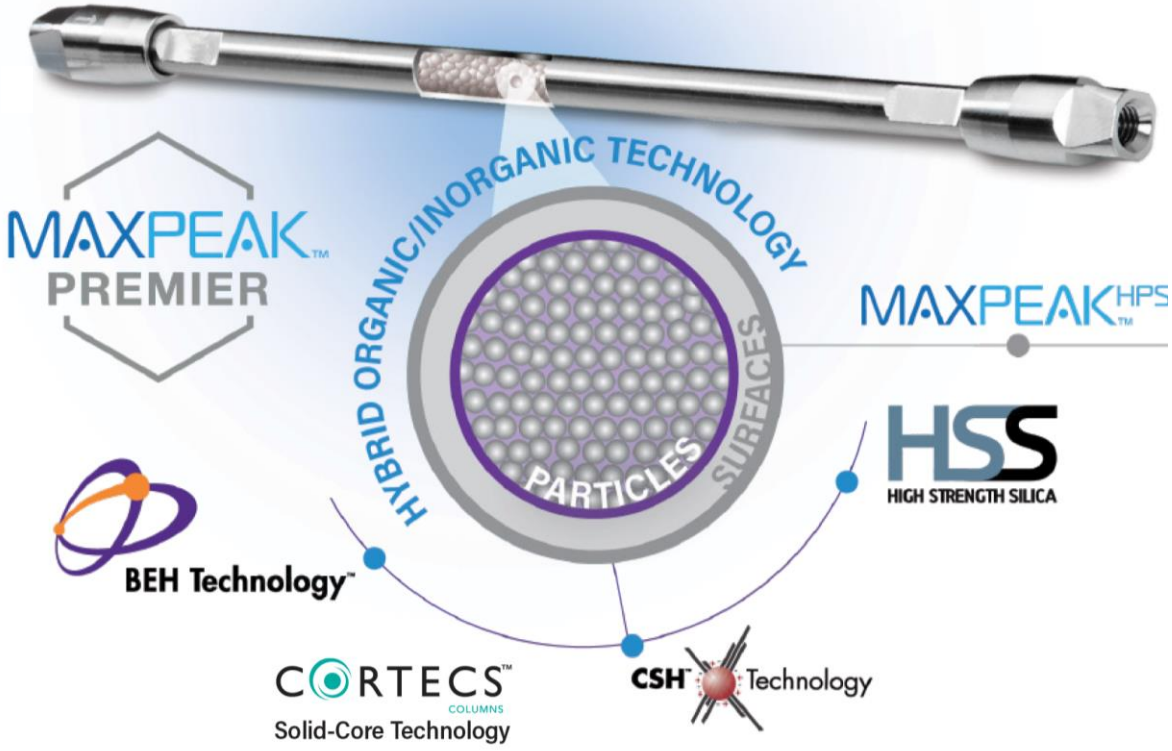
MAXPEAK™  
HIGH PERFORMANCE SURFACES



Odstranění problémů s analyty citlivými na kovy

Reverzně-fázové aplikace

# Co je MaxPeak High Performance Surfaces technologie?



# MaxPeak Premier kolony jsou dostupné pro všechny moderní částice **Waters™**

Acquity  
UPLC™

Acquity PREMIER

Bridge  
COLUMNS

Bridge PREMIER  
COLUMNS

XSELECT  
Columns

XSELECT PREMIER  
Columns

CORTECS  
COLUMNS

CORTECS PREMIER  
COLUMNS

## Hybrid



**BEH™ Technology**  
**FULLY POROUS**

**Unparalleled pH stability**

Mobile phase and temperature versatility

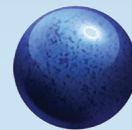


**CSH™ Technology**  
**FULLY POROUS**

**Controlled surface charge**

Unparalleled peak symmetry for bases in formic acid

## Silica



**HSS Technology**  
**FULLY POROUS**

**Mechanical stability of pure silica particle**

Increased retention



**CORTECS SOLID-CORE TECHNOLOGY**

**High efficiency and resolution**

High speed optimized for backpressure

**Fully Scalable:** UPLC ↔ UHPLC ↔ HPLC

## Jaké jsou ohlasy ze světa?

MaxPeak High-Performance Surfaces have been recognized by the Biotech Breakthrough Awards as the **overall biopharma innovation of the year, 2021**. Judged by a panel of technical, business, academic and marketing experts from within the industry, MaxPeak High-Performance Surfaces have been identified as **solving a persistent problem that has plagued analytical scientists for years**. By removing unwanted interactions in LC-MS, MaxPeak High-Performance Surfaces **bring greater confidence to analytical scientists**, and help them to ensure the safety and efficacy of medicines as required by regulatory authorities around the world.





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“Astonishing separation with maximal resolution and confidence.”

Rating: 4.7 ★★★★★

Application Area: LC-MS

"Waters LC columns have been my choice for a long time now. When the PREMIER column was presented I finally found the absolute best solution for my LC ECD system. All previous problems disappeared, now I **do not need to wash my column for 3 days to eliminate contamination from metal particles**, the system is stable for a long period, and we reduce polishing gold flow cell from once a week to once a month. Peaks are narrower and I can see and quantify impurities with confidence. PREMIER column are proof that **Waters is truly is pioneer in liquid chromatography, and all others follow its footprints.**"

Review date: 08 Apr 2021 | ACQUITY Premier Columns

“Great and reliable results.”

Rating: 4.0 ★★★★★

Application Area: Peptides, proteins

"For the analysis of peptides, I consider that it gives me very good and accurate results. **For me the most important advantage of this product is not only the reliable results but also the after-sales care.**"

Review date: 08 Apr 2021 | ACQUITY Premier Columns

“Smooth method transfer and significantly lower carry-over for sticky peptides.”

Rating: 5.0 ★★★★★

Application Area: Peptide analysis

"At our lab we were already using the Waters Acquity HSS-T3 column for the analysis of peptides with very satisfying results. For some peptides, significant carry-over was visible. To minimize carry-over, the Acquity Premier HSS-T3 column was purchased. For us, it was **very important that the column would give similar retention times** since we are analyzing a lot of peptides in one single run in a routine method. **With the Acquity Premier column, the retention times were identical to the Acquity HSS-T3 column**, moreover the carry over was significantly lower. Price of the Acquity Premier column is comparable to other UHPLC columns."

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„MaxPeak Premier Columns mitigate risk. They offer change, without change. They help us sleep better at night.“

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Application Area: Peptide analysis

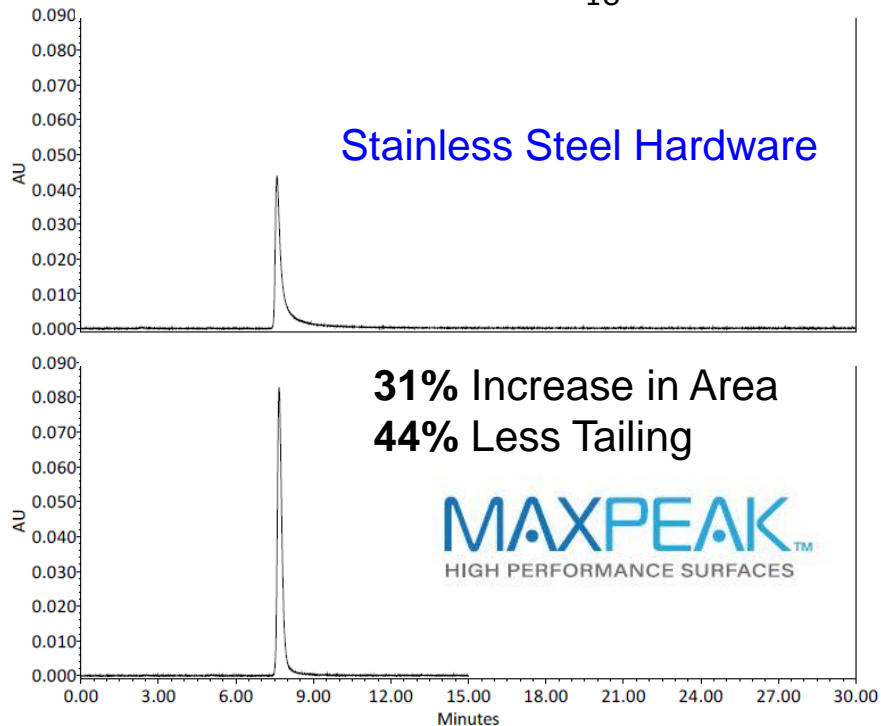
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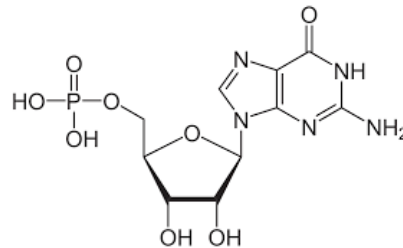
# Výhody MaxPeak HPS Hardwaru

## Lepší tvar píků a lepší citlivost

Atlantis Premier BEH C<sub>18</sub> AX



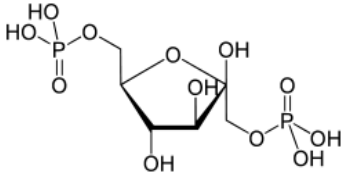
- Zlepšená výtěžnost a tvar píku pro analyty citlivé na kovy
- Guanosine 5'-monophosphate



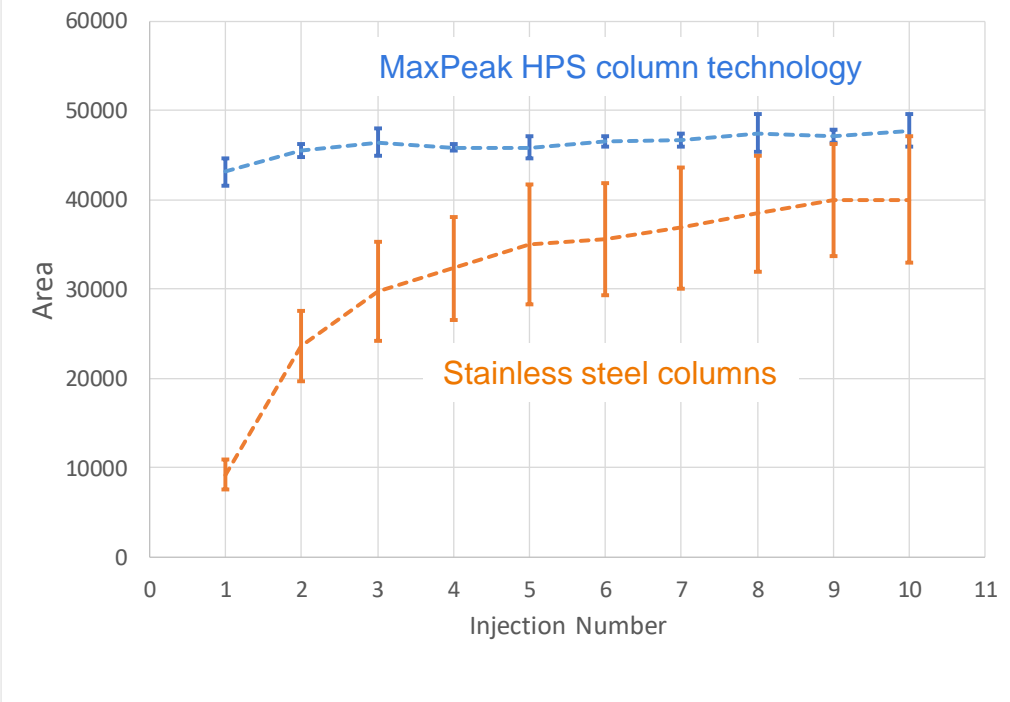
- Conditions:
  - 100% 10mM ammonium formate, pH 3
  - 200  $\mu$ L/min, PDA 254 nm

# Výhody MaxPeak HPS Hardwaru

*Konzistentní výsledky od prvního nástřiku*



- Opakovatelné výsledky nástřik od nástřiku
- 1.7 μm Atlantis BEH C<sub>18</sub> AX packed into standard 2.1 x 50 mm stainless steel or MaxPeak HPS technology columns (6 of each)
- 0.2 μg fructose 1,6-bisphosphate injected
- Mobile phase: 10 mM ammonium formate pH 3.00 (aq)
- 30°C
- ESI- detection



# MaxPeak Premier kolony: Stejné částice, Stejná selektivita

Zlepšení výkonu a snížení rizika

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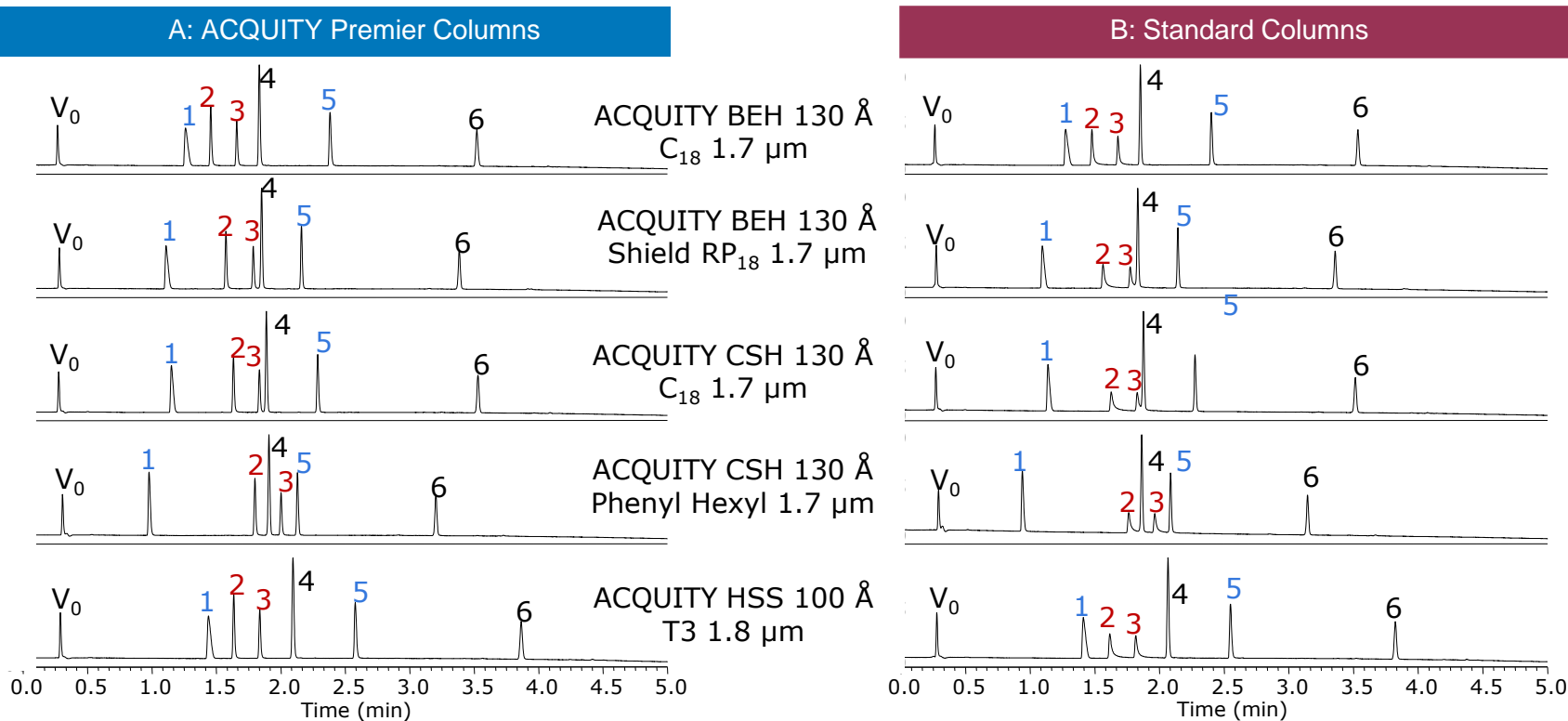
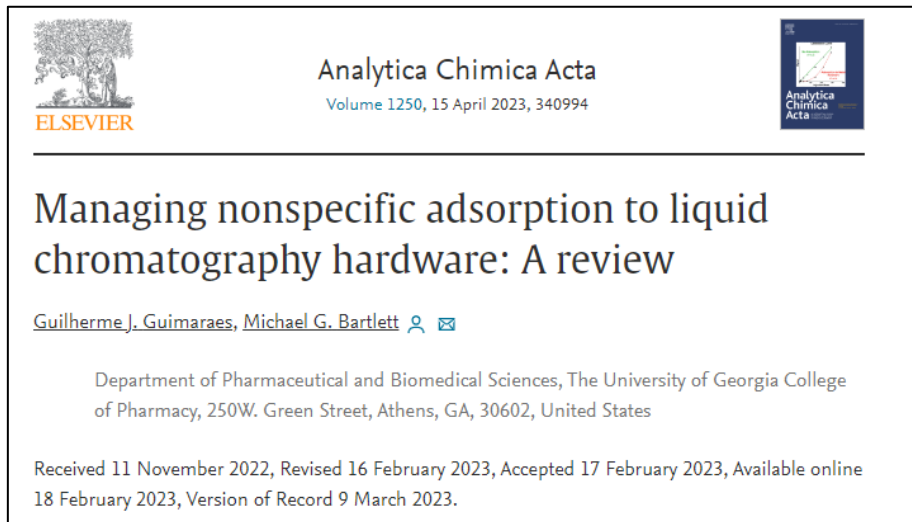


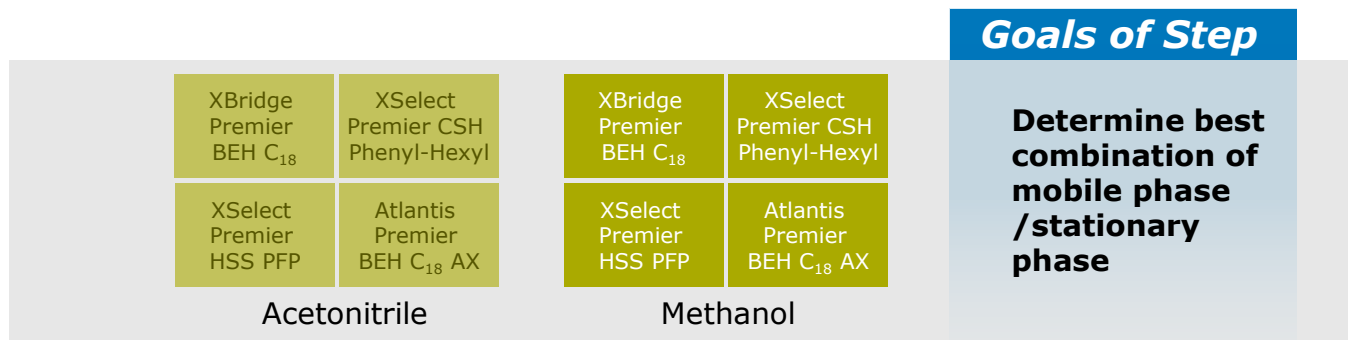
Figure 2: Comparison of separations achieved using PREMIER vs standard columns. Peak Identification: V<sub>0</sub> :thiourea, 1: metoprolol, 2: hydrocortisone phosphate, 3: dexamethasone phosphate, 4: prednisone, 5: amitriptyline, 6: dipropyl phthalate.



- Volba alternativních materiálů namísto hardwaru z nerezové oceli při konstrukci systémů kapalinové chromatografie odhalila, do jaké míry ovlivňuje nespecifická adsorpce reprodukovatelnost LC metod.
- Ačkoliv pro analýzu analytů citlivých na kovy neexistují žádné zvláštní požadavky, je v nejlepším zájmu chromatografisty dopřát těmto analytům bioinertní prostředí během LC analýz.

## Column Screening

4-6 Columns  
ACN & MeOH



Column 1 XBridge Premier BEH C <sub>18</sub>	Column 2 XSelect Premier CSH Phenyl-Hexyl	Column 3 XSelect Premier HSS PFP (fluoro-phenyl)	Column 4 Atlantis Premier BEH C <sub>18</sub> AX
Popular ligand chemistry for method development	<ul style="list-style-type: none"> <li>CSH (charged surface hybrid) particle provides slight IEX functionality</li> <li>Bonded Phenyl-Hexyl group provides unique selectivity, especially when comparing organic modifiers, acetonitrile vs methanol</li> </ul>	<ul style="list-style-type: none"> <li>HSS (high strength silica) particle has higher silanol activity compared to hybrid particles and should improve retention</li> <li>Bonded PFP ligand provides multiple secondary interactions including <math>\pi</math>-<math>\pi</math> interactions, hydrogen bond interactions, etc.</li> </ul>	<ul style="list-style-type: none"> <li>Uses BEH (bridged-ethylene hybrid) particles</li> <li>Mixed mode bonding allow for both reversed-phase and ion exchange (Anion Exchange) retention mechanism</li> </ul>

# Jak vám MaxPeak Premier HPS pomůže urychlit vývoj metody o 30%?

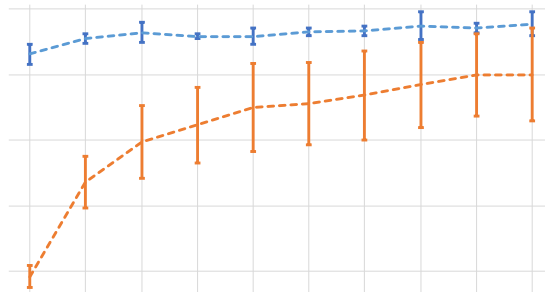
Waters™

## Začněte s menšími částicemi



Moving from a 5µm particle to a 3.5µm particle reduces run time by 30%.

## Vyhnete se pasivaci & opakovaným nástřikům



Save hours by removing the need to passivate your LC column and run conditioning injections

## Zvyšte důvěru ve své výsledky

- ↗ Peak area
- ↗ Assay precision
- ↘ Peak tailing
- ↘ Data processing

Additional time savings through better data and increased confidence

Snižte rizika při vývoji metod HPLC a eliminujte pochybnosti o výsledcích



Děkuji Vám za účast. Co dál?



**3.5 um částice**

..již brzo !!

Otázky?



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