Chromatography Solutions

Addressing ion suppression from HPLC columns

Matt James[1], Tony Edge[1], and Geoff Faden[2]

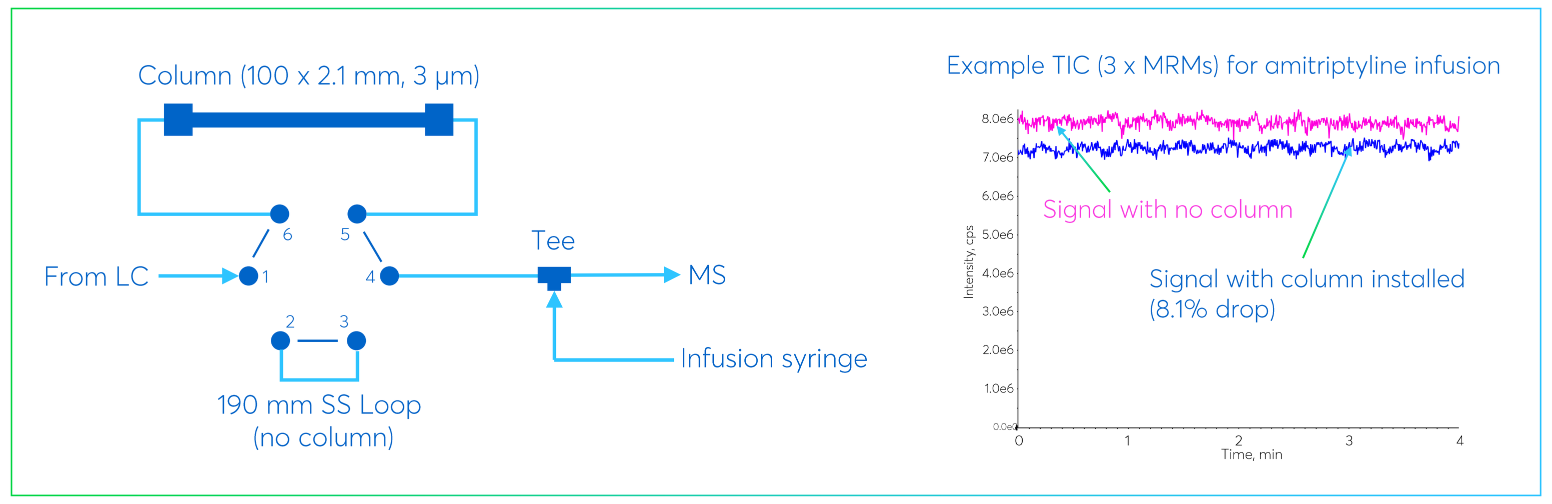
[1]Avantor, Theale, Reading, Berkshire RG7 4PE, UK, [2]MAC-MOD Analytical Inc., 103 Commons Court, PO Box 587, Chadds Ford, PA 19317 USA

1. Background

- LC-MS allows quantitative and qualitative measurement of the LC eluent.
- Electrospray ionisation (ESI) is the most commonly used interface between the LC and MS.
- Two fundamental processes for the technique:
  - 1. Desolvation
  - 2. Charge Transfer
- Efficiency of both processes (and therefore signal intensity) are impacted by co-eluting species resulting in:
  - Ion suppression/enhancement
- Separation of components by LC helps reduce this.
- Signal intensity is also affected by:
  - Mobile phase
  - System background components
  - Components bleeding from the LC column
- Often, the influence of the LC column bleed is not considered.
- This poster assesses the impact of different column stationary phase chemistries on MS signal intensity and specifically the comparison of short chain and novel long chain ligands.

2. Methods

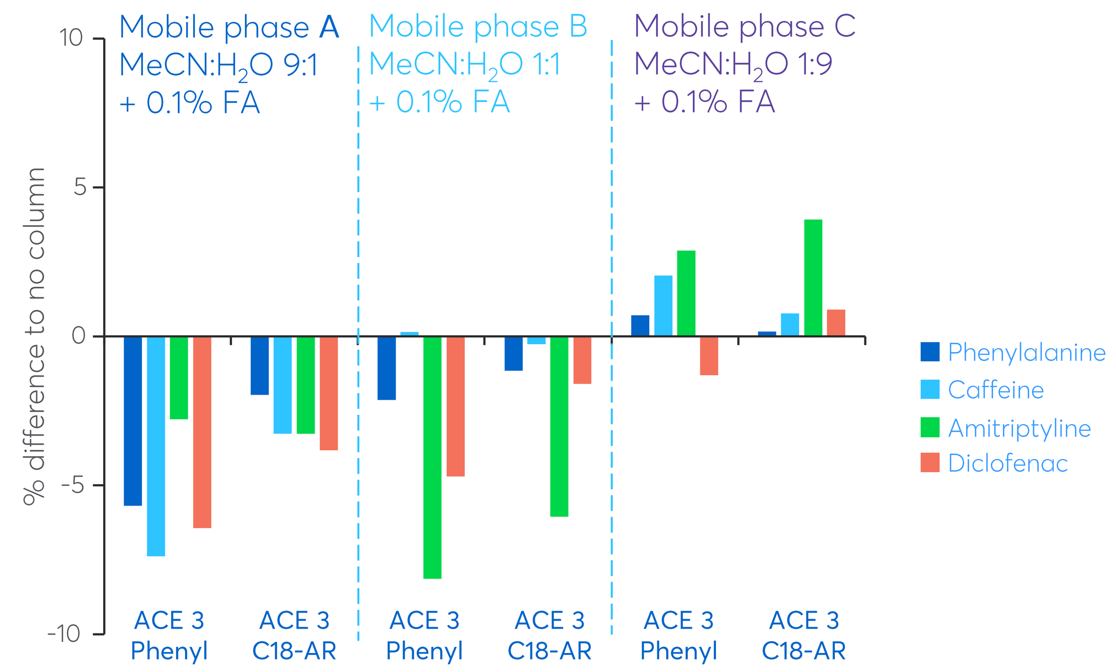
- Comparison between direct infusion of analytes into an LC eluent stream directed to the MS with and without a column, via a 6-port, 2-position integrated valve:



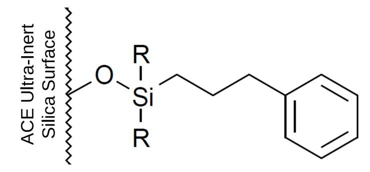
- LC Flow rate: 0.475 mL/min, Infusion flow rate: 0.025 mL/min, Temperature: ambient.
- Columns initially flushed with 95% organic (20 column volumes).
- MS optimisation performed for each analyte (3 x MRMs) & mobile phase. TIC (*m/z* 100-1,000) also recorded to monitor bleed.
- Initially, small molecules were assessed on Phenyl and C18-AR phases.
- 18 PFAS components (EPA 537.1 test mix) were then assessed.

3. Small molecules

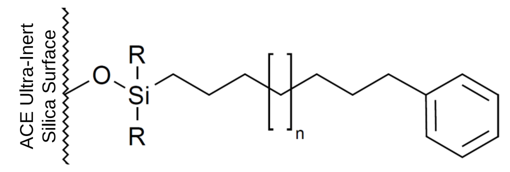
- Presence of LC column results in higher signal suppression in higher organic conditions.
- Some enhancement of signal observed at low organic content (possibly due to retention of mobile phase background components on column).
- Suppression effects observed were lower for the longer chain ligand (C18-AR).
- Supports the theory that increased stability of longer chain ligands leads to less bleed from column.
- Ligand bleed not observed on either phase, but background ion abundance was higher with the phenyl phase.



Avantor® ACE® Phenyl Propyl spacer



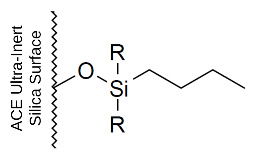
Avantor® ACE® C18-AR Proprietary Extended Stability Spacer



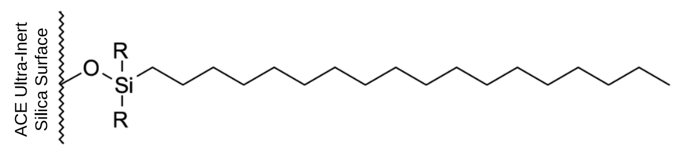
4. PFAS (Per/polyfluoroalkyl substances)

- Impact of LC stationary phase on signal intensity of 18 PFAS compounds was assessed.
  - Short- and long-chain ligands with same functionality compared.
  - 2 mobile phases:
    1: MeOH:H_2O 9:1 v/v + 10 mM ammonium acetate
    2: MeOH:H_2O 1:1 v/v + 10 mM ammonium acetate
- To compare data, recorded data was normalised to recorded background signal and expressed as a percentage.
  - $\%_{long\ chain} - \%_{short\ chain}$ plotted (+ve result indicates higher response with long chain ligand).

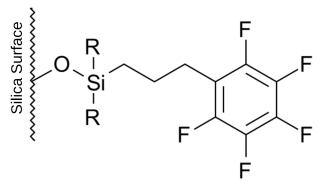
Avantor® ACE® C4



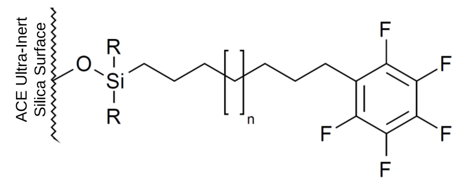
Avantor® ACE® C18

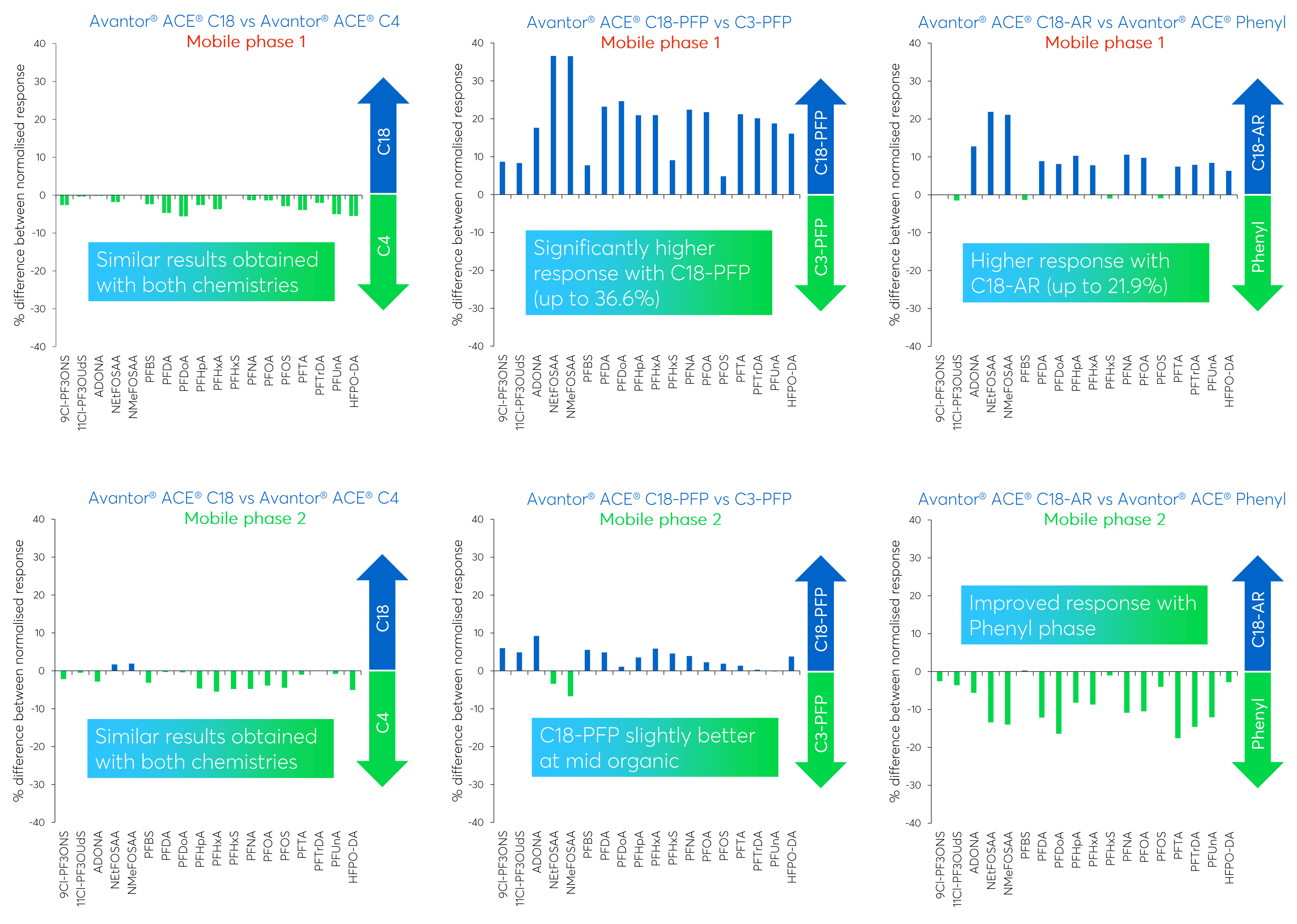


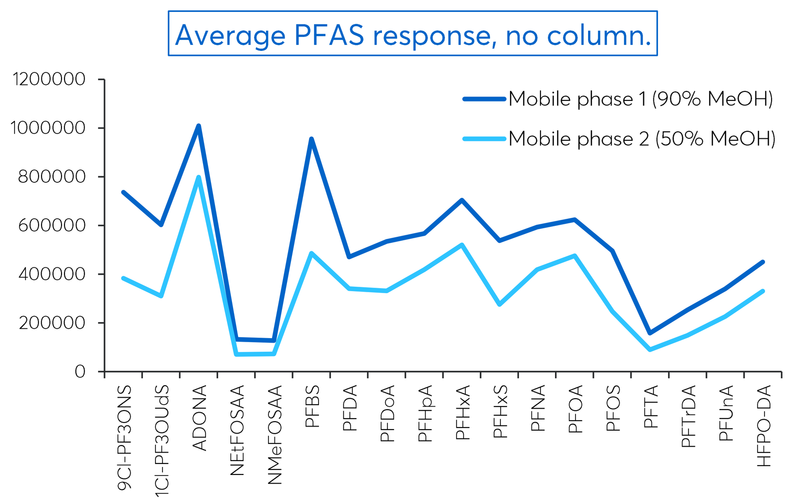
Commercially available competitor C3-PFP Propyl spacer



Avantor® ACE® C18-PFP Proprietary Extended Stability Spacer







Impact of mobile phase composition:

- With no column, PFAS response was up to 2x higher in high organic mobile phase.
- With chromatographic separation, longer chain ligands would result in elution under higher % organic conditions, providing further signal increase vs short chain ligands.

5. Conclusions

- Impact of the LC column on MS signal intensity was assessed for a variety of long- and short-chain stationary phase ligands.
- In general, longer chain ligands gave an improved signal response vs short chain ligands.
  - Particularly under highly organic eluent conditions.
  - Results suggest that this is largely due to ion suppression caused by increased bleed from shorter chain ligands.
- For PFAS, MS signals are improved under high organic conditions, further favouring use of stationary phases bonded with more retentive long chain ligands.
- The use of columns bonded with higher stability long chain ligands can potentially provide improved LC-MS sensitivity.