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Serial versus parallel columns using isocratic elution: A comparison of multi-column approaches in mono-dimensional liquid chromatography

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ABSTRACT

When a new separation problem is faced with high-performance liquid chromatography (HPLC), the analysis is addressed conventionally with a single column, trying to find out a single experimental condition aimed to resolve all compounds. However, in practice, the system selectivity may be insufficient to achieve full resolution. When a separation fails, the usual practice consists of introducing drastic changes in the chromatographic system (e.g. use of another column, solvent or pH). An alternative solution is taking benefit of the combined separation capability of two or more columns, which can be attained in multiple ways, such as diverse modalities of two-dimensional HPLC, or mono-dimensional HPLC with serial or parallel columns. In this work, the separation performance offered by the serial coupling of columns of different nature and length, operated at varying mobile phase composition in isocratic elution, is compared with the results offered by parallel columns. The resolution capability of both approaches is characterised through the limiting peak purities. It is demonstrated that serial columns of different lengths perform as new columns that increase enormously the probabilities of success. The potential of the approach is illustrated through the separation of 15 sulphonamides. In spite of the poor individual performance of the four selected columns (phenyl, cyano and two C18 columns, with nearly null resolution for the cyano column), it was found that the serial coupling of the phenyl and cyano columns of appropriate lengths succeeded in the full resolution of the 15 compounds in 20–25 min, and the serial coupling of the two C18 columns yielded acceptable resolution in less than 20 min.

1. Introduction

High-performance liquid chromatography (HPLC) is currently the most widely used analytical separation technique, due to its applicability, robustness and sensitivity, responding to multiple problems in environmental, pharmaceutical, clinical and food control, as well as in genomics, proteomics, lipidomics and metabolomics. The advances in this field are the result of fundamental research in several directions, which have gradually addressed increasingly complex problems [1–3]. However, HPLC with a single column still presents challenges in terms of resolution, analysis time and baseline drift in gradient elution, due to the limited chemistry of conventional stationary phases. The combination of retention mechanisms by coupling two or more columns in mono- [4–12], or multi-dimensional [13–16] configurations, is currently the best solution to solve this drawback, which has opened enormously the range of resolutions. It is possible to achieve almost continuous transitions between the selectivity of two or more stationary phases by combining columns. However, being the combination of different columns in multi-column HPLC, a highly powerful tool to resolve complex samples, its success requires the assistance of an interpretive optimisation of the separation conditions [7,9,12,17].

We will refer here to the combination of columns in mono-dimensional HPLC. The idea of serially coupling stationary phases (Fig. 1a) to analyse complex samples appeared early in the development of chromatography [4,18,19]. However, this approach has been only feasible when the connection between columns has been improved to avoid the undesirable presence of void volumes and changes in the packing material close to the junctions. We have recently proposed a reliable and flexible system, to connect conventional columns from different manufacturers through zero dead volume fingertight couplers, screwed directly to the columns [8].

Also, along the last decade, we have reported a different separation approach, based on the complementarity concept, which has succeeded in the resolution of highly complex samples [20]. In this approach, a separation condition focuses on the resolution of some compounds in the sample, while the other compounds remain

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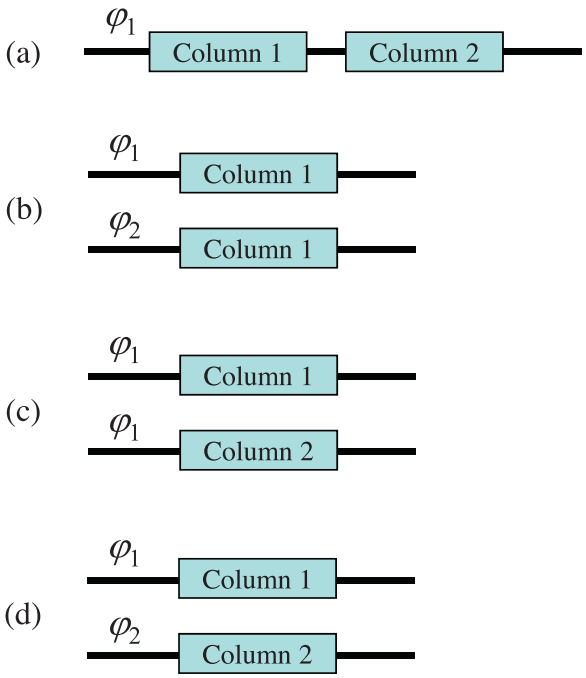

Fig. 1. Column/solvent configurations studied in this work: (a) serially coupled columns, (b) two identical parallel columns with independent mobile phases, (c) two parallel columns of different nature with a common mobile phase and (d) two parallel columns of different nature with independent mobile phases.

unresolved, but are optimally resolved in a second (or subsequent) condition(s). Consequently, the separation space increases, and so the chances of success. The selection is done in such a way that when the results of the optimal complementary separation conditions are considered altogether, all compounds are maximally resolved. In previous work, we have checked the capability of complementary mobile phases (Fig. 1b), containing the same or two different organic solvents, to improve the resolution of a complex sample. In this work, we extend this development to the optimisation of two different parallel columns, sharing the same (Fig. 1c) or different (Fig. 1d) mobile phase compositions.

The capability of both mono-dimensional multi-column approaches (serial and parallel columns) to resolve a complex sample is compared, using pairs of combined columns, and considering both column nature and lengths together with the mobile phase composition. In order to find out the maximal performance of the systems in both approaches, a rigorous optimisation of the experimental factors based on the modelling of the retention and peak shape was carried out. It is shown how the combination of two serially coupled columns, both offering very poor resolution when used as single columns, can yield outstanding full resolution, highly extending the separation power of the parallel columns.

2. Experimental

For evaluating the performance of the different approaches, a set of 15 sulphonamides studied in previous work [8,9,12] was considered: (1) sulphacetamide, (2) sulphachloropyridazine, (3) sulphadiazine, (4) sulphadimethoxine, (5) sulphaguanidine, (6) sulphamerazine, (7) sulphamethazine, (8) sulphamethizole, (9) sulphamethoxazole, (10) sulphamonomethoxine, (11) sulphanilamide, (12) sulphapyridine, (13) sulphaquinoxaline, (14) sulphathiazole and (15) sulphisoxazole. The chromatographic behaviour of these compounds was examined with columns of different lengths containing four different stationary phases, supplied by Advanced Chromatography Technologies Ltd. (ACE, Aberdeen, Scotland, UK): phenyl, cyano, C18-HL and C18-AQ. Isocratic runs were carried out with acetonitrile/water mobile phases in the 10–20% (v/v) range, buffered at pH 3.5. The chromatographic system consisted of an isocratic pump operated at a flow rate of 1 mL/min, autosampler, thermostated column compartment set at 25 °C, and variable wavelength UV–vis detector set at 254 nm, all from Agilent (Waldbronn, Germany). Other details can be found elsewhere [8,9].

The retention and peak shape behaviours needed to simulate the expected chromatograms under any configuration (single, serial and parallel columns) were modelled assisted by MATLAB 2014b (The MathWorks Inc., Natick, MA) routines. This information was used to evaluate the limiting resolutions and the expected optimal separations.

3. Results and discussion

3.1. Columns and optimisation of the resolution

The below discussion intends to offer some light on the behaviour found with serial columns, where only the nature and length of the coupled columns or these together with the mobile phase composition are optimised. For this purpose, several configurations of serial and parallel columns were compared. In order to facilitate the comparisons, the studies were carried out using isocratic elution with mobile phases containing only one factor (the organic solvent content) and attending exclusively to the resolution (except in Section 3.5, where the analysis time was also considered). Four stationary phases, which showed very poor performance when used as single columns, were selected deliberately to demonstrate the capability of the multi-column approaches, and highlight their differences: phenyl, cyano, and two C18 columns (C18-HL and C18-AQ, the former with a high surface area and carbon load, and the latter with an integrated polar functionality). The retention and peak profile behaviours (required to predict the resolution in the single and combined columns of any length) were modelled with single columns of the following lengths (arbitrarily selected): 5 cm phenyl, 11 cm cyano, 3 cm C18-HL and 5 cm C18-AQ.

In serially coupled columns, where the mobile phase elutes the solutes sequentially (Fig. 1a), the retention is the sum of the contributions of each column, being independent of their coupling order in isocratic elution [8,9]. Meanwhile, in the complementarity approaches, where two mobile phases are optimised for the same column (Fig. 1b), or two different parallel columns are optimised using the same or different mobile phases (Fig. 1c and d, respectively), the retention time for each solute is simply the retention time in the column (or mobile phase) where it is best resolved. The effect of the mobile phase composition on retention was considered using a quadratic dependence of the retention factor with the organic solvent content. The peak profiles were predicted as explained in Ref. [9].

The optimisation of the resolution along this work was made based on an exhaustive examination of synthetic designs of separation conditions (e.g. column nature and length, and mobile phase composition). For these conditions, the corresponding chromatograms were predicted and the separation quality evaluated. Finally, the separation conditions offering the maximal separation quality were selected. The chromatographic resolution was measured using the peak purity concept, which is the peak area fraction free of overlap for a given compound, considering all other compounds as interferences [17]. For a fully overlapped peak and a peak resolved up to the baseline, the elementary peak purity for a solute j is $p_j = 0$ and 1, respectively. The global resolution (P) was calculated as the product of the elementary peak purities; therefore, the range of variation is also $0 \leq P \leq 1$. Limiting peak purities