Chromatography Solutions

The use of Short 10 mm Columns for Rapid LC-MS Residue Testing

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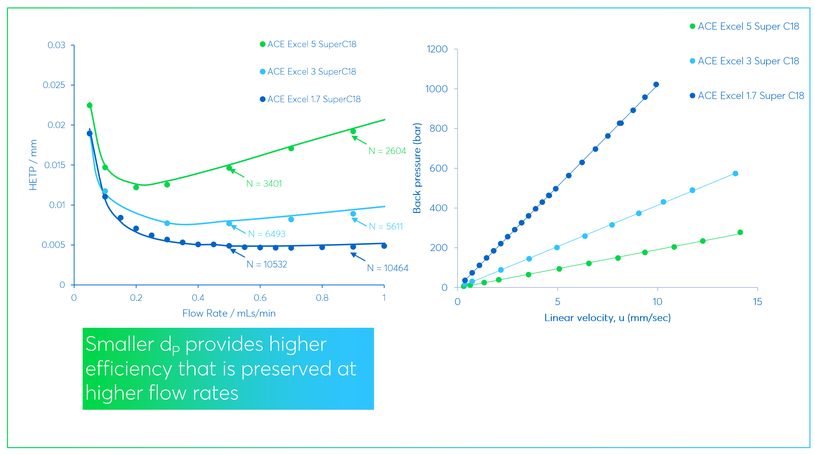
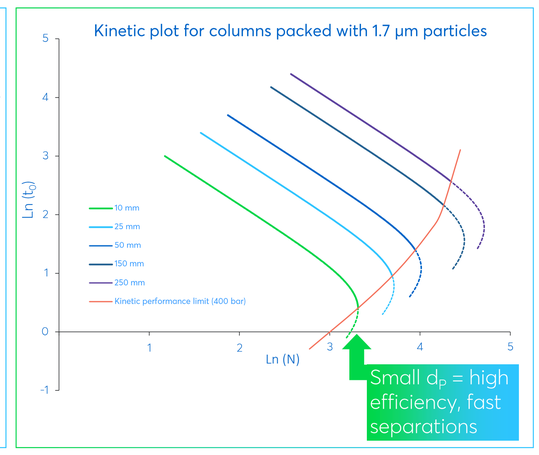
1. Background

- Higher throughput LC-MS analysis is required in many environmental labs:
  - Increasing contaminants of concern / stricter regulatory limits.
  - Large scale monitoring campaigns
- Samples typically involve complex matrices, e.g. soils, wastewater etc.
- LC-MS separations can substantially reduce sample complexity:
  - Fast/simple sample prep strategies employed.
  - Typically employ short LC columns (e.g. 50 x 2.1 mm).
  - High sample throughput.
  - LC can now be a bottleneck.
- Improved MS performance (sensitivity & data acquisition) opens new possibilities for fast separations and increased throughput:
  - Opportunities for faster separations using specially designed high throughput columns.

2. Development of Avantor® ACE® HTP-MS column design

- Requirements for high throughput:
  - High flow rate/linear velocity.
  - Short column length.
  - Maximise chromatographic efficiency.
- Consider dispersion theory (van Deemter) & kinetic performance.

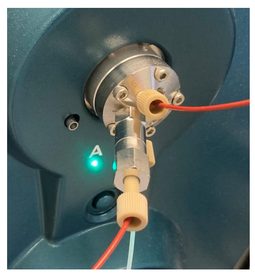




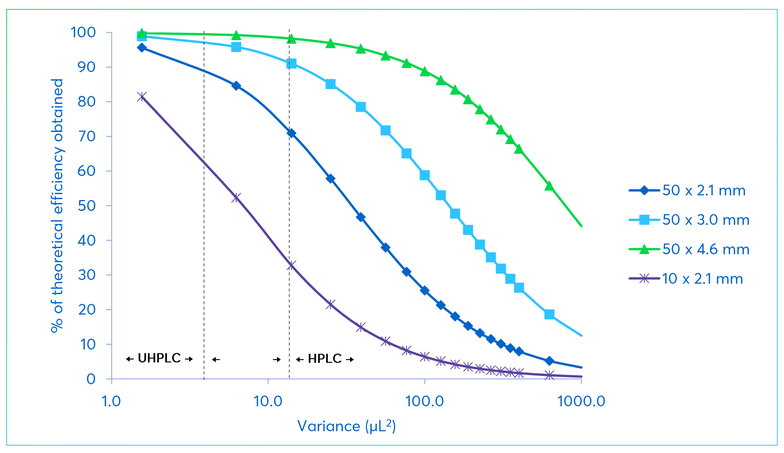
- System pressure limits restrict performance obtainable for small particles.
- Short columns with small particles provide fast, high efficiency separations.

3. 10 x 2.1 mm columns and considerations for use

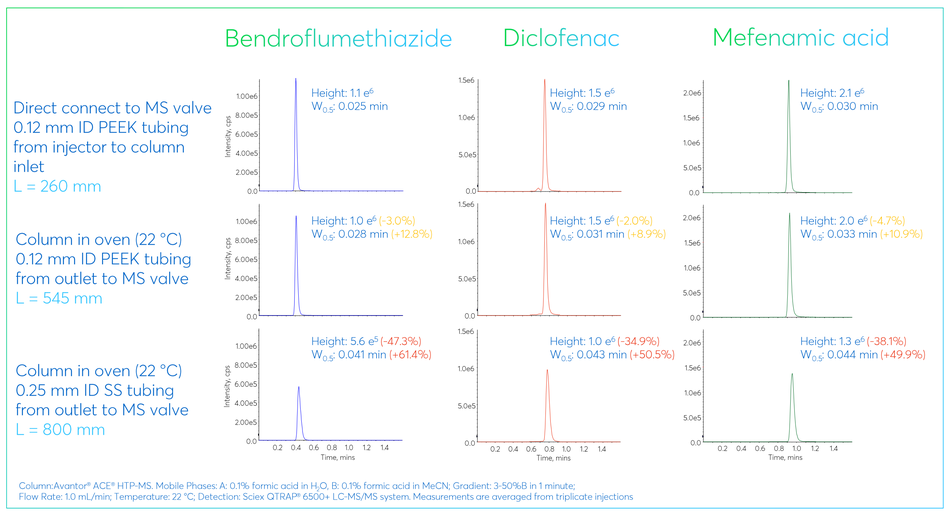
- Avantor® ACE® HTP-MS: 10 x 2.1 mm cartridge style column:
  - 2 µm particles, 1,000 bar limit.
  - Male outlet (for connection to grounded inlet).
  - Cartridges individually QC tested.



- Extra column dispersion:
  - Highly impactful for small format columns.
  - Minimise wherever possible.

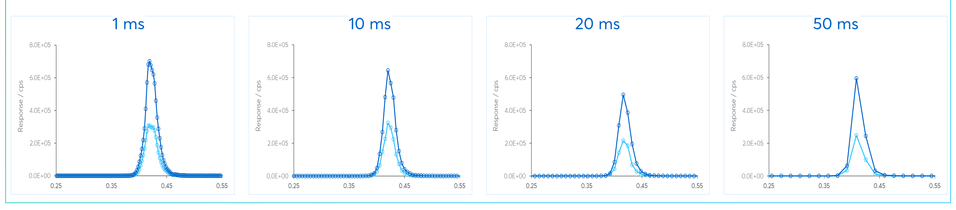


- Assessment of impact of tubing configuration on performance:
  - Maximum performance when post column dispersion is minimised.
  - Avantor® ACE® HTP-MS can be used in the column oven with suitably optimised connections.



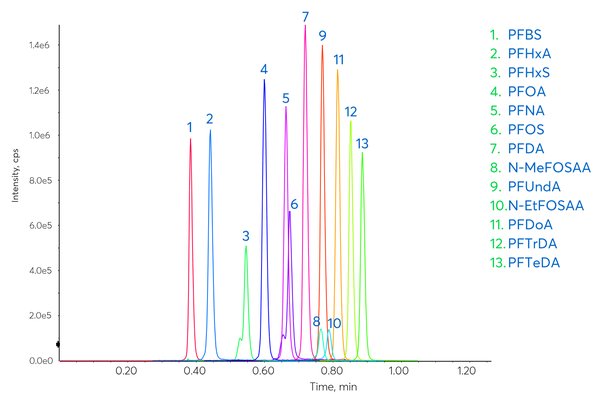
- Detector data rates:
  - Fast detector sampling rates/dwell times are required for small volume peaks.
  - >10-15 data points per peak for accurate quantitation.

Bendroflumethiazide dwell time (Quant 419.9 → 289.0, Qual 419.9 → 327.9):



4. Applications

PFAS Analysis



- Analysis of 13 PFAS compounds by LC-MS/MS
- 1 minute gradient @1.0 mL/min.
- 28 samples per hour
  - 0.3 min post gradient re-equilibration
  - 30 s injector cycle time

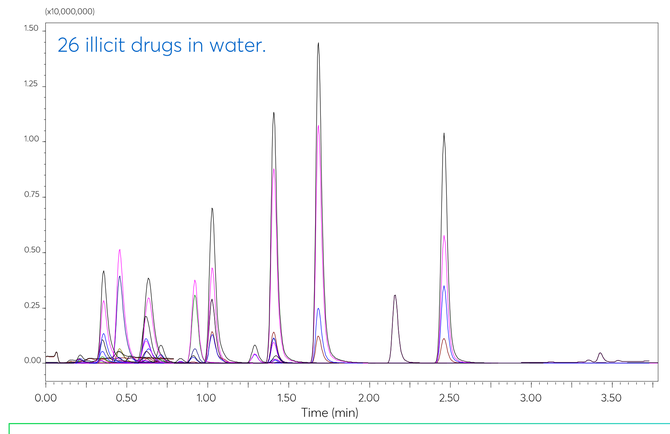
Screen 28 samples per hour

Column: Avantor® ACE® HTP-MS, 2 µm, 10 x 2.1 mm
Mobile Phases: A: 20 mM ammonium acetate in H_2O
B: MeOH
Flow Rate: 1.0 mL/min
Temperature: 22 °C
Injection Volume: 1 µL
Detection: Sciex QTRAP® 6500+ LC-MS/MS system.
Ionisation mode: ESI, negative mode; Source temperature: 450 °C; Curtain gas: 38 psig; Ionspray™ source voltage: -4500 V; Ion source gas 1: 40 psig; Ion source gas 2: 70 psig
Sample: 10 ng/mL

Gradient:

Time (mins)	% B
0	5
0.05	40
1.00	100
1.20	100
1.30	5
1.60	5

Illicit Drugs in Water



- 2.8 minute gradient @0.5 mL/min.
- Methodology can be applied to analysis of contaminants of concern in wastewater samples.
- Method details: Avantor® ACE® Application Note #7600.
- 135 simultaneous contaminants have been analysed in a single run using this type of methodology.
  - *J. Haz. Mat* **398** (2020) 122933

26 illicit drugs as part of 135 contaminant screen in 4.0 minutes

Column: Avantor® ACE® HTP-MS, 2 µm, 10 x 2.1 mm
Mobile Phases: A: 0.1% formic acid in H_2O
B: 0.1% formic acid in MeOH:MeCN (1:1 v/v)
Flow Rate: 0.5 mL/min
Temperature: Ambient
Injection Volume: 10 µL
Detection: Shimadzu LCMS 8060
Ionisation mode: +/- switching; Nebulising gas flow: 2 L/min; Heating gas flow: 15L/min; Interface temperature: 400 °C; DL temperature: 250 °C; Heat block temperature: 400 °C; Drying gas flow: 3 L/min
Sample: 5,000 ng/L standard Mix in ultrapure water

Gradient:

Time (mins)	% B
0	10
0.2	10
3	60
3.01	100
4	100
4.01	10

Data courtesy of Dr Leon Barron & Dr Keng Tiong Ng

5. Conclusions

- Improved MS performance provides scope for reducing LC-MS run times in high throughput settings.
- Avantor® ACE® HTP-MS 10 mm columns provide an effective solution.
- Use of 10 x 2.1 mm columns requires consideration of:
  - Extra column dispersion.
  - MS detector sampling rates/dwell times.
- High efficiency, ultra fast LC-MS analyses are achievable environmental analysis.