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RESEARCH ARTICLE

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Reducing pressure and increasing peak capacity by incorporating an adaptable flow stream splitting platform to high-performance liquid chromatography columns: A study on C18 silica-based monoliths

Ross Andrew Shalliker[1,2,3]

[1]School of Science, Western Sydney University, Parramatta, Australia

[2]Australian centre for Research on Separation Science, Western Sydney University, Parramatta, Australia

[3]Chromaspeed, Pty Ltd, Tonsley, Adelaide, Australia

Correspondence
Ross Andrew Shalliker, School of Science, Western Sydney University, Locked Bag 1797, South Penrith Distribution Centre, South Penrith, 1797, NSW, AUSTRALIA.
Email: r.shalliker@westernsydney.edu.au

In this study, an adaptable end-column platform was fitted to a commercially available monolith, which enabled the column to be fitted with a flow-splitting device. A variety of flow-splitting adapters could be incorporated into the platform, and in this study, a radial flow stream splitter was utilized. The advantage of the radial flow stream spitter was that it overcame issues relating bed density variations that could cause bands to distort in the radial cross-section of the column. Using propylbenzene as a test standard in isocratic elution mode, height equivalent to a theoretical plate curves were constructed across ten flow rates, and it was found that the column efficiency improved by as much as 73%. Furthermore, the dual outlet flow splitter enabled a very substantial reduction in column back pressure, with the decrease being consistently between 20 to 30% depending on the column length. Additionally, sensitivity increased by 45%, consistent with the observed increase in efficiency. The adaptable end-column platform could be retrofitted to almost any commercial column with the expectation of gaining efficiency, sensitivity, and reducing back pressure.

KEYWORDS
C18 silica monoliths, chromatography, high-performance liquid chromatography, radial flow stream splitting

1 | INTRODUCTION

Irrespective of whether the chromatography column is prepared from fully porous particles, superficially porous particles, or as a monolith the goal is to produce the most efficient separation device. Substantial advances have been made in particle design, that is, superficially porous particles [1–3], and the manner in which particles are packed into tubes. Also, substantial improvements have been gained in monoliths [4–6], although these gains have come at the detriment of requiring higher pressure to drive solvent through the reduced interstitial space.

There are a number of factors that detract from the performance of the perfect column. The first is that columns must be used in an instrument and irrespective of how well the system is designed, there is always a loss in performance due to system dead volume. This is especially the

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Article Related Abbreviations: WBOC, wide bore outlet column; WBOF, wide bore outlet end fitting.

case as the size of the particle and the column decreases, especially for earlier eluting compounds, as the dead volume becomes a more significant component of the peak volume. The second is that columns, both particle-packed and monoliths, have some degree of radial heterogeneity. This heterogeneity leads to a distortion of the band profile, with generally a tailing region since the solute generally travels slower near the column wall. The cause of radial heterogeneity in packing and the problems that result has been discussed in considerable detail and hence do not warrant further discussion here [7 and references cited therein]]. The third factor relates to columns operating under high pressure and/or flow. Viscous frictional heating effects lead to axial and radial temperature variations [7 and references cited therein, 8]. It is the radial temperature variation, that is, of the biggest concern since otherwise ideal cylindrical-shaped plugs distort into parabolic profiles since the bed is generally warmer in the radial center of the column and cools towards the wall. As such, the flow is faster in the radial center than the wall and hence solute travels at a slightly higher velocity in the radial central region of the column. The end result is similar to a column that has a radial heterogeneous stationary phase density; this is a more important factor in columns packed with sub-2-micron particles. It might appear that even if the column was perfectly packed, using such a column at high velocities and high pressures would subsequently decay the performance, furthermore, the manufacturer cannot control how any particular laboratory uses the column, that is, to what extent does the end user manage the extra-column dead volume.

In recent times, there has been a drive toward making columns using additive manufacturing [9] and chemical etching techniques [10], the latter being limited to nano- and micro-scale devices. In the case of additive manufacturing, however, there is hope that advances in 3D printing may be applicable for larger scale format columns, say 2.1–4.6 mm id, although their construction is not without issue. Presently, the resolution of the printing system is insufficient to prepare separation devices with a structured array, that is, less imperfect than a "modern" particle-packed column. Furthermore, until the resolution of the printing system is able to generate a secondary inner porous region in the order of nano-scale dimensions, these separation devices will largely be limited to low surface area platforms. Another issue is that the additive manufacturing process will need to be able to provide different surface chemistries so that selectivity options are available.

With all these limitations, and the solution to the problem requiring a very significant degree of workaround, perhaps the easiest solution is to simply modify how mobile phase and solute exit the column so as to mitigate against the deleterious effects of heterogeneous flow. In that manner, the most efficient region of flow only is processed by the detector. The focus of the present study is to illustrate exactly that point. Here we employ a new type of outlet end-fitting platform that counteracts the deleterious effects of radial heterogeneity, whether that be heterogeneity generated through the bed structure or heating effects, which makes no difference. This device segments the flow, such that the radial central section of the solute band is directed to the detector, and the flow near the wall region is sent to either a secondary detector, recycled, or to waste. The outcome is a substantial improvement in separation performance. In this research, the fitting of the outlet platform to a monolith is illustrated and the performance gain is demonstrated. These monoliths were commercially sourced. While the work in this research relates to just two specific versions of the device a variety of fittings are available that enable a variety of opportunities for flow stream manipulation, that is, radial flow stream splitting, or zero dead volume axial flow stream splitting. Subsequently increasing the performance of HPLC columns, especially in the case where radial flow stream splitters are utilized to minimize the effects of radial heterogeneity of the column bed or eliminate radial heating effects in UHPLC operations. In essence, the key feature of the column end fitting design is that the column end fitting can accommodate a variety of 'devices' that can be incorporated directly against the column outlet frit, which minimizes and/or eliminates extra column dead volume. This new concept is referred to as the wide bore outlet column (WBOC), which is illustrated in Figure 1 [11].

The photograph in Figure 1A from left to right shows the original Merck end fitting, the Chromaspeed wide bore outlet (WBOF) end fitting, the inner side of an insert that fits into the WBOF, a single port insert, a dual port insert, and a 3-port insert.

The photograph in Figure 1B illustrates examples of the variety of inserts that can be fitted to the WBOF, that is, attached to the monolith. The photograph in Figure 1C illustrates the same fittings, but with the WBOF attached to the column outlet without an insert in the WBOF. The frit, which is a standard, polyetheretherketone encased stainless steel frit, at the outlet of the column is exposed, but the bed is secure, so changing from one insert fitting to another does not result in damage to the column bed.

The design of the WBOF, which subsequently converts practically any conventional column to a WBOC differs from that of a conventional column in that the frit is held to the column using a retaining device (the Wide bore outlet fitting—WBOF), and this retaining device has an id, that is, similar to the id of the column itself. When the WBOC is 'open', that is, there is no insert fitted to the WBOF, the frit of the column on the external surface is exposed, but the bed is intact and in no danger of damage. The entire radial

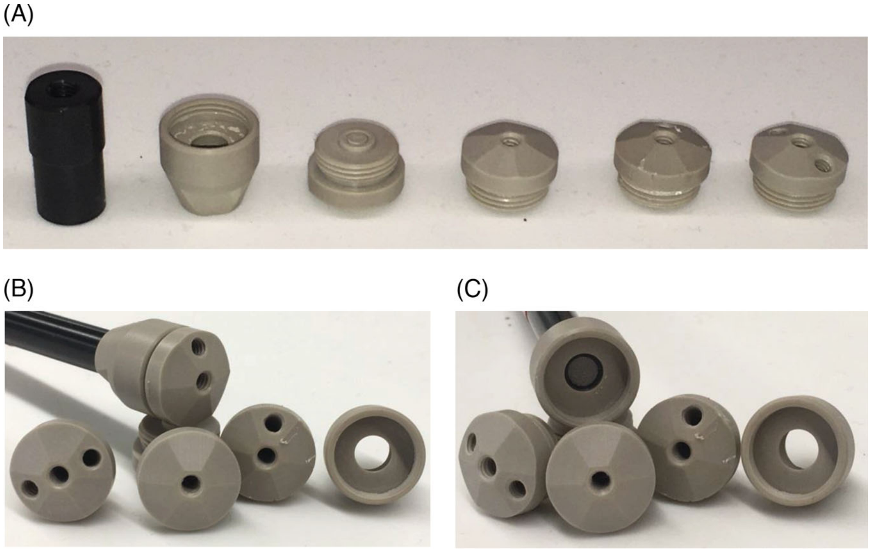

FIGURE 1 Photograph of the prototype Chromaspeed end fittings attached to a Merck Monolith.

cross-sectional surface of the frit is visible, yet it remains tightly sealed on the bed—see photograph in Figure 1C. In this mode, the column is not operational as the fluid leaving the column cannot be captured with any meaningful level of separation efficiency. However, inside the outlet fitting (WBOF) of the WBOC other devices can be coupled or inserted in a manner whereby they make direct contact with the frit. These inserts offer a variety of flow opportunities; in the most basic design, the insert contains a single flow channel located in the radial central section of the column. With this insert the column functions as a regular conventional column. In other more important designs, the insert can be a flow splitter, either splitting flow in the axial flow direction (not discussed in this research), or in the radial flow direction (the focus of this study), and the splitting process, in either case, is not limited to simply a two-directional flow split; in the current design, up to six flow splits (7-port) have been allowed for. In prior studies, we demonstrated radial flow stream splitting and the advantages it offers on a column that was referred to as a parallel segmented flow column [7]. While very substantial gains in performance were obtained, the column could only be used as a parallel segmented flow column, since the frit in that design was a three-piece frit with an outer porous region and a radially central region, the two separated by an impermeable plastic ring (in this design the frit is a single stainless steel frit, encased in polyetheretherketone, which bares against the column tube for the purpose of sealing without leakage). When the three-piece frit of the earlier design was used, the result was that if the flow was directed only through the radial central outlet port of the parallel segment flow column, the efficiency was very poor. Furthermore, the end fitting could not be changed by end users as there was a very high risk of damage to the column since the bed would be open upon removal of the end fitting. Such is not the case with the WBOC as discussed here.

In this study, the performance of the WBOF design concept, coupled to a conventional column to thus produce a WBOC, in two modes of operation is reported: (1) as a conventional column with a single port outlet located at the radial center of the column outlet, and (2) as a column in which radial flow splitting has been achieved using an insert with two outlet flow channels—one located at the radial center of the column outlet, the other located near the wall region of the column outlet. The photographs in Figure 1 illustrate these columns.

2 | MATERIALS AND METHODS

2.1 | Chemicals

HPLC-grade methanol was purchased from Fisher Chemicals (Loughborough, UK). Milli-Q water (18.2 MΩ cm) was prepared in-house and filtered through a 0.2 µm filter. Propylbenzene as a test solute was purchased from Sigma Aldrich (Dorset, UK). All mobile phases were prepared volumetrically.

2.2 | Equipment

Chromatographic investigations were performed on an Ultimate 3000 RSLC instrument with UV detection, running Chromeleon 7.0 software.

Two chromolith high-resolution silica-based C18 monolithic columns (1: 50 × 4.6 mm and 1: 100 × 4.6 mm) were sourced from Merck (Darmstadt, Germany). These columns were tested as received and then fitted with the WBOF that was machined by Chromaspeed Pty Ltd (Tonsley, SA., Australia). With these fittings attached the monolith could function as a WBOC, which was then kitted out with either a single port insert or dual port radial flow insert depending on the test being undertaken. Irrespective of the column, that is, the original Chromolith or the Chromaspeed modified monolith, the system dead volume remained constant. That is to say, the exact precolumn tubing and the exact post-column tubing leading to the detector were used. In total, the volume of the precolumn tubing was 1.9 μl, the volume of the post-column tubing was 2.5 μl and the volume of the flow cell was 2.3 μl.

When required, the flow ratio through the dual port radial flow stream fitting insert was measured by mass.

2.3 | Column efficiency and asymmetry measurements

All performance metrics were measured using the Chromeleon 7.0 software. Theoretical plates were calculated using the second-moment method, which is sensitive to peak tailing and asymmetrical behavior.

Plates measured using the second-moment method were calculated according to Equation (1),

$$N = \left(\frac{\mu_1'}{\mu_2'}\right)^2 \tag{1}$$

where N is the number of theoretical plates, μ'_1 is the first central moment or retention time, and μ'_2 is the second central moment or SD of the peak. This method is more susceptible to indicating tailing, co-elution, or asymmetrical phenomena. Band variance and hence the number of theoretical plates was not corrected for the contributions made by the system itself. The results are reported in a manner that would be expected from an end user, rather than from a research scientist attempting to maximize column performance.

The asymmetry (tailing factor) = a/2b, where a is the peak width measured at 5% height and b is the width of the first portion of the peak measured at 5% of the peak height.

The operating pressure was recorded based on the pressure response of the instrument. No correction was made for the system itself.

2.4 | Standard and sample preparation and chromatographic conditions

The standard propylbenzene solution (0.45 mg/ml) was prepared in 100% methanol. Chromatographic behavior was assessed under isocratic conditions in a 20/80 water/methanol mobile phase (retention factor of propylbenzene = 1.4). A range of flow rates was utilized, as identified in the relevant sections of this text. When the monolithic column was operated in radial flow stream splitting mode, the flow through the radial central port was adjusted by varying the pressure restriction of the tubing attached to the peripheral port. The ratio of flow was measured by mass. Injections were performed in duplicate and the results are reported as the average. The column was operated at an ambient room temperature of ∼22°C. Injection volumes were set at 5 μl. Detection was set to 254 nm.

Green tea samples were prepared from tea bags in 50 ml of hot water (in-house heated water for the preparation of tea/coffee is available at WSU on-tap). The tea bag was agitated periodically over a 10-min period. The tea sample was injected without further dilution, but filtered through a 0.2 μm nylon filter. The tea was analyzed using gradient elution conditions. When tested in radial flow stream splitting mode the flow through the radial central port to the detector was 40%. Other than the flow ratio setting, separation conditions on all columns were identical, specifically, the initial mobile phase composition was 95/5 water/methanol, followed by a linear gradient running to 100% methanol at a rate of 10% per minute. The gradient was initiated at the time of injection. At 100% methanol, the composition was held for 3 min and then returned to the initial conditions in 1 min. Prior to any injection, all columns were equilibrated with five column volumes of the initial mobile phase composition. The mobile phase flow rate was 2 ml/min. The sample injection volume was 5 μl and detection was set to 254 nm.

3 | RESULTS AND DISCUSSION

3.1 | The performance of a Merck monolith (50 × 4.6 mm format)

3.1.1 | Efficiency

The plots in Figure 2A illustrate the efficiency of the original Merck monolithic column and the modified Merck monolith fitted with the WBOF and operating with a single outlet port insert and a dual outlet radial flow stream splitting insert. For ease of discussion, the monoliths that are fitted with the WBOF are referred to as the "'Chromaspeed Monolith" for the case of the monolith fitted with a single port insert, and the "Chromaspeed HP Monolith" for the monolith fitted with the dual-port radial flow stream splitting insert. The term "Chromaspeed" reflects the manufacturer of the WBOF and inserts. The efficiency

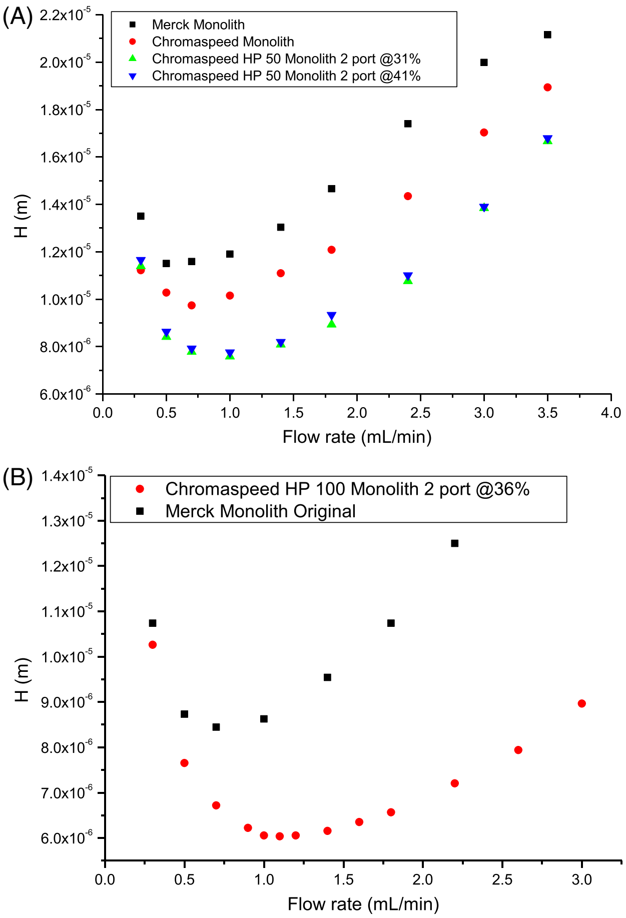

FIGURE 2 Height equivalent to a theoretical plate (HETP) plots for the monoliths: (A) 50 mm length monolith—Merck original, wide bore outlet column (WBOC) with Chromaspeed single port fitting (Chromaspeed monolith), Chromaspeed HP monolith with dual port radial flow splitting operating at 41% and 31% segmentation ratios and (B) 100 mm length monolith—Merck original (red trace) and the Chromaspeed HP 100 monolith with the dual port outlet fitting operating with a 36% segmentation ratio.

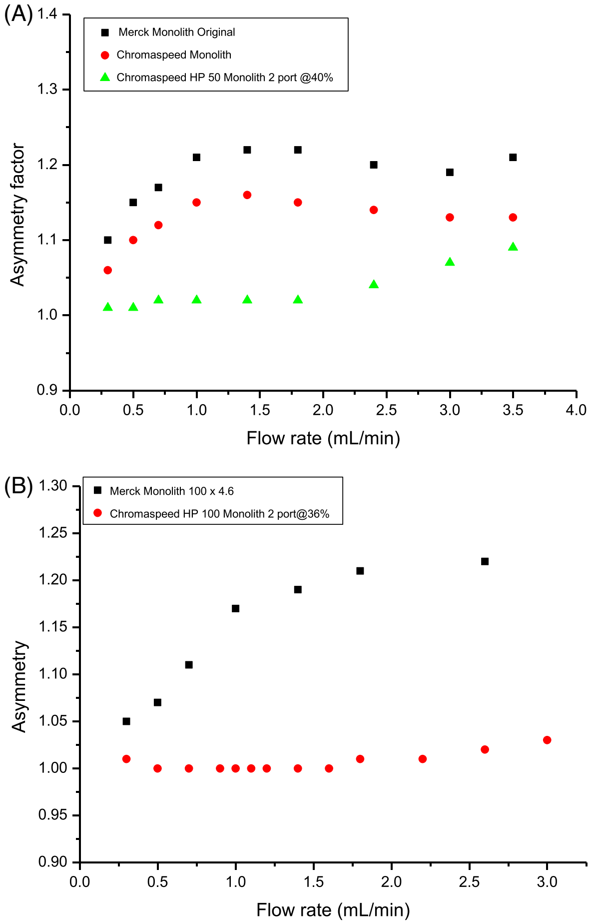

FIGURE 3 Plots of asymmetry as a function of mobile phase flow rate on the various monoliths. (A) 50 mm length monolith—Merck original, Chromaspeed monolith with a single port fitting, Chromaspeed HP 50 monolith with dual port radial flow splitting operating at 41% segmentation ratio, and (B) 100 mm length monolith—Merck original and the Chromaspeed HP 100 with dual port outlet fitting operating with a 36% segmentation ratio.

is plotted as a function of the mobile phase flow rate. These results show that even when the WBOF with a single port insert was fitted to the original Merck monolith there was a gain in efficiency. This was most likely due to the higher quality frit used in the WBOF than that supplied with the Merck column. When the single port insert was replaced with the dual port radial flow stream splitting insert there was a very substantial gain in operational efficiency. In this instance, the dual port insert allows a portion of the mobile phase exiting the column to be channeled to waste, and only the radial central portion of the mobile phase is allowed to enter the detector. In that way, the most uniform portion of the solute band is detected. In the experiments discussed in Figure 2A, 41% and 31% (two examples shown) of the total flow has been allowed to enter the detector, and the rest was directed to waste. In these cases, the gain in efficiency was around 52% in the number of theoretical plates at the comparative optimum flow rate for the respective Merck monolith (original) and the Chromaspeed HP 50 monolith with the 2-port radial flow stream splitting operating with a segmentation ratio of 40%. The greatest efficiency gain was 64% at the flow rate of 1.8 ml/min. These gains were consistent at both 41% and 31% flow split ratios.

3.1.2 | Asymmetry

Figure 3A illustrates the symmetry of the propylbenzene band eluting from each of the monolithic columns. On the original Merck monolith, the average asymmetry factor was 1.19, compared to 1.03 on the Chromaspeed HP 50 monolith with the 2-port radial flow splitter. The

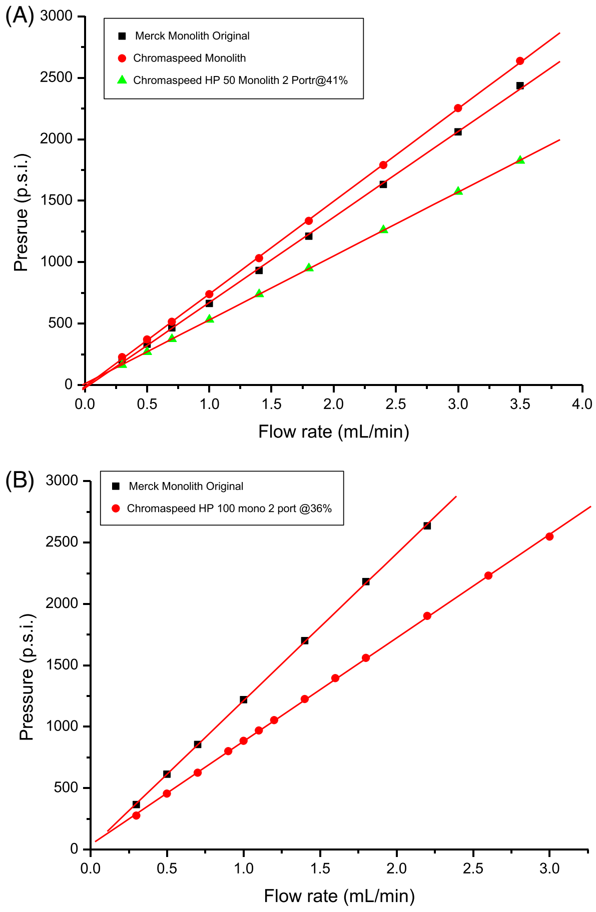

FIGURE 4 Plots of pressure as a function of flow rate for each of the monolith (A) 50 mm length monolith—Merck original, Chromaspeed monolith with a single port fitting, Chromaspeed HP 50 monolith with dual port radial flow splitting operating at 41% segmentation ratio and (B) 100 mm length monolith—Merck original and the Chromaspeed HP 100 with dual port outlet fitting operating with a 36% segmentation ratio.

single-port Chromaspeed monolith performed marginally better than the original Merck monolith.

3.1.3 | Pressure

An operational advantage of the radial flow stream splitting end fitting is that it enables the column to function at a lower pressure than a conventional single port outlet column. Figure 4A illustrates the relationship between column pressure and flow rate for each of the monolithic columns. The single port Chromaspeed monolith had an average of 10% greater pressure compared to the original Merck monolith—most likely due to the frit that was utilized in the Chromaspeed end fitting. However, when the WBOF Chromaspeed monolith was fitted with the 2-port radial flow splitter (aka Chromaspeed HP 50 monolith) the pressure decreased. In fact, the pressure was on average 21% lower than the original Merck monolith, across all flow rates tested.

Perhaps an obvious reason as to why the end fitting with radial flow stream splitting enables a reduction in pressure might be that the flow between the column and the detector is reduced according to the flow split ratio, and hence as a consequence, there is a reduction in the pressure in the post-column tubing. However, such reasoning would assume that the peripheral port had no tubing attached that directed flow to waste. This is not the case, tubing is attached to the peripheral port, in part to direct flow to waste, but also to enable the balance of the appropriate flow segmentation ratio. Hence, if the reduction in pressure was related to the reduction in flow through the post-column tubing the dual port Chromaspeed radial flow spitting column should experience an increase in pressure since more (not less) tubing is added to the system. As an example, at the flow rate of 1.0 ml/min using a 100% methanol mobile phase with 100% of the flow through the central port (peripheral port closed), operating with a 15 cm long section of a 0.07″ id tube connected to the center port the pressure was 322 p.s.i. When the flow ratio was adjusted to 65% through the center (i.e., 0.65 ml/min), with the same 0.07″ tubing connected to the center, and an additional 75 cm length of 0.07″ tubing connected to the peripheral port the pressure was reduced to 294 p.s.i.—a reduction of 9% despite the effective tubing length increasing from 15 cm in total to 90 cm in total.

3.1.4 | Sensitivity

Perhaps a question that could be raised is that if about 60% of the flow is directed to waste, would there be an observed decrease in sensitivity? However, this is not the case, as illustrated in Figure 5A which details the sensitivity (based on peak height) for each of the monolithic columns. The Chromaspeed HP 50 monolith fitted with the 2-port radial flow splitter operating with a segmentation ratio of 41% had an average increase in sensitivity of 21%, with the greatest gain being 31% at the highest flow rate tested (3 ml/min). The reason behind the increase in sensitivity is that the band reaches the detector in a more concentrated plug since only the radial central region of the sample is taken to the detector, and it is not diluted by the inefficient tailing section of the peak.

3.1.5 | Robustness

Since chromatographic columns are required to operate in continual modes of analysis, an important aspect of their

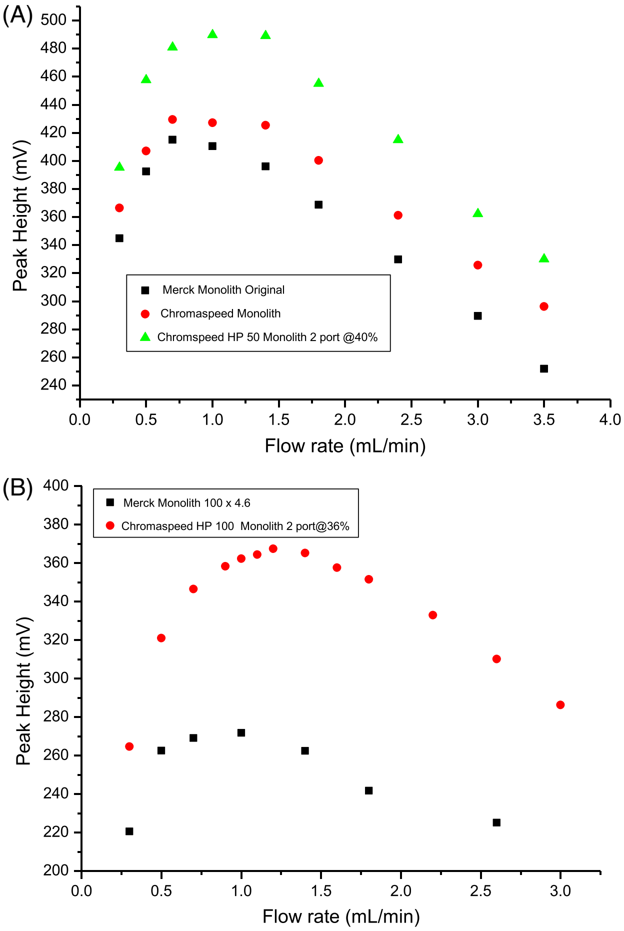

FIGURE 5 Plots of sensitivity as a function of flow rate for each of the monolith (A) 50 mm length monolith—Merck original, Chromaspeed monolith with a single port fitting, Chromaspeed HP 50 monolith with dual port radial flow splitting operating at 41% segmentation ratio and (B) 100 mm length monolith—Merck original and the Chromaspeed HP 100 with dual port outlet fitting operating with a 36% segmentation ratio.

operation is quantitative reliability. Subsequently, a series of tests were undertaken to assess robustness in operation. This entailed reproducibility in retention time, peak height and peak area measurements, and the number of theoretical plates. All experiments detailing reproducibility were undertaken at 1.8 ml/min. The data in Tables 1–4 detail the reproducibility in retention time (Table 1), peak area (Table 2), peak height (Table 3), and the number of theoretical plates (Table 4). Across all these tests there was effectively no significant difference in the reproducibility in the retention time for any of the monoliths, however, in regards to peak area, peak height, and the number of theoretical plates, the RSD was always lower for the Chromaspeed HP 50 monolith fitted with the 2-port radial flow stream splitter. Albeit, these gains in performance metrics were unlikely to be statistically significant.

TABLE 1 Reproducibility in retention time.

Merck monolith	Chromaspeed monolith	Chromaspeed HP monolith @41%
0.908	0.905	0.904
0.907	0.907	0.904
0.908	0.907	0.905
0.908	0.907	0.909
0.906	0.908	0.909
0.907	0.908	0.908
0.907	0.908	0.908
0.907	0.909	0.908
0.912	0.908	0.908
Mean = 0.908	**Mean = 0.907**	**Mean = 0.907**
RSD = 0.19%	**RSD = 0.12%**	**RSD = 0.23%**

TABLE 2 Reproducibility in area.

Merck monolith	Chromaspeed monolith	Chromaspeed HP monolith @41%
120 851	131 117	138 100
121 004	130 732	136 752
121 130	130 697	137 902
119 558	131 103	139 469
118 308	130 623	139 062
117 514	130 034	138 304
116 480	128 836	137 674
117 077	128 431	138 045
123 350	128 287	137 535
Mean = 119 474	Mean = 129 984	Mean = 138 094 (Gain = 16%)
RSD = 1.92%	RSD = 0.89%	RSD = 0.58%

TABLE 3 Reproducibility in height.

Merck monolith	Chromaspeed monolith	Chromaspeed HP monolith @41%
356.36	404.7	450.43
356.26	399.57	449.36
358.67	398.83	451.63
353.64	400.29	456.18
350.01	399.54	453.6
347.43	398.77	451.8
346.68	394.13	450.29
347.93	392.71	450.26
364.54	392.63	449.38
Mean = 353.50	**Mean** = 397.91	**Mean** = 451.44 **(Gain = 28%)**
RSD = 1.71%	**RSD = 1.01%**	**RSD = 0.49%**

TABLE 4 Reproducibility in number.

Merck monolith	Chromaspeed monolith	Chromaspeed HP monolith @41%
3451	4142	5440
3449	4063	5543
3517	4076	5486
3505	4100	5536
3485	4135	5506
3477	4186	5497
3548	4111	5530
3566	4125	5492
3522	4089	5508
Mean = 3502	**Mean** = 4114	**Mean** = 5504 **(Gain = 57%)**
RSD = 1.16%	**RSD = 0.92%**	**RSD = 0.57%**

3.1.6 | Complex real samples: gradient elution

As a final assessment of the performance of the monolithic columns fitted with the WBOC fitting and the dual port insert, the analysis of a complex sample was tested in gradient elution mode. Separations of green tea samples are illustrated in Figure 6A,B. These separations were undertaken on the Merck monolith—original and the Chromaspeed HP 50 monolith operating with a 40% segmentation ratio with a void volume of ~0.37 ml. Similar to the performance gain in isocratic elution mode, the performance of the Chromaspeed HP 50 monolith was also superior to that of the Merck monolith. This is highlighted in the expanded region of the chromatogram shown in Figure 6B, which illustrates the separation between the two most abundant components between 4.75 and 5.5 min. There is substantially less tailing and as a consequence greater sensitivity in the analysis conducted using the Chromaspeed HP 50 column. The gain in sensitivity was approximately 35% for the component eluting at 5.2 min.

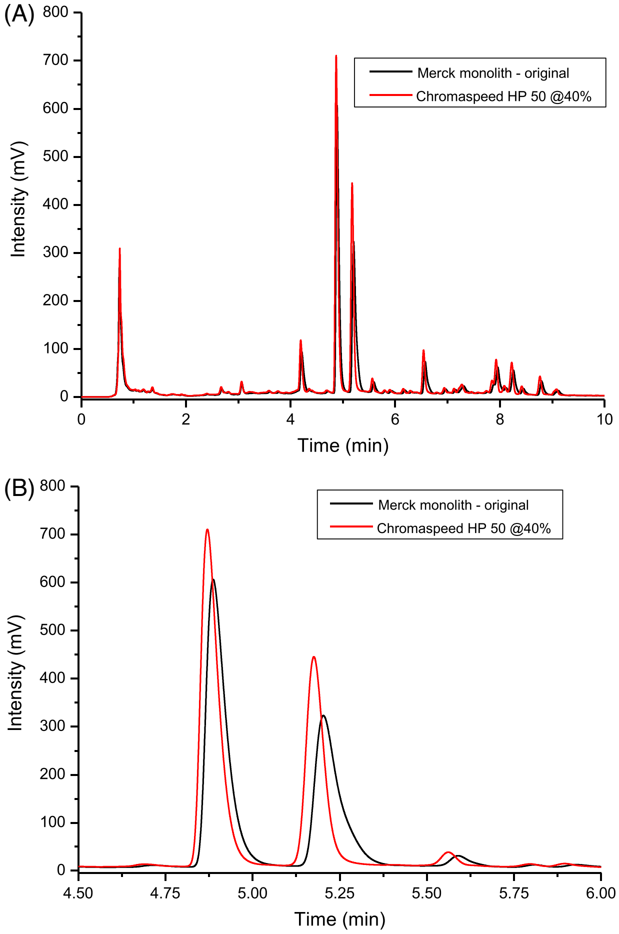

FIGURE 6 (A) Separation of green tea on the monolithic columns using gradient elution. The black trace is the original Merck monolith, and the red trace is the Chromaspeed HP 50. (B) The expanded region of the chromatogram is shown in Figure (A).

3.2 | Merck monolith, and Chromaspeed HP monolith (100 × 4.6 mm formats)

3.2.1 | Efficiency

The gains in performance for the short 50 mm version of the monolith were impressive when the Chromaspeed dual port insert was fitted to the column. Hence, the study was extended to evaluate the efficiency of 100 mm format beds. The plots in Figure 2B illustrate the efficiency of each 100 mm long monolithic column as a function of the mobile phase flow rate. While the Chromaspeed HP 50 monolith fitted with the 2-port radial flow stream splitter showed gains of up to 51% compared to the original Merck monolith, with respect to the number of theoretical plates, the gains obtained for the longer 100 mm column (Chromaspeed HP 100 monolith) were even greater; up to 73% recorded at the highest flow rate tested. Furthermore, the gain in efficiency increased as the flow rate increased. Thus, the Chromaspeed HP 100 monolith shows increasing benefits as the flow rate increases.

3.2.2 | Asymmetry

As was the case in the 50 mm bed, the peak symmetry was greatly improved when the dual port radial flow stream splitter was fitted to the Merck monolith. Figure 3B details the asymmetry differences between the original Merck monolith and the Chromaspeed HP 100 monolith fitted with the 2-port radial flow stream splitter, and operating with a 36% segmentation ratio. The Merck column had an average asymmetry value of 1.15 whereas the Chromaspeed HP 100 monolith had an average asymmetry value of 1.00.

3.2.3 | Pressure

As was the case with the 50 mm bed, the pressure drop across the 100 mm bed was also less when the dual port radial flow stream splitter was utilized. The data in Figure 4B details the relationship between pressure and flow rate for the original Merck monolith and the Chromaspeed HP 100 monolith fitted with the 2-port radial flow stream splitter. The Chromaspeed HP 100 monolith operated with an average reduction in pressure of 27%. Given the reduction in pressure, the length of the Chromaspeed HP 100 monolith could be increased by 10%. At say the highest flow rate tested here – 3.0 ml/min, this would yield around 12 300 plates. Subsequently, considering the gain in efficiency, the Chromaspeed HP 100 monolith operating at its highest flow rate would be approximately 4%–5% more efficient than the Merck monolith (when operating at its optimum flow rate of 0.7 ml/min). This translates to an analysis that would be approximately 4-times faster using the Chromaspeed HP 100 monolith.

3.2.4 | Sensitivity

Figure 5B shows the relationship between sensitivity and flow rate on each of the monoliths. The Chromaspeed monolith fitted with the 2-port radial flow stream splitter showed an increase in sensitivity that was as high as 45%. The average gain was 32%. Indeed, the sensitivity on the Chromaspeed HP 100 monolith at the highest flow rate was higher than on the Merck monolith operating at its most sensitive flow rate – 0.7 ml/min.

4 | CONCLUDING REMARKS

Radial heterogeneity of the monolithic column is an important contributor to the limitation in the performance of the efficiency of the column. But this limitation can be overcome by removing from the flow stream the portion of the band that relates to the tailing section of the peak. If this section of the band does not pass through the detector, a very efficient separation is observed. In order to enable the wall region of the flow, which is the region responsible for the severe tailing effect, a radial flow stream splitter can be added to the column. The WBOF provides a simple solution to be able to modify the column, and then apply a variety of WBOC inserts to the column. The case in point discussed here was the addition of a dual port radial flow stream splitting insert. The end result was that the efficiency of the monolithic column was increased by as much as 75%, with a reduction in pressure of around 27%. Because only the uniform radial central region of the eluting sample band enters the detector, the sensitivity is also increased, effectively by as much as 45%.

Finally, it is important to reflect on the development of HPLC and UHPLC columns over the decades. In unidimensional HPLC/UHPLC, the limit in performance is restricted by the available pressure in the system. Increasing the permeability of the column bed, that is, utilizing core-shell technology or monoliths has helped reduce pressure, but then to further gain separation power—peak capacity, there is the need to either increase the column length or decrease the particle diameter—or both, or decrease the domain size of the interstitial space. The result is a subsequent increase in pressure, with peak capacity thus being limited by pressure and/or time. In contrast, using the WBOF to convert a conventional column to a WBOC with radial flow stream splitting capabilities, has enabled an increase in separation power, that is, an increase in peak capacity, while at the same time returning a decrease in pressure. This is the only technology available that affords such an advantage: Increase in efficiency, decrease in pressure, irrespective of the nature of the bed.

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CONFLICT OF INTEREST STATEMENT

Shalliker is a Professor of Chemistry at Western Sydney University and Co-founder of Chromaspeed Pty Ltd.

DATA AVAILABILITY STATEMENT

Data are available on request from the author.

ORCID

Ross Andrew Shalliker https://orcid.org/0000-0003-1443-2632

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