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Solvent minimization in two-dimensional liquid chromatography

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ABSTRACT

An algorithm was developed for the minimization of consumption of organic solvent in comprehensive two-dimensional liquid chromatography (2DLC). It was shown that one can reach higher peak capacities only by using more eluent. The equilibration volume of the second dimension, however, did not affect the solvent consumption significantly. Calculations confirmed that the same target peak capacity could be achieved by consuming significantly different volume of organic modifier depending on the number of fractions analyzed in the second dimension suggesting that 2D separations can be optimized for eluent consumption. It was shown that minimization of eluent usage requires the use of small and high efficient columns in the second dimension. A simple equation was derived for the calculation of the optimal number of collected fractions from the first dimension that allowed the minimization of eluent usage, cost and environmental impact of comprehensive 2DLC separations.

1. Introduction

Determination of organic compounds in various matrices is usually carried out by the means of liquid chromatography. Chromatographic procedures are used in wide area of research, medical, industrial, food and environmental analysis [1]. As a result, very large number of chromatographic analyses performed worldwide. In some cases, solvent employed as mobile phase can be more toxic than the species being determined. As a result, side effects of chromatographic methods can generate greater environmental and human impact than the problem analyzed [2]. Considering that it is not uncommon for a single pharmaceutical company to have more than 1000 HPLC instruments and that a single liquid chromatography can potentially generate 1–1.5 L of liquid waste daily [3], the environmental impact of solvents should be taken into account during method development.

The idea of green analytical chemistry and green chromatography has been introduced at the end of the last century [4] based on the 12 well-known principles of green chemistry [5]. The concept of green analytical chromatography can be summarized in the three "R"s: Reduce, Replace, Recycle [3]. For years, the green approach alone could not gain a solid ground in the practice of HPLC. Fortunately, reduction of eluent consumption in liquid chromatographic separations has gained attention due to the worldwide acetonitrile shortage happened last decade and the increasing cost of waste disposal. Nowadays, the interest in green analytical techniques is growing. Books were published [6–8], special issues of journals and reviews were dedicated to green analytical chemistry [9–12] and green chromatography [3,13,14] recently.

In bioanalysis, high-performance liquid chromatography (HPLC) is often called to resolve highly complex samples containing hundreds or even thousands of components in a very wide range of concentrations. Despite the continuous progress in column and instrument technologies, these separations exceed the possibilities of conventional chromatographic methods [15]. Two-dimensional liquid chromatography (2DLC) is being developed to improve the separation power of chromatography [16–18]. In these systems, the separation of solutes is implemented through two columns sequentially. Small-volume aliquots from the first column are collected and analyzed by the second column. Successful implementation of 2DLC requires the separation mechanism in the two dimensions be orthogonal. Two separations are said to be orthogonal if there is no relationship between the retention data of the different components of the sample in the two separations [19]. Another parameter that significantly affects the separation power of 2DLC systems is the number of fractions collected from the first dimension. This problem was studied thoroughly by different theoretical approaches [20–22] and statistical methods [23] as well. The studies showed that undersampling the first dimension reduces the effective peak capacity, i.e. gives an effective peak capacity that is

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smaller than the value could be achieved with more fractions collected. As a conclusion, the optimal sampling time was suggested to be two standard deviations (2σ) [20,21] assuming that peak shapes are Gaussian. Accordingly two fractions per peak should be collected if the peak width is considered to be 4σ.

The main advantage of comprehensive 2DLC is the large separation power that allows the analysis of hundreds of compounds from a single sample. On the other hand, however, more complex instrumentation is required, and data handling [24] and optimization of the operating conditions can be challenging due to the many parameters one must consider simultaneously [25–28]. Since the number of fractions analyzed in the second dimension is large and the flow rate applied during the analyses is high, the eluent consumption of a 2DLC separation is rather high. Accordingly, the environmental impact and the cost of analysis of one sample are also high. The role of comprehensive two-dimensional liquid chromatography is now important in the separation of proteins and metabolites. This approach is fast becoming widely used in the analysis of other complex samples containing nonvolatile compounds, including low molecular weight extracts from plants, pharmaceuticals, and polymers as well as many environmental samples [16,17]. Even if the value of information produced by 2DLC exceeds the supposed environmental impact and the additional costs, it should be considered if the same separation efficiency and the same results could be obtained with decreased waste generation. The aim of this work was to develop a general framework for the minimization of solvent consumption of comprehensive 2DLC separations taking into account the separation power needed for the analysis of the given sample solution.

2. Theory

The total consumption of organic modifier of a two-dimensional chromatographic method, V_{2D}^{org}, consists of the consumption of first dimension and that of all of the runs in the second dimension. Since usually much more eluent is consumed in the second dimension, the contribution of the first dimension can be neglected in most cases.

$$V_{2D}^{org} = V_1^{org} + \mu V_2^{org} \approx \mu V_2^{org} \tag{1}$$

where μ is the number of fractions analyzed in the second dimension.

Accordingly, the value of V_{2D}^{org} depends mainly on the number of fractions analyzed and the eluent consumption of one separation cycle in the second dimension.

In the practice of 2DLC, reversed-phase linear gradient runs are applied in the second dimension in most of the cases. The consumption of organic modifier of a separation cycle is

$$V_2^{org} = V_{2,grad}^{org} + V_{eq}^{org} \tag{2}$$

where $V_{2,grad}^{org}$ is the organic modifier consumption of the gradient run, and V_{eq}^{org} is the volume of organic modifier used during column equilibration and any additional processes taking place between two subsequent injections in the second dimension.

Peak capacity, n, is defined as the maximum number of compounds that can be separated on a column with unit resolution [29]. The peak capacity of a linear gradient run can be estimated after Gilar's simplified relationship [30] as

$$n_2 = 1 + \frac{\sqrt{N}}{4}\frac{B\,\Delta C}{B\,\Delta C(t_0/t_g)+1} \simeq \frac{\sqrt{N}}{4}\frac{B\,\Delta C}{B\,\Delta C(V_{0,2}/V_{2,grad})+1} \tag{3}$$

where N is the number of theoretical plates, ΔC the change of concentration of the organic modifier during the gradient, t_g the gradient time, F the flow rate, t_0 the dead time, $V_{0,2}$ the dead volume, $V_{2,grad}$ the volume of eluent used in the second dimension (including water, organic modifier, etc.) and B the slope of the $\ln k$ vs. C plot of the components (with k the retention factor). Note that in the right hand side of Eq. (3), t_0/t_g was substituted by $V_0/V_{2,grad}$ and "1" was neglected.

Note, that in classical definitions of peak capacity, resolution and number of theoretical plates, chromatographic peak widths, w, assumed to be four Gaussian standard deviations (4σ). In order to be coherent with these definitions, the same assumption is used throughout this paper.

$$w = 4\sigma \tag{4}$$

where σ is the standard deviation of chromatographic peak.

In case of linear gradient, $V_{2,grad}^{org}$ is a certain factor of $V_{2,grad}$.

$$V_{2,grad}^{org} = \kappa\, V_{2,grad} \tag{5}$$

Value of κ depends on the mode of separation (reversed phase, hydrophilic interaction), the initial fraction of stronger eluent component, φ_0, the time of analysis and ΔC. For a linear gradient, the gradient program is

$$\varphi = \varphi_0 + \Delta C\frac{t}{t_g} = \varphi_0 + \Delta C\frac{V}{V_{grad}} \tag{6}$$

where V stands for eluent volume.

For a reversed-phase gradient, where the organic modifier is the stronger eluent component, κ can be calculated as

$$\kappa_{RP} = \frac{1}{V_{grad}}\int_0^{V_{grad}}\left(\varphi_0 + \Delta C\frac{V}{V_{grad}}\right)dV = \varphi_0 + \frac{\Delta C}{2} \tag{7}$$

Note that the sum of φ_0 and ΔC is smaller than or equal to one. In reversed-phase gradient runs, value of κ is usually between 0.3 and 0.5.

Eq. (3) can be simplified by combining together the constant terms as

$$n_2 \simeq \frac{a\,V_{2,grad}}{b + V_{2,grad}} = \frac{a\,V_{2,grad}^{org}}{\kappa\, b\, V_{0,2} + V_{2,grad}^{org}} \tag{8}$$

where n_2 is the peak capacity of the second dimension, a and b are constant parameters.

The peak capacity of a multidimensional separation, n_{2D}, is the product of that of individual dimensions. Taking into account the effect of undersampling, n_{2D} can be expressed as

$$n_{2D} \simeq n_1' n_2 \simeq \frac{n_1\,\rho}{\sqrt{\rho^2}}\frac{a\,V_{2,grad}^{org}}{\kappa\, b\, V_{0,2} + V_{2,grad}^{org}} \tag{9}$$

where n_{2D} stands for the two-dimensional peak capacity, n_1 the nominal, n_1' the limiting peak capacity of the first dimension, β is 3.424 [23], and ρ is the number of fractions collected per peak

$$\rho = \frac{\mu}{n_1} \tag{10}$$

Note again, that peak width is assumed to be 4σ. Accordingly, sampling time, t_s, is

$$t_s = \frac{4\,\sigma}{\rho} \tag{11}$$

By rearranging Eq. (2) for $V_{2,grad}^{org}$ and substituting it into Eq. (9) the separation power of the 2D system can be expressed as a function of total eluent consumption.

$$n_{2D} \simeq \frac{n_1\,\rho}{\sqrt{\rho^2+\beta}}\frac{a\left(\left(V_{2D}^{org}/\mu\right) - V_{eq}^{org}\right)}{\kappa\, b\, V_{0,2} + \left(V_{2D}^{org}/\mu\right) - V_{eq}^{org}} \tag{12}$$