High Throughput LC/MS using Active Flow Technology Chromatography Columns

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Overview

Purpose: To investigate the gains in sensitivity and throughput with a virtual 3.0 mm ID column running in curtain flow mode.

Methods: The virtual 3.0 mm ID column was compared to a 4.6 mm ID column (run in standard and post-column split modes) and a 3.0 mm ID column run in standard mode, using a mixture of testosterones. The study was carried out in LC/ESI-MS with SRM.

Results: Significant improvements in peak height and signal-to-noise ratios were measured when using the virtual 3.0 mm ID column set-up. Run time was reduced by half.

Introduction

In Active Flow Technology (AFT) the flow of the mobile phase is dynamically managed as it passes through the column to eliminate wall effects, minimise solute band broadening, maximise signal response or maximise theoretical plates.

Within the set of column platforms that are described by AFT is a column that we have referred to as the curtain flow column or 'virtual column' [1]. In this configuration the mobile phase flow is managed at both the column inlet and outlet. The sample injection plug is constricted to the radial central region of the column and only the radial central region of the bed is utilised for detection purposes, thus eliminating the wall effect. This process contributes to improvements in signal intensity in the UV [2] and increases the number of theoretical plates [2]. These improvements arise because the process of the curtain flow injection, and the parallel segmented flow outlet fitting on these columns overcomes much of the flow heterogeneity that occurs in the column due to packing irregularities and the wall effect.

While gains in sensitivity have been shown in UV [2, 3] so far very little is known with respect to other detection techniques. In this work we investigate the performance of the virtual columns in MS detection with regard to sensitivity and sample throughput.

Methods

Columns: Thermo Scientific™ Hypersil GOLD™ 3 μm, 30 x 4.6 mm
Hypersil GOLD 3 μm, 30 x 3.0 mm
Hypersil GOLD 3 μm, 30 x 4.6 mm, Curtain flow hardware

Mobile phase: water / methanol (40:60) + 0.1% formic acid

Flow rate delivered to MS: 1 mL/min

Injection volume: 10 μL (4 μL for 3.0 mm ID column)

Test probes: Testosterone, epitestosterone, 11-ketotestosterone, 19-nortestosterone (at 0.5, 1, 5, 10, 50 and 100ng/mL concentrations)

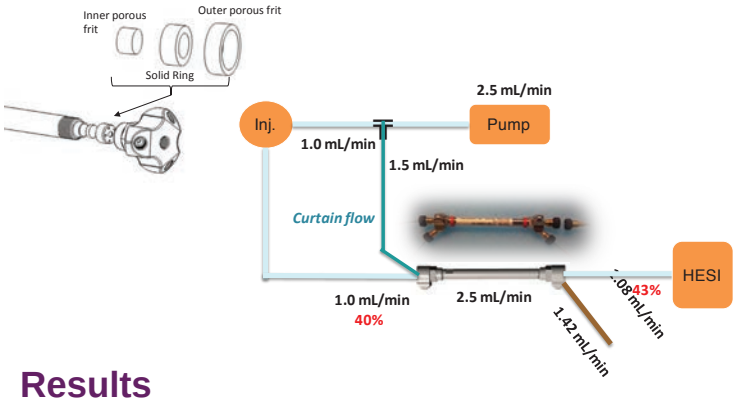
TABLE 1 – 3. ESI and MS/MS detection parameters.

+ESI Parameters	
Sheath gas	60
Auxiliary gas	20
Ion sweep gas	0
Vaporiser temperature (°C)	400
Capillary temperature(°C)	385
Spray voltage (V)	3500
Probe position	C, 1.75, II

Collision gas	1.5
Scan width	0.03
Scan time	0.03
Peak width Q1, Q3	0.7, 0.7

Parent mass	Product masses	Collision energy	S-lens
289.1	79.1	40	80
	97.07	20	
	109.09	23	
275.4	79.05	38	77
	109.07	26	
	239.18	14	
303.1	91.04	44	83
	105.07	36	
	121.05	26	

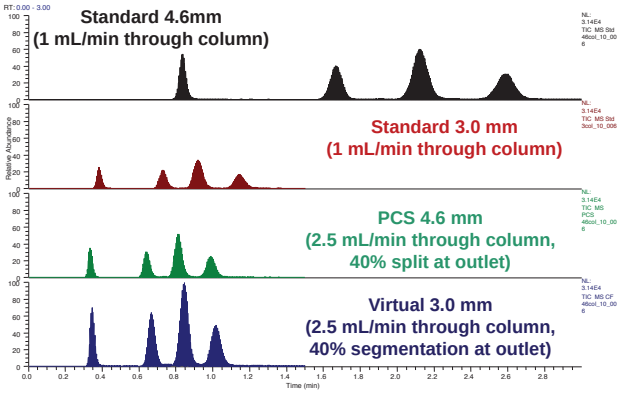
FIGURE 1. Illustration of the parallel segmented flow fittings and the curtain flow configuration.



Results

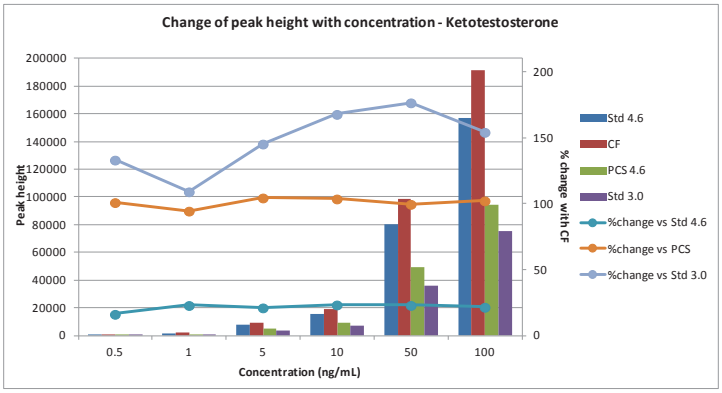
1) Chromatographic comparison

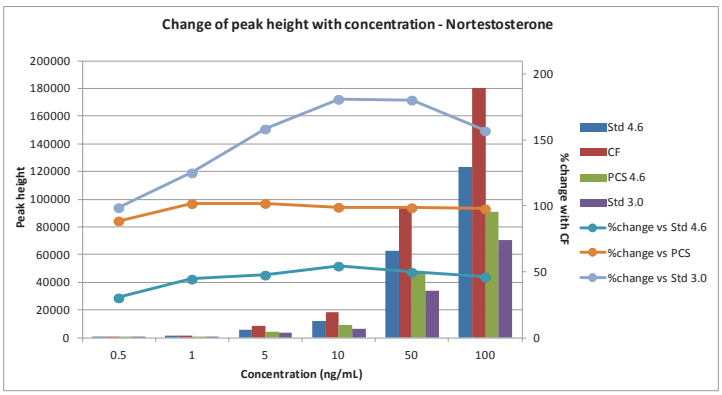
FIGURE 2. Comparison of normalised TICs obtained using the four different chromatographic configurations: (a) Standard 4.6 mm ID column configuration (b) Standard 3.0 mm ID column configuration (c) 4.6 mm ID column with post-column split (d) Virtual 3.0 mm column–4.6 mm ID column run in curtain flow mode

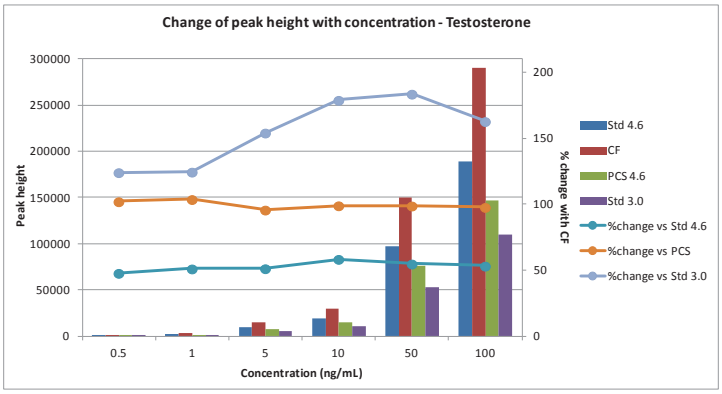


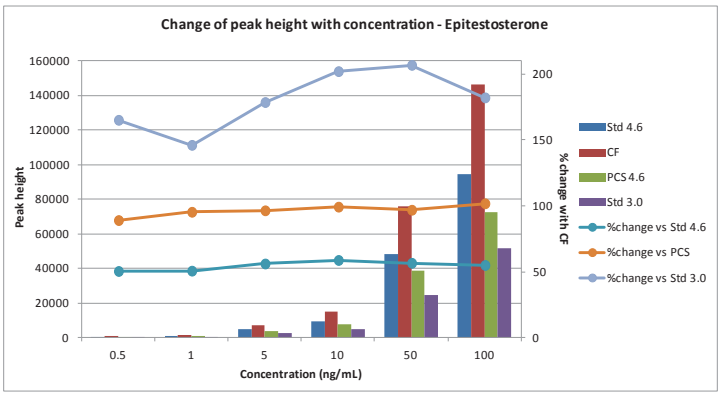
2) Peak height comparison

FIGURE 3. The variation of peak height for the four test compounds across the tested concentration range for the four chromatographic arrangements. Overlaid on all the individual graphs are the improvement seen when using the curtain flow approach to the other techniques employed.









The key observations from the plots in Figure 3 are:

• The curtain flow column (CF) always exhibits better peak height than all other configurations

• The highest gains of the curtain flow column (CF) are against the standard 3.0 mm ID column (Std 3.0)

TABLE 4. Average % increase in peak height when using curtain flow column.

	Std 4.6	PCS 4.6	Std 3.0
Ketotestosterone	21	101	148
Nortestosterone	46	98	150
Testosterone	53	100	155
Epitestosterone	55	96	180

• The % changes against Std 4.6 and Std 3.0 increase with increasing capacity factor (k'), but the %change against PCS 4.6 stays constant.

3) Signal-to-noise ratio comparison

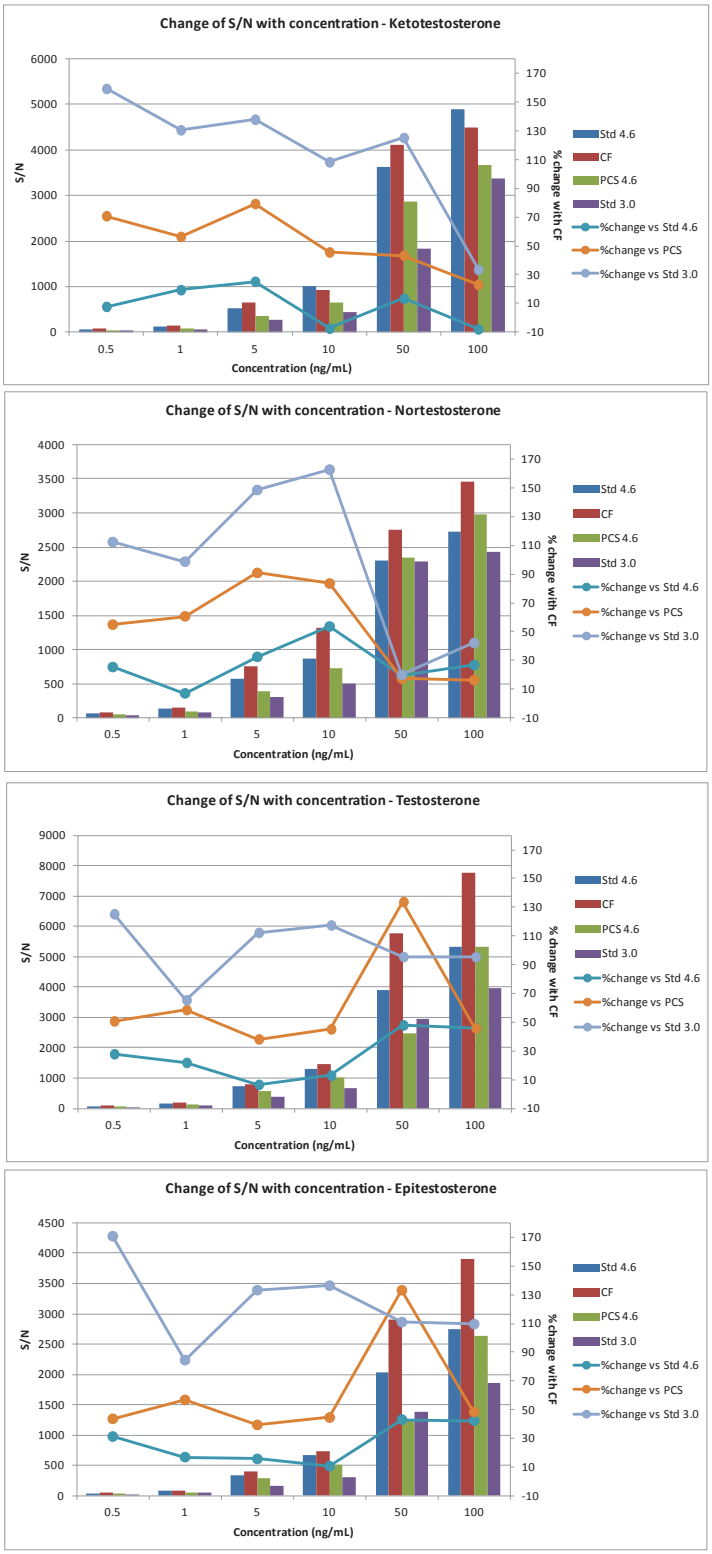
The key observations from the plots in Figure 4 are:

• With 3 exceptions for ketotestosterone and nortestosterone, the curtain flow column (CF) always exhibits better signal-to-noise than all other configurations

TABLE 5. Average % increase of signal-to-noise ratio when using curtain flow column.

	Std 4.6	PCS 4.6	Std 3.0
Ketotestosterone	8	53	116
Nortestosterone	27	54	97
Testosterone	27	62	102
Epitestosterone	27	61	124

FIGURE 4. The variation of signal to noise for the four test compounds across the tested concentration range for the four chromatographic arrangements. Overlaid on all the individual graphs are the improvement seen when using the curtain flow approach to the other techniques employed.



Conclusion

The curtain flow column ('virtual 3 mm column') tested demonstrated the following improvements when compared to the standard 4.6 mm ID column (Std 4.6) the 4.6 mm post-column split (PCS 4.6), and the standard 3.0 mm ID (Std 3.0):

- Reduction of run time reduced by half (versus Std 4.6)
- Versus Std 4.6 peak height increases by half and signal-to-noise increases by a quarter
- Versus PCS 4.6 peak height doubles and signal-to-noise increases by half
- Versus Std 3.0 peak height troubles and signal-to-noise doubles

Therefore, the virtual 3.0 mm column has clear sensitivity advantages over the standard 3.0 mm ID column.

References

1. R.A. Shalliker, M. Camenzuli, L. Pereira, H.J. Ritchie, *J. Chromatogr. A*, 1262 (2012) 64-69
2. M. Camenzuli, H. J. Ritchie, J.Ladine, R. A. Shalliker, *J. Chromatogr. A*, 1232 (2012) 47-51
3. D. Foley, L. Pereira, M. Camenzuli, T. Edge, H. Ritchie, R.A. Shalliker, Microchem. J. , 110 (2013),127-132

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