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Approaches to comprehensive multidimensional liquid chromatography systems

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ABSTRACT

This work compares the performance of the three different schemes implementing two-dimensional liquid chromatography (2D-LC) in terms of the peak capacity that they can generate and of the time that they need to complete a two-dimensional analysis. We discuss in detail how time is spent in these two-dimensional liquid chromatography × liquid chromatography (LC × LC) schemes and how to compare them. Keeping constant the characteristics of the first-dimension separation, we systematically varied those of the second-dimension separation and of its coupling to the first-dimension. In the process, five systems were created, based on the principles of the three known implementations of comprehensive 2D-LC. This work demonstrates an original method for the selection of the best comprehensive 2D-LC approach, depending on the desired peak capacity and on time constraints. The decision to use a 2D-LC method arises from the need to achieve a given resolution (i.e., a target peak capacity) within as short a time as possible or to reach the highest possible resolution in a given analysis time. Using the most appropriate schemes, we suggest how it is realistically possible to generate peak capacities ranging from 266 in just over 20 min or about 2800 in 2.3 h. When the time available for a two-dimensional separation is very short and the desired peak capacity cannot be achieved in 1D-LC, an on-line 2D-LC approach is unquestionably best. However, if a longer analysis time is acceptable, a 10-fold increase in the peak capacity can be obtained at the cost of a mere 7-fold increase in total analysis time.

1. Introduction

Comprehensive, two-dimensional liquid chromatography implemented by coupling two separations in time presents three available schemes: on-line; stop-and-go; and off-line [1]. The on-line coupling consists of the second-dimension being carried out in real time with the first-dimension. This system requires that the second analysis be completed during the time needed to collect the fraction, transfer and analyze it, and restore the column to the initial conditions of the analysis. This constraints the second-dimension separation to be completed in what is typically a very short amount of time, resulting in a limited separation power. However, this scheme yields a peak capacity of the two-dimensional separation that is an order of magnitude larger than that of the unidimensional separation performed with the first column alone in the same time. Thus, most separations with analysis times less than 2-h and peak capacities between 500 and 1000 have been obtained with this scheme [2–8].

The stop-and-go scheme involves stopping or pausing elution from the first-dimension column while a fraction is transferred to and analyzed on the second-dimension column, then resuming the elution in the first-dimension. This somewhat alleviates the time constraints of the second-dimension, but results in excessively long peak parking times, which decreases the efficiency of the first-dimension separation [9–11]. Recently, Bedani et al. investigated in detail the use of stop-and-go elution out of the first column when they coupled size-exclusion chromatography (SEC) and reverse-phase liquid chromatography (RPLC) [12]. However, this technique was previously used by Blahová et al. [13] and notably by Yates and coworkers in the form of MudPIT to generate high peak capacities (*ca.* 2500) with very long analysis times (*ca.* 28 h) [14–18].

The last scheme is the off-line system in which fractions eluted from the first-dimension column are collected and stored indefinitely, until their injection onto the second-dimension column. There is no time constraint for either dimension and therefore, virtually no high limit to the separation power available. Separations of this type done in our lab have achieved peak capacities of *ca.* 7000 at a cost of a long analysis time (27 h) [19].

Each approach has distinct features; particular considerations can make the use of one of them more advantageous than that of the other two for some specific applications. To compare this set of systems, analysts value the peak capacity generated by each and the amount of time necessary to generate that peak capacity. Also, knowing the characteristics of the separation implemented in each dimension (i.e., the peak capacities generated by the first- and second-dimension columns, the additional time required for

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ancillary tasks, like the injection and the re-equilibration, the overall peak capacity desired, the time constraint, etc.) a separation approach can be recommended via simulation. The peak capacity achieved in multidimensional separations compared to the time that is needed scales quite differently in unidimensional and two-dimensional separations. In unidimensional separations, long, slow gradient elutions are used most often and peak capacity increases but slowly toward a limit with this technique. In contrast, in two-dimensional chromatography, the second-dimension separation is usually performed with a gradient elution that is very fast, with an analysis time on the order of a few seconds in the on-line scheme, rarely more than a few minutes with the off-line scheme. Then, an increase in the second-dimension gradient time from a few seconds to a few minutes increases the peak capacity considerably. This is obviously desirable when large peak capacities are needed. It also offers the ability to optimize the number of fractions transferred from the first-dimension to the second-dimension column.

The goal of this work was to compare the potential performance of the three known methods of implementation of 2D-LC. We needed to explore how time is used in two-dimensional liquid chromatography × liquid chromatography (LC × LC) systems and how a comparison of the performance of these schemes was possible. The origins, natures, and characteristics of the separation power of each of these implementations and of the time needed to implement them will be discussed. Comments on which one is most appropriate for different applications will be made. We propose a method for the selection of the best comprehensive 2D-HPLC approach, based on the peak capacity desired and time constraints.

2. Theory

2.1. Peak capacity in gradient elution

The separations of complex mixtures are usually carried out in gradient elution because the range of retention factors experienced for these mixtures is usually very large. This imposes obvious requirements on the retention mechanisms and on the stationary phases selected for the two-dimensions of a 2D-LC analysis. Several parameters influence the peak capacity, n_c, in gradient elution, including the gradient time, t_G, the relationship between the retention factor, k, of the components of interest under isocratic conditions and the mobile phase composition, the initial value of this composition and its range of variation, $\Delta\varphi$ during the gradient. In the case of linear gradient runs (i.e., when φ changes linearly with t), Gilar et al. [20] derived a simplified relationship based on the linear dependence of $\log k$ on φ, with a slope S:

$$n_c = 1 + \frac{\sqrt{N}}{4}\frac{S\Delta\varphi}{1 + S\Delta\varphi t_0/t_G} \quad (1)$$

where n_c is the peak capacity, N the column efficiency, and t_0 the hold-up time. The derivation was made with the assumption that the widths of the eluted peaks were constant. However, peak widths under gradient elution conditions in RPLC can often vary significantly [21], depending upon the molecular diffusivity, the mass transfer kinetics across a given column, and certain thermodynamic effects [22]. Eq. (1) can be simplified as:

$$n_c = 1 + \frac{at_G}{b + t_G} \quad (2)$$

where a and b are constants. Eq. (2) can be fitted by data from n_c vs. t_G experiments and a and b iterated to approximate gradient elution peak capacities in one-dimension.

2.2. Two-dimensional peak capacity

In order to compare different two-dimensional systems, we use two main parameters that we describe with a novel terminology. The parameters that are measured to qualify the column performance are the peak capacity and the cost which we must pay to achieve a certain separation power, time. Later, the time factor will be discussed further to reflect the varying ways in which it may be measured (see Section 2.4). Originally, Giddings proposed to define the peak capacity as the number of peaks with unit resolution that could be placed in a defined chromatographic time (e.g., between the two peaks corresponding to the first and the last eluted components of interest in the analysis). This conditional, sometimes also referred to as the sample peak capacity, is used as a metric for the chromatographic space used in a separation. It is given by Eq. (3).

$$n_c^* = \frac{t_{r,n} - t_{r,1}}{W_{4\sigma}} \quad (3)$$

where $t_{r,1}$ and $t_{r,n}$ are the retention times of the first and last eluted compounds respectively, and $W_{4\sigma}$ is the average (baseline) peak width. For effective gradient separations, the peak capacity, n_c, can be approximated as [20,23]:

$$n_c \approx \frac{t_G}{W_{4\sigma}} \quad (4)$$

In two-dimensional chromatography, two separate, independent separation systems are combined to achieve a higher separation power. Thus, if both separation systems are used to their potential, the overall peak capacity of the 2D-LC system considered would be equal to the product of their peak capacities,

$${}^2n_c \approx {}^1n_{c,1} \times {}^1n_{c,2} \quad (5)$$

where left-handside superscript 1 and 2 refers to the unidimensional and the two-dimensional peak capacities, respectively, while the right-handside subscripts 1 and 2, in ${}^1n_{c,1}$ and ${}^1n_{c,2}$ refer to each of the first- and second-dimension columns used in the 2D-LC system considered, respectively. However, Eq. (5) is valid only if the retention mechanisms implemented in the two columns are completely independent, so that the entire separation space is used [24,25], and if there is no loss of the first-dimension peak capacity due to back mixing during the transfer of the effluent to the second-dimension [26]. For there to be no loss of the first-dimension peak capacity during the storage and the transfer of the effluent fractions of the first-dimension column to the second-dimension one, an infinite number of fractions (each of infinitesimal volume) should be collected. Therefore, the collection of any finite number of fractions collected creates an undersampling and limits the actual peak capacity generated in the first-dimension [27]. The number of fractions collected from the first-dimension, f, can be expressed as a function of the first-dimension peak capacity and the fraction collection ratio, r as:

$$f = {}^1n_{c,1} \cdot r \quad (6)$$

When gradient elution is performed in the first-dimension, the peak width along the first-dimension remains approximately constant, as should the frequency of fraction collection. The limiting peak capacity of the first-dimension is:

$${}^1n'_{c,1} = \frac{{}^1n_{c,1}}{\beta} \quad (7)$$

where β is the "first-dimension broadening factor" [28] calculated as:

$$\beta = \sqrt{1 + 0.214\left(\frac{t_s}{\sigma_1}\right)^2} \quad (8)$$