

Ultra-fast LC-MS Analyses using Short 10 mm Columns

Matt James¹, Tony Edge¹, David Dunthorne¹, Mark Fever¹, Katie Lawlor^{2,3}, Lewis Couchman², Keng Tiong Ng⁴, Leon Barron⁴ and Geoff Faden⁵

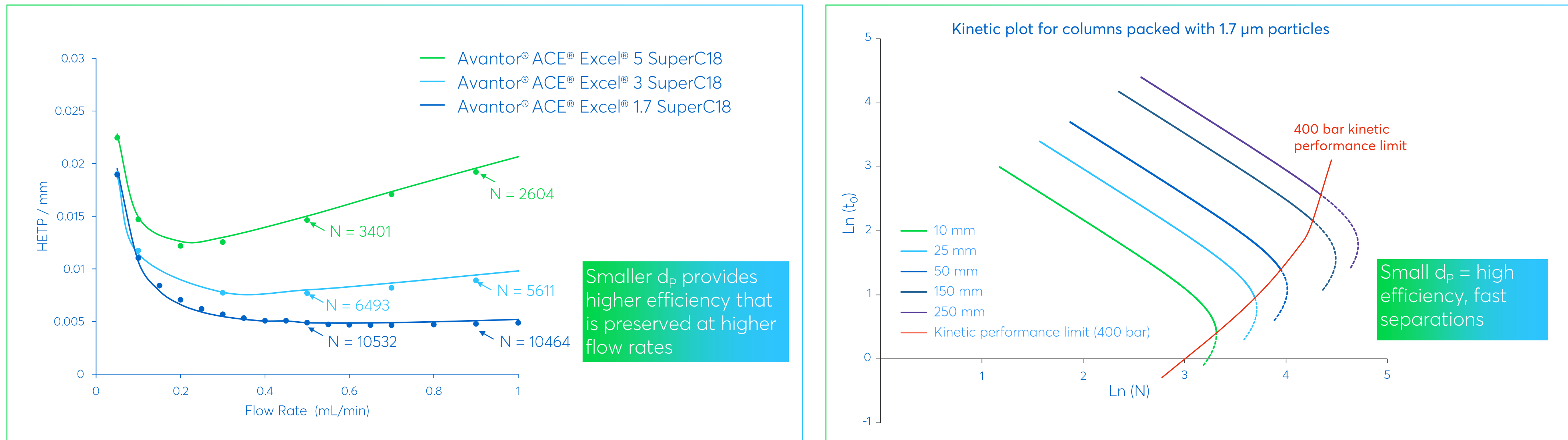
¹Avantor, Theale, Reading, Berkshire RG7 4PE, UK, ²Analytical Services International, St. George's - University of London, UK, ³Department of Analytical, Environmental and Forensic Sciences, King's College London, London, UK, ⁴Environmental Research Group, School of Public Health, Imperial College London, UK, ⁵MAC-MOD Analytical Inc., 103 Commons Court, PO Box 587, Chadds Ford, PA 19317 USA

1. Background

- Higher throughput LC-MS analysis is required in many different labs:
 - Clinical, bioanalytical, forensic, toxicology, and drug discovery.
 - Other sectors (e.g. environmental, F&B etc.) also face similar demands:
 - Increasing contaminants of concern / stricter regulatory limits.
- In many of these settings, samples involve complex matrices, e.g. blood, urine, wastewater etc.
- LC-MS separations can substantially reduce sample complexity:
 - Fast/simple sample prep strategies employed.
 - Typically use short LC columns (e.g. 50 x 2.1 mm).
 - High sample throughput.
- Improved MS performance (sensitivity & data acquisition) opens new possibilities for fast separations:
 - LC can now be a bottleneck.
 - Opportunities for faster separations on smaller format columns are possible.

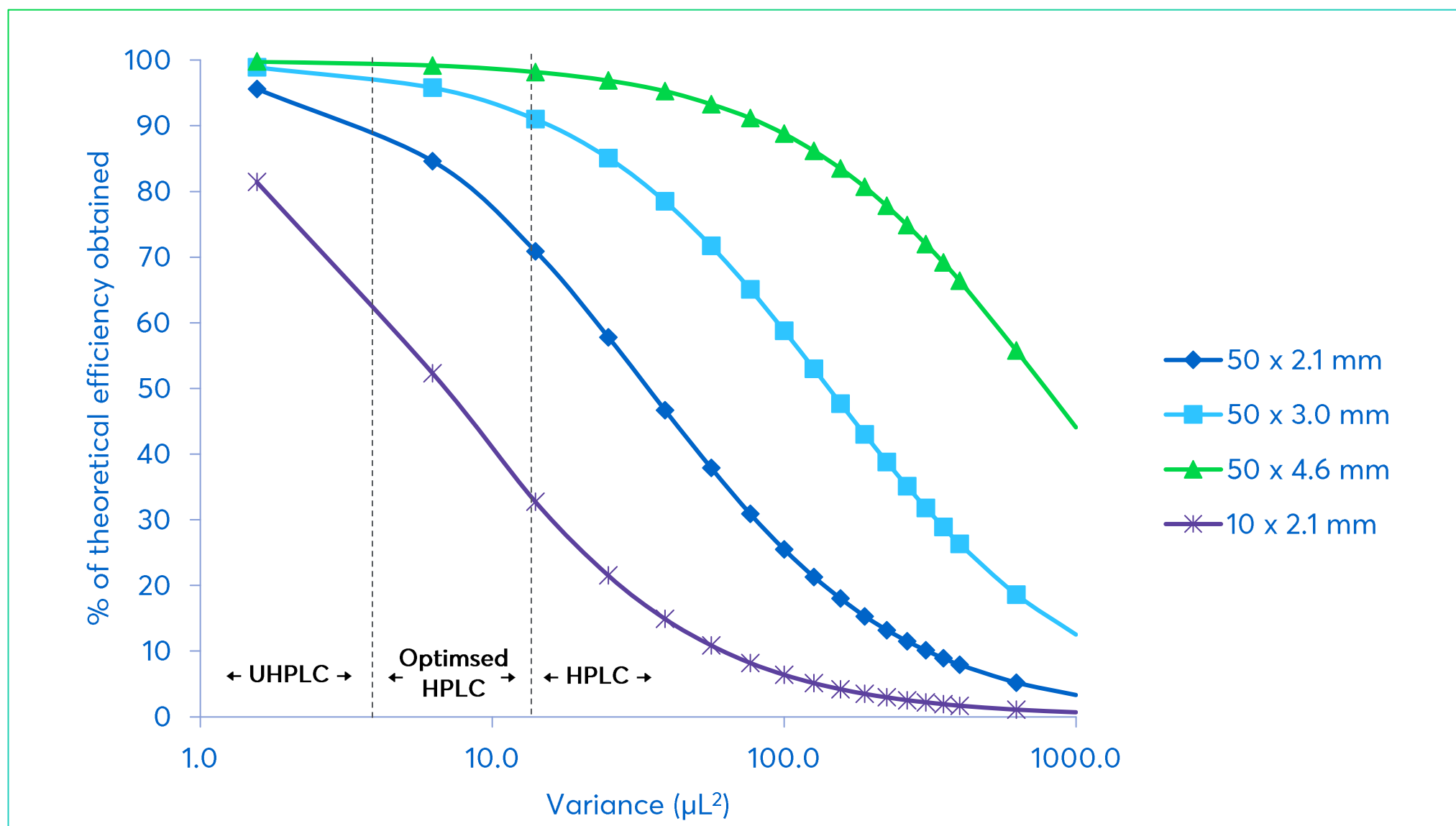
2. Development of Avantor® ACE® HTP-MS column design

- 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.

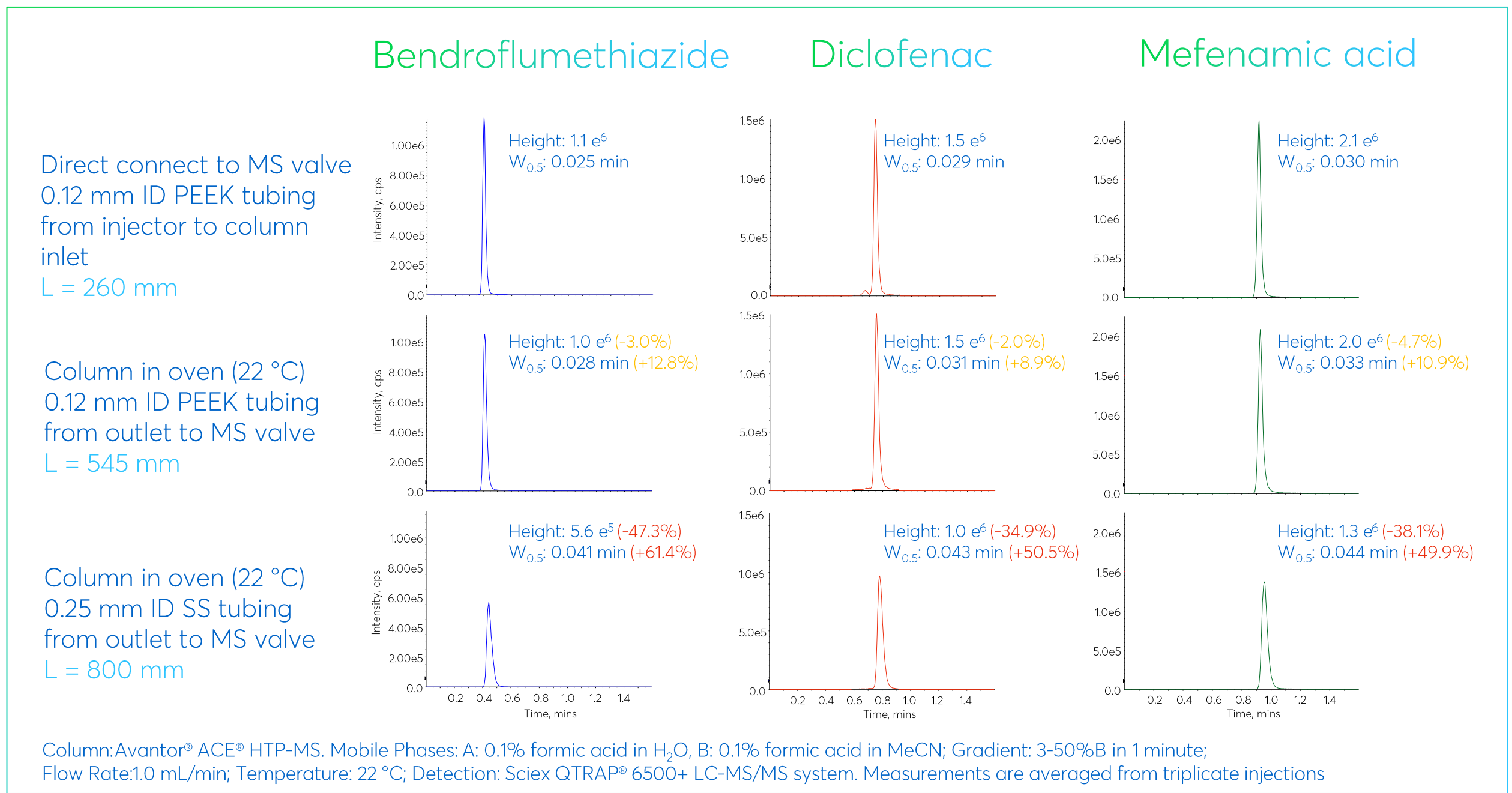


3. 10 x 2.1 mm columns and considerations for use

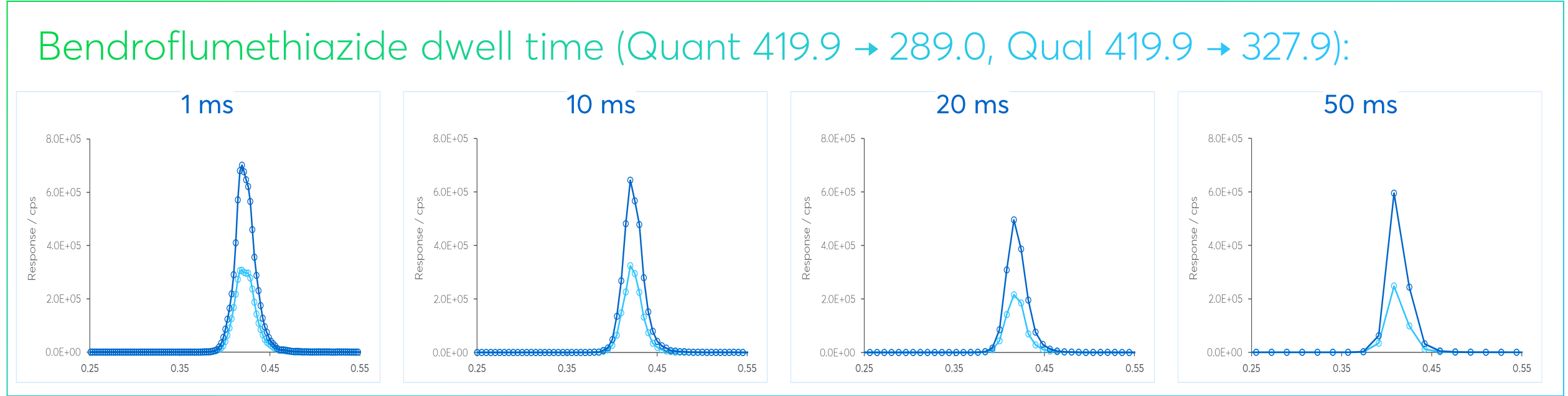
- Avantor® ACE® HTP-MS: 10 x 2.1 mm cartridge style column:
 - 2 μm particles, 1,000 bar limit.
 - Male outlet (for connection to grounded MS inlet).
 - Cartridges individually QC tested.
- Extra column dispersion:
 - Highly impactful for small format columns.
 - Minimise wherever possible.



- 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.

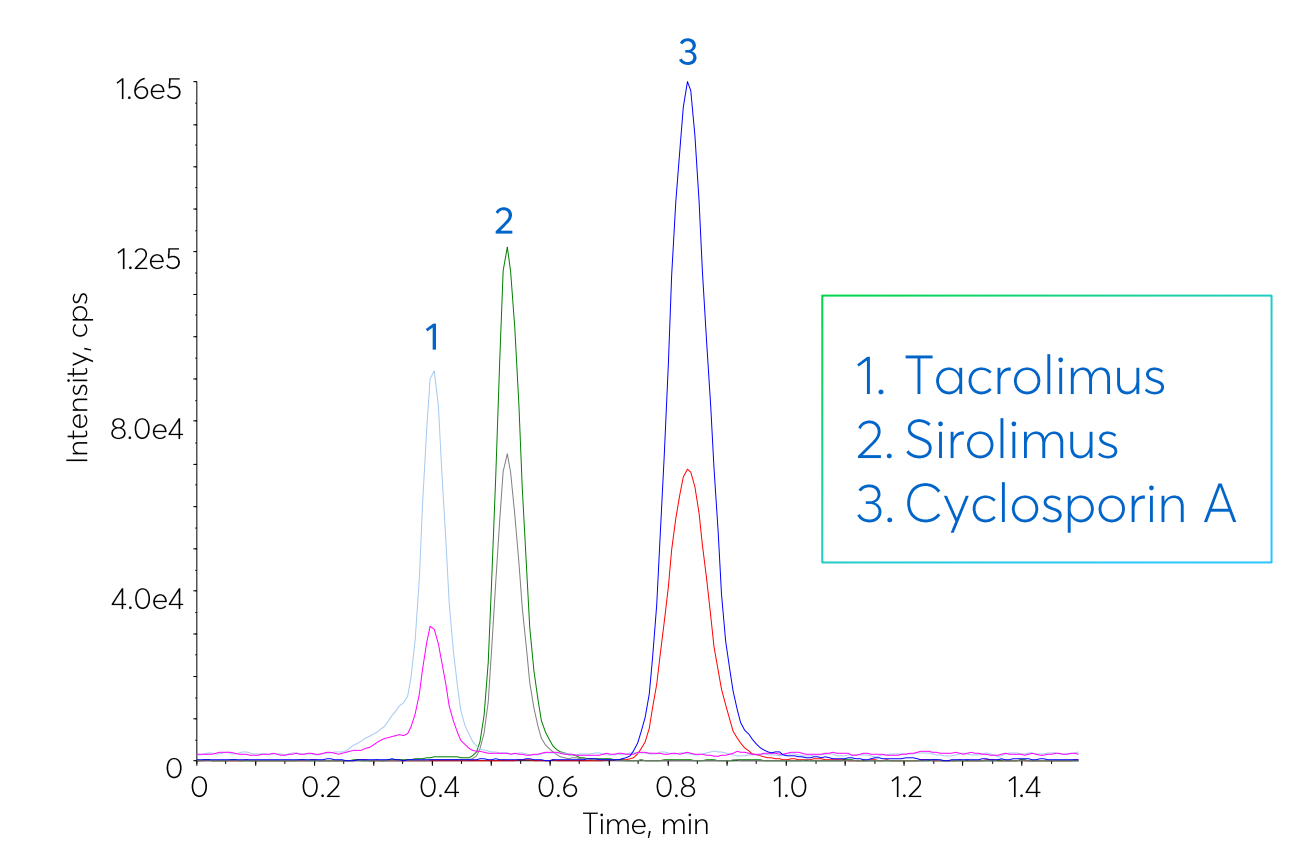


5. Conclusions

- 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 for clinical and environmental analysis.

4. Applications

Immunosuppressants @ 80 °C

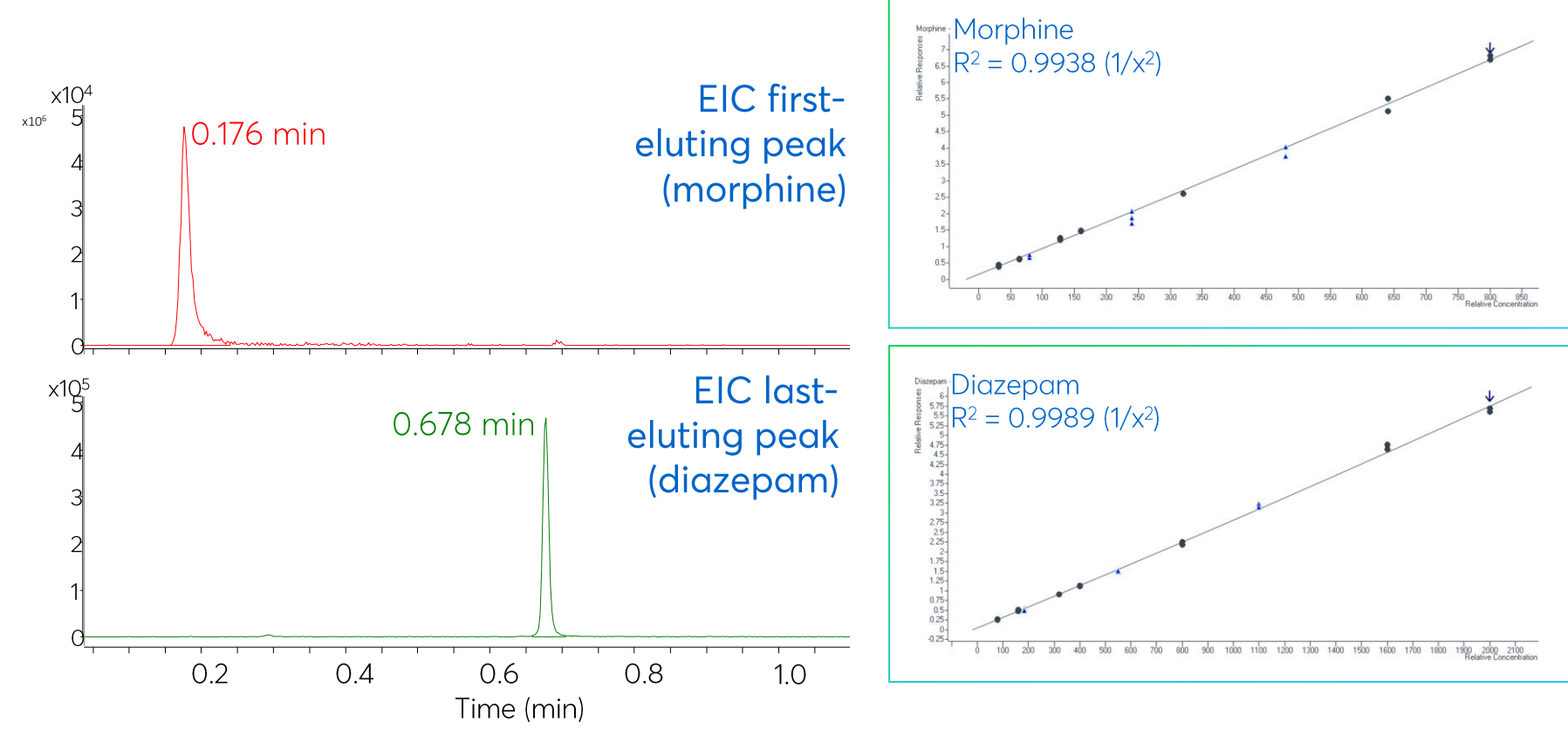


Good peak shape and performance obtained. Analysis in <1.4 mins.

- Isocratic analysis, 0.4 mL/min.
- Temperature control via column oven required.
- Method details: Avantor® ACE® Application Note #7490.

Analyte	MRM
Tacrolimus	+821.3 amu → +768.4 amu +821.3 amu → +796.3 amu
Sirolimus (Rapamycin)	+931.4 amu → +864.4 amu +931.4 amu → +892.4 amu
Cyclosporin A	+1219.7 amu → +1184.6 amu +1219.7 amu → +125.1 amu

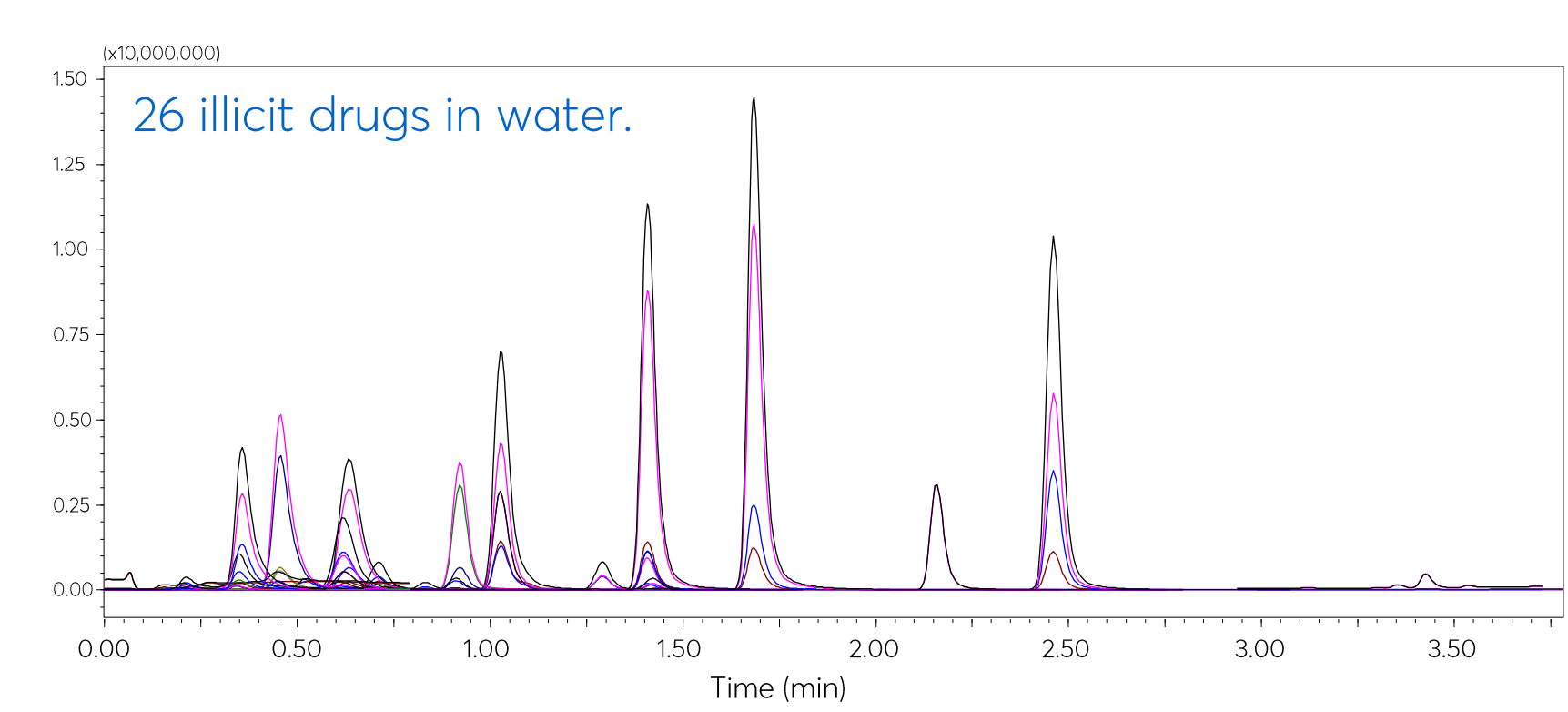
Drugs-of-Abuse in Plasma



16 analytes in 1 minute vs 22 minutes for standard method

- UK Section 5A Road Traffic Act drugs (minus THC).
- Existing method: 16 analytes in 22 mins.
- Using HTP-MS: 16 analytes 1 min.
 - 0.7 minute gradient
 - Flow rate: 1.5 mL/min
 - Extracts from fluoride-oxalate preserved whole blood via liquid-liquid extraction
- Developmental separation (non-validated) with 1 minute analysis time.
- Excellent quantitative performance.

Illicit Drugs in Water



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

- 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.
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