Precision and Reliability: an Intercontinental Study of Curtain Flow Chromatography

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Overview

Purpose: To compare analytical figures of merit for Active Flow Technology (AFT) in two independent laboratories.

Methods: Curtain flow ('virtual') columns were compared to conventional columns for signal response, noise magnitude, LODs and LOQs, by two independent laboratories, using the same chromatographic conditions.

Results: The outcome in the analytical performance for these curtain flow columns followed the same trends.

Introduction

Active Flow Technology is a novel column technology that yields significantly improved chromatographic performance. In AFT the flow of the mobile phase is dynamically managed as it passes through the column, to eliminate wall effects and minimise solute band broadening, therefore maximizing signal response or maximizing theoretical plates [1]. In parallel segmented flow (PSF) the mobile phase is managed at the column outlet only, in curtain flow (CF) it is managed both at the column inlet and outlet (Figure 1) to create a "virtual" column (Figure 2) inside the analytical column [2]. The "virtual" column has a narrower internal diameter, the dimensions of which are related to the volumetric ratio of flow exiting the column through the centre, relative to the flow exiting through the peripheral zones.

In the work presented herein we report an inter-laboratory study undertaken in two laboratories to measure key analytical figures of merit at the analytical scale. Curtain flow columns were compared to conventional columns for signal response, noise magnitude, LODs and LOQs. The 2 fold gains in sensitivity with curtain flow were found to be comparable between the 2 laboratories. Furthermore, the data acquired for curtain flow columns exhibits excellent precision compared to conventional columns, enabling lower LOQs to be achieved.

Methods

1) Performance of Parallel Segmented Flow

Columns:

Thermo Scientific™ Hypersil GOLD™ 5 µm, 100 x 4.6 mm
Hypersil GOLD 5 µm, 100 x 3.0 mm
Hypersil GOLD 5 µm, 100 x 2.1 mm
(in standard, PSF and CF configurations)

Mobile phase: 70:30 methanol:water

Segmentation ratio at column outlet: 10 to 55% every 5%; 21, 43% for flow equivalence to 2.1 and 3.0 mm ID columns.

Injection volume: 5 µL

Test solutes: Theophylline, toluene, propylbenzene and butylbenzene.

2) Curtain Flow Linearity and precision

Columns:

Hypersil GOLD 5 µm, 50 x 4.6 mm
Hypersil GOLD 5 µm, 50 x 3.0 mm
Hypersil GOLD 5 µm, 50 x 2.1 mm
(in standard, PSF and CF configurations)

Mobile phase: 80:20 methanol:water

Segmentation ratio at column outlet: 22, 44%

Curtain flow at column inlet: 40% into the central port

Injection volume: 5 µL (2 µL and 1 µL for 3.0 mm and 2.1 mm)

Test solutes: Theophylline, toluene, propylbenzene and butylbenzene.

FIGURE 1. Illustration of the AFT column inlet and column outlet.

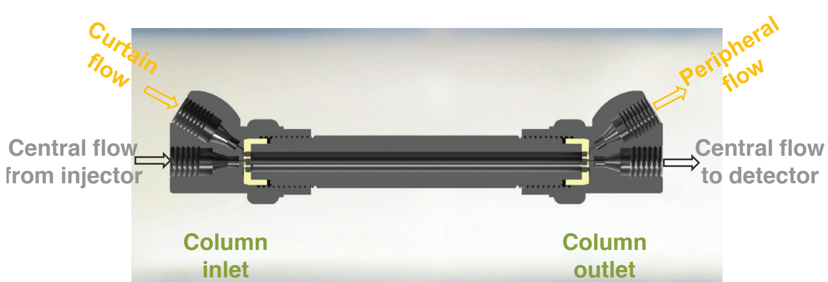
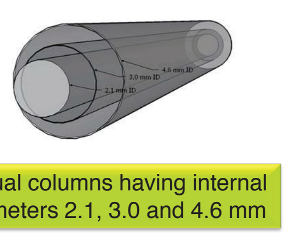


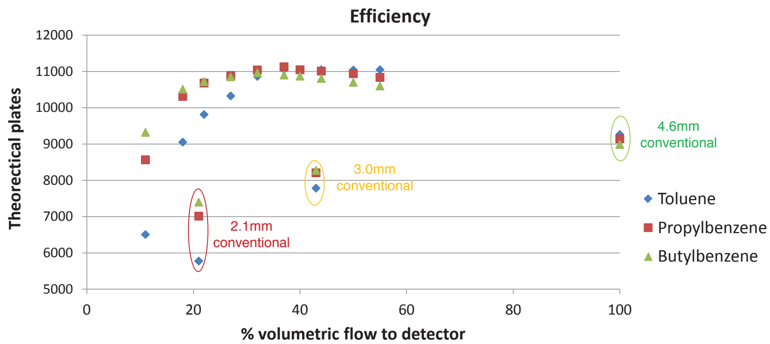
FIGURE 2. Illustration of the 'virtual' column.



Results

1) Performance of Parallel Segmented Flow (PSF)

FIGURE 2. Comparison in N values obtained on the 4.6 mm ID Parallel Segmented Outlet flow column and the 4.6, 3.0 and 2.1 mm ID conventional columns.



N.B. The data obtained on the 2.1 mm and 3.0 mm ID columns are centred on the 21% and 43% volumetric flow positions to correspond to the equivalent flow through the 4.6 mm ID column at that specific segmentation ratio.

- With PSF improvements in efficiency of up to 30%, 30% and 45% over conventional 4.6, 3.0 and 2.1 mm ID columns respectively were observed.
- The most efficient segmentation ratio occurs at 37% of volumetric flow delivered to the detector

TABLE 1. Injection-to-injection reproducibility.

Column Frit 1:3	Rt (min) (r.s.d.%)	N (r.s.d.%)	Height (r.s.d.%)
25%			
Toluene	1.792 (-)	9684 (0.61)	33.23 (0.016)
Propylbenzene	3.186 (0.018)	10658 (0.34)	30.88 (0.022)
Butylbenzene	4.595 (0.013)	10737 (0.30)	18.47 (0.23)
41%			
Toluene	1.778 (0.032)	10830 (0.26)	34.93 (0.092)
Propylbenzene	3.169 (0.018)	10997 (0.32)	31.43 (0.097)
Butylbenzene	4.577 (0.025)	10820 (0.04)	18.68 (0.11)
Conventional			
Toluene	1.781 (0.032)	9263 (0.85)	33.18 (0.36)
Propylbenzene	3.169 (0.018)	9142 (0.26)	29.44 (0.25)
Butylbenzene	4.572 (0.022)	8989 (0.37)	17.27 (0.14)

- The data is reproducible with %RSD below 0.4%

2) Curtain Flow Linearity and Precision

TABLE 2. Analytical figures of merit: linearity, LODs and LOQs. (a) Lab 1; (b) Lab 2.

(a)

Column	Slope	r^2	Gain in Signal Intensity (×)	LOD (mM)	LOQ (mM)
50 × 2.1 mm	6584	1.000		4.4	14.5
50 × 3.0 mm	7090	1.000	1.07[1]	4.5	15.0
50 × 4.6 mm	7473	1.000	1.1[1]	5.0	16.6
CF22	17936	1.000	2.7[1] 2.4[2]	1.7	5.6
CF44	14793	0.999	2.1[3] 2.0[2]	1.9	6.4

(b)

Column	Slope	r^2	Gain in Signal Intensity (×)	LOD (mM)	LOQ (mM)
50 × 2.1 mm	6658	0.999		6.4	21.4
50 × 3.0 mm	7719	0.999	1.16[1]	2.6	8.7
50 × 4.6 mm	8288	0.999	1.2[1]	2.5	8.3
CF22	18681	0.999	2.8[1] 2.3[2]	2.8	9.4
CF44	16432	0.999	2.1[3] 2.0[2]	1.8	5.8

[1]Relative comparison against the 2.1 mm ID conventional column format
[2]Relative comparison against the 4.6 mm ID conventional column format
[3]Relative comparison against the 3.0 mm ID conventional column format

- All calibration curves in both laboratories were linear with r^2 values equal or greater than 0.999
- In both laboratories the slope of the calibration curves obtained on the CF columns was greater than on the conventional columns, indicating a greater sensitivity to changes in sample concentration
- The signal intensity measured for the curtain flow columns was always substantially greater than any of the other columns (1.1 to 2.8 fold higher)
- LODs and LOQ were significantly lowered with the curtain flow column configuration in both laboratories. Up to 3 fold gains in LOQs were observed with the a 22% outlet segmentation ratio, compared to the 4.6 mm ID column

FIGURE 3. Calibration curves for butylbenzene on conventional and PSF columns. (a) Lab 1; (b) Lab 2.

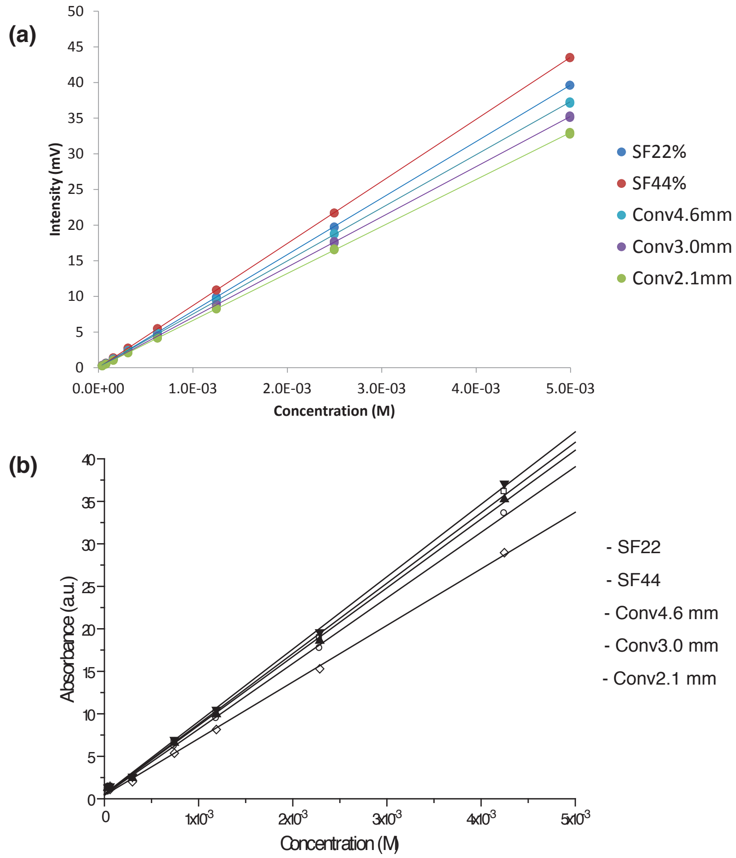
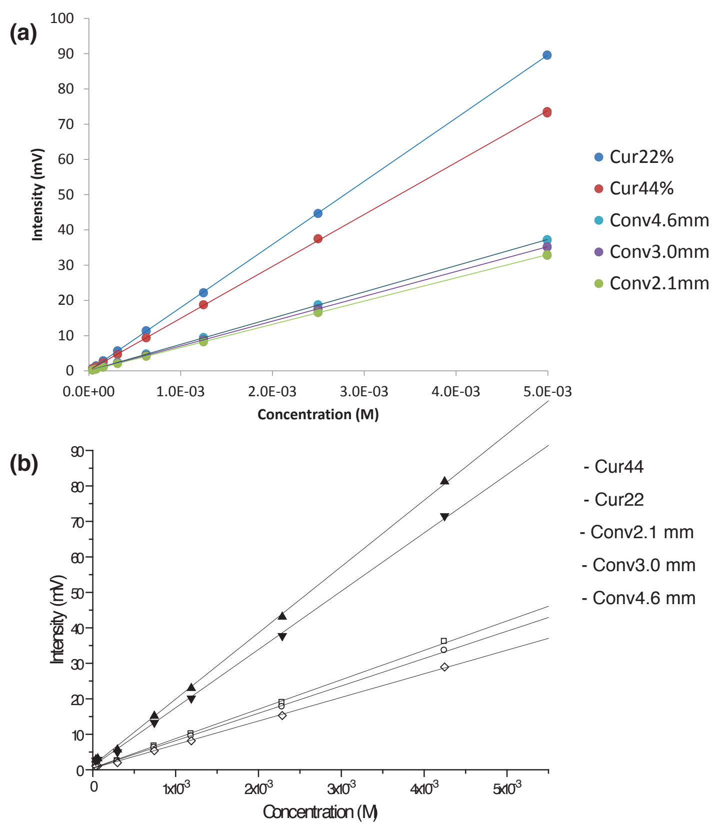


FIGURE 4. Calibration curves for butylbenzene on conventional and CF columns. (a) Lab 1; (b) Lab 2.



- Calibration curves follow the same trend in both laboratories.

Conclusion

- The efficiency of the PSF columns is greatly improved over that of comparable conventional bore columns (45% versus the conventional 2.1 mm column).
- CF columns provide significantly higher signal intensity than conventional columns.
- CF columns provide a much greater degree of discrimination in standard and sample concentration measurements than conventional columns.
- CF columns provide significantly improved LODs and LOQs (at least 2 fold improvement versus the conventional 2.1 mm column).
- The analytical performance for the PSF and CF columns followed the same trends in both laboratories demonstrating that AFT is transferable and reliable.

References

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