Optimisation of gradient elution with serially-coupled columns. Part I: Single linear gradients☆

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ABSTRACT

A mixture of compounds often cannot be resolved with a single chromatographic column, but the analysis can be successful using columns of different nature, serially combined through zero-dead volume junctions. In previous work (JCA 1281 (2013) 94), we developed an isocratic approach that optimised simultaneously the mobile phase composition, stationary phase nature and column length. In this work, we take the challenge of implementing optimal linear gradients for serial columns to decrease the analysis time for compounds covering a wide polarity range. For this purpose, five ACE columns of different selectivity (three C18 columns of different characteristics, a cyano and a phenyl column) were combined, aimed to resolve a mixture of 15 sulphonamides using acetonitrile–water gradients. A gradient predictive system, based on numerical integration, was built to simulate chromatograms under linear gradient profiles. Two approaches were compared: the optimisation of the combination of columns pre-selecting the gradient profile, developed by De Beer et al. (Anal. Chem. 82 (2010) 1733), and the optimisation of the gradient program after pre-selecting the column combination using isocratic elution, developed for this work. Several refinements concerning the gradient delays along the solute migration and peak half-width modelling were included to improve the realism of the predictions. Pareto plots (expressed as analysis time versus predicted global resolution) assisted in the selection of the best separation conditions. The massive computation time in the gradient optimisation, once the column combination was optimised, was reduced to ca. 3 min by using genetic algorithms.

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1. Introduction

Recent advances in column technology have allowed the development of successful functional hybrid column systems, built up by serial coupling of columns containing different stationary phases. The combination of separation mechanisms through columns connected in series is a simple option to improve the resolution, since the overall selectivity will be usually better compared to any single column (i.e. single stationary phase). Even poor separations with a variety of different single columns may succeed when these are combined. Basic requirements for the success of the approach are the proper connection of the columns, with zero dead volume (ZDV) junctions, and a robust and practical system for binding the columns.

In 2005, under a patent, Bischoff Chromatography commercialised a new system of serially-coupled columns, under the trademark "PopLink columns" [1], giving new life to the idea of tuning the selectivity by combining different stationary phases [2,3]. The "PopLink columns" kit consists of PEEK holders that maintain tightly pressed stainless steel cartridges of several lengths differing in one cm, filled with stationary phases of different nature. Recently, we demonstrated the possibility of using with good results conventional short columns linked with ZDV fingertight column couplers, screwed directly to the columns without the need of external holders [4]. This approach allows coupling any already existing or newly branded conventional column available in the market. Chen et al. have also proposed assembling conventional short columns with standard stainless steel tubing of 105 mm length and 0.12 mm i.d. [5]. These authors reported poorer predictions with this system compared to the "PopLink columns", and suggested shorter tubing or one-piece couplers to improve the predictions by decreasing the void volume in the connections.

The "PopLink columns" approach was developed fixing the mobile phase composition and optimising the combination of column segments (nature and length), based on the PRISMA approach

[6]. We have also developed, on a different basis, reliable algorithms to optimise serially-coupled columns [4,7]. We first improved the predictions of chromatographic resolution by including an algorithm to predict the peak profiles (width and asymmetry), with results virtually matching the experimental separations [4]. In a second contribution, we developed an approach that optimised simultaneously the mobile phase composition and the stationary phase nature and length [7], which is the most powerful approach to finely tune the selectivity with serially-coupled columns in isocratic elution.

A drawback of the optimisation approach run at fixed mobile phase composition is the requirement of column segments of multiple lengths to achieve an extensive range of combinations of stationary phases, from which the best is selected. Interestingly, the inclusion of the mobile phase composition in the optimisation (which can be finely tuned) reduces the number of column lengths needed, without loss of performance. This has evident consequences in the practical implementation of the serially-coupled columns approach [7].

The initial development of the approach associated to isocratic elution [3,4,6,7] is not surprising, attending to the attractive results that perform closer to two-dimensional liquid chromatography in terms of resolution, and to gradient elution in terms of analysis time, using a simpler and more accessible instrumentation to most laboratories. The optimisation approach implemented with isocratic elution is also relatively simple, especially if it is only based on the prediction of retention. However, mixtures of compounds differing significantly in their polarity require still a gradual increase in the elution strength, in order to obtain practical analysis times and an adequate distribution of peaks along the separation space. This is usually achieved through the application of gradients of organic solvent. Therefore, in spite of the complexity of implementing gradients with serially-coupled columns, several authors have been interested in its development.

Initially, a multi-step isocratic methodology was proposed for the coupled columns [8,9]. The approach reported by De Beer et al. [9] was inspired in the "PopLink columns" optimisation [6], and can be summarised as follows: (i) after analysing the solute mixture using a linear gradient with a conventional single column, solutes were grouped according to their polarity; (ii) independent optimisations at fixed mobile phase composition (isocratic elution), selected from the linear gradient, were then developed to obtain the best column combination for each group of solutes; (iii) among these column combinations, a common combination for all groups was selected; (iv) finally, a multi-step isocratic gradient was built using the selected common column combination and the particular mobile phase composition for each group of solutes.

In a further development, also based on the "PopLink columns" principle, a gradient function (with single or multiple linear segments) was pre-selected, the optimisation being restricted to the selection of the best column combination (nature and length) [10–12]. In order to improve the reliability of the predictions, a linear relationship between the peak standard deviation and the retention time (considering Gaussian chromatographic peaks) was further considered to improve the prediction of peak profiles [5].

In this work, a further step in the systematic method development for serially-coupled columns is proposed: the optimisation of single linear gradient programs. In Part II of this work, multi-linear gradients including isocratic steps will be addressed. Gradient simulations are enhanced considering the formation of skewed peak profiles along the elution through the serially-coupled columns. Several refinements concerning the gradient delays along the solute migration are also included in the approach, which largely improve the realism of the predictions.

2. Theory

In order to optimise rationally serially-coupled columns using gradient elution, the first step is modelling the retention behaviour for each compound in the sample as a function of the solvent content (φ, usually expressed as percentage v/v). It is also convenient to have a system to forecast peak profiles in order to enhance the reliability of the predictions. Predictions of retention time in isocratic or gradient elution, for a given solute in a column assembly, require combining the predictions attained for each column segment.

2.1. Isocratic elution for serially-coupled columns

When several columns are serially coupled, the total retention time for a solute j ($t_{\mathrm{Rc},j}$) is a linear combination of the extra-column contribution (t_{ext}) and the retention times for each column i ($t_{\mathrm{R},ij}$) in the assembly [4,6]:

$$t_{\mathrm{Rc},j} = t_{\mathrm{ext}} + \sum_{i=1}^{nc} t_{\mathrm{R},ij} = t_{\mathrm{ext}} + \sum_{i=1}^{nc} t_{0\mathrm{int},i}(1 + k_{ij}) = t_0 + \sum_{i=1}^{nc} t_{0\mathrm{int},i} k_{ij} \tag{1}$$

where nc is the total number of combined columns, $t_{0\mathrm{int},i}$ the intra-column dead time for each coupled column i (i.e. the column segment dead time without considering the extra-column contribution), t_0 the total dead time for the whole assembly, and k_{ij} the retention factor for solute j in the column segment i, which is invariant with the column length and should be calculated according to:

$$k_{ij} = \frac{t_{\mathrm{R},ij} - t_{0\exp,i}}{t_{0\exp,i} - t_{\mathrm{ext}}} \tag{2}$$

$t_{0\exp,i}$ being the experimental dead time for column i. The final expression in Eq. (1) including the $t_{0\mathrm{int},i}k_{ij}$ terms has the advantage of being directly related to the solvent content for each column segment. In this work, we have used the quadratic model to describe the retention:

$$\log k_{ij}(\varphi) = c_{0,ij} + c_{1,ij}\varphi + c_{2,ij}\varphi^2 \tag{3}$$

where $c_{0,ij}$, $c_{1,ij}$ and $c_{2,ij}$ are coefficients with particular values for each column i and solute j. The individual predictions are initially performed at the length at which the models were obtained (i.e. a reference column with length $l_{r,i}$), and should be scaled up (or down) to the length of the target segment i (l_i) through the column dead time ($t_{0\exp,i}$).

The dead time has been observed to depend not only on the column length, but also slightly on the mobile phase composition, owing to changes in the solvent viscosity and the residual interactions of the dead time marker with the stationary phase, among other reasons. In this work, the following equation is used to estimate the dead time for a column of length l_i:

$$t_{0\exp,i}(\varphi, l_i) = t_{\mathrm{ext}} + t_{0\mathrm{int},i}(\varphi, l_i) = t_{\mathrm{ext}} + (d_{0,i} + d_{1,i}\varphi + d_{2,i}\varphi^2)\frac{l_i}{l_{r,i}} \tag{4}$$

where $d_{0,i}$, $d_{1,i}$ and $d_{2,i}$ are the fitting parameters for describing the variations in the intra-column dead time with the mobile phase composition in a reference column of the same type (and length $l_{r,i}$). Other details can be found in Ref. [7].

The peak profiles were predicted considering the relationships between the left (A) and right (B) half-widths and the retention times. For column i and solute j [13,14]:

$$A_{ij} = a_{0i} + a_{1i}t_{\mathrm{R},ij} + a_{2i}t_{\mathrm{R},ij}^2 \tag{5}$$

$$B_{ij} = b_{0i} + b_{1i}t_{\mathrm{R},ij} + b_{2i}t_{\mathrm{R},ij}^2 \tag{6}$$