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Using active flow technology columns for high through-put and efficient analyses: The drive towards ultra-high through-put high-performance liquid chromatography with mass spectral detection

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ABSTRACT

The performance of active flow technology chromatography columns in parallel segmented flow mode packed with 5 μm Hypersil GOLD particles was compared to conventional UHPLC columns packed with 1.9 μm Hypersil GOLD particles. While the conventional UHPLC columns produced more theoretical plates at the optimum flow rate, when separations were performed at maximum through-put the larger particle size AFT column out-performed the UHPLC column. When both the AFT column and the UHPLC column were operated such that they yielded the same number of theoretical plates per separation, the separation on the AFT column was twice as fast as that on the UHPLC column, with the same level of sensitivity and at just 70% of the back pressure. Furthermore, as the flow velocity further increased the performance gain on the AFT column compared to the UHPLC column improved. An additional advantage of the AFT column was that the flow stream at the exit of the column was split in the radial cross section of the peak profile. This enables the AFT column to be coupled to a flow limiting detector, such as a mass spectrometer. When operated under high through-put conditions separations as fast as six seconds, using mobile phase flow rates in the order of 5–6 mL/min have been recorded.

1. Introduction

An ever increasing priority in analytical chemistry and the fields of science that it supports is improving the work-place productivity, part of which necessitates decreasing the time required to undertake an assay. In any assay that involves liquid chromatographic separation there are at least two key factors that limit through-put, which are, the availability of pressure to drive solvent through the chromatographic bed, and the lower minimal column format (particle size and column dimensions) that can provide for the required separation power, with consideration to the decay in separation efficiency due to extra column effects. These concepts have been discussed in detail by numerous researchers, notably by Guiochon in his land mark paper that described the limits of unidimensional HPLC [1], and more recently Gritti and Guiochon [2] and De Vos et al. [3]. While Ref. [1] highlighted that the analyst can achieve almost any degree of separation provided they are able to pay for the outcome with the currency of time, i.e., employ long efficient columns operating at low flow rates potentially yielding plate numbers around the one million mark per separation [4,5]. That being said, most analytical requirements do not necessitate one-million plates, rather, there are a great many separations that are in contrast quite simple, and high efficiency at high through-put is the more pressing challenge. This was the primary objective in the work by De Vos et al. [3], which demonstrated the importance of minimising the extra column dead volume and operating the column at very high back pressure.

A common factor in all high through-put assays is that the column must have a small void volume, and ideally the separation performance be highly efficient. Hence the system dead volume must also be minimised so as to not inadvertently loose separation power. High efficiency may be obtained in a number of ways, either, the use of efficient monoliths having a high bed permeability so as to avoid high pressure environments, or the columns may be packed with core shell particles, which provide higher efficiency than a comparable sized fully porous particles, and hence they also function at lower back pressures than fully porous particles. Fully porous particle packed columns nevertheless are probably

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the most widely used column technology in UHPLC environments orientated towards high through-put applications, since they are more robust than core shell particles and can be utilised at higher back-pressures, and hence potentially they can be employed in very high through-put applications beyond the capacity of the core shell particle [3].

When considering the demands of a high through put assay, the detection method must also be part of the equation. As MS is now emerging as the go-to detector because it offers in itself a degree of separation power, high through-put assays often incorporate MS detection. Yet an important limitation with respect to the MS detector is the ability of the MS to process the volumetric flow of mobile phase, especially since factors that have led to more efficient chromatographic separations, i.e. core shell particles, monoliths and small sub-2 μm particles have resulted in the use of higher flow rates, and this has resulted in the scale down in the geometrical format of the HPLC column, since post column flow stream splitting processes are an inconvenient method to hyphenate HPLC or UHPLC with the MS detector. Hence this is an additional driving factor to decrease the column internal diameter; the 2.1 mm i.d. format being preferred, but sometimes 1 mm i.d. columns are employed. When 2.1 mm i.d. columns are utilised in preference to a 4.6 mm i.d. column, for example, the solvent load to the MS is decreased by approximately 5-fold, but achieving the same linear velocity through the column. While this appears to be a suitable solution to solvent removal prior to the mass spectrometer the price paid for compatibility is a compromise in the performance of the chromatographic column [2], primarily as a result of wall effects [6] and the management of extra-column dead volume contributions [2,3], which become extremely problematic when scaling down the column to enable high through-put [3].

It is well known that the wall effect is an important contributor to band broadening in the chromatographic migration process [6], and this has been discussed by numerous researchers in the field of column technology, and need not be discussed here in further detail, suffice to say, the historical accounts of the understanding of the heterogeneity in the chromatographic bed can be found in references cited in the recent review by Shalliker and Ritchie [7]. Importantly, however, Gritti and Guiochon [2] showed that column internal diameter was an extremely important factor as the loss in optimal column performance in a 2.1 mm i.d. column format compared to the 4.6 mm i.d. column format (based on the reduced plate height, h) for superficially porous particles, with diameters between 1.7 and 2.7 μm, was between 13 and 42% depending on particle size, column length and manufacturer. This loss was attributed largely to the long range eddy dispersion term, a factor dependent in part, on the homogeneity of the radial packing density. Although this length scale of the eddy dispersion term is generally small with respect to the radius of the column, it is between 10 and 15% of the radius on narrow diameter columns (2.1 mm), while around 2% on 4.6 mm i.d. columns [2]. Hence, a reduction in the column radius results in a relative increase in the contribution made by the wall effect [2]. Another factor that significantly affects the performance of the 2.1 mm i.d. format is related to the extra-column dead volume contributions, which rapidly decreases the efficiency as the peak volume decreases. Although this is not an insurmountable limitation, it nevertheless requires substantial care to minimise its effects, and it is often overlooked, and places practical limitations to the employment of columns with internal diameters less than 2.1 mm, unless equipment specifically designed for the task is employed. De Vos et al. [3] for example, demonstrated that the peak variance for columns 150 mm in length, packed with 1.5 μm particles operating at 1500 bar could be decreased by around 15% by using 75 μm connective tubing, but at the cost of additional back pressure. Further down-scaling of the column geometry in order to facilitate greater separation speed therefore would place further requirement to the minimisation of the extra-column dead volume effect, ultimately this places a limit on the concept of efficiency and through-put.

For the most part, the operators of mass spectrometers that require some form of separation ignore the loss in the separation performance they observe when using narrow bore columns because of the very big advantage associated with the reduced solvent load that is presented to the MS detector. This is especially so when 4.6 mm i.d. columns are employed since, these analytical scale columns cannot be utilised in a high through-put scenario that approaches the speed of the 2.1 mm format column.

Recently, a new suite of column technologies has been developed, which is referred to as active flow technology (AFT), as reviewed in Refs. [7] and [8] and the concept of a 'virtual' column being demonstrated in Ref. [9]. The primary advantage of these columns is that they unite together the benefits of both large and smaller internal diameter columns, and they are particularly suited to analyses that involve high speed chromatographic separation coupled to MS detection, and it is this column technology that is the focus of this communication. While the details of how these AFT columns operate have been demonstrated in Refs. [7–9] (and references cited therein), it is nevertheless important to point out three major advantages of this column technology; (1) they enable an increase in detection sensitivity compared to conventional columns, (2) they provide higher separation efficiency than conventional columns, and (3) they reduce the volume load of solvent to the detection source since an 'on-column' flow stream splitting process is utilised – a major benefit when the MS is employed. An obvious question in regards to the on-column flow stream splitting process is, how does this differ from a post column flow stream split? This phenomenon is easiest to explain by realising the principle of the AFT 'on-column' split is a radial flow stream splitting process, which effectively samples the most efficient portion of the mobile phase flow stream and that portion is sampled to the detector. That is, the centre portion of the peak only or effectively the peak apex. In a post column flow stream spitting process, the entire peak is sampled along the axial direction and this includes the dilute diffuse tailing section of the peak. To aid in this description Fig. 1 illustrates the difference between the on-column radial sampling process (AFT), versus the post column flow stream split that samples in the axial direction. The former – 'on-column' AFT split samples the central zone of the bed, whereas the post column flow stream split samples a portion of the flow stream effectively across the entire radial cross section of the column. Hence post column flow stream splitting will always result in a loss in sensitivity as an axially homogeneous portion of the mobile phase is sampled. Whereas the AFT splitting process cuts out the 'heart' of the peak and a gain in signal response is observed. The only time whereby the AFT splitting process and the post column splitting process would yield the same outcome would be if the chromatography column were absolutely perfect, and at present this is not the case. Perfect also implies that the sample is loaded in equal concentration across the radial cross section at the column inlet, and unfortunately frits are not perfect in that regard either [10].

In the context of the present communication, we discuss here the benefits of active flow technology columns in high performance liquid chromatography and we have compared AFT columns packed with 5 μm particles to the modern UHPLC column packed with 1.9 μm particles. Ultimately we hope that the reader will understand that the benefits of the AFT column are especially important in high through-put applications that utilise MS detectors. The AFT columns utilised in this study have been restricted to the parallel segmented flow (PSF) mode, packed only with 5 μm particles, and