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Some barriers are good…

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Days in the life of a method development scientist

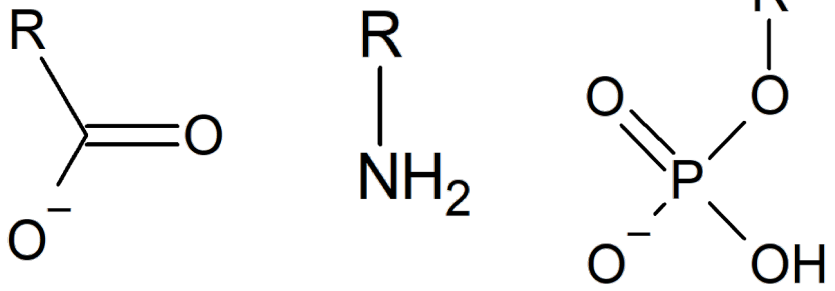
We receive a molecule for which we need to develop a method….

We start off with our favorite method/column/mobile phase combination…

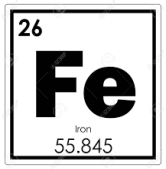
- Great day 
  - The method works perfectly, and no modifications are required
- Good day 
  - The method gives promising results but needs some simple modifications/changes
- Nightmare 
  - We don’t see our compound at all, even though we think we should

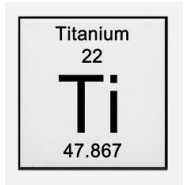
Analytes can be lost due to metal interactions

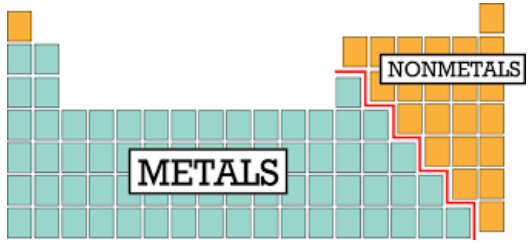
Analytes



Metal Surface







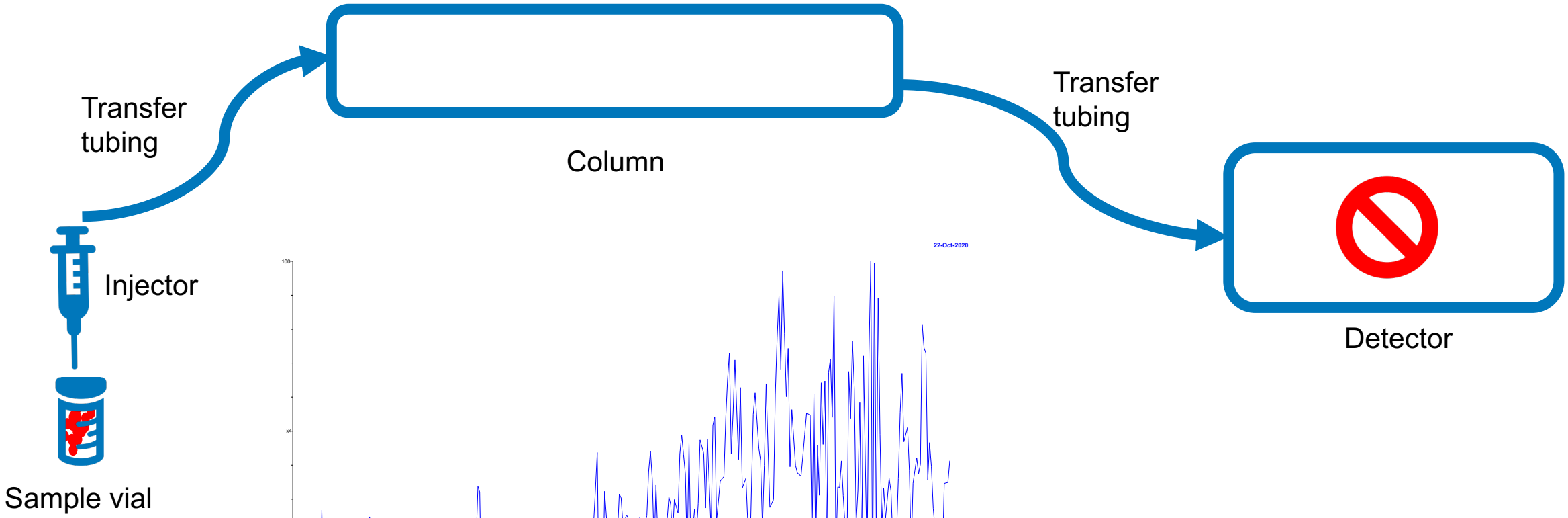
How do we try to solve this problem?

- Passivation of surfaces with acid or sacrificial sample
- PEEK or PEEK lined steel
- Titanium
- Industrial coatings

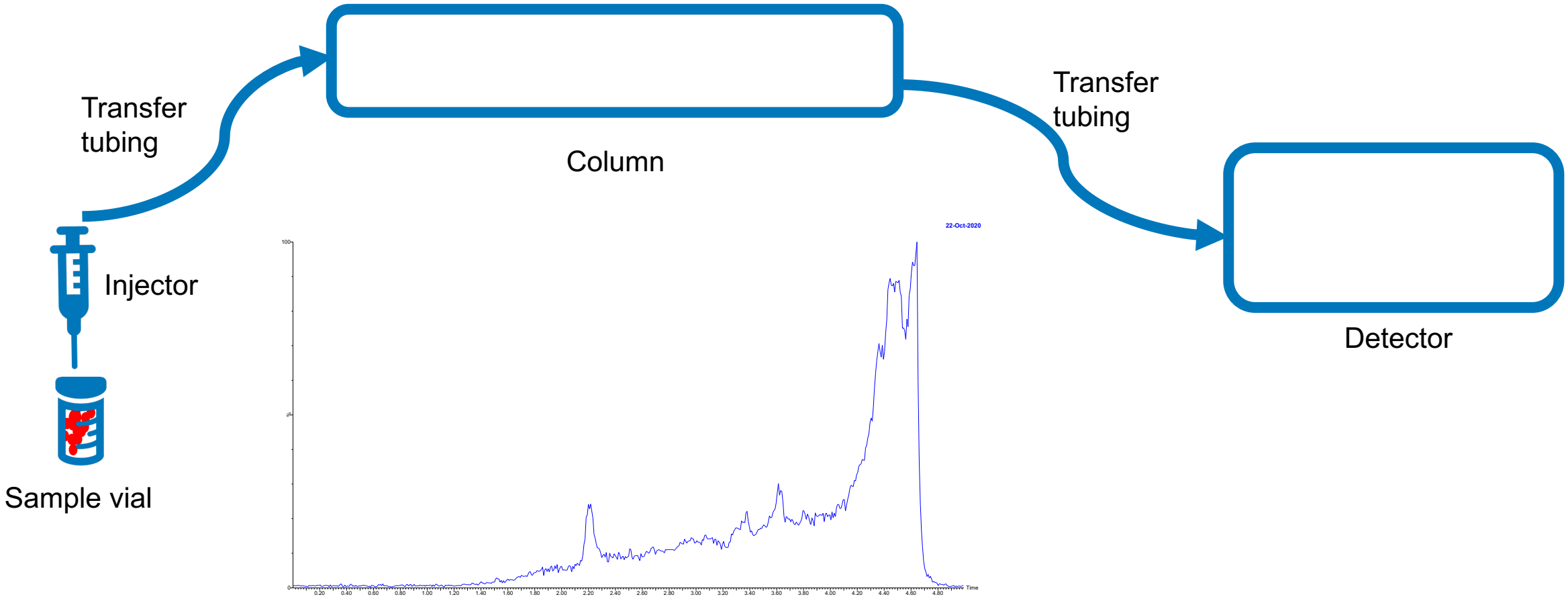
But these solutions are reversible, non-permanent or affect selectivity..

What we really need is a permanent, inert surface technology that is ready to use out of the box..

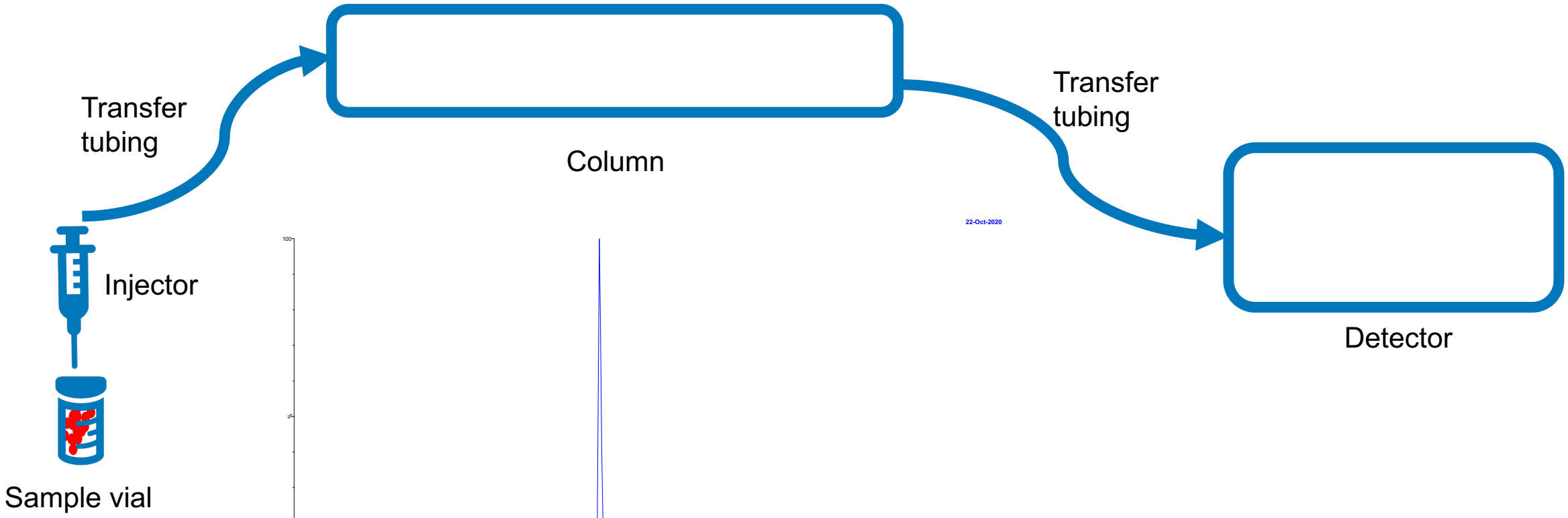
Metal sensitive analyte loss at low concentrations



Metal sensitive analyte loss at mid concentrations



Metal sensitive analyte loss at high concentrations



Hybrid Organic/Inorganic barrier technology

Metal sensitive small molecules

Small molecule compound mix

Useful tool in method development

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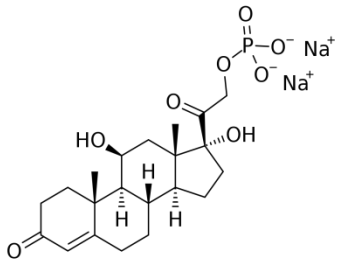
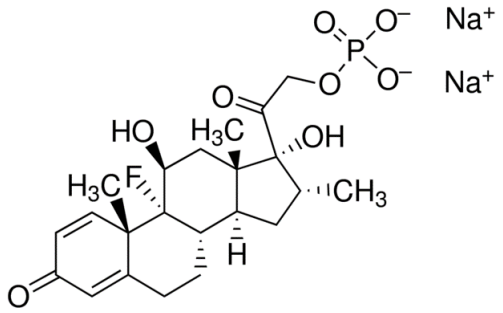
Acids

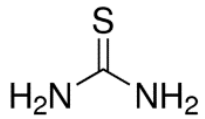


Hydrocortisone sodium phosphate
pK_a (strongest acid) 1.2; logP 1.15

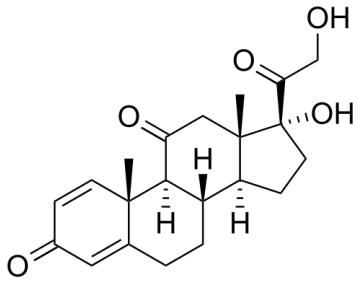


Dexamethasone sodium phosphate
pK_a (strongest acid) 1.2, logP 1.56

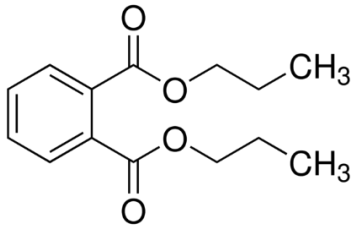
Neutrals



Thiourea
logP -1.08

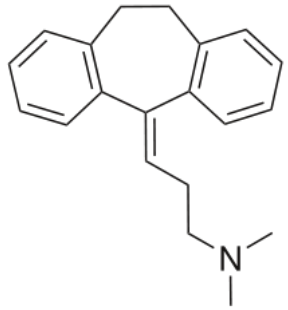


Prednisone
logP 1.46

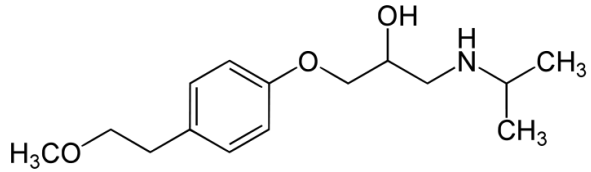


Dipropyl phthalate
logP 3.27

Bases



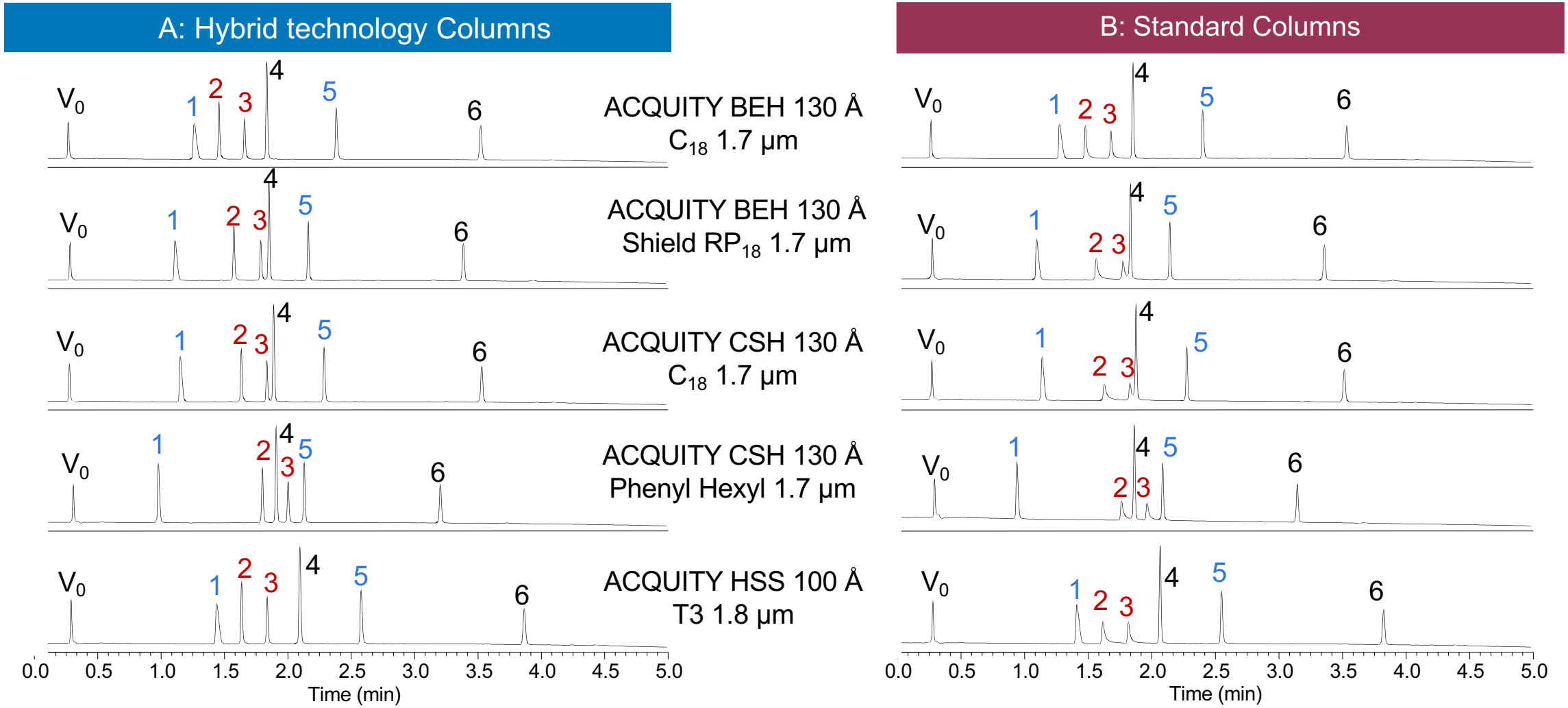
Amitriptyline
pK_a 9.4, logP 4.92



Metoprolol
pK_a 9.7, logP 2.15

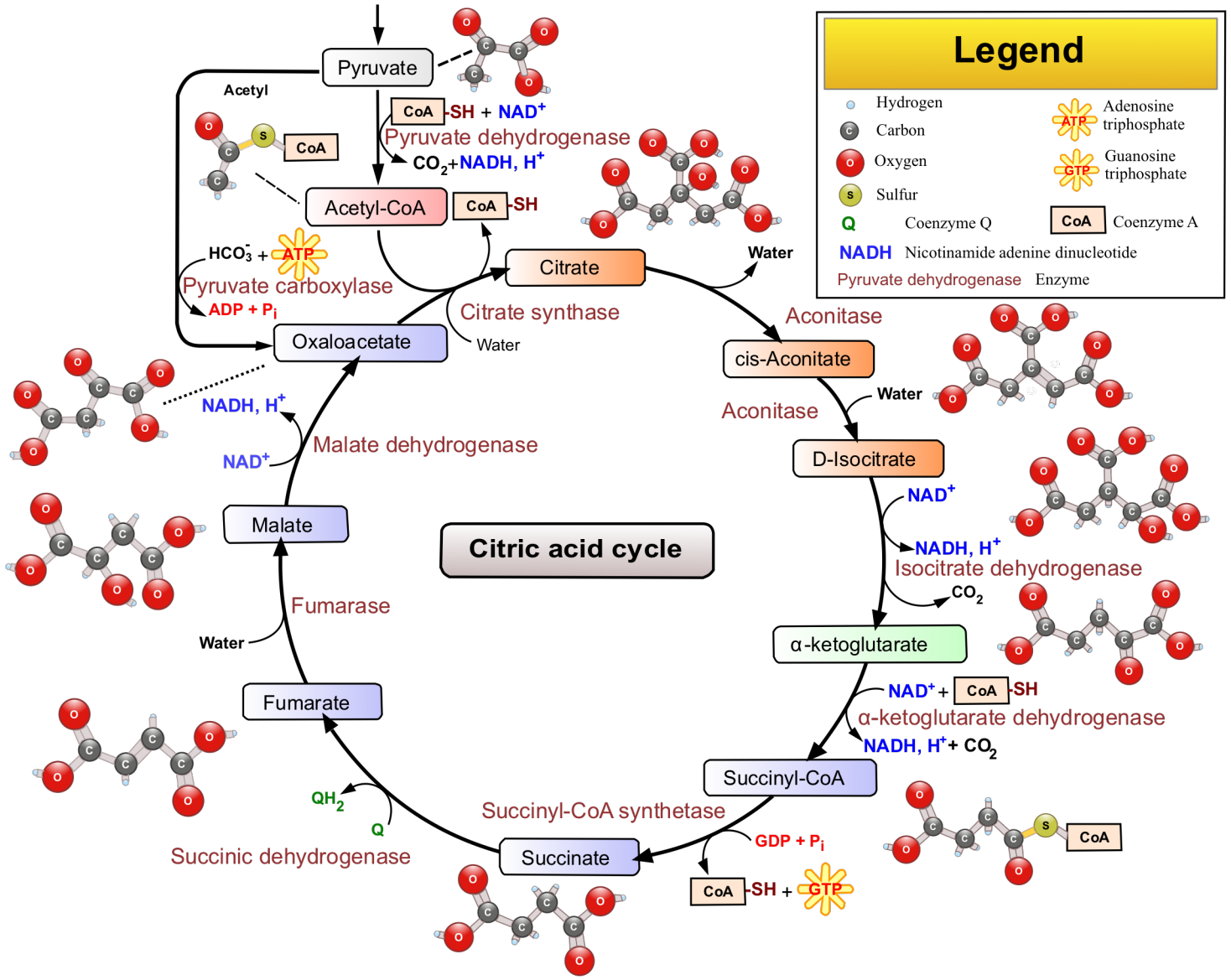
Small molecule compound mix

Useful tool in method development



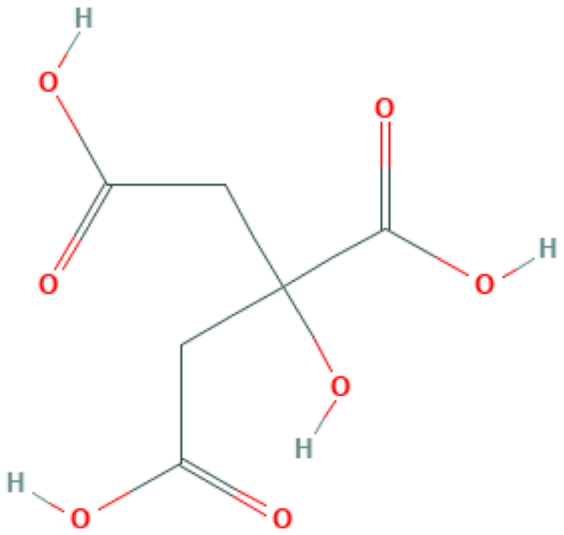
V_0 :thiourea, 1: metoprolol, 2: hydrocortisone phosphate, 3: dexamethasone phosphate, 4: prednisone, 5: amitriptyline, 6: dipropyl phthalate.

Citric Acid Cycle

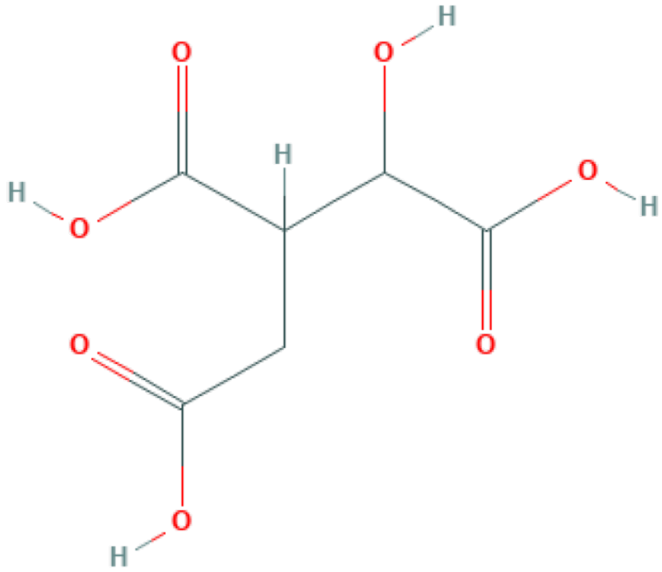


Small organic acids

Critical metabolites



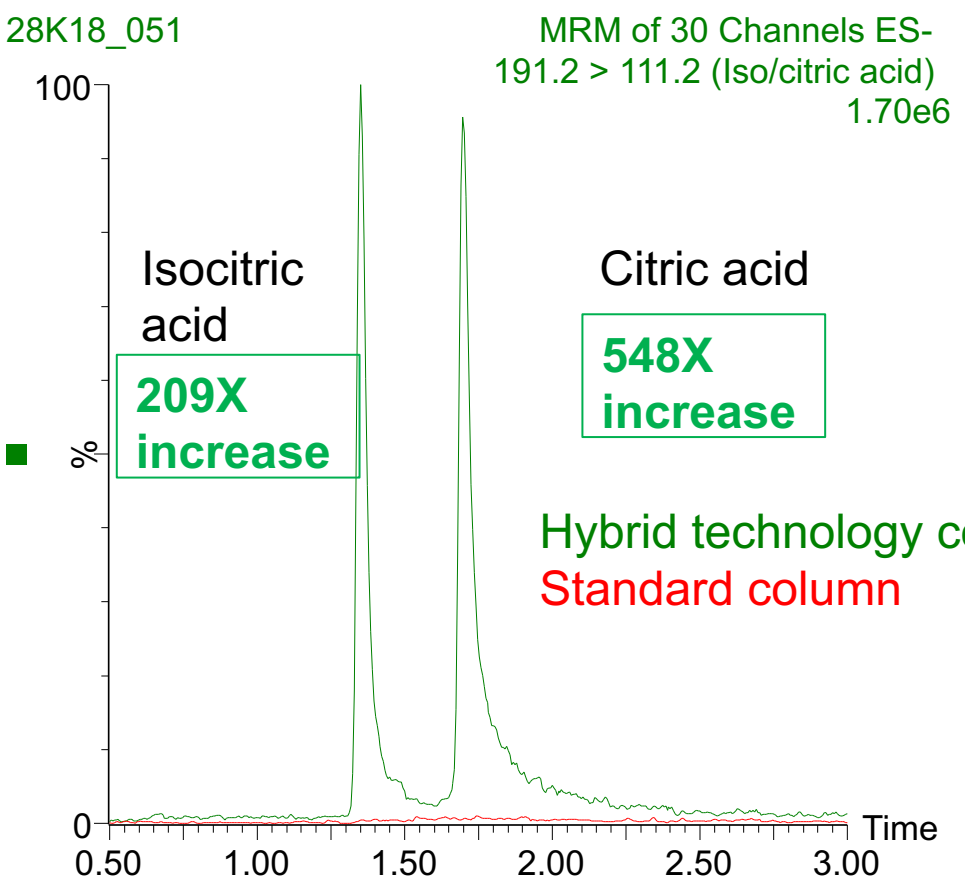
Citric Acid



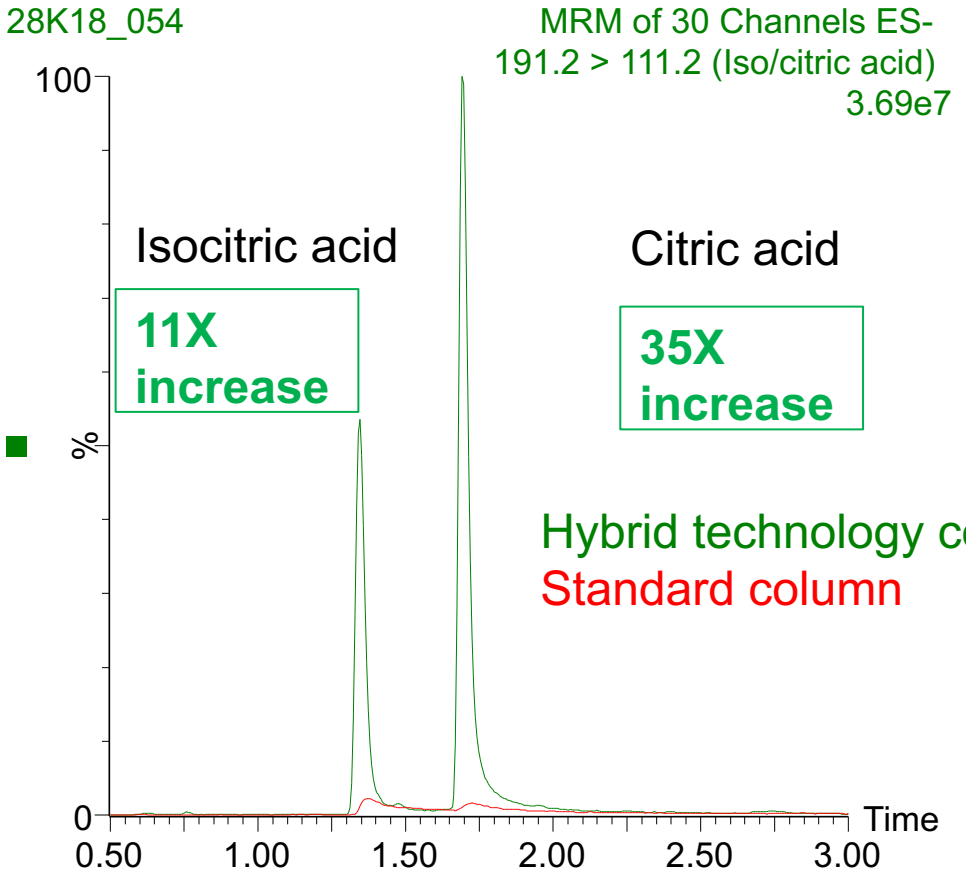
Isocitric Acid

Hybrid barrier technology columns

Making the impossible, possible for organic acids

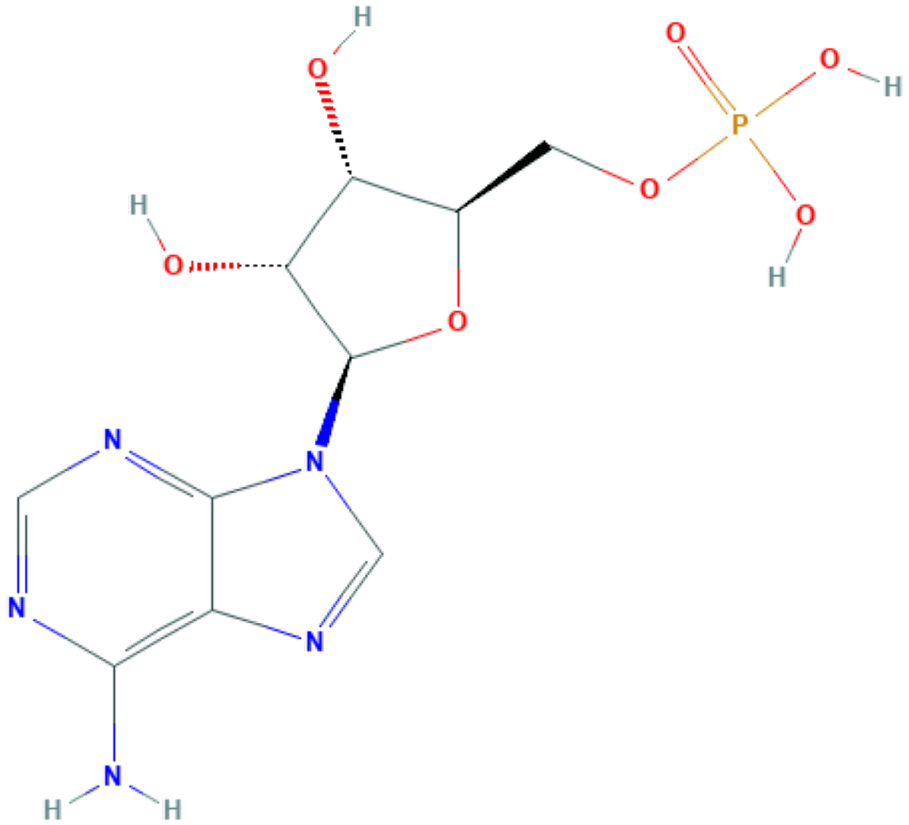


1 uM Standard concentration

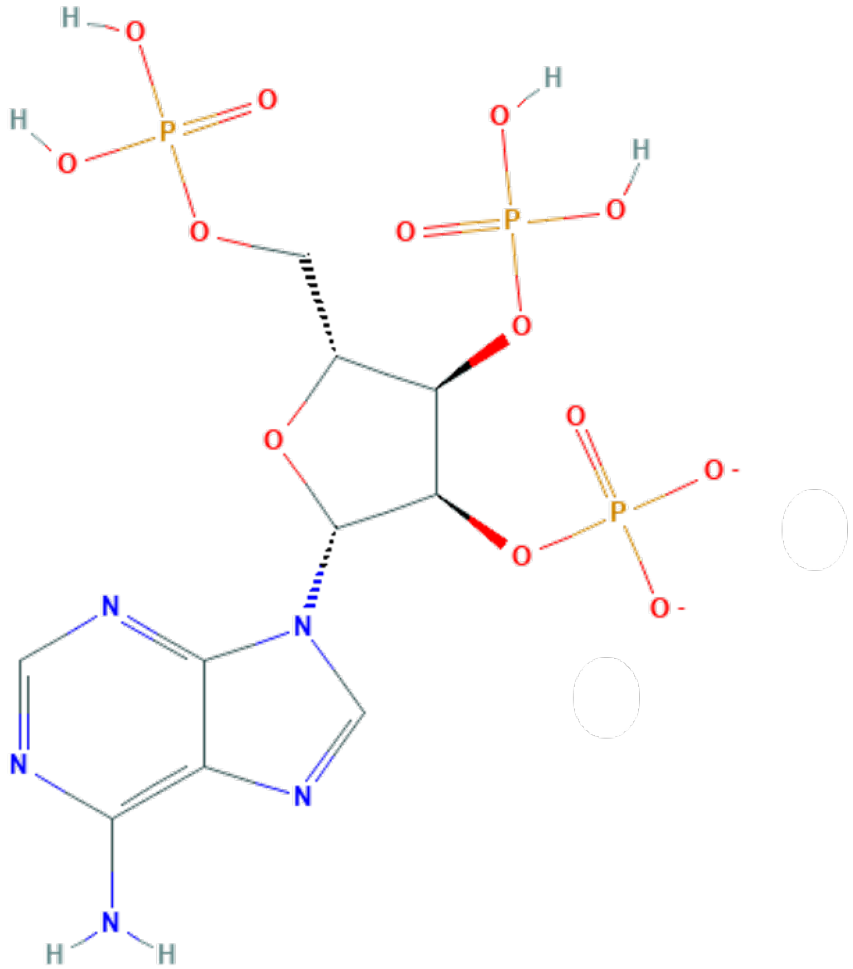


10 uM Standard concentration

Phosphorylated compounds



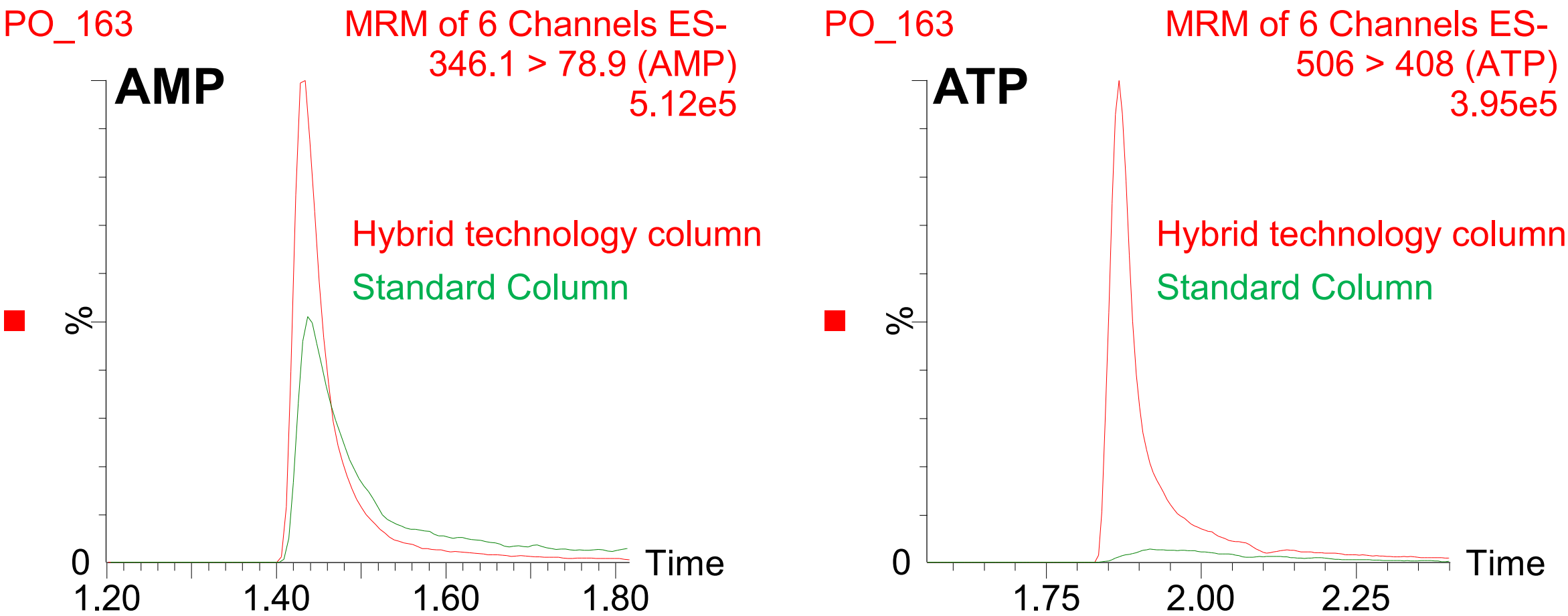
Adenosine mono phosphate (AMP)



Adenosine tri phosphate (ATP)

Hybrid barrier technology columns

Performance improvements for phosphorylated compounds



Separation on the Atlantis BEH C_{18} AX. Ref. Waters Tech Brief: 720006745EN

Hybrid barrier technology columns

Dependable performance from the 1st Injection

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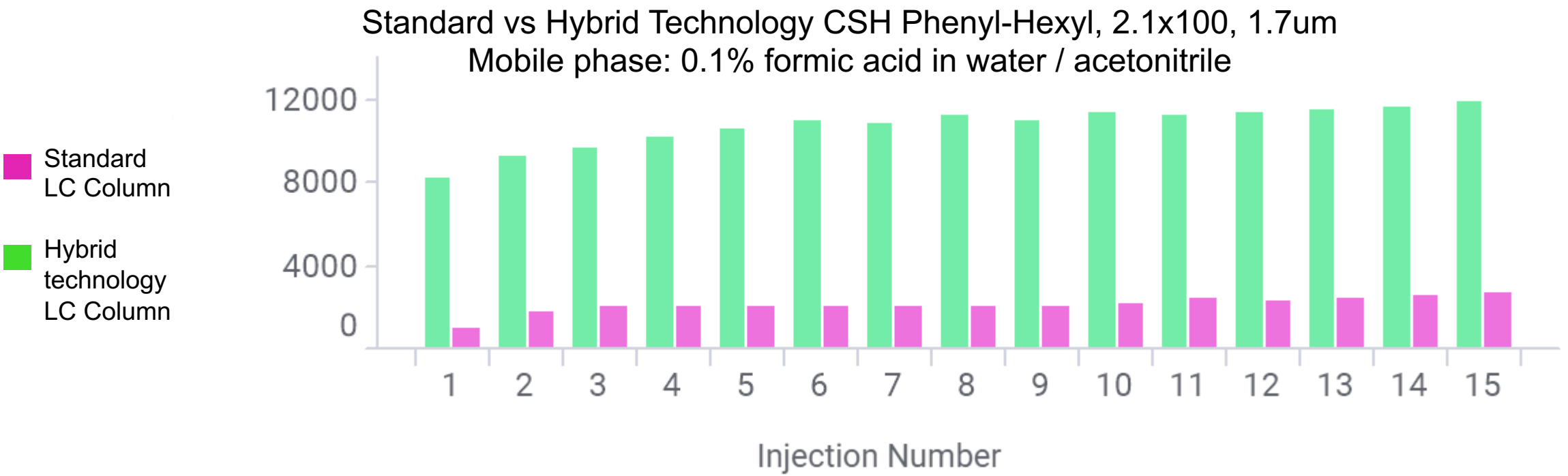
Improved Area Counts without Passivation or Additives



Citric Acid injections plotted versus number of injections / 3 µL injections of a 100 µM solution

Oligonucleotides

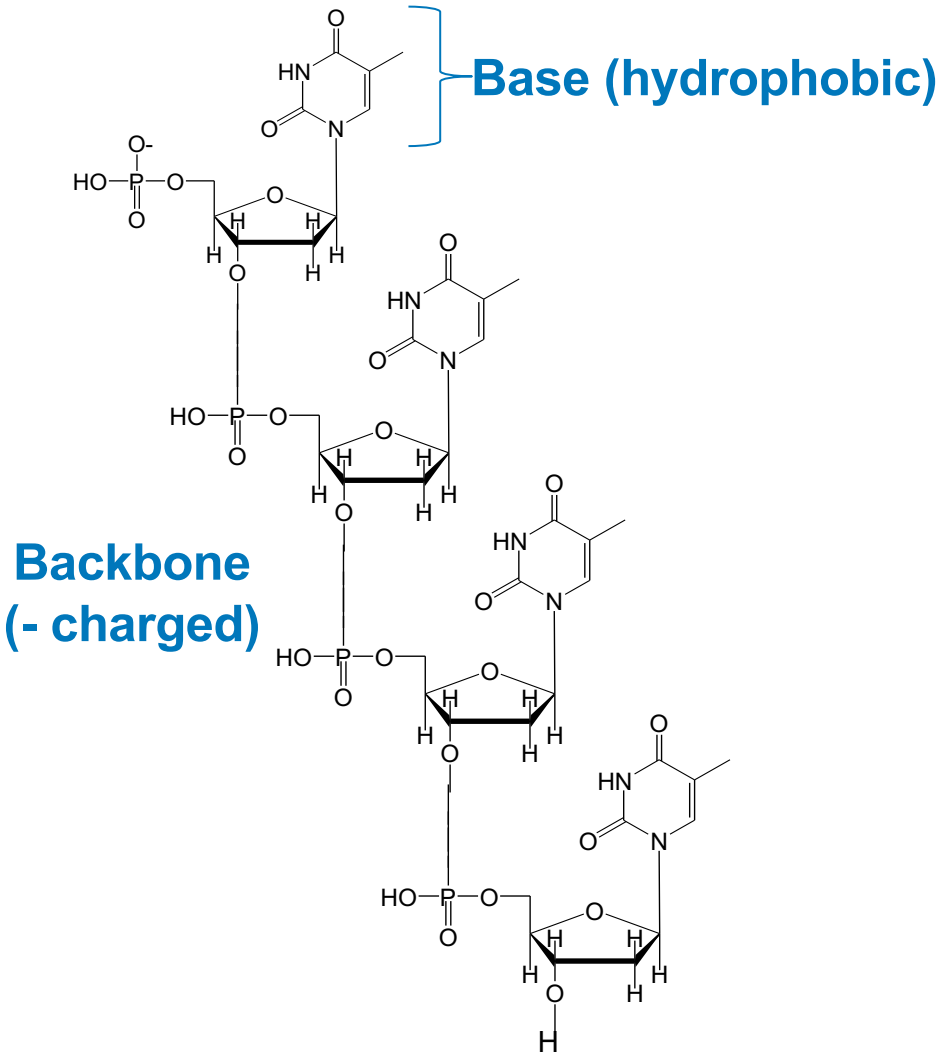
Oligonucleotide LC analysis

The Challenge of Non-Specific Adsorption

- Reversed-phase with ion-pairing
  - Good resolution
  - Requires efficient columns
  - Volatile mobile phases
  - MS compatibility

- Negatively charged nucleic acids interact (chelate) with metallic surfaces such as stainless steel or titanium, or more accurately with the oxide layer present on the metal surfaces

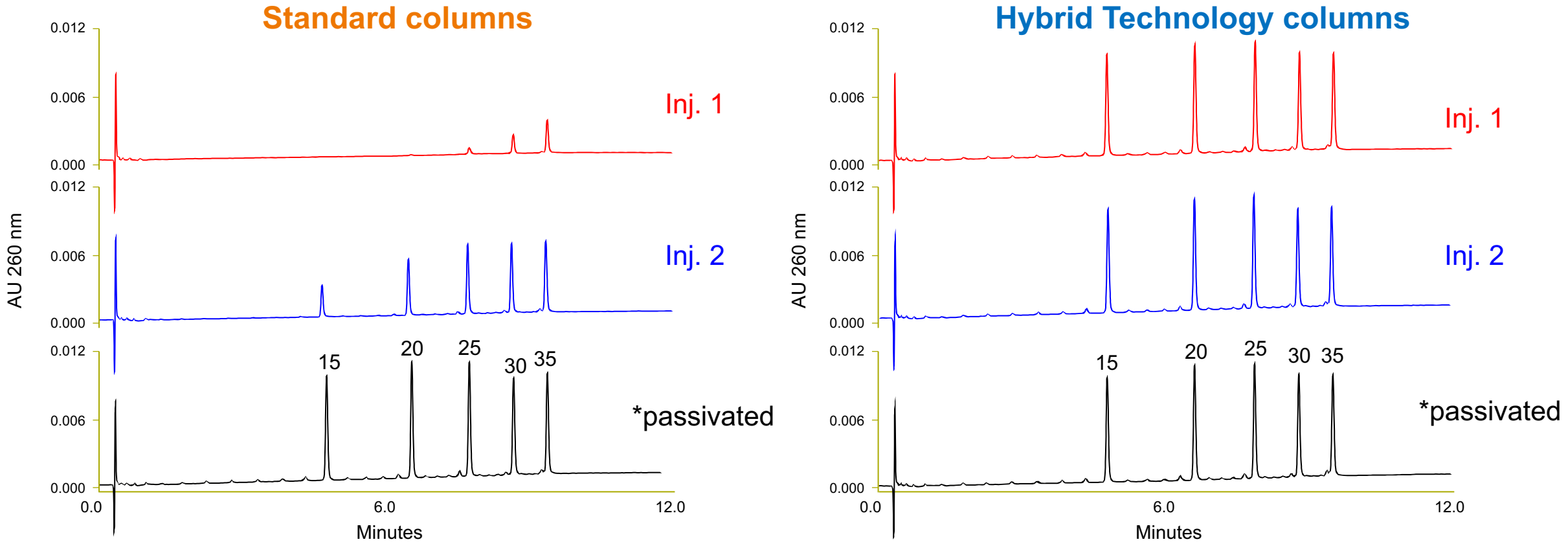
- The adsorption on metallic surfaces may contribute to peak tailing, recovery loss, sample carryover and lengthy passivation times



Hybrid barrier technology columns

Performance improvements for Oligonucleotides

15-35mer Oligonucleotide Standard (oligodeoxythymidines)



Injection of 2 µL of standard diluted in water, 10 pmol of each oligonucleotide injected on column
*passivation with 500 pmol injection of 35 mer, followed by “post passivation” injection of 10 pmol of standard

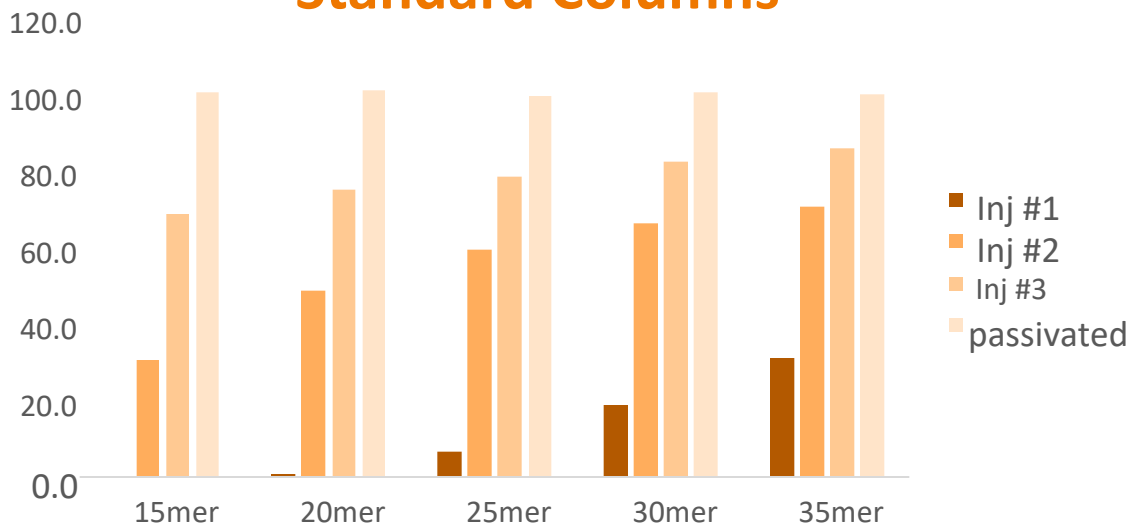
Hybrid barrier technology columns

Performance improvements for Oligonucleotides

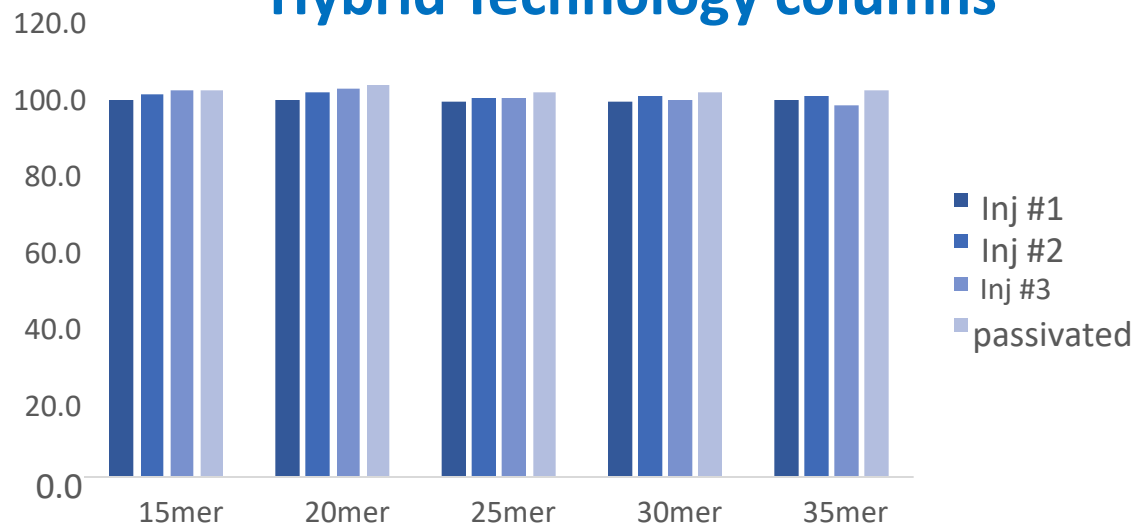
Out of the box performance for all oligonucleotides from injection #1

15-35mer oligonucleotides in Hexylammonium acetate, pH 6

Standard Columns



Hybrid Technology columns



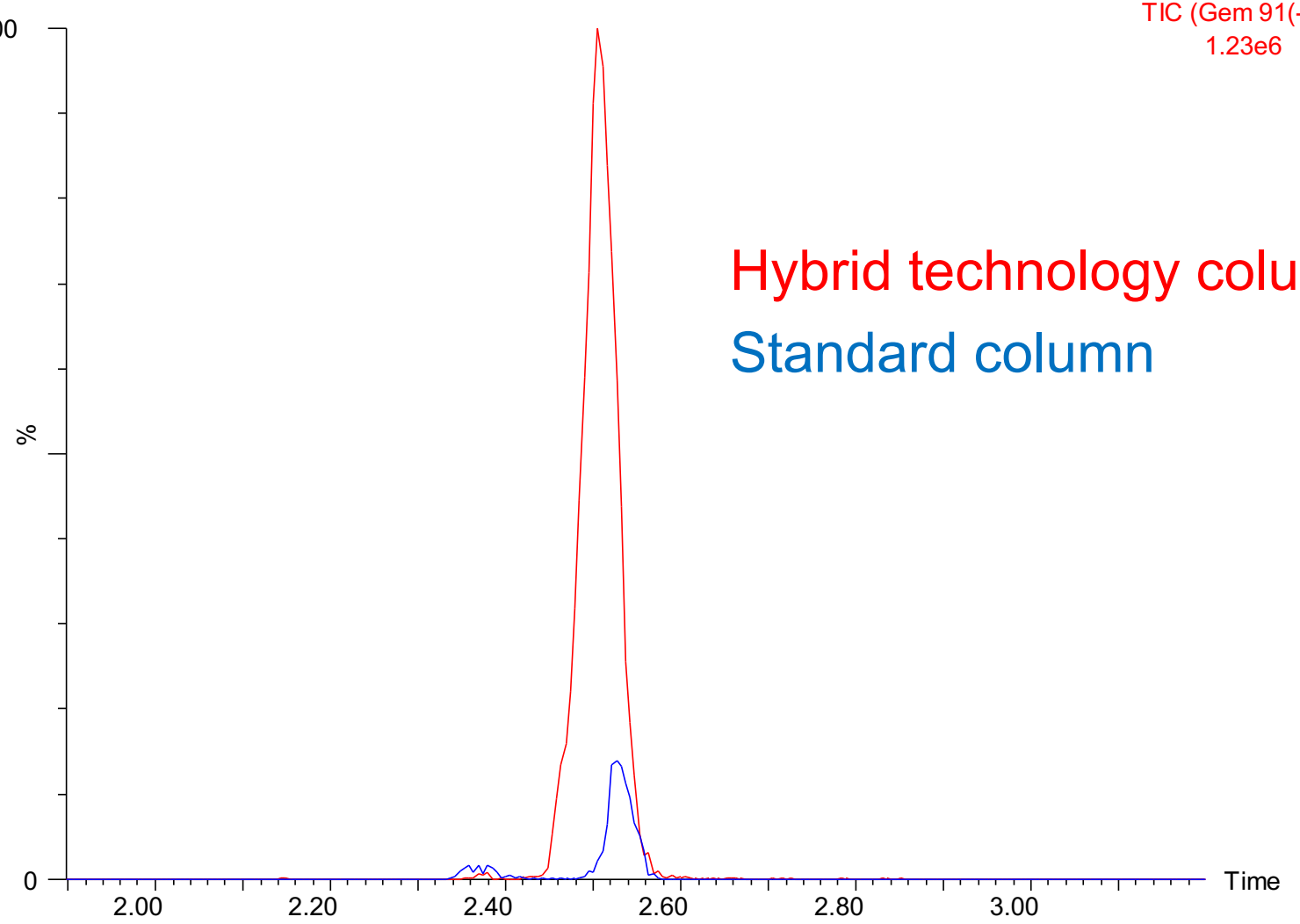
Injection of 2 µL of standard diluted in water, 10 pmol of each oligonucleotide injected on column
*passivation with 500 pmol injection of 35 mer, followed by “post passivation” injection of 10 pmol of standard

Hybrid barrier technology columns

Performance improvements for Oligonucleotides

GEM91 - Antisense oligonucleotide phosphorothioate

10x improvement in signal



Hybrid technology column

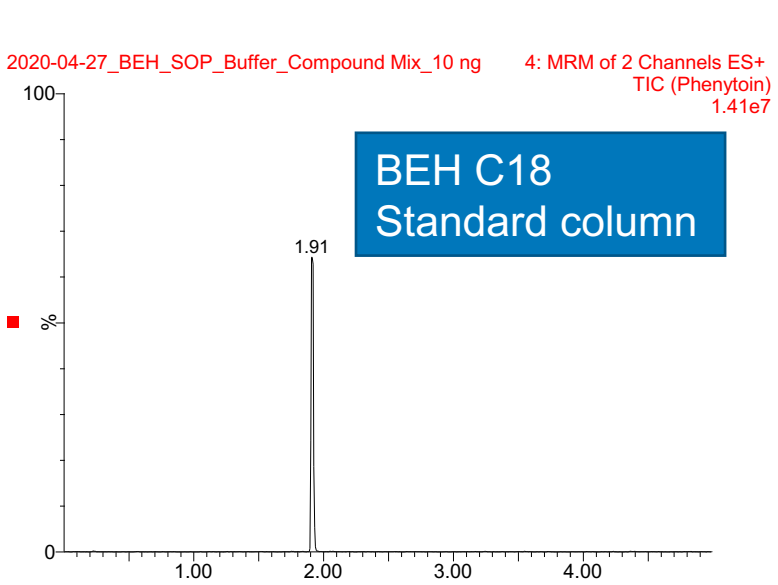
Standard column

UPLC Oligonucleotide C18 column

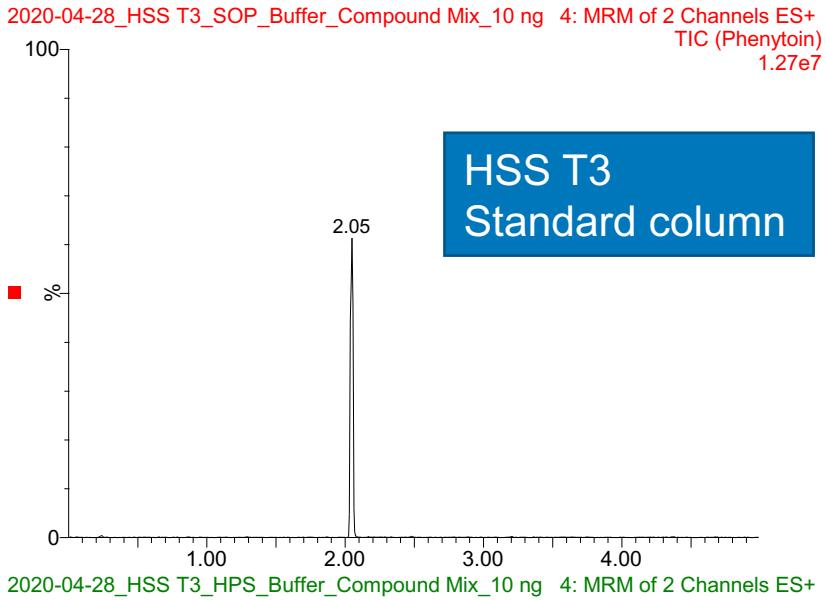
Metal insensitive compounds

Hybrid barrier technology columns

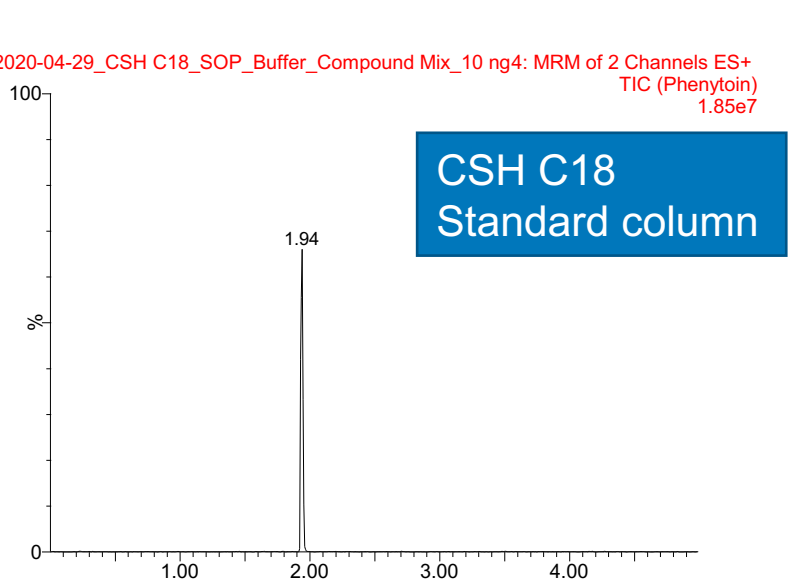
Performance improvements for metal insensitive compounds (PHENYTOIN)



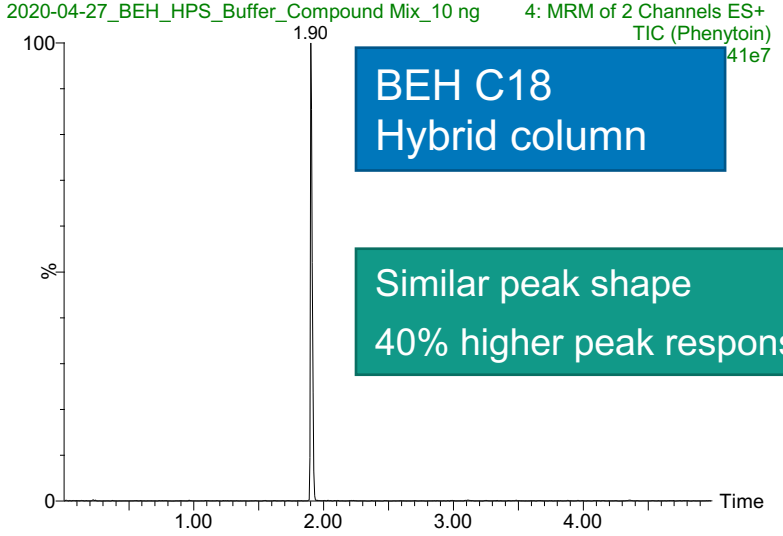
BEH C18
Standard column



HSS T3
Standard column



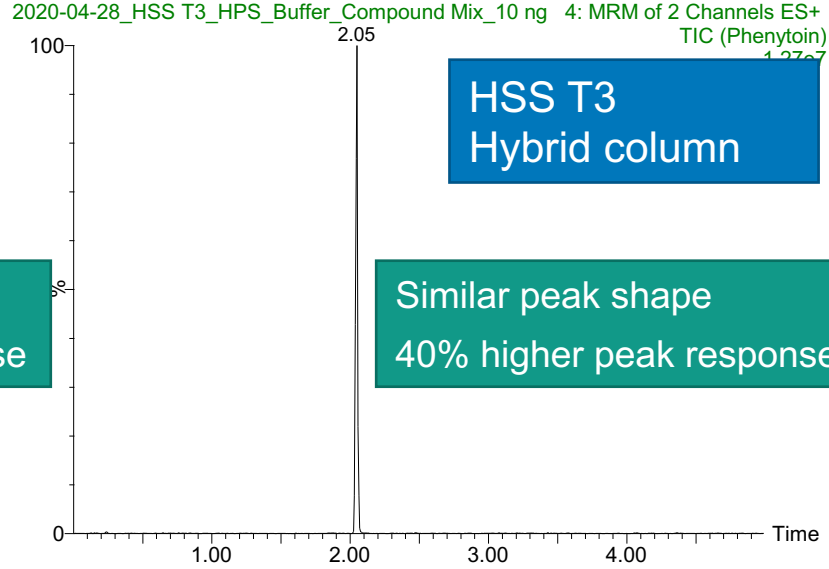
CSH C18
Standard column



BEH C18
Hybrid column

Similar peak shape

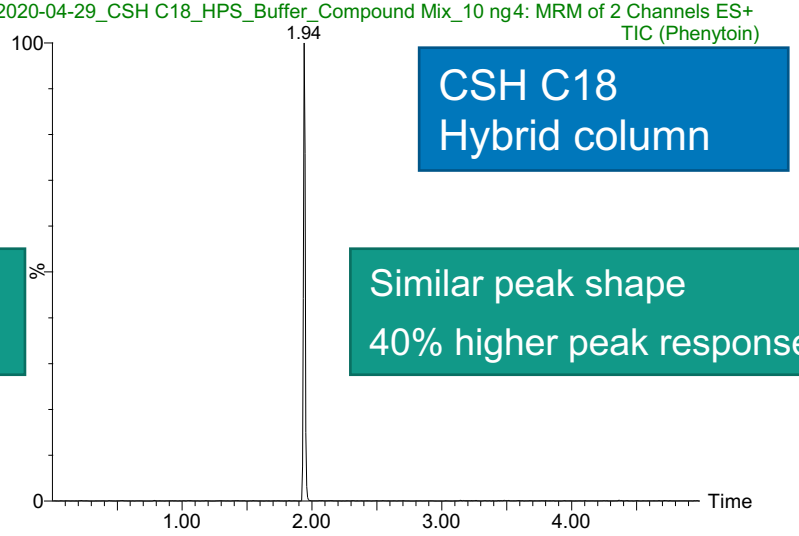
40% higher peak response



HSS T3
Hybrid column

Similar peak shape

40% higher peak response



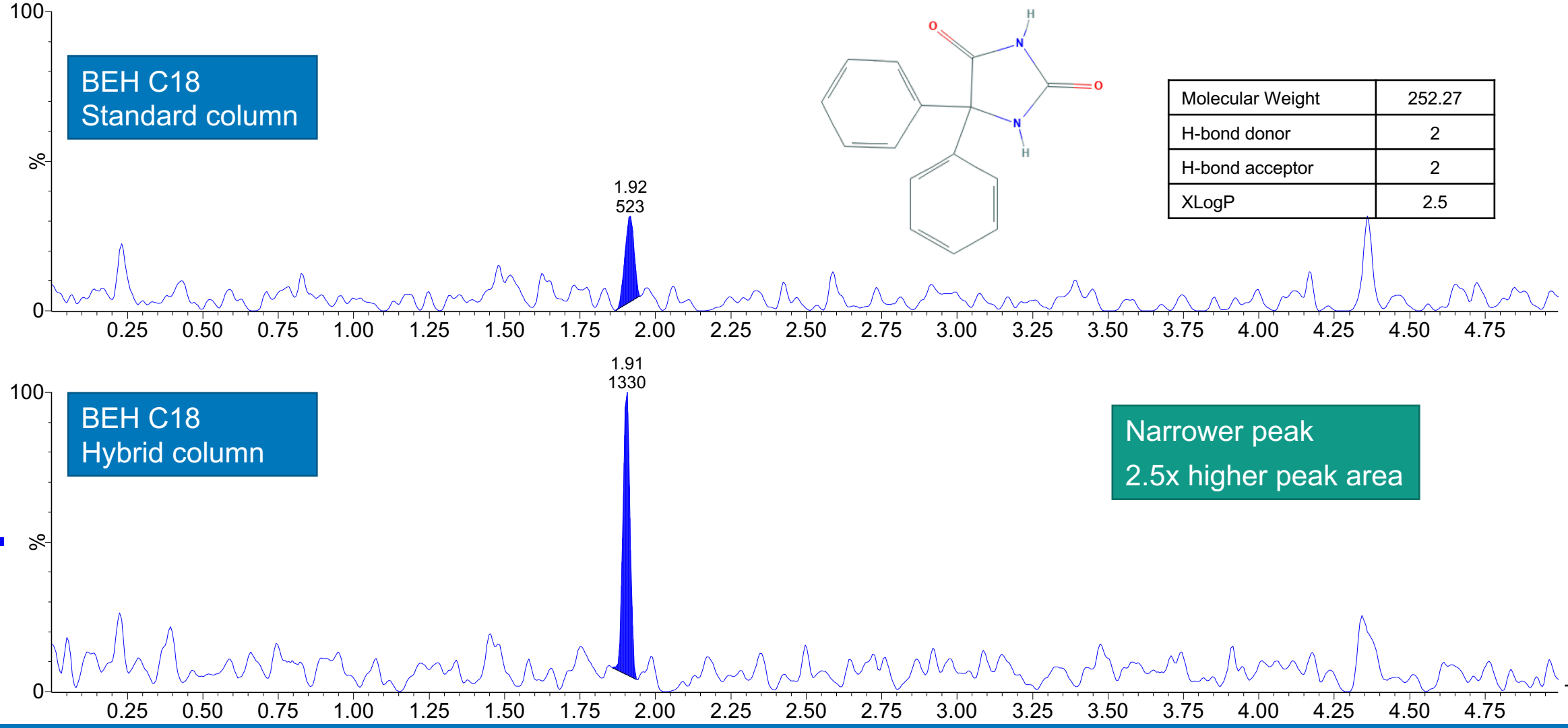
CSH C18
Hybrid column

Similar peak shape

40% higher peak response

Hybrid barrier technology columns

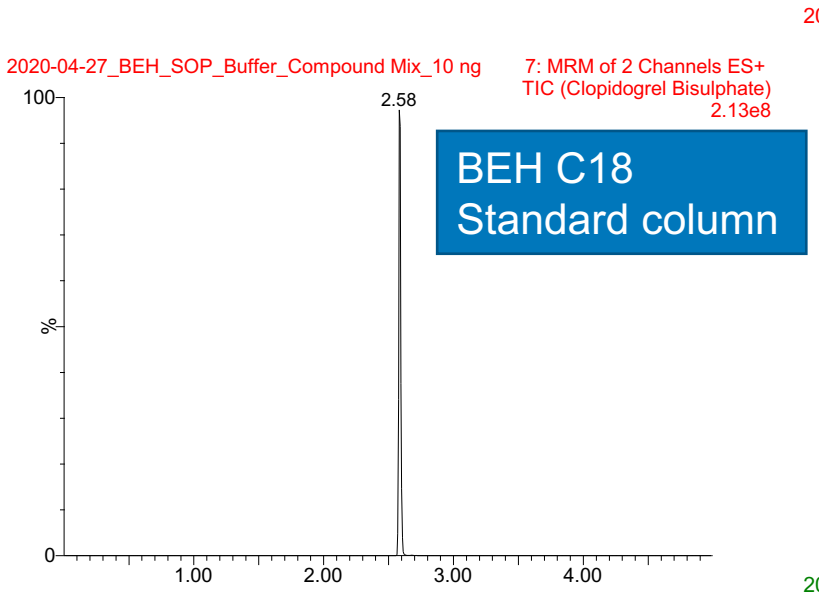
Performance improvements for metal insensitive compounds (PHENYTOIN)



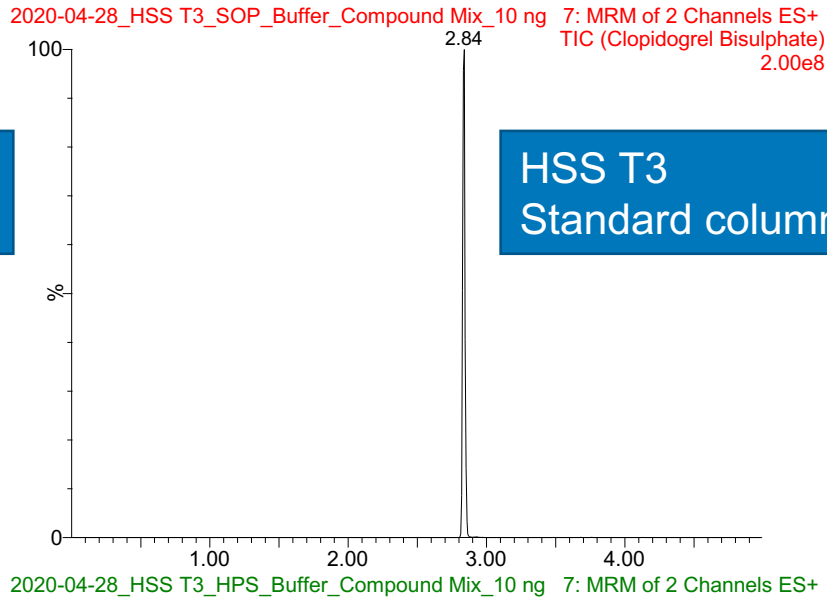
Molecular Weight	252.27
H-bond donor	2
H-bond acceptor	2
XLogP	2.5

Hybrid barrier technology columns

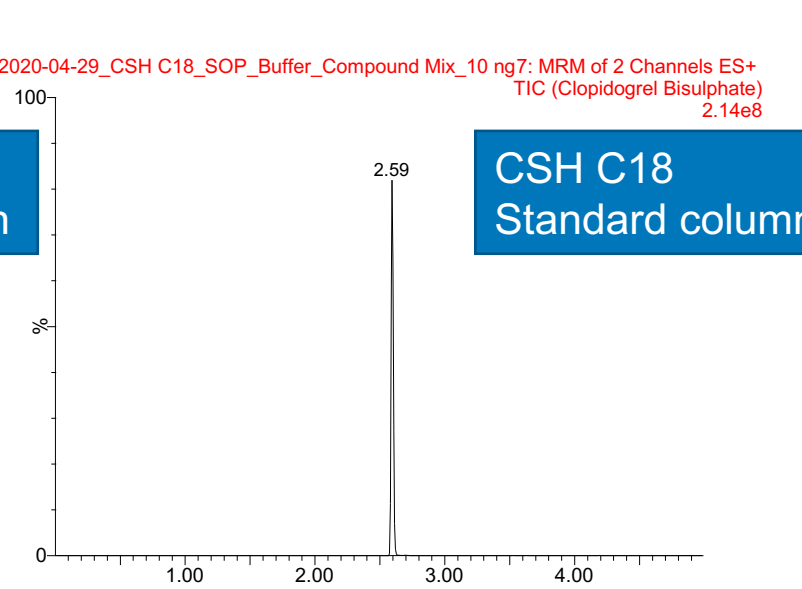
Performance for metal insensitive compounds (CLOPIDOGREL BISULPHATE)



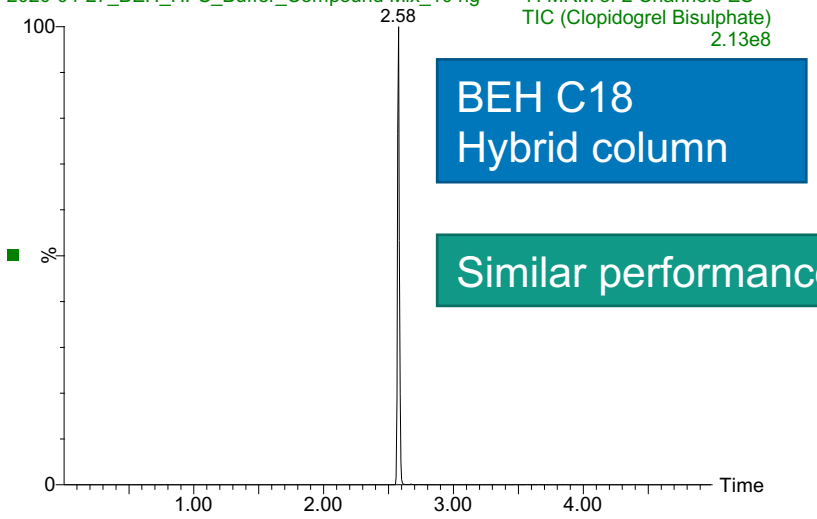
BEH C18
Standard column



HSS T3
Standard column

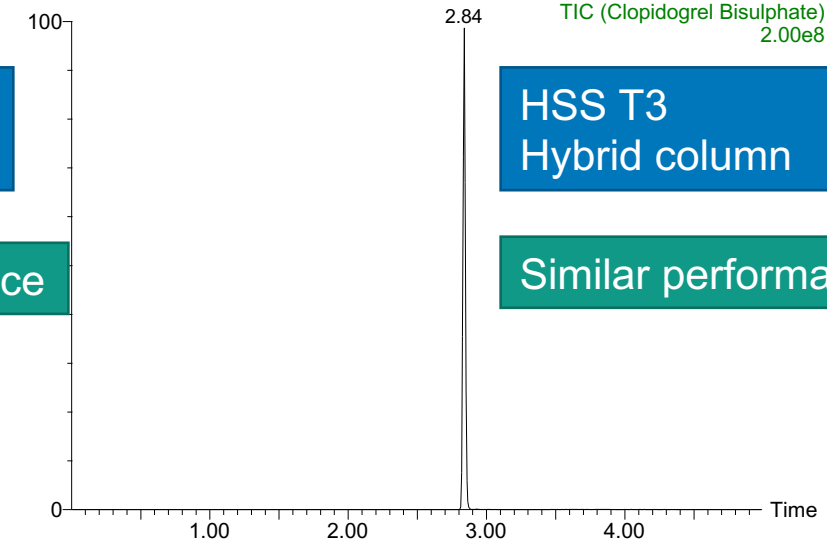


CSH C18
Standard column



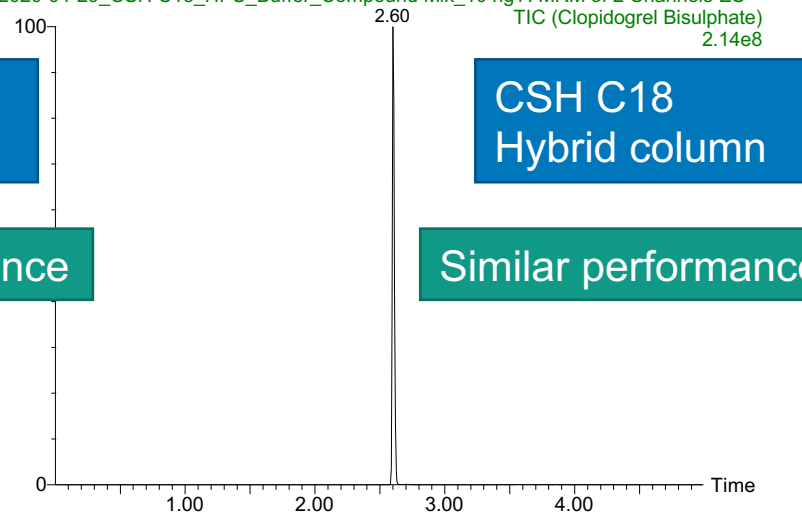
BEH C18
Hybrid column

Similar performance



HSS T3
Hybrid column

Similar performance



CSH C18
Hybrid column

Similar performance

Conclusions

- The new hybrid organic/inorganic technology provides an inert barrier which prevents unwanted analyte-column interactions without affecting chromatography

- Columns with this technology
  - Reduce metal interactions and significantly improve performance for metal chelating compounds
  - Can also improve chromatographic performance for some compounds which don't have strong metal interactions
  - Show comparable chromatographic performance to standard columns for most metal insensitive compounds
  - Can lead to increased reproducibility and robustness, especially at the lower concentration levels

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