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Review Article

Application of chemometry for optimization of liquid chromatographic parameters

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Liquid chromatography is one of the most common separation techniques. Optimization of the experimental conditions is a complicated process due to the large number of the variables, which must be simultaneously treated. This mini review summarizes some of the chemometric approaches used in the literature to separate mixtures.

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Optimization of chromatographic conditions

Micellar liquid chromatography (MLC) has been extensively utilized lately as a useful chromatographic method due to several reasons; such as its ability to separate ionic and nonionic compounds, direct injection of biological fluids such as serum and plasma without complicated extraction procedures. Other advantages include the enhancing effect of micelle formation on luminescence intensity, low cost, low volatility of mobile-phase constituents and being environmentally benign (Hernandez *et al.*, 1992).

Since large number of the variables must be simultaneously treated; optimization of the experimental conditions in MLC is complicated. Retention of the analytes as well as extent of the surfactant monomers that are adsorbed onto the stationary phase are affected by variations of the experimental parameters. Consequently, such factors affect retention in an interdependent and nonlinear way (Rukhadze *et al.*, 1998; Gianotti *et al.*, 2005, Marengo *et al.*, 1996). As a result, multivariate analysis is applied usually.

For optimization of different chromatographic parameters, multivariate analysis, experimental design, and multi-criteria decision-making (Smilde *et al.*, 1986) are employed usually.

Taking the determination of caffeine in presence of non steroidal anti inflammatory drugs (El Sherbiny *et al.*, 2014) as an example, *face centered cube response surface* experimental

design was used for optimization. Stepwise multiple linear regression (MLR) was used subsequently to choose the most significant effects and also to calculate the coefficients that relate such effects with retention time. To evaluate the optimal points, the Pareto-optimal approach was applied.

On the other hand, optimization of the separation conditions for 27 molecules was investigated by Mbinze *et al*. (2012). To establish a design space (DS); Design of experiments (DoE) was utilized (Lebrun *et al*., 2008), which was dependant on using a predictive statistical model of the retention times. DoE with predictive probability is able to optimize the separation and also to estimate the robustness of the method over the experimental domain.

Simultaneous determination of caffeine, paracetamol and p-aminophenol was conducted using a reversed phase liquid chromatographic method (Crevar *et al.,* 2008), full factorial design of 8 experiments was used. Where; all possible combinations of selected factors are covered by the number of experiments utilized. When the number of factors is limited, full factorial design is significant. This experiment was conducted using three variables which were found to influence the outcome significantly.

Trial and error methodology was the basis in HPLC separations for decades. The traditional approach is based upon measuring the effect of the specified factors by Changing One Single factor at a Time (COST), keeping other factors constant (Singh *et al.,* 2004). The technique is known also as OFAT (Singh *et al.,* 2004; Fonner *et al*., 1970; Shek *et al*., 1980). After applying it for many experiments, COST methods were found to be inefficient and time consuming since they require effort (planned experiments) and time without being capable of identifying the optimum conditions (Singh *et al.,* 2004; Tye, 2004). These remarkable disadvantages forced scientists to utilize experimental design as a more efficient optimization technique. The basic concept of these techniques which are known as Design of Experiments (DoE), includes the application of experimental design and consequently generation of graphic outcomes and mathematical equations (Singh *et al.,* 2004).

Through employment of several combinations of factors, statistical experimental design transforms experimental data to be mathematical equations which are known as models; in order to be able to predict and optimize the responses. DoE was found to be important in the development and optimization of various mixtures applying HPLC as a separation technique (Wang *et al*.**,** 2006a; Wang *et al*.**,** 2006b; Sivakumar *et al*., 2007; Nemutlu *et al*., 2007).

Multivariate optimization

Before the researchers start to optimize various experimental parameters, it is essential to identify the crucial factors that affect the quality of the delivered outcomes. For instance, when CAF was determined with NSDIs (El Sherbiny *et al.,* 2014), the effect of six independent factors on the separation parameters was thoroughly studied using a two-level fractional factorial design. Factors selection for optimization procedure was mainly based on preliminary experiments. The six factors selected in this work were considered to influence the outcome dramatically. It is worth to mention that identification of significant main effects rather than interaction effects is performed through application of screening designs . Hence, they are in most cases first-order designs with low resolution (Singh *et al*., 2004). The simplest form of design applied for screening k number of factors is the two-level factorial designs (2^k) (http://www.itl.nist.gov/ div898/handbook/05/07/2008).

The following equation represents the mathematical model revealing the effect of main and interaction effects (Barmpalexis *et al.*, 2009):

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j$$

Where *X* is the factor examined, *n* is the number of factors, *Y* is the measured response, and β_0, βi, βij are the coefficients for each main or interaction effect. Based on preliminary experiments, the high and low levels of factors could be determined, putting in consideration that all experiments are performed randomly and in triplicate.

Factorial design

In most cases, the experiments utilized for optimization are performed on three levels corresponding to each of the studied factors. Face centered cube response surface experimental design are usually applied. This design is gaining the reputation of being one of the most suitable experimental designs for modeling and optimization. It is well known that a full factorial design using six variables and two levels would need 64 experiments. To reduce the experiments number, a two-level fractional experimental design which is consisting of 2^{6-3} experiments might be utilized. Such design allows the preliminary assumption of the main factor effects confounded with the second-order interaction; however this leads to a partial loss of information. Central point Experiments are repeated to estimate the experimental error and to test system reproducibility.

Fold-over fractional factorial design

To determine the typical factors which affect separation, a complementary set of experiments known as fold-over design is usually carried out. This design is used to differentiate between the principal effects and the second-order interactions. Combination between results (fold-over and fractional factorial design) confirm the observed results.

Star design

Presence of quadratic significant effects could be evaluated by means of *F*-test which compares the variation between the experimental retention time in the central point and factorial design (Marengo *et al.*, 2009). *F*-test is calculated according to the following equation:

$$F = \frac{(\bar{y_0} - \bar{y_f})^2}{S_{pe}^2 \left(\frac{1}{n_0} + \frac{1}{n_f} \right)}$$

Where; S_{pe}^2 is the purely experimental variance. $\bar{y_0}$, $\bar{y_f}$, n_0, and n_f are average response of the replicated central, factorial design experiments, number of experiment in the central point, and in the factorial design, respectively. Quadratic effects must be included in the regression models if it was found that the calculated *F*-values are greater than the critical *F*-value. This is the main reason for the addition of star design experiments to the factorial experimental design to produce a composite design. Best regression models are produced by a variable selection algorithm. Separation optimization is achieved through grid search program.

Application of this program allows the prediction of the retention times of all studied analytes for every mobile phase composition.

Optimization of design and analysis

Statistical analysis tools; (analysis of variance (ANOVA)) is usually applied to identify significant effects.

Chromatographic optimization function (COF) is calculated according to the equation (Barmpalexis *et al.*, 2009):

$$COF = \sum_{i=1}^{K} A_i \ ln\left(\frac{R_{si}}{R_{sid}}\right) + B(t_M - t_L)$$

Where *Rsi* is the resolution of the *i*th pair, *Rsid* is the desired resolution for the specific pair, *Ai* and *B* are weighted parameters, t_M is the the desired maximum analysis time, and t_L is the actual time of the last eluted peak. COF is used since it has the capability to reduce data from each chromatogram to a single number which can be applied in optimization.

Greater COF value represents satisfactory peak resolution and low elution time. It is important to notice that COF is satisfactory only when all peaks have the same relative order of retention in all conditions (Barmpalexis *et al.*, 2009). Response transformations are made when necessary. When an independent factor has a *p*-value < 0.05, it imposes a significant effect on a given response.

Optimization using central composite design

By understanding factor's main and interaction effects, Central composite design could be applied to optimize an HPLC separation (Sivakumar *et al.*, 2007).

To clarify the results, predicted models are presented as perturbation plot. For an optimization design, this graph shows the changes in the response when each factor moves from a chosen reference point, when all of the other factors are kept constant at the reference value (Sivakumar *et al.*, 2007). If a steep slope or curvature in a factor takes place, it indicates that the response is sensitive to that factor.

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