# The Use of Short 10 mm Columns for Rapid LC-MS Analyses

Matt James[1], Arianne Soliven[1], Colin Pipe, David Dunthorne, Mark Fever, Tony Edge*, Avantor, 1 The Markham Centre, Station Road, Theale, Reading, RG7 4PE, UK.
[1]Joint first authorship *Corresponding author anthony.edge@avantorsciences.com

## Abstract

This brief communication demonstrates the utilisation of a short 10 mm length, narrow bore 2.1 mm internal diameter (ID) column for rapid LC separations coupled with mass spectrometry (MS) detection for high sample throughput (HTP). We demonstrate the capabilities for three applications that differ in sample and separation complexity; a sample separated via isocratic separation conditions and two samples that require differing gradient conditions. For all three cases, high laboratory productivity rates were calculated based on the number of samples that can be analysed in a 24-hour time frame and ranged from 464-1200 samples.

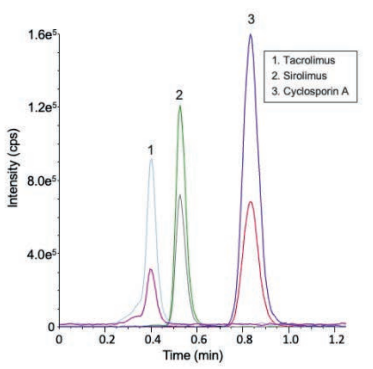
## Introduction

Rapid LC-MS assays are valuable for analytical laboratories that have a large number of samples to analyse and/or increase laboratory productivity [1]. The MS detector's speed and selectivity for ionised species can facilitate the use of direct injection HTP assays [2,3]. However, direct injection-MS analyses are prone to sample matrix effects, where the presence of competing ionised species can lead to suppression and in some cases the enhancement of the signal response [4]. Hence, the integration of a front-end liquid chromatography column separation provides an additional degree of resolution/peak capacity to separate targeted analytes or isobaric species from the sample's matrix, before ionisation, desolvation and MS detection [5].

The column length selection is a critical practical parameter for maximising the peak capacity for the separation of complex low molecular weight samples [6]. On the other hand, to develop rapid LC HTP assays, we must reduce the column volume used in the LC front-end separation strategy [7]. Moreover, for gradient elution conditions, reducing the sum of the gradient time and re-equilibration 'total cycle time', can be achieved by employing low-volume, highly efficient columns [8]. In this short communication, we highlight how to exploit short 10 mm columns to achieve rapid LC-MS analysis for three different low molecular weight applications, with differing sample complexities.

## Experimental

The short 10 mm length column with a narrow bore 2.1 mm ID, was packed with 2-micron particles ($d_p$) to provide sufficient plates and minimise the column volume. The cartridge style small column format is housed within hardware that facilitates the use of standard 1/16-inch fittings and outer-diameter connection tubing. For optimum use, the data acquisition rate must be increased. For quantification purposes, we recommend collecting at least 10-15 data points across each peak by applying fast detector sampling rates/dwell times for the small volume peaks [9]. The column separation and MS detection details are listed in the respective application (*Figures 1-3*). For all post-column fluidic connections, 0.12 mm ID PEEK tubing was used. Standards were of high analytical grade and solvents were of HPLC grade and were sourced via VWR International, UK – part of Avantor®, except for PFAS standards, which were sourced through Wellington Laboratories, US.



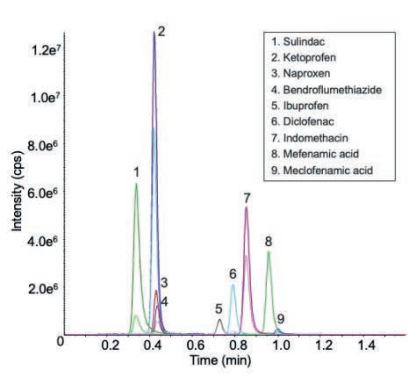

| | |
|---|---|
| Column: | Avantor® ACE® HTP-MS, 2 µm, 10 x 2.1 mm |
| Mobile Phase: | 10 mM ammonium formate in $H_2O$/MeCN (46:54 v/v) |
| Flow Rate: | 0.4 mL/min |
| Injection: | 0.1 µL |
| Temperature: | 80°C |
| Detection: | Sciex QTRAP® 6500+ LC-MS/MS system. |
| Ionisation mode: | ESI, positive mode |
| Dwell | 60 ms (fixed for all transitions, ~390 ms scan time with 5 ms pauses) |
| Source temperature: | 350°C |
| Curtain gas: | 33 psig |
| Ionspray source: | 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 mobile phase |

| Analyte | MRM | Optimised MS Parameters | | |
|---|---|---|---|---|
| | | Declustering potential (V) | Collision energy (V) | Cell exit potential (V) |
| Tacrolimus | +821.3 amu → +768.4 amu | 56 | 27 | 42 |
| | +821.3 amu → +786.3 amu | 56 | 23 | 42 |
| Sirolimus (Rapamycin) | +931.4 amu → +864.4 amu | 76 | 23 | 48 |
| | +931.4 amu → +882.4 amu | 76 | 17 | 46 |
| Cyclosporin A | +1219.7 amu → +1184.6 amu | 1 | 47 | 50 |
| | +1219.7 amu → +425.1 amu | 1 | 73 | 28 |

*Figure 1: isocratic separation conditions were employed for the HTP separation of three immunosuppressants.*

## Discussion

To demonstrate the high throughput capabilities when exploiting the use of the 10 mm short length column, we highlight the application of the HTP-MS column for separations that vary in sample and separation complexity (*Figures 1-3*). In *Figure 1*, isocratic separation conditions were employed for the HTP separation of three immunosuppressants. The additional advantage of exploiting high-temperature liquid chromatography was used (compatible with both the sample, stationary phase, and instrumentation). An isocratic peak width of 0.25 min was achieved. The sample complexity does not require the use of gradient elution, hence there is no need to re-equilibrate the column with a fixed mobile phase composition flowing through the column from injection to injection. A total cycle time of <1.2 min was achieved in the final assay, representing a lab productivity rate equivalent to analysing approximately 1,200 samples in a 24-hour time frame. This is the maximum sample throughput if operated with stacked injections (no injection cycle time).




| | |
|---|---|
| Column: | Avantor® ACE® HTP-MS, 2 µm, 10 x 2.1 mm |
| Mobile Phases: | A: 0.1% formic acid in $H_2O$<br>B: 0.1% formic acid in MeCN |

| Time (mins) | % B |
|---|---|
| 0 | 30 |
| 1 | 50 |
| 1.1 | 100 |
| 1.5 | 100 |
| 1.6 | 30 |
| 2.6 | 30 |

| | |
|---|---|
| Flow Rate: | 1.0 mL/min |
| Temperature: | 22°C |
| Detection: | Sciex QTRAP® 6500+ LC-MS/MS system.<br>Ionisation mode: ESI, +/-<br>Dwell = 12-20 ms (MRM dependant, based on target scan time of 100 ms)<br>Source temperature: 550°C<br>Curtain gas: 40 psig<br>Ionspray™ source voltage: 4500 V<br>Ion source gas 1: 70 psig<br>Ion source gas 2: 70 psig |
| Sample: | Standards at a concentration of 500 ng/mL<br>10 µg/mL for ibuprofen and naproxen |

*Figure 2: a series of nine different low-molecular-weight nonsteroidal anti-inflammatory drugs (NSAIDs), were separated using the 10 mm length column hyphenated to the MS.*

In the second application (*Figure 2*), a series of nine different low-molecular-weight nonsteroidal anti-inflammatory drugs (NSAIDs), were separated using the 10 mm length column hyphenated to the MS. The sample complexity was higher compared to the simpler first application and therefore required gradient elution. The gradient separation

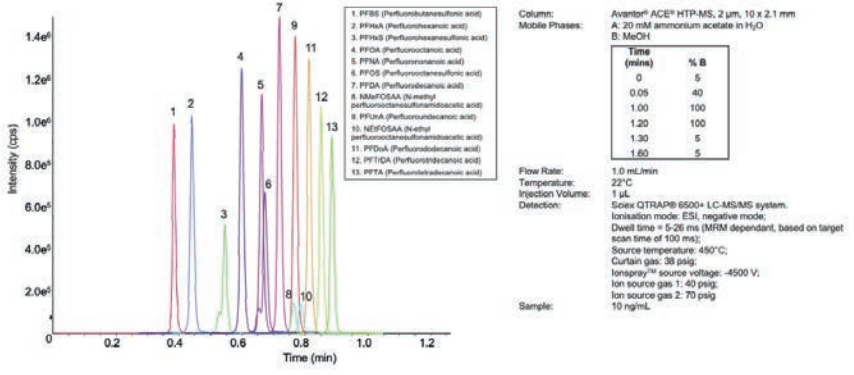



*Figure 3: The gradient separation of 13 perfluoroalkyl substances (PFAS).*

method included a long 1-minute post-gradient isocratic step to re-equilibrate the column with the initial mobile phase conditions. The injection cycle time for this gradient separation was 0.5 min. Hence, the total analysis time for each sample was 3.1 min (injection to injection), representing a laboratory productivity rate of 464 samples analysed within 24 hours. Note, the injection cycle time can be used to the analyst's advantage, to effectively minimise/eliminate the gradient's final re-equilibration step and further increase sample throughput.

The third application was focused on the gradient separation of 13 perfluoroalkyl substances (PFAS) shown in *Figure 3*. A steep gradient was employed to benefit from the gradient compression effect on the peak shape, resulting in very narrow peaks approximately 0.05 min in width. The short 10 mm column separation prior to MS detection provides a peak capacity advantage with respect to contaminant analysis versus a direct injection i.e. chromatographically separate target analytes and resolve from interfering matrix components. The total analysis time for the third application was 2.1 min, hence 685 samples can be analysed per 24-hour day targeting PFAS contaminants.

## Conclusion

We demonstrate the utilisation of a short length (10 mm) column, hyphenated with MS, to achieve rapid high-resolution high-throughput separations, for three applications with differing sample complexity. One isocratic elution method for a simple three-compound mixture and two different gradient elution methods for two other samples of higher complexity. The three applications highlight the potential to analyse between 464 to 1200 samples per 24-hour time frame using a 10 mm length LC column.

## References

*1. Ultra-fast LC–MS/MS in therapeutic drug monitoring: Quantification of clozapine and norclozapine in human plasma. Couchman et al., Drug Testing and Analysis 10 (2018) 323–329.*

*2. High-throughput multi-residue quantification of contaminants of emerging concern in wastewaters enabled using direct injection liquid chromatography-tandem mass spectrometry. Ng et al., Journal of Hazardous Materials 398 (2020) 122933.*

*3. Direct injection liquid chromatography-tandem mass spectrometry as a sensitive and high-throughput method for the quantitative surveillance of antimicrobials in wastewater. Li et al., Science of the Total Environment 900 (2023) 165825.*

*4. Evaluation of direct sample injection as a fast, no-sample handling, approach for the LC-MS/MS monitoring of pharmaceuticals in different water matrices. Simarro-Gimeno et al., Microchemical Journal 193 (2023) 108985.*

*5. Current state-of-the-art of separation methods used in LC-MS based metabolomics and lipidomics. Harrieder et al., Journal of Chromatography B 1188 (2022) 123069.*

*6. Optimization of gradient reversed phase chromatographic peak capacity for low molecular weight solutes. Soliven et al., Journal of Chromatography A 1273 (2013) 57–65.*

*7. The theory and practice of ultrafast liquid chromatography: A tutorial. Wahab et al., Analytica Chimica Acta 1151 (2021) 238170.*

*8. Comparison of core–shell particles and sub-2 m fully porous particles for use as ultrafast second dimension columns in two-dimensional liquid chromatography. Haidar Ahmad et al., Journal of Chromatography A (2015) 31–38.*

*9. The myth of data acquisition rate. Felinger et al., Analytica Chimica Acta 854 (2015), 178-182.*