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Enhanced separation performance using a new column technology: Parallel segmented outlet flow

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ABSTRACT

A new column technology – termed parallel segmented outlet flow was employed here to illustrate gains in separation performance that are achievable by the active management of flow as it exits from the outlet of the chromatography column. Parallel segmented outlet flow requires a column be fitted with an outlet fitting that separates flow from the central region of the column from that of wall region. Each region of flow is able to be processed independently, such that post column detection emulates end column localised detection. As a result of this flow segmentation and the subsequent more efficient means of detection, column efficiency was observed to increase by more than 20%, with gains in sensitivity by as much as 22%, and a decrease in peak volume by up to 85%.

1. Introduction

It is now well known that solute transport through chromatography columns is not uniform: Variations in packing density [1–19], both radially and axially, and the generation of thermal gradients due to frictional heating [20–29] lead to the distortion of the band profile. While the variation in bed density in the axial direction is important since it increases the reduced plate height, its effect on modern HPLC columns is small, as high performance columns are now packed in short column formats and this axial heterogeneity extends in scales larger than the current dimensions [4]. Hence individual beds are now more likely to be essentially uniform in the axial direction. However, radial heterogeneity is far more detrimental to column performance since variations in the packing density across the column radius lead to variations in flow velocity. The bed density usually increases from the column centre to the wall region and as consequence flow is faster in the central region than near the wall. The solute band thus migrates along the column in a parabolic profile. Further degradation in separation performance occurs as a result of the 'wall effect' whereby a higher than average void fraction occurs at the wall because the particles and wall surface cannot bend to accommodate the other. Hence solute migrating in this section of the column region does so at higher velocity than the bulk. A recent study undertaken by Bruns and Tallarek [30] verified that the porosity of the bed was greatest in the vicinity immediately adjacent to the wall, and this was due to geometrical packing restrictions. They also showed that the bed displayed significant radial heterogeneity, which was caused by friction between particles during the slurry packing process.

Band distortion is enhanced further by the frictional heating associated with solvent being forced through finely divided powders at high pressures. Gritti and Guiochon, for example showed that the temperature in the central region of a bed may be as much as 1.5 °C higher compared to the wall region, even at pressures of only 290 bar [31]. The end results of these contributing factors to flow migration is a variation in flow velocities dependent on the path of migration, hence an increase in band broadening, Perhaps the most significant development in the quest for improvements in separation efficiency of liquid chromatographic columns has been in the implementation of core shell particle technology in sub – 3 μm particles. Within the last five years the number of publications that have utilised this technology exceeds 70[1] and is growing exponentially. The main advantage of these columns stems from their reduced mass transfer term of the Van Deemter equation. The surface of these particles is also rough, a consequence of the manner in which they are synthesised, and some consider that this feature is an important aspect in the preparation of high quality beds. When utilised in systems that can deliver flow at high pressures, and optimised for the minimisation of extra column dead volumes, in excess of 250,000 plates per metre can be achieved, with corrected reduced plate heights often less than 1.5 (corrected for extra column band broadening). However in systems

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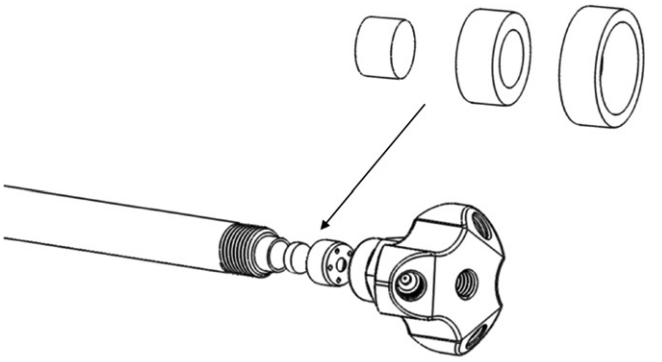

Fig. 1. Illustration of the column outlet showing the three peripheral exit ports and the single central exit port. The annular frit is expanded and inserts into a cap located within the outlet cap.

where the extra column volume is not minimised, the realisation of the true column efficiency may be decreased by more than 20–30%.

In addition to the necessity to 'fine tune' the performance of the modern HPLC system in order to minimise extra column band broadening, the efficiency of these columns are limited by frictional heating of mobile phase as it traverses the finely divided particles within the column at high pressures. As a consequence the central radial region of the bed is at a higher temperature than the wall region, and this leads to heterogeneity in the flow path of a migrating bed, resulting in a decrease in separation performance. The dilemma that the chromatographer thus faces is how therefore to practically utilise the true performance of these columns.

It is important to realise that as a consequence of the radial variation in flow velocity the parabolic profile that is established in fact resembles that of a bowl [32]. Hence the separation problem reduces to the requirement of separating the surfaces of stacked bowls, rather than separating all bowls across all dimensions. It is in fact this aspect of the separation that end column detection processes exploit. That is, a localised region of flow passes through a discrete detection point such that effectively a heart-cut section of the entire sample plug is observed. Provided the discrete detection point is small enough with respect to the bulk flow the parabolic nature of the entire band is not seen. Hence, fewer plates are required to separate the surfaces of two bowls than the two bowls entirely across all dimensions.

The aim of the current work is to illustrate a column design that allows for the processing of flow streams in a manner that emulates end column detection, but with the convenience of a post column bulk detector. This process involves the segmentation of flow streams, separating the flow near the wall from that of the flow in the central section of the bed. The current paper discusses this process in analytical scale column formats.

2. Experimental

2.1. Chromatography columns

Reversed phase Hypersil Gold chromatography columns (100 mm × 4.6 mm, 5 μm) were supplied by ThermoFisher Scientific (Runcorn, Cheshire, United Kingdom). Specialised parallel segmented flow outlet fittings were made especially for this work by ThermoFisher Scientific. These fittings were designed to fit exactly, existing ThermoFisher Scientific columns. Details of these fittings are shown in Fig. 1 and will be described later in the text.

2.2. Chemicals and reagents

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

2.3. Reagents

Standard test compounds were prepared in mobile phase at concentrations of 0.015 g/L (toluene), 0.045 g/L (propyl benzene) and 0.060 g/L butyl benzene.

2.4. Chromatographic separation

Chromatographic experiments were undertaken on a Shimadzu analytical HPLC system, comprising a Shimadzu LC-20ADvp quaternary pump, Shimadzu SIL-10ADvp auto injector, Shimadzu SPD-M10Avp PDA 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 was 2 μL and UV detection was performed at 250 nm. The reproducibility in peak area, peak height and N (exponentially modified Gaussian fit) was approximately 2% for duplicate injections.

3. Results and discussion

The diagram in Fig. 1 is an illustration of the chromatography column kitted out for operation in an outlet parallel segmented flow mode, which allows separation of the flow between the wall and central regions. The basis of this new design consists of an annular frit whereby the central portion of frit was 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. The frit is housed in an outlet fitting that has multiple exit ports: a central port to capture flow eluting from the central, inner frit portion of flow, and three exit ports to capture flow eluting from the outer annulus or wall region of the frit. The purpose of this outlet fitting was therefore to segment flow into two portions: (1) the central portion of flow and (2) the peripheral or wall region portion of flow. Either of these flow portions could then be processed further, i.e., passed through a detector or the sample collected in a fraction collector. The segmentation ratio (central flow:wall flow) could be easily adjusted by regulating the pressure in any of the respective outlet ports, simply by the addition of differing lengths of tubing, post detector – for example.

To illustrate the basic function of this new column design separation performance was assessed using three test solutes; toluene, propyl benzene and butyl benzene. The performance of a regular column was tested in comparison to a column fitted with the new outlet fitting illustrated in Fig. 1. A chromatographic elution profile of the three test solutes eluting from a regular Hypersil Gold HPLC column is illustrated in Fig. 2a (solid trace). In comparison the separation of the three test solutes eluting from a Hypersil Gold column fitted with the parallel segmented outlet fitting is illustrated also in Fig. 2a (dashed trace). In the case of the separation that was achieved with the parallel segmented outlet fitting, the segmentation ratio was 45:55 (centre flow:wall flow). Each separation was essentially coincident in time, but the separation observed in the parallel segmented mode of operation had a sensitivity approximately 20% greater than the regular column. In addition, the band width (in units of time, centralised to the peak maxima and height normalised) of butyl benzene eluting via the parallel segmented

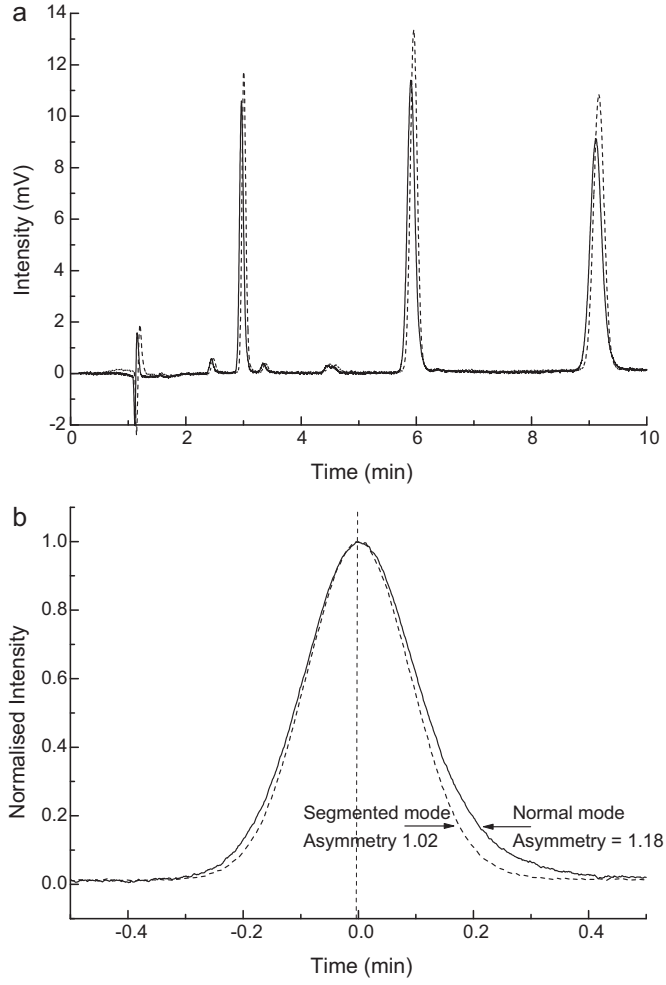

Fig. 2. (a) Chromatograms of the three component mixture eluting from the column operating in normal mode (solid) and parallel segmented mode (45:55 central:peripheral) (dotted). *Elution order*: toluene, propyl benzene, butyl benzene. Mobile phase 30/70 water/methanol, flow rate 1.0 mL/min, injection volume 2 μL, detection 250 nm. (b) Normalised band profiles of butyl benzene eluting from the column operating in normal mode (solid) and parallel segmented mode (45:55 central:peripheral) (dotted). Mobile phase 30/70 water/methanol, flow rate 1.0 mL/min, injection volume 2 μL, detection 250 nm.

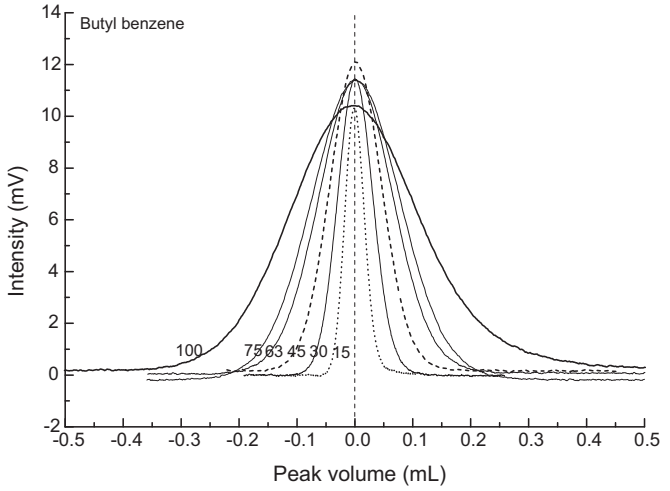

Fig. 3. Band profiles of butyl benzene eluting from the column operating in normal mode (100) and parallel segmented modes 75–15% solvent exiting via central port as marked. Band width expressed in units of volume. Mobile phase 30/70 water/methanol, flow rate 1.1 mL/min, injection volume 2 μL, detection 250 nm.

outlet mode of operation was narrower than on the regular column, with less peak asymmetry also (Fig. 2b). A summary of the performance gains using parallel segmented outlet flow, obtained at the most efficient segmentation ratio with respect to N (45% through column centre – see later discussion) are given in Table 1, An important feature not immediately apparent from the chromatograms illustrated in Fig. 2 is the difference in the peak volume of each of the three test solutes. Since the segmentation outlet fitting serves to separate flow from the centre portion of the column from that of the wall region of the column, the passage of solvent thus exiting the column via the central exit port, passing through the detector is at a lower flow rate, containing a lower volume of solute than would otherwise be observed if there were no segmentation and thus the requirement to separate the entire solute 'bowl' rather than just the discrete solute 'surface'. Thus the solute is contained in a smaller volume, dependent on the segmentation ratio. Fig. 3 illustrates this effect. The bold profile (labelled 100) represents the profile of the butyl benzene band following elution from a regular column at a flow rate of 1.0 mL/min. In contrast, the dotted line (labelled 15) illustrates the band profile for butyl benzene following elution with 15% parallel segmented flow exiting the column from the central exit port. The peak volume is substantially smaller (820 μL for 100% of the sample exiting column through central port, compared to 127 μL for 15% of the sample exiting the column through the central exit port). Furthermore, the sensitivity of these bands are exactly the same, even though a smaller mass of substance is obviously eluted when a 15% segmentation ratio is applied, since only a small surface of the overall band plug is processed by the detector. This equality in sensitivity comes about because the concentration of the elution profile in the case of the 15% segmentation ratio, exiting via the parallel segmented outlet fitting is not diluted by the diffuse tail region. At higher segmentation ratios, i.e., 45%, the sensitivity was observed to be greater than when 100% of the sample was processed through the detector. The relative change in sensitivity as a function of the segmentation ratio is shown in Fig. 4a for each of the three compounds. For the more strongly retained propyl- and butyl-benzenes, the maximum sensitivity was observed at the segmentation ratio of 45:55 (column central zone: wall zone). The gain in sensitivity was 22% relative to that of the normal mode of operation for the butyl benzene band and 15% for propyl benzene. For toluene, however, the maximum gain in sensitivity was observed at a ratio of 65:35 (centre:wall) where a 13% gain was observed. It appears that performance related to sensitivity is dependent on the retention factor, and perhaps also on the design of the frit (the latter aspect is the focus of following studies).

In addition to the gain in sensitivity and the reduction in peak volume, outlet parallel flow segmentation serves to increase the efficiency of the separation, since only a surface of the overall sample band is separated. The plot in Fig. 4b shows the relationship between the number of theoretical plates and the segmentation ratio relative to the operation of the column in a normal mode. For the butyl benzene band, the gain in efficiency was as much as 42% (at a segmentation ratio of 45:55 – centre:wall) compared to that of the normal mode of operation. The gain in N was slightly less for propyl benzene and toluene, and in the case of toluene, the most efficient segmentation ratio was at 65:35 (centre:wall). The plot in

Table 1
Performance summary: data for butyl-benzene obtained at the most efficient parallel segmented flow mode – 45% through column centre.

Column	N (% gain)	Peak height (mV) (% gain)	Peak volume at elution (mL)
Normal	6826	9.85	820
Parallel flow segmented	9812 (43)	11.95 (21)	315

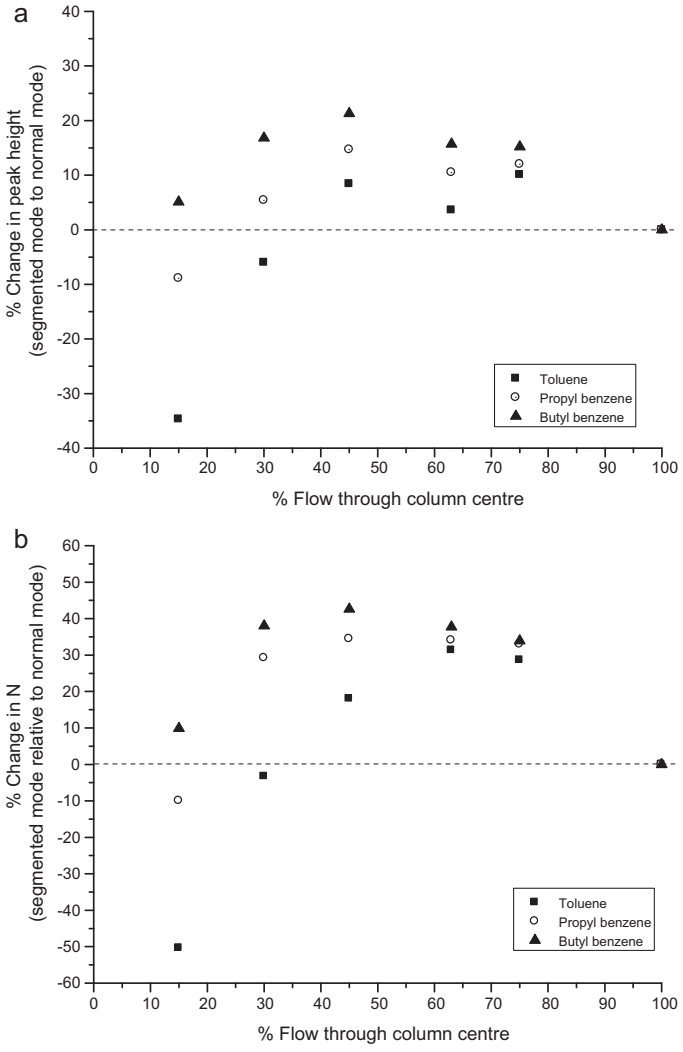

Fig. 4. (a) Relative change in peak sensitivity as a function of segmentation ratio (flow exiting column from central port). '0%' change in peak height represents elution from the column operated in a normal mode. (b) Relative change in N as a function of segmentation ratio (flow exiting column from central port). '0%' change in N represents elution from the column operated in a normal mode.

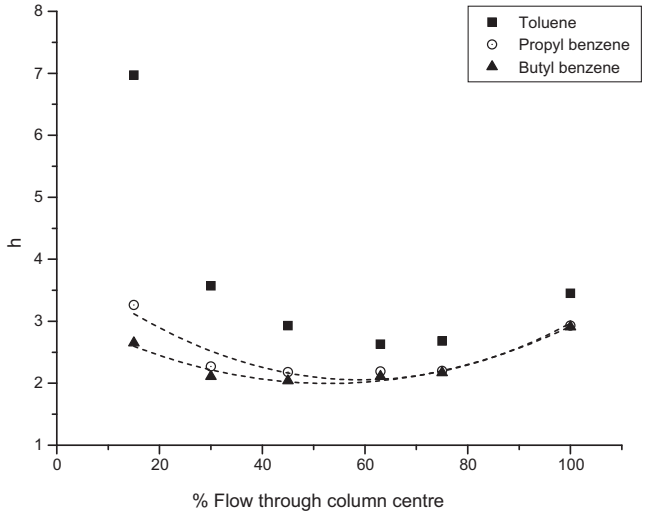

Fig. 5. Reduced plate height as a function of segmentation ratio (flow exiting column from central port).

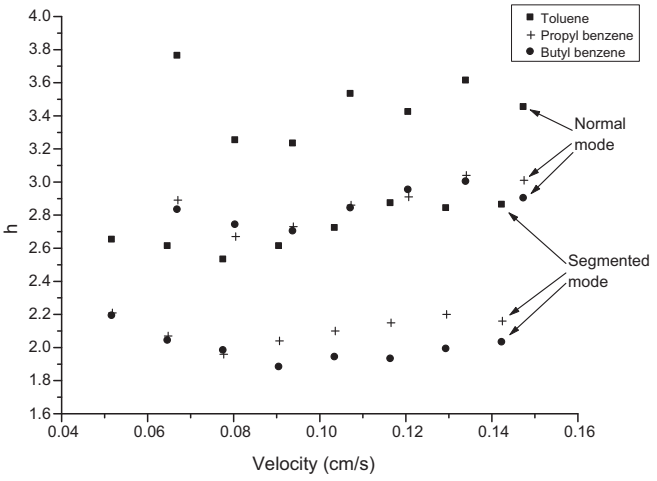

Fig. 6. Plot of reduced plate height as a function of flow velocity for the column operating in both the normal and parallel segmented modes. Segmentation ratio 45:55 (central:wall). Injection volume 2 μL, mobile phase 30:70 water:methanol.

Fig. 5 shows the measure of the reduced plate height as a function of the segmentation ratio at the flow rate of 1.1 mL/min. For both propyl and butyl benzenes, reduced plate heights as low as 2 were observed.

The efficiency of the separation was tested as a function of the flow velocity using the most efficient segmentation ratio as determined from the data presented in Fig. 6 for the propyl- and butyl-benzene standards. (i.e., 45:55 central:wall). A total of 7 flow velocities were tested on both the parallel flow segmented column and a normal column. In the case of the parallel segmented outlet flow column the optimum flow rate was 0.7 mL/min, while that for the normal column was 0.8 mL/min. However, the minimum h value was 1.88 for the parallel flow segmented column and 2.70 for the normal column. These reduced plate heights were not corrected for the extra column dead volume contributions to band broadening, and we should note, that in the chromatographic system employed here, we deliberately added a 60 cm section of 5/1000 capillary between the injector and the column (8 μL). We did that so as to illustrate the performance that may be achieved in a typical QA environment whereby columns are typically temperature regulated in a variety of column heater formats, which by necessity require additional dead volume to be added to the system to accommodate the column. In actual fact, the addition of this tubing was not detrimental to performance (h remained constant to within 1%) even when up to 1.5 m (20 μL) of dead volume was added (results not shown). Furthermore, the values of N on the parallel flow segmented mode were measured without taking into consideration the reduction in flow rate through the detector flow cell. Such a reduction results in an apparent increase in band width relative to the elution volume of the sample from the column. If this aspect of elution is corrected, i.e., the sample is assumed to elute from the column in a 'virtual' column whose dimensions are consistent with the segmentation ratio, then the reduced plate height is actually ~5% lower, that is 1.81 compared to 1.88. This correction, however, does not constitute a correction in band variance associated with the extra column tubing, which remains uncorrected.

3.1. Advantages of flow segmentation

(1) Efficiency: An increase in efficiency of around 42%, consistent with the findings observed here, would result in the possibility of reducing the column length by approximately 20% for the same degree of resolution as a regular column. This would

further translate to a 20% reduction in solvent consumption, and a 20% saving in analysis time.

(2) Detection: An increase in sensitivity is particularly important as we seek to gain more from less. Here a 20% gain in sensitivity was observed with UV detection. However, it is not just the gain in sensitivity that is important. For volume dependent detectors, such as aerosol type detectors, ELSD and MS, the removal of solvent is important. Hence lower volumetric flow rates translate to decreased solvent removal requirements. Thus decreasing the amount of solvent leaving the central zone of the column yields a more efficient desolvation process. This may negate the need to further flow split a solvent line post column, resulting in less extra-column band broadening and greater efficiency and sensitivity in the MS detector etc. In addition, since flow is processed separately in the wall and central zones there remains the possibility that multiple detection modes could be employed simultaneously that collect flow from these separated portions (study currently in progress).

(3) Peak volume: A very important aspect of this new column design is the ability to remove solute from the column in a smaller peak volume than in a conventional HPLC column. In doing so, this type of column is more suitable to operations involving coupled column chromatography, such as on-line comprehensive 2DHPLC. A requirement of on-line comprehensive 2DHPLC is that adequate sampling is undertaking in the first dimension and hence transported to the second dimension. In order to provide full realisation of the chromatographic information the sample volume should be equivalent to a single standard deviation of a typical elution band. In the case of the conventional column used here that would amount to a sample volume of 165 μL (total peak volume = 820 μL), whereas, for the parallel flow segmented mode of operation, at maximum sensitivity, the transport volume between dimensions would be reduced to 58 μL (peak volume = 290 μL). As a result of this decrease in transport volume between dimensions the efficiency of the second dimension will be higher (since efficiency decreases as injection volume increases). This in turn would result in a higher system peak capacity. Furthermore, solvent compatibility between dimensions will be greater, with less chance in the occurrence of viscous fingering (injection volume dependent), solvent mismatch associated with thermodynamic differences between the injection plug and the mobile phase, and less chance of changes in selectivity as a consequence of a large bolus plug of the first dimension mobile phase traversing the second dimension. In addition, the solute eluting from the first dimension is more concentrated (high peak sensitivity) hence greater sensitivity will also be observed in the second dimension. In turn, since shorter, more efficient columns can be employed in both dimensions, less band broadening will be observed, further increasing sensitivity, and being able to employ shorter columns in the second dimension there is a reduced risk of the wrap around effect being realised. These aspects translate to improved operations in 2DHLC.

(4) System dead volume tolerance: In this study reduced plate heights as low as 1.88 were observed using systems with up to 20 μL of pre-column extra dead volume added, plus a flow cell with a volume of 8 μL. Such performance could not be realised in the new core shell technologies utilising traditional column formats. Future work will focus on performance gains obtainable using superfine particles packed in columns fitted with this new outlet fitting design.

4. Conclusion

The active management of flow via a segmentation outlet fitting on a conventional HPLC column yielded very substantial gains in separation performance. Gains were observed in the number of theoretical plates (22%), sensitivity (22%), more efficient peak elution volumes (85% reduction for the same level of detection sensitivity) and potentially more efficient coupling in 2D systems.

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References

[1] T. Yun, G. Guiochon, J. Chromatogr. A 760 (1997) 17.
[2] R.A. Shalliker, B.S. Broyles, G. Guiochon, J. Chromatogr. A 888 (2000) 1.
[3] B.G. Yew, J. Ureta, R.A. Shalliker, E.C. Drumm, G. Guiochon, AIChE J. 49 (2003) 642.
[4] V. Wong, R.A. Shalliker, G. Guiochon, Anal. Chem. 76 (2004) 2601.
[5] C.E. Schwartz, J.M. Smith, Ind. Eng. Chem. 45 (1953) 1209.
[6] J.H. Knox, G.R. Laird, P.A. Raven, J. Chromatogr. 122 (1976) 129.
[7] U. Tallarek, K. Albert, E. Bayer, G. Guiochon, AIChE J. 42 (1996) 3041.
[8] D.S. Horne, J.H. Knox, L. McLaren, Sep. Sci. Technol. 1 (1966) 531.
[9] C.H. Eon, J. Chromatogr. 149 (1978) 29.
[10] J.E. Baur, E.W. Kristensen, R.M. Wightman, Anal. Chem. 60 (1988) 2334.
[11] J.E. Baur, R.M. Wightman, J. Chromatogr. 482 (1989) 65.
[12] T. Farkas, J.Q. Chambers, G. Guiochon, J. Chromatogr. A 679 (1994) 231.
[13] T. Farkas, M.J. Sepaniak, G. Guiochon, AIChE J. 43 (1997) 1964.
[14] T. Farkas, G. Guiochon, Anal. Chem. 69 (1997) 4592.
[15] K. Miyabe, G. Guiochon, J. Chromatogr. A 830 (1999) 29.
[16] R.A. Shalliker, V. Wong, B.S. Broyles, G. Guiochon, J. Chromatogr. A 977 (2002) 213.
[17] R.A. Shalliker, B. Scott Broyles, G. Guiochon, J. Chromatogr. A 994 (2003) 1.
[18] J.A. Abia, K.S. Mriziq, G.A. Guiochon, J. Chromatogr. A 1216 (2009) 3185.
[19] B.G. Yew, E.C. Drumm, G. Guiochon, AIChE J. 49 (2003) 626.
[20] R. Endele, I.N. Hal, K. Unger, J. Chromatogr. 99 (1974) 377.
[21] M. Martin, C. Eon, G. Guiochon, J. Chromatogr. 99 (1974) 357.
[22] H.-j. Lin, S. Horváth, Chem. Eng. Sci. 36 (1981) 47.
[23] H. Poppe, J.C. Kraak, J. Chromatogr. 282 (1983) 399.
[24] M. Martin, G. Guiochon, J. Chromatogr. A 1090 (2005) 16.
[25] G. Desmet, J. Chromatogr. A 1116 (2006) 89.
[26] A. de Villiers, H. Lauer, R. Szucs, S. Goodall, P. Sandra, J. Chromatogr. A 1113 (2006) 84.
[27] F. Gritti, G. Guiochon, J. Chromatogr. A 1166 (2007) 47.
[28] K. Kaczmarski, F. Gritti, G. Guiochon, J. Chromatogr. A 1177 (2008) 92.
[29] F. Gritti, G. Guiochon, J. Chromatogr. A 1131 (2006) 151.
[30] S. Bruns, U. Tallarek, J. Chromatogr. A 1218 (2007) 1849.
[31] F. Gritti, G. Guiochon, J. Chromatogr. A 1138 (2007) 141.
[32] R.A. Shalliker, B.S. Broyles, G. Guiochon, Anal. Chem. 72 (1999) 323.