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The design of a new concept chromatography column

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Active Flow Management is a new separation technique whereby the flow of mobile phase and the injection of sample are introduced to the column in a manner that allows migration according to the principles of the infinite diameter column. A segmented flow outlet fitting allows for the separation of solvent or solute that elutes along the central radial section of the column from that of the sample or solvent that elutes along the wall region of the column. Separation efficiency on the analytical scale is increased by 25% with an increase in sensitivity by as much as 52% compared to conventional separations.

Introduction

The heterogeneity of the chromatographic column[1–6] and the temperature differential between the column centre to that of the column wall,[7] are now well documented. The end result is that solute plugs migrate along the column in a parabolic-shaped band, rather than as flat discs.[8,9] These parabolic band profiles generally present a faster and more efficient migration in the centre of the column, and slower and less efficient migration near the wall.[8–10] With the aid of on-column visualisation[8–10] these parabolic-shaped plugs were shown to be hollow, much like that of a soup bowl, or cup. The ramifications of such a migration process is that there is a much greater demand on the number of theoretical plates to separate bowls than there is to simply separate a series of flat discs that are broadened only by axial dispersion. Hence factors that lead to the generation of parabolic sample bands demand that chromatography columns be packed with more plates; that is columns are made longer, or packed with smaller diameter particles. Both of these factors lead to the generation of higher pressure, which can in turn, as the particle size decreases, result in a greater differential in the heat generation between the radial central region of the column compared to the wall. Hence the parabolic migration profile is further encouraged. Furthermore, the higher demand on column efficiency leads to increased analysis time and cost.

There has been little change in the design of HPLC chromatography columns since the time particles were first packed into stainless steel tubes, except to say, the particles themselves have changed dramatically over that time. The basic nature of the column has always been a tube (highly polished) with a frit at the inlet and outlet contained inside a header or end fitting. Flow enters the header through the centre, and exits the column through the centre of the outlet fitting.

The present study, however, describes a new concept in the design of the HPLC column and introduces new analytical separation technology. Here the chromatography column has been modified to include a specially designed header fitting that allows for sample to be introduced to the top of the column as a central injection.[11,12] A curtain flow of mobile phase restricts the solute migration to the central region of the bed and a special outlet fitting separates sample eluting from the central radial section of the column from sample that elutes in the wall region of the column. The principles of the infinite diameter column are applied,[11,12] but in a manner which facilitates automated injection.

Experimental

Chromatography column

Reversed phase Hypersil Gold chromatography columns (100 × 4.6 mm, P_d 5 μm) were supplied by ThermoFisher Scientific (Runcorn, Cheshire, United Kingdom). Specialised curtain flow inlet and segmented flow outlet fittings were made especially for this work by ThermoFisher Scientific. Details of these fittings are illustrated in Fig. 1.

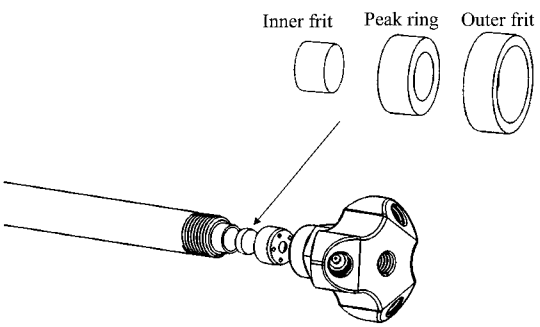

Fig. 1 Illustration of the column header showing the three peripheral entry ports and the single central entry port. Sample is loaded *via* the central port, a curtain mobile phase flows through the peripheral ports. The same fitting is applied to the outlet of the column. The figure also illustrates the annular frit design, where an inner frit section is separated from an outer frit section by a PEEK ring.

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Chemicals and reagents

All mobile phases were prepared from HPLC-grade solvents purchased from Merck (Kilsyth, Victoria, Australia). All chemicals were commercially available. Toluene, propylbenzene and butylbenzene were purchased from Sigma Aldrich (Castle Hill, New South Wales, Australia). Milli-Q water (18.2 MΩcm^{-1}) was prepared in-house and filtered through a 0.2 μm filter.

Reagents

Standard test compounds were prepared in mobile phase at concentrations of 0.30 g L^{-1} (toluene), 0.90 g L^{-1} (propylbenzene) and 1.20 g L^{-1} butylbenzene.

Chromatographic separation

All chromatographic experiments were conducted using a Shimadzu LC-20ADvp chromatographic system (Shimadzu, Rydalmere NSW, Australia) equipped with a Shimadzu LC-20ADvp quaternary pump, Shimadzu SIL-10ADvp auto injector, Shimadzu SPD-M10Avp photo diode array detector and a Degassex model DG-440 inline degasser unit (Phenomenex, Lane Cove NSW, Australia). Analysis was conducted under isocratic conditions using a mobile phase of 30 : 70 water: methanol. The injection volume used was 2 μL and UV detection was performed at 250 nm. In all experiments undertaken, whether using normal, conventional HPLC columns, or the newly designed curtain flow model described herein, the extra column dead volumes through which sample was transported was identical. Furthermore, the frit in the newly designed curtain flow column was of the exact material (thickness and porosity) as the normal HPLC column. Hence the addition of the curtain flow header and parallel segmented outlet fitting did not introduce additional dead volume to the system.

Results and discussion

At the core of active flow management (AFM) is a new column format where the eluent flow in the wall region is separated from the eluent flow in the radial central region of the column. Sample is introduced into the radial central region of the column and a curtain flow of mobile phase prevents its migration to the wall during passage along the column. This is distinct from the manner in which sample is introduced into a normal chromatography column, whereby the sample is introduced into the central region of the inlet frit, but the frit then distributes sample across the radial cross section of the column inlet, from the centre to the wall. In this new column design, a segmented outlet fitting ensures that the sample eluting in the central region of the column is separated from sample in the wall region. Thus sample from the central section can be passed through a detector in a plug that is not diluted by the entire bulk flow of eluent from the column. The principles of the infinite diameter column are thus applied and maintained throughout elution and detection. The diagram in Fig. 1 illustrates the design of the chromatography column. At the heart of this new design is an annular frit contained within a head fitting that has multiple entry ports. The central portion of the annular frit is separated from an outer portion of frit by a solid PEEK ring. This frit prevents cross dispersion of solute between the radial directions that separate the wall zone from the central zone. Sample is introduced to the column *via* the central entry port, whereas, mobile phase is introduced to the column through a series of entry ports in the peripheral region of the column. A means of providing mobile phase to both the central entry port and the peripheral ports is most conveniently achieved using a separate pump to deliver these two different solvent streams to the column inlet. At the column outlet a segmentation fitting is employed. The construction of this fitting is the same as that for the inlet. The purpose, however, of this fitting is to maintain the separation of analyte that migrates through the radial central region of the column from that of the sample that migrates along the outer region of the column. Sample eluting from the central region thus exits the column *via* the inner annulus of the outlet frit through the central outlet port to the detector.

The chromatograms in Fig. 2 compare the separation of the three test solutes eluting under a normal mode of operation compared to that of the separation achieved with the AFM mode of operation. In the AFM mode of operation, the ratio of flow (central to peripheral) was 45 to 55 at both the column inlet and outlet. These flow ratio conditions (central:peripheral) were derived by systematically assessing the chromatographic data obtained using different inlet and outlet central:peripheral flow ratios. The optimum was selected as the 45 : 55 (central:peripheral) based on two criteria: (1) The maximum in column efficiency, and (2) the minimum variation in replicate analysis. The data in Table 1 illustrates some of the most important optimisation conditions tested, and this data includes the variation in the result (expressed as a %) from that of the mean of duplicate analyses. In general, the maximum sensitivity also coincided with these two criteria. At the optimum flow ratio conditions, the maximum variation in retention time for the curtain flow mode of operation was less than 0.2% for retention time, 0.4% for peak height, 0.4% for peak area, and 1.7% in N. These variations were consistent with the operation of a conventional HPLC column.

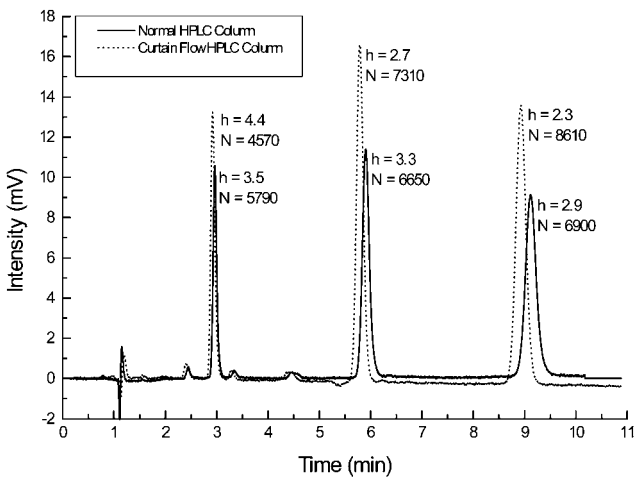

Fig. 2 Chromatograms of the three component mixture eluting from the column operating in normal mode (solid) and curtain flow inlet – parallel segmented flow outlet (dotted) Flow ratios for the curtain flow mode were 45 : 55 central:peripheral at the column inlet – curtain flow, and 45 : 55 parallel segmentation ratio central:peripheral at the column outlet. Elution order; toluene, propyl benzene, butyl benzene. Mobile phase 30/70 water/methanol. Total flow rate through the column was 1.1 mL min^{-1} (*i.e.*, 0.5 mL min^{-1} through centre, 0.6 mL min^{-1} through peripheral), injection volume 2 μL, detection 250 nm.

Table 1 Figures of merit in the optimisation of curtain flow and parallel segmented flow conditions. t_r = retention time, H = peak height, N = number of theoretical plates, h = reduced plate height. All data collected at 0.8 mL min^{-1}. Data presented as the mean of duplicate injections with %RSD

Solute (conditions)	t_r (min) (%RSD)	H (mV) (%RSD)	Area (%RSD)	N (%RSD)	h	Asymmetry
Toluene[a]	4.06 (0.2)	27.42 (1.3)	274855 (0.4)	3172 (1.3)	6.3	1.24
Propyl benzene[a]	7.9 (0.2)	13.27 (3.3)	201928 (1.0)	5663 (3.9)	3.5	1.10
Butyl benzene[a]	12.07 (0.06)	9.44 (3.6)	205389 (0.5)	6694 (9.6)	3.0	1.08
Toluene[b]	3.98 (0)	12.57 (0.23)	101538 (0.4)	4790 (1.7)	4.2	1.2
Propyl benzene[b]	7.95 (0.02)	20.00 (0.3)	262578 (0.3)	7787 (0.02)	2.6	1.08
Butyl benzene[b]	12.3 (0.3)	19.13 (0.2)	368596 (0.1)	8927 (0.3)	2.2	1.04
Toluene[c]	3.92 (0)	8.48 (2.3)	66171 (2.6)	4827 (1.7)	4.1	1.24
Propyl benzene[c]	7.76 (0.1)	13.42 (2.0)	177482 (1.3)	7007 (0.8)	2.8	1.13
Butyl benzene[c]	11.96 (0.2)	13.03 (0.2)	255974 (1.8)	7746 (2.7)	2.6	1.10
Toluene[d]	3.93 (0.1)	9.77 (2.5)	75919 (2.7)	5023 (3.9)	4.0	1.2
Propyl benzene[d]	7.87 (0.2)	14.95 (2.8)	198075 (2.6)	7478 (0.2)	2.7	1.13
Butyl benzene[d]	12.22 (0.2)	14.15 (3.0)	279560 (3.0)	8296 (0.5)	2.4	1.06
Toluene[e]	3.94 (0)	8.51 (7.7)	67325 (2.3)	5142 (1.1)	3.9	1.2
Propyl benzene[e]	7.92 (0)	12.97 (2.1)	178026 (1.9)	6984 (0.8)	2.9	1.13
Butyl benzene[e]	12.29 (0.1)	12.22 (1.8)	252908 (1.0)	7424 (1.7)	2.7	1.09

[a] = 20% flow through central zone at inlet, 45% flow through central zone at outlet. [b] = 45% flow through central zone at inlet, 45% flow through central zone at outlet. [c] = 80% flow through central zone at inlet, 45% flow through central zone at outlet. [d] = 45% flow through central zone at inlet, 80% flow through central zone at outlet. [e] = 80% flow through central zone at inlet, 80% flow through central zone at outlet.

There are two important points to note in the separations illustrated in Fig. 2. (1) The retention times between both modes were almost coincident, and (2) the sensitivity of the AFM mode was much greater than the normal mode of operation – details of which will be discussed later in this text.

An important aspect of the separations illustrated in Fig. 2 that is not immediately obvious is that there is a reduction in the peak volume for sample that elutes in the AFM mode compared to that in the normal mode of operation. The chromatograms in Fig. 2 are presented with respect to the units of time, however, since only a portion of the flow that elutes from the column is passed through the detector, a more appropriate measure of the separation performance can be gauged by evaluating the chromatographic profiles as a function of peak volume. For example, Fig. 3 illustrates the elution profile of butyl benzene in a normal mode of operation compared to the elution profile of butyl benzene in the AFM mode of operation. In this comparison, the central retention time has been set to zero for ease of comparison. Quite clearly there is a substantial reduction in peak volume, which is proportional to the ratio of central to peripheral flow at the column outlet – we call this the segmentation ratio. As the amount of flow eluting from the central section is decreased, the volume of the peak decreases accordingly. This feature could be used to fine tune the efficiency of solute transport to say a MS detector or the second dimension of a two-dimensional separation. Nevertheless in the specific example illustrated in Fig. 3 the volume of the peak was reduced from 820 μL in the normal mode of operation to 290 μL in the AFM mode of operation: A reduction by 64%.

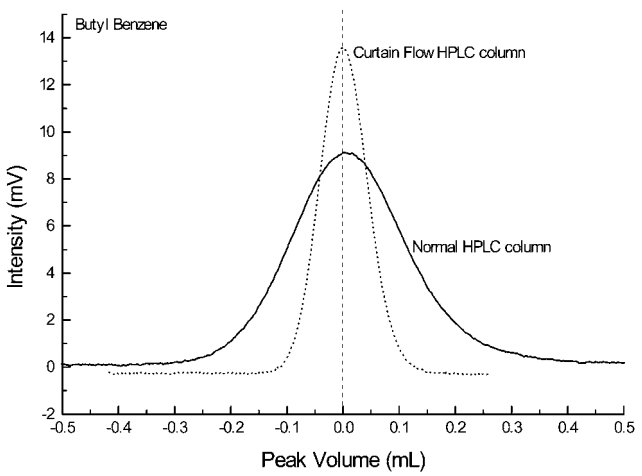

Fig. 3 Band profiles of butyl benzene eluting from the column operating in normal mode (100) and curtain flow inlet - segmented flow outlet (45 : 55 central:peripheral at both inlet and outlet). Band width expressed in units of volume centred around the elution of the peak maxima. Mobile phase 30/70 water/methanol, flow rate 1.1 mL min^{-1}, injection volume 2 μL, detection 250 nm.

Importantly, even though 55% of the solvent flow was not passed through the detector (*i.e.* only the central mobile phase zone was detected, which was 45% of the total) the peak sensitivity was not decreased. Rather there was a substantial increase in sensitivity, approximately 52% for the butyl benzene band illustrated in Fig. 3. This increase in sensitivity is a result of the entire sample being loaded into the central region of the column and eluting according to the principal of the infinite diameter column,[9,13] whereby approximately 60% of the sample eluted from this central region and was detected *via* the UV detector connected to the central exit port. In the flow geometry employed for the separation in Fig. 3 approximately 40% of the sample eluted from the peripheral ports (this was not detected). However, by varying the flow ratios at both the inlet and the outlet, the amount of sample that elutes through the central exit port can be varied, but at the cost of separation efficiency. Importantly, for aspects related to sensitivity, the outlet of the column also utilised the services of a segmented flow fitting, and this meant that the flow exiting the column from the central zone was not diluted by the entire bulk flow (wall plus central flows). Hence at the column exit, the solute was not diluted by the bulk flow. This gain in sensitivity would not be realised without both these factors being applied. The sensitivity gain was, however, not uniform across the entire separation; gain decreased as the retention factor decreased. For example, the gain in sensitivity was 25% for toluene (retention factor - $k \sim 1.5$), 47% for propyl benzene ($k \sim 4.0$), and 52% for butyl benzene ($k \sim 6.5$).

Another advantage of the AFM mode of separation was a gain in separation efficiency, as measured by the number of theoretical plates and expressed as the reduced plate height (reduced plate height (h) for each peak is included in Fig. 2). For example, the increase in N (AFM compared to normal mode) was 25% for butyl benzene and 10% for propyl benzene. There was, however, a reduction in efficiency for toluene (21%). There is an optimisation factor related to the portion of flow through the central region of the column to that of the peripheral region, and hence continued studies will focus on this aspect of the AFM separation mode to seek improvement.

The advantages of AFM separations are four-fold:

- The gain in sensitivity increases the limits of detection (effectively by more than 50% in some instances) and this may be especially important in 2DHPLC where sample dilution into the second dimension is often a limiting factor in the application. Note there was no distinguishable difference in the magnitude of the baseline noise between data obtained on either the curtain flow HPLC column or the normal – conventional HPLC column.
- The reduction in peak volume will allow for more efficient transport to a MS, since the solvent removal requirement will be drastically reduced.
- The reduction in peak volume will allow for improved transport of solute from the first dimension to a second dimension in 2DHPLC modes of operation. In the example used here for butyl benzene, the peak width in a normal mode of operation was 820 μL and only 290 μL in the AFM mode of operation. For comprehensive 2D analysis sampling at 5σ with respect to sample population, this would equate to a transfer volume of 168 μL in the normal mode of operation, but only 68 μL for the AFM mode. This reduced solvent load will minimise mismatch phenomena, such as thermodynamic differences between different solvent environments, and minimises the effects of viscous fingering.[14] Furthermore, a decrease in the volume of transport between the two dimensions will lead to a greater efficiency in the separation in the second dimension, and this will increase the overall two-dimensional peak capacity.
- The increase in separation efficiency (as a measure of the number of theoretical plates) obtained using the curtain flow mode, compared to the normal flow mode was 25%. This is a substantial gain and thus will allow for a decrease in column length by approximately 10% for constant resolution. As such there would be a 10% reduction in pressure. Subsequently there would be a 10% increase in analysis through-put.

Conclusions

The new concept chromatography column that enabled active flow management yielded a greater degree of separation efficiency. This efficiency gain was not simply measured by the number of theoretical plates, but rather also by virtue of a decrease in the peak volume following elution and an increase in the peak height due to sample concentration in the central region of the column. Continued studies aim to optimise the process of active flow management in separations by HPLC.

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