Chromatography Solutions

Technical note #031

Fast LC-MS/MS Analysis using Avantor® ACE® HTP-MS Columns

INTRODUCTION

Many bioanalytical laboratories across multiple industrial sectors are required to analyse increasingly high numbers of samples by LC-MS daily. A majority of these samples will be complex matrices such as biological fluids, typically plasma or urine. The use of LC-MS, whether that be high resolution mass spectrometry (LC-HRMS) or tandem mass spectrometry (LC-MS/MS), substantially reduces the complexity of the data analysis, since the resulting chromatograms will generally only contain peaks related to the specific analytes of interest. Importantly matrix components would not be directly detected. Another advantage of these approaches to detection, is the ability to set different data channels specific to an individual analyte, which allows for quantification of different analytes even if co-elution is occurring. The specificity that LC-MS technology offers is a tremendous advantage over less specific detectors such as UV, however there are challenges. Ion suppression and isobaric interferences must be addressed, which is why it is important to perform chromatography prior to MS detection. In many cases, the use of very short packed bed lengths (i.e., considerably less than 50 mm) can provide sufficient chromatography for matrix removal and target analyte retention, whilst achieving substantial increases in sample throughput compared to standard format LC-MS columns (e.g., 50 x 2.1 mm).

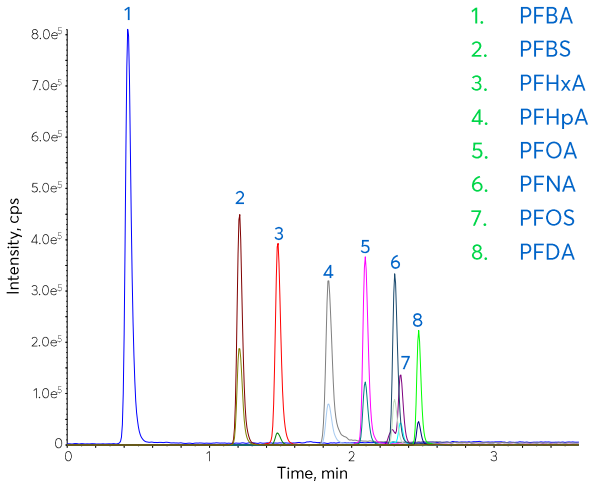
Figure 1: Avantor® ACE® HTP-MS column hardware.

It is not only bioanalytical labs, such as clinical, forensic and toxicology labs, where there has been an increase in sample turnaround, environmental labs face similar challenges, with increasing numbers of environmental contaminants of concern (e.g., pharmaceuticals, pesticides, herbicides, illegal drugs, and their metabolites) continually being identified. [1] For many LC-MS applications, short column formats, such as 50 x 2.1 mm, have been routinely used to achieve the required separation in reasonable time frames. In many cases, a targeted approach is used for MS detection, where compound specific precursor to product ion transitions are monitored for target analyte identification and quantification. In the case of tandem MS, this targeted approach imposes limitations in terms of reducing chromatographic run times, as it is only feasible to monitor a limited number of transitions in a given time frame. Analyses that target many different analytes (potentially hundreds in one run) are therefore likely to have undesirably long run times. However, advances in tandem mass spectrometer performance (i.e., improved sensitivity and faster data acquisition capabilities) over recent years, provides renewed opportunity to reduce analytical run times, and therefore overall cycle times, through use of specially designed, high throughput columns.

This technical note summarises the Avantor® ACE® HTP-MS column technology and provides example applications to demonstrate how this new format LC-MS column can be used to develop faster LC-MS methods.

AVANTOR® ACE® HTP-MS COLUMNS

One solution for achieving faster chromatography for LC-MS methods is to use shorter column lengths (e.g., 30 mm). However, this can prove to be an expensive option when "crude" sample preparation approaches, such as "dilute and shoot" are used, as matrix components and particulates can accumulate at the column head, impacting the column lifetime. Alternatively, guard cartridges (typically around 5 mm in length) have been successfully utilised. However, the short packed bed length may not provide sufficient retention for successful matrix removal (particularly when using "dilute and shoot" approaches for sample preparation) or adequate analyte retention and separation. Additionally, guard cartridges are typically not individually QC tested, so variability in packed bed quality and overall performance may be encountered if they are used for high throughput analysis. The Avantor® ACE® HTP-MS column format utilises a cartridge style holder together with 10 mm packed cartridges (Figure 1), to provide a cost effective solution, whilst providing enough packed material to achieve successful separations. The low dead volume holder is designed for easy connection and features a replaceable PEEK ferrule. The column can be connected to the MS to minimise post column dispersion, or be situated in the column oven as per a conventional column. If connected directly to a MS, it is essential that it is connected to a suitable grounded inlet, to avoid potential for electric shock.



Column:	Avantor® ACE® HTP-MS
Particle Size:	2 µm
Dimensions:	10 x 2.1 mm
Mobile Phase:	A: 20 mM ammonium acetate in H_2O B: 20 mM ammonium acetate in MeOH/H_2O (90:10)
Gradient:	

Time (mins)	% B
0	0
0.1	25
2.1	75
2.2	100

Flow Rate:	0.5 mL/min
Injection:	0.5 µL
Temperature:	22 °C
Detection:	Sciex QTRAP® 6500+ LC-MS/MS system. Ionisation mode: ESI, negative mode Source temperature: 450 °C Curtain gas: 38 psig Ionspray™ source voltage: -3500 V Ion source gas 1: 40 psig Ion source gas 2: 70 psig
Sample:	12 ng/mL for each analyte

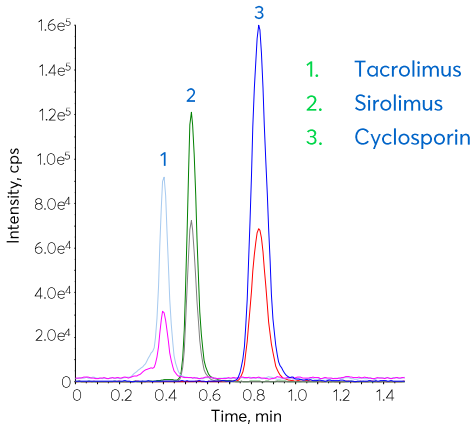
Figure 2: LC-MS/MS analysis of perfluoro alkyl substances using an Avantor® ACE® HTP-MS column (Avantor® ACE® Application note #7500).

The replaceable 10 x 2.1 mm cartridges are packed with high performance 2 µm silica UHPLC particles. These are bonded with a proprietary stationary phase, suitable for the retention of hydrophobic and moderately polar analytes and is compatible with 100% aqueous mobile phases. The cartridges are pressure rated to 1,000 bar and are individually QC tested to ensure robustness and reproducibility. The 10 mm cartridge length allows the use of short gradient and post gradient re-equilibration times to dramatically reduce LC-MS method cycle times.

EXAMPLE APPLICATIONS

Perfluoro alkyl substances (PFAS) have been used in a wide variety of industrial and manufacturing applications for over 70 years due to their water repellent and high thermal stability and extremely resistant to degradation. However, these properties mean that they are highly persistent in the environment and can accumulate within the body and have been linked to adverse health effects. [2] In recent decades, they have therefore emerged as environmental and biological contaminants of concern and are therefore regulated and routinely analysed to monitor for exposure and risk. LC-MS/MS is typically employed to achieve the low-level (part-per-trillion range) quantitative determination of PFAS. [3] Figure 2 demonstrates the use of an HTP-MS column for the separation of a range of PFAS analytes. The high-efficiency particles, coupled with the low-dead volume design means that full resolution of seven of the eight analytes is readily achieved in less than 2.5 minutes on the 10 mm column length. For LC-MS/MS determination, providing the analytes are not isobaric and show no interference effects with one another, full chromatographic resolution is often not a requirement, due to the specificity of the MS detector when operated in MRM mode. This method could therefore readily be adapted using a steeper gradient profile to sacrifice resolution and further reduce run time if required.

For applications requiring control of the column temperature, the HTP-MS column can be situated in a column oven, as per standard format LC columns. For this approach, it is important to consider that smaller volume columns are more prone to extra column band broadening, which leads to reduced chromatographic performance. [4, 5] It is therefore essential to use appropriate internal diameter tubing post column (i.e., < 0.005 in., 0.12 mm) to reduce the potential impact of these effects and minimise the tubing length where practical. Figure 3 shows data obtained for the separation of three cyclic immunosuppressant drugs on an HTP-MS column. At room temperature, these moderate molecular weight analytes showed very poor peak shape, with little resolution, potentially due to a combination of slow molecular diffusion [6] and the presence of multiple structural forms. Elevated column temperature was found to be necessary for these analytes to achieve good chromatographic performance and peak shape. The HTP-MS column was therefore sited in a column oven with 0.12 mm ID PEEK tubing used post column. Using this approach, the LC-MS/MS analysis was successfully performed in less than 1 minute with the HTP-MS column maintained at a temperature of 80 °C.



Column:	Avantor® ACE® HTP-MS
Particle Size:	2 µm
Dimensions:	10 x 2.1 mm
Mobile Phase:	10 mM ammonium formate in H_2O/MeCN (46:54 v/v)
Flow Rate:	0.4 mL/min
Injection:	0.1 µL
Temperature:	80 °C
Detection:	Sciex QTRAP® 6500+ LC-MS/MS system. Ionisation mode: ESI, positive mode Source temperature: 350 °C Curtain gas: 33 psig Ionspray™ source voltage: 5500 V Ion source gas 1: 30 psig Ion source gas 2: 70 psig.
Sample:	Mixture containing tacrolimus (10 ng/mL), sirolimus (42 ng/mL) and cyclosporin A (277 ng/mL) in 10 mM ammonium formate in H_2O/MeCN (46:54 v/v).

Figure 3: Rapid LC-MS/MS analysis of immunosuppressants using an Avantor® ACE® HTP-MS column.

CONCLUSION

The Avantor® ACE® HTP-MS column has been designed to provide a cost-effective solution for high throughput LC-MS analysis. The low-dead volume, cartridge style format together with high efficiency 2 µm UHPLC particles, allows fast, high resolution separations to be developed with reduced cycle times. The applications presented in this article demonstrate how rapid, high throughput LC-MS/MS separations are achievable using this new format column.

REFERENCES

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4. Avantor® ACE® Knowledge Note #0017 "How to Determine Extra Column Dispersion and Extra Column Volume".

5. S. Fekete, I. Kohler, S. Rudaz and D. Guillarme, "Importance of instrumentation for fast liquid chromatography in pharmaceutical analysis," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 87, pp. 105-119, 2014.

6. Avantor® ACE® Knowledge Note #0010 "Chromatographic band broadening and the van Deemter equation".