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Effect of chromatographic parameters and detector settings on the response of HILIC–evaporative light-scattering detection system using experimental design approach and multicriteria optimization methodology

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ABSTRACT

Four polar compounds, i.e. pantothenic acid, inositol, taurine and caffeine were used as probe solutes in conjunction with chemometric methods to find out meaningful implications of chromatographic conditions and detector settings on the system performance. Putting a premium on the conditions of hydrophilic interaction liquid chromatography (HILIC) and settings of evaporative light-scattering detection (ELSD), we scrutinize the importance of certain factors on signal-to-noise ratio and its variability. The application of a central composite design reveals that caffeine, which sublimes, differentiates from the relatively thermosensitive pantothenic acid as well as from inositol and taurine, which are thermostable, do not sublime and have high melting points. It seems that prior knowledge of solute characteristics is critical to estimate the chromatographic response as a function of chromatographic conditions and detection settings. Reducing the responses to just one by combining them "ad hoc", results in an overall desirability function, which brings out the global optimal chromatographic conditions and detector settings.

1. Introduction

During the past 25 years, evaporative light-scattering detection (ELSD) has moved into the mainstream of detection choices for HPLC separation. The great interest in ELSD-based analytical procedures can be charted in surveys of international literature using "evaporative light scattering" as a keyword. Several reviews discussing the operational uses, advantages and limitations of this technique in various fields have also been published [1,2]. HPLC–ELSD is an especially useful 'quasi universal' detector for poor UV absorbing compounds or with mobile phases bearing UV chromophores with the caveat that it misses truly volatile sample constituents. However, not all chromatographers are well versed in the details of detector's operation that make it unique among detector alternatives.

Hydrophilic interaction liquid chromatography (HILIC) can be described as a variant of normal-phase chromatography, where a hydrophilic stationary phase is used in combination with a mostly organic mobile phase and elution is performed by increasing the water concentration. HILIC has been introduced by Alpert [3] and used to analyze different types of carbohydrates. Since HILIC is employed at relatively high mobile phase organic proportions facilitating rapid solvent evaporation, the additional benefit of high ELSD sensitivity was obtained. In this context, significant applications have been reported related to the developed combination of HILIC and ELSD [4–7].

An effective approach to experimental planning is to study factor effects, simultaneously, by setting a statistical experimental design. Fundamentals and applications of statistical designs and response surface techniques for the optimization of chromatographic systems for compound separations have been reported by Ferreira et al. [8]. In certain cases, it is necessary to consider, at the same time, analytical aspects such as reducing signal variability, minimizing noise contribution, maximizing the signal/noise ratio, etc. It is common practice that multicriteria decision-making may be applied in case that several responses have to be considered simultaneously. This usually requires finding optimal compromises among the total number of responses taken into account. A large number of multicriteria decision-making techniques are available in the literature [9].

Vander Heyden et al. studied the operation of ELSD by the use of a three-level screening design for drift-tube temperature, gas flow rate and gain to obtain the fingerprint chromatogram of *Ginkgo biloba*, without paying regard to the influence of chromatographic parameters [10]. Koupparis et al. performed a brief classical study to appraise the influence of solvent composition of mobile phase on ELSD response of amikacin, using a flow injection set-up, after replacing the analytical column by a stainless steel coil [11]. In

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the study herein, we investigate the effect of conditions when developing a chromatographic method with the general-purpose ELSD, the operational requirements of which mandate the knowledge of its critical settings. We assess how typical HILIC conditions and ELSD settings influence the response of model compounds, using as tool of statistical experimental design. A fractional factorial $2^{(7-4)}$ design was firstly used to evaluate the significance of factors followed by a fractional central composite design to appraise systematically the chromatographic system. This design not only helps shorten the time needed to complete the study but also provides a better understanding of the relative importance and inter-relationship of the experimental factors. Finally, the desirability function was employed in the optimization stage of the proposed procedure, as a multicriteria tool, to maximize the individual responses and reduce the variability of the signals.

2. Theoretical aspects

2.1. Chromatographic and detector conditions to optimize

The mechanism of ELSD is complex, although the operation principle mainly consists of three successive processes:

I. nebulization of the chromatographic effluent,
II. evaporation of the mobile phase and
III. detection of the non-volatile residual particles, by means of the measurement of the scattered light.

The composition of the mobile phase affects the ELSD response in a dual way: firstly, it controls the aerosol mean droplet diameter produced by the nebulization process [12] and secondly, it affects the extent of droplets condensation on the walls of the nebulization chamber [13,14]. Apart from the mobile phase composition, the droplet distribution strongly depends upon mobile phase and nebulizing gas flow rates. This dependence is highly interactive.

The particle size is influenced by several other factors, including solute concentration along the peak profile. Larger particles scatter more light and longer residence times of the particles in the light beam – affected by the gas flow rate – enhance scattering [15].

More attention has been paid to the ELSD temperature settings and the melting point (and in rare cases, the molar heat of vaporization) of the analytes. Solutes in solid state scatter light more efficiently than in the liquid state. Therefore, the response decreases when the drift-tube temperature increases and solutes display varying responses depending upon their melting point or molar heat of vaporization [16]. The useful temperature range for the evaporation stage (drift tube) of an ELSD is a matter of distinction between the various instruments on the market. One way to accomplish efficient evaporation at lower temperatures is to lengthen the drift tube. In general, the recommendation for setting the drift-tube temperature is to use the lowest temperature that yields an acceptably low-noise baseline response and reveals all analytes of interest.

Mobile phase additives are highly relevant to many separation processes. Under HILIC conditions, solvent modifiers prepared from crystalline salts are another source of elevated and noisy ELSD baselines. When using a modifier in the mobile phase, a lower temperature sometimes introduces unacceptable noise in the baseline because the evaporative burden is increased.

Finally, the gain of ELSD controls the detector signal amplification to ensure the detection of small peaks. But gain not only amplifies the signal; it amplifies the noise as well. Therefore, chromatographic signal-peak itself is influenced in a different fashion than a calculated signal-to-noise ratio response, which essentially contains the analytical information.

It is evident that the simultaneous and successful handling of the settings of detector in connection with the chromatographic conditions can constitute a tough undertaking, especially for an inexperienced user dealing with analytes of diverse physicochemical properties.

2.2. Experimental design—multicriteria decision-making

Chemometrics offer a sound theoretical basis for the study of chemical and analytical systems as well as processes [17,18]. Chemometric tools, through the development of mathematical models, can assess the statistical significance of the independent factor effects being investigated and evaluate their interaction effects. The use of experimental design allows the simultaneous study of multiple factors, which lead to a significant decrease in the number of necessary experiments [19].

To detect and quantify non-linearity, the effect of each of the factors is usually studied at three or more favorably, at five levels. The exponential increase in the experimental effort with the number of factors is dealt with the employment of a response surface method. The trade-off among the factors is analyzed through response surface methodology, using a central composite design (CCD). Furthermore, the effort to study or optimize a system is greatly reduced by fractional factorial experimental CCD, considering curved responses as well as factor interactions without significant loss of information [20,21].

The selection of response is a critical step in the optimization process since the main aim of optimization is to find the experimental conditions, which provide the best response. More analysts find it imperative to evaluate alternative analytical procedures according to multiple criteria. Multicriteria decision-making is applied when several responses have to be considered at the same time. This methodology is based on constructing a desirability function for each individual response. Derringer functions or desirabilities d_i, are the main tool of one of the most important multicriteria decision-making methods [22]. Each individual desirability function is a continuous function chosen from among a family of linear or exponential ones and varies from zero (undesirable response) to 1 (optimal response). Based on these individual desirability functions, the overall desirability function is estimated as the weighted geometric average of the individual ones. In this way, the multicriteria problem is reduced to a single criterion problem of D optimization.

3. Experimental

3.1. Chemicals and reagents

Ammonium formate, caffeine and pantothenic acid were obtained from Sigma–Aldrich Hellas (Athens, Greece). Taurine and *m*-inositol were purchased from Merck (Schuchardt, Germany) while formic acid was obtained from Scharlau Chemie (Barcelona, Spain). HPLC grade acetonitrile (ACN) was purchased from Fisher Scientific (Leicester, UK). All chemicals and solvents were of analytical-reagent grade.

Stock solutions of caffeine, pantothenic acid, taurine and *m*-inositol (1000 mg/L) were prepared individually by dissolving appropriate amounts in double-distilled water in volumetric flasks of 10 mL. All stock solutions were stored refrigerated in dark vials. Composite standard solutions (20 mg/L) were prepared daily by appropriate dilution of the concentrated stock solutions with the mobile phase. The concentrations of the analytes were chosen so as when gain parameter is set at its maximum value, the peaks are measured within scale.

3.2. Instrumentation-chromatographic analysis

Liquid chromatographic analysis was conducted on a Shimadzu (Duisburg, Germany) HPLC system consisting of CBM-20A pump, a CTO 10AS column oven and an ELSD-LT II detector. Injections were made through a manual 7725i Rheodyne (Cotati, CA, USA) injector using a 20 μL sample loop. A GL Sciences (Tokyo, Japan) HILIC Inertsil, diol column (150 mm × 4.6 mm, 5 μm particle size) was used for the separation of analytes. LC-solution software Version 1.21 SP1 was used for data analysis and processing.

The mobile phase of the HPLC system consisted of aqueous ammonium formate at various concentrations, adjusted to pH 4.0 (Solvent A) and ACN–water 90:10 (v/v) containing ammonium formate (Solvent B), at concentrations equal to those in solvent A. The pH of solvent A was adjusted with formic acid. The aqueous and organic mobile phases were filtered before use, through a 0.45-μm nitrocellulose membrane and a 0.45-μm Teflon membrane, respectively. Different mixtures of hydro-organic mobile phases were delivered isocratically.

Each baseline noise was derived from the chromatographic area just after the elution of the respective peak, although noise amplitude is almost unaltered throughout the chromatogram.

3.3. Statistics

The experimental design matrices were constructed and the results were evaluated using the Statistica 7.0 software (StatSoft, Inc., Tulsa, OK, USA).

4. Results and discussion

Four polar compounds were selected to be studied, i.e. caffeine, pantothenic acid, inositol and taurine, based on the following criteria:

I. their different physicochemical properties (caffeine: mp 235 °C, sublimes; pantothenic acid: mp 137 °C, thermosensitive; inositol: mp 224 °C, thermostable; taurine: mp > 300 °C, thermostable),
II. their ability to separate in a single chromatogram under a wide range of HILIC chromatographic conditions,
III. the absence of strong absorbing UV chromophores for the three out of the four target analytes and
IV. their possible occurrence as components in real samples (e.g. energy drinks).

Several chromatographic conditions and detector settings can be considered as critical in order to assess their significance on the analytical signal. The employment of a flow-injection analysis system for injecting the individual analytes, seemingly, would be a rapid and more convenient way to study the system. Because the concentration of a solute is changing over the duration of the chromatographic peak, the particle size, which is proportional to the concentration, changes over the course of peak, too. To compensate for these issues and attain pragmatic conditions, a chromatographic system connected to an ELSD was employed.

An estimation of the ratio of signal compared to baseline is a way to measure system performance. This comparison relates the height of the signal to that of noise and not to the peak area measurements that are typically employed for quantification with ELSD. In this sense, there are rarely published procedures for linking noise and peak area [23]. However, many analysts face a trade-off between obtaining a more meaningful smooth baseline and having the data output truly representative of the peak area. Our experiments evidenced that peak broadening and asymmetry due to the chromatographic system were reduced under the employed chromatographic conditions, as exemplified by the calculated low peak widths at half-height (0.13–0.31 min) and tailing factors (1.1–1.3). As such, these peaks are less prone to false integration of peak area in chromatography. Hence, the signal-to-noise ratio based on peak area (Sa/N) constitutes the main goal of the study herein and is used for reasons of comparison toward simultaneous maximization of peak area and suppression of noise.

Table 1
Independent factors and levels used in the factorial experimental $2^{(7-4)}$ design.

Factor	Code	Level		
		Low (−1)	Center (0)	High (+1)
ACN in mobile phase, %	Var1	70	77.5	85
Drift-tube temperature, °C	Var2	30	50	70
Mobile phase flow rate, mL/min	Var3	0.5	1.25	2.0
Buffer, 10 mM	Var4	Acetate	Acetate	Formate
Nitrogen flow rate, L/min	Var5	2.2	2.8	3.4
Photomultiplier gain	Var6	4	8	12
Dummy	Var7	−1	0	+1

4.1. Factor screening

Because of the significant number of chromatographic and detection parameters to be tested a screening design of the selected factors preceded the surface modeling study to define their experimental domain, under HILIC conditions. The need for factors screening was suited by a factorial experimental $2^{(7-4)}$ design (two-level fractional design), which assumes that the interactions can completely be ignored; so, the main effects were calculated with a reduced number of experiments.

The levels of factors (+1, −1) were chosen in such a way to avoid peak broadening and to adequately measure their effects on the Sa/N of the analytes. These levels encompass the experimental conditions likely to be encountered according to the literature, preliminary studies and taking into account the limitations of the chromatographic system. For instance, the utilization of highly organic mobile phases, under HILIC conditions, offers the benefit of operating at elevated flow rates with acceptable backpressure levels, as long as the optimum of salt content in the mobile phase has been established [24]. Nonetheless, a fairly low ACN content in the mobile phase in connection with an elevated flow rate can readily result in peak overlapping. Likewise, ELSD basic settings, as described before, can be set in limited ranges based on the manufacturing specifications. Taking into consideration the above, Table 1 gives the examined levels for the six variables considered along with one dummy to estimate the experimental error. Every three experiments, a nominal experiment was performed (three in total) where all factors were at level 0, in order to estimate the experimental error. The 8 runs plus 3 center points were randomly carried out in order to nullify the effect of extraneous or nuisance factors (See Table S1 in Supplementary Material for the factorial experimental $2^{(7-4)}$ design matrix, which consisted of seven factors at two levels).

The effects of the studied factors on the Sa/N of the individual peaks in the screening experiment are portrayed in Fig. 1, in the form of bar charts (Pareto). The bar lengths are proportional to the absolute values of the estimated main effects. Those, which exceed the reference line corresponding to 95% confidence interval, are significant as regards the chromatographic Sa/N. Inspection of the results of this first study expectedly revealed that not all factors are significant for the whole set of the target analytes. Photomultiplier gain (Var6) and buffer composition (Var4) are marginally significant for taurine, while the factorial experimental $2^{(7-4)}$ design demonstrated significant effects for drift-tube temperature (Var2),

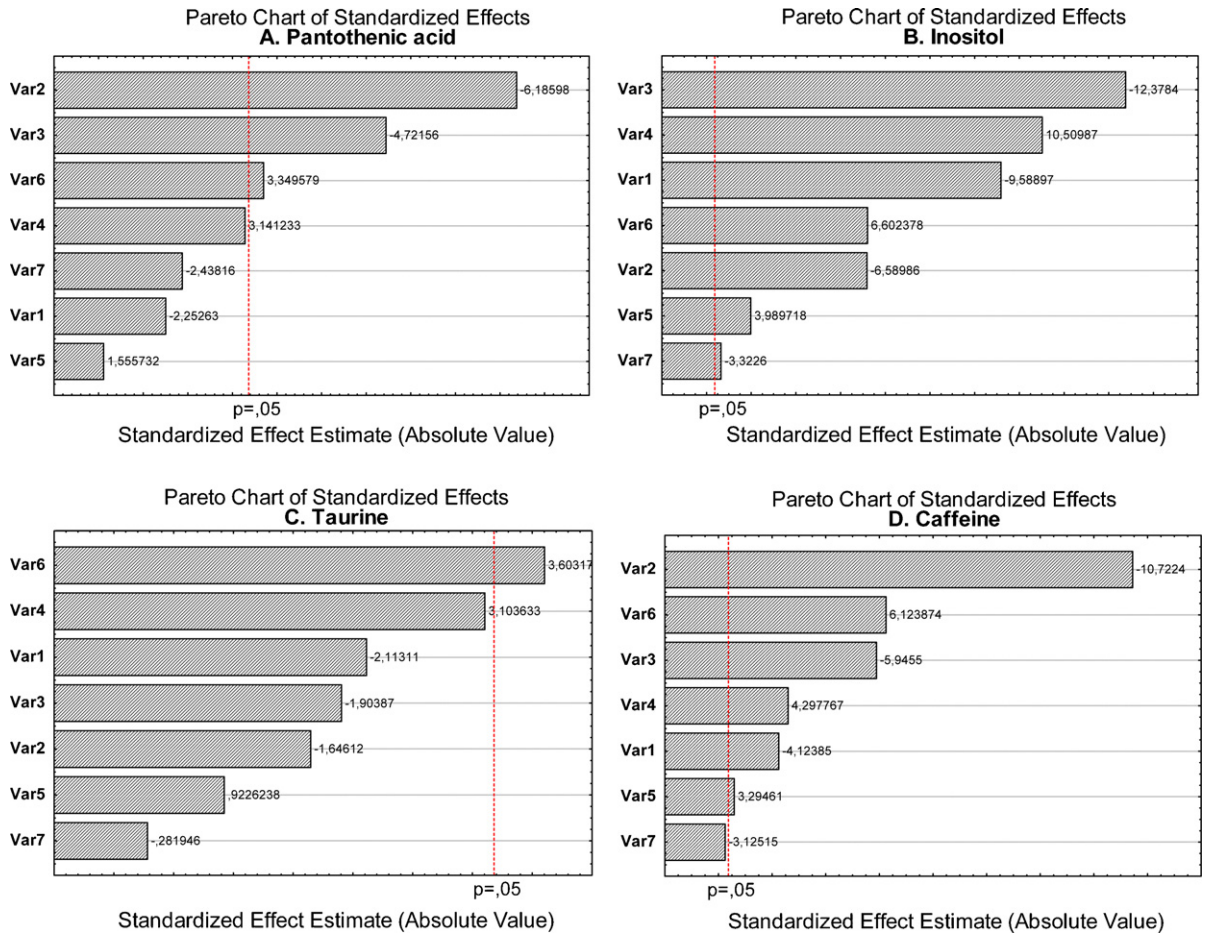

Fig. 1. Standardized main effect Pareto charts for the factorial experimental $2^{(7-4)}$ design of screening experiment. Vertical line in the chart defines 95% confidence level. Variable coding: Var1, ACN in mobile phase, %; Var2, drift-tube temperature, °C; Var3, mobile phase flow rate, mL/min; Var4, formate concentration, mM; Var5, nitrogen flow rate, L/min; Var6, gain.

mobile phase flow rate (Var3), gain and marginally for buffer composition in the case of pantothenic acid. Yet, all factors are statistically significant for caffeine and inositol.

The sign of the main effects showed whether the considered chromatographic response would improve or decrease on passing a given factor from the low to the high level and determined the definite experimental domain to be explored. The charts indicated that, in general, a better Sa/N can be obtained by decreasing ACN in mobile phase, drift-tube temperature and mobile phase flow rate, all of which have negative effects. In contrast, buffer composition, nitrogen flow rate and gain have positive effects.

4.2. Fractional factorial CCD

In pursuit of figuring out the influential behavior exerted by the factors and the interactions among them, a fractional factorial CCD was elaborated further. Although not all factors under consideration are significant for the whole set of the target analytes, they were retained for the subsequent study in order to scrutinize their effects and interpret interactions, since all analytes elute in a single chromatographic run and only little extra workload was required. The experimental domain of the drift-tube temperature was slightly modified toward lower values (negative effect) as compared to that of the screening experiment. In addition, as the effect of buffer composition is positive and due to lower signal noise, we opted for formate buffer, at different concentrations. The rest of the factors were studied after they had been maintained at the same levels as in screening experiment due to the chromatographic and detector limitations, already mentioned above. A rotatable $2^{(6-1)}$ fractional CCD (number of blocks: 1; design generator: 123456) was contemplated, which was composed of a total of 48 randomized chromatographic runs (32 cube points, 12 star and 4 center points) [19]. The independent factors and their levels are provided in Table 2 (See Table

Table 2
Independent factors and levels used in the rotatable fractional CCD.

Variable	Level			Star points ($\alpha = 2.3784$)	
	Low (−1)	Center (0)	High (+1)	$-\alpha$	$+\alpha$
ACN in mobile phase, %	74	77.5	81	69.2	85.8
Drift-tube temperature, °C	38	48	58	24	72
Mobile phase flow rate, mL/min	0.93	1.25	1.56	0.50	2.0
Formate buffer concentration, mM	9.3	12.5	15.6	5	20
Nitrogen flow rate, L/min	2.6	2.9	3.2	2.2	3.6
Photomultiplier gain	6	8	10	4	12

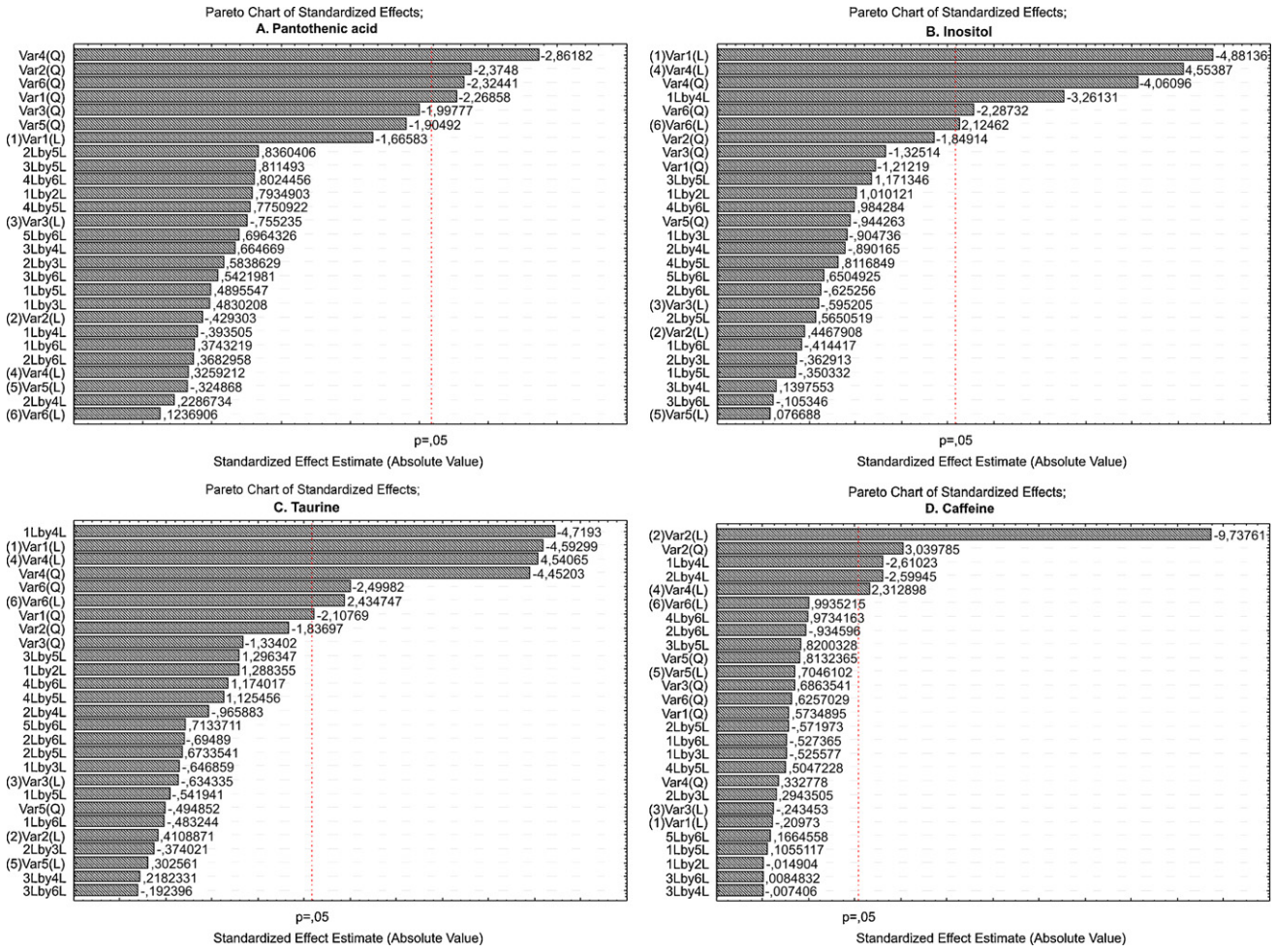

Fig. 2. Pareto chart obtained from $2^{(6-1)}$ fractional CCD for the study of (A) pantothenic acid, (B) inositol, (C) taurine and (D) caffeine. Variable coding as in Fig. 1. L: linear, Q: quadratic.

S2 in Supplementary Material for $2^{(6-1)}$ rotatable fractional CCD matrix).

Again, the Pareto charts (Fig. 2A–D) provided a means to understand the relative importance of the experimental factors on Sa/N. Inspection of the significant effects brings out the diverse behavior on Sa/N of each of the model compounds. Evidently, few interactions of the independent factors displayed significant effects ($p < 0.05$). Amongst all factor effects, the concentration of formate buffer (Var4), the percent of ACN in mobile (Var1), the gain (Var6) and drift-tube temperature (Var2) were the most significant with respect to the Sa/N of specific target analytes.

Plotting the instrumental response as a function of all factors enables visualization of the mutual interactions between the independent factors and helps further discussion. As the applied fractional CCD produced a vast number of figures containing response surface and iso-response plots, only the most significant of them are shown in the publication and Supplementary Material. For the independent factors not shown in the graphs, constant values (the center points of the considered intervals) are imposed on them.

For pantothenic acid (Fig. 2A), the squared term of formate content was the factor with the largest effect, at a 95% confidence level. This was followed by the squared terms of drift-tube temperature, gain and ACN percent in mobile phase. Less relevant appear to be the flow rates of mobile phase and nitrogen. The surfaces obtained are "mound shaped" (see Supplementary Material) indicating that all factors involved acquire maxima at center values with significant decrease in both sides of the experimental domains. Pantothenic acid is thermosensitive; therefore, increased temperatures reasonably result in lower peak values. Practically, out of a certain temperature range (approx. 38–58 °C), the Sa/N of pantothenic acid deteriorates because the system fails to reach a compromise between complete solvent evaporation and high Sa/N.

For inositol (Fig. 2B), the linear term of ACN in mobile phase followed by the linear and squared terms of formate content, the interaction term of (ACN in mobile phase) × (formate content) and the squared and linear terms of gain proved to be statistically significant, at a 95% confidence level. Response surfaces of Fig. 3A–C show the effects of these factors on the Sa/N of inositol. The calculated response increases significantly with the formate content when the ACN content diminishes. High Sa/N can also be achieved through decreasing the ACN content in mobile phase with moderate increase of gain, beyond the center point. However, the formate content and gain result in maximum response when both are simultaneously at center values, followed by a decline in both sides of the experimental domain.

For taurine (Fig. 2C), again, the linear term of ACN in mobile phase, the linear and squared terms of formate content, the interaction term of (ACN in mobile phase) × (formate content) proved to be highly statistically significant followed by the linear and squared terms of gain, at a 95% confidence level. Not surprisingly, the surface response patterns are similar to those of inositol, already described (see Supplementary Material). This is ratified by the fact that both the model analytes are highly polar and thermostable.

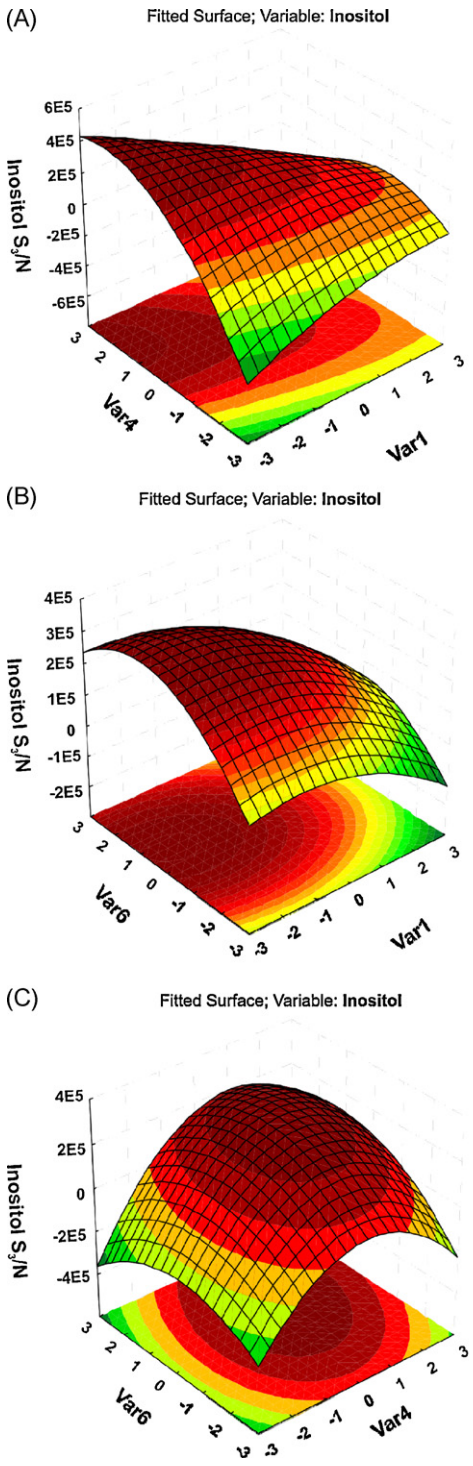

Fig. 3. Response surface plot showing the effect of significant factors on the Sa/N of inositol. Other independent factors are set at the center points of the considered intervals. Variable coding as in Fig. 1.

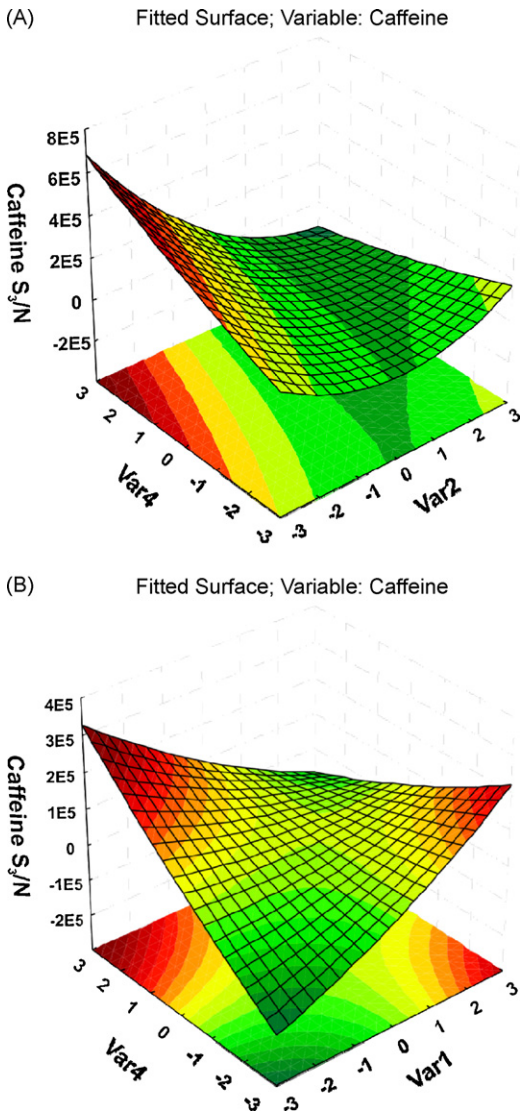

Fig. 4. Response surface plots showing the effect of significant factors on Sa/N of caffeine. Other independent factors are set at the center points of the considered intervals. Variable coding as in Fig. 1.

The linear term of drift-tube temperature was by far the most statistically significant factor on caffeine Sa/N (Fig. 2D) followed by the respective squared term. Caffeine is also influenced by the formate content and its interaction terms with drift-tube temperature and ACN in mobile phase. The three-dimensional surfaces illustrated in Fig. 4, indicate the complexity of interactions of factors and that optima lie far from center values. The drift-tube temperature demonstrated quadratic behavior on Sa/N (Fig. 4A). There is a small region, as shown on fitted response surfaces, where high concentration of formate and low drift-tube temperature maximize the Sa/N. There are also several points that correspond to the highest Sa/N in two small regions, as shown on the surface response and iso-response projection of Fig. 4B. It can be seen that the displacements from the center of surface improve the response toward the direction: (i) of high ACN and low formate content and mainly (ii) of low ACN and high formate content in the studied experimental domain. In either event, it is worth quoting that for caffeine, the response Sa/N increases steeply without leveling off and highest responses are achievable when the statistically significant factors obtain values beyond the designated experimental range.

4.3. Appraisal

Photomultiplier gain manifests itself as the factor of great significance for pantothenic acid, inositol and taurine but not for caffeine. This is not surprising in view of the augmentation of chromatographic signal with gain but also of the different manner that noise is influenced under several experimental conditions. It has been reported that the dependence of signal and photomultiplier gain is logarithmic for the chromatographic separation of antiepilep-

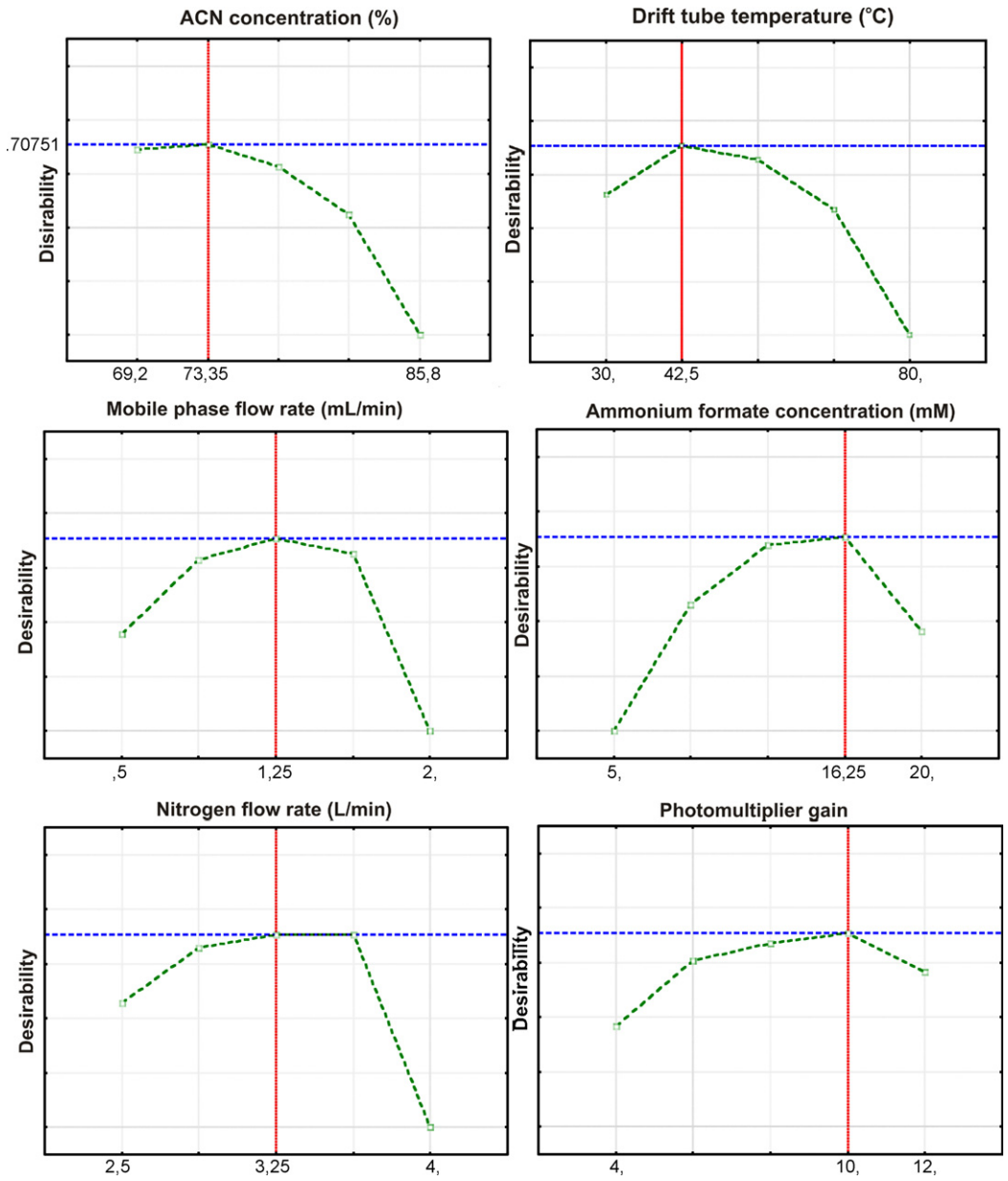

Fig. 5. Profiles for predicted values and desirability function approach for all target analytes.

tic drugs using gradient elution with ammonium acetate (0.01 M), ethanol and *iso*-propanol [25]. In our study, plots of log signal as a function of photomultiplier gain for the four model compounds, showed a linear dependence. Besides, plots of log (Sa/N) as a function of gain follow second-order polynomial equations.

Predictably, drift-tube temperature greatly affects the Sa/N of caffeine and less pantothenic acid. The sublimation of the former and thermosensitive nature of the latter justify this bearing. As far as ACN content in connection with gain is concerned, the tendency for Sa/N of pantothenic acid, inositol and taurine is to increase with decreasing the ACN content, at high gain. This corroborates the affirmation that the higher the water percent in the mobile phase the lower the noise amplitude of ELSD.

As for the concentration of formate buffer, almost invariably, enhanced Sa/N is attained at modest to high salt concentrations levels. The intensity of the scattered light is proportional to the size of the analyte particles and the presence of higher content of formate buffer presumably increases the size of particles. Finally, the Sa/N is practically independent from the nitrogen flow rate in the parametrical range under scrutiny. The non-significance of nitrogen flow rate could be attributed to the high percentage of ACN in the mobile phase, which in the studied range of mobile phase flow rate, does not give rise to variations in droplet distribution. It is thought that large droplets are formed at low gas flow rate, which results in spiked and noisier signals. On the other hand, droplets that are too small to carry sufficient analyte do not contribute to the detection of signal.

4.4. Multicriteria optimization using Derringer's desirability function

The experimental conditions, where the optima are found for each individual response are rather contradictory. So, it is required to look for a certain compromise, which can be used as the criterion on which the systems' performance is judged. In order to reach a compromise among the responses, which could better satisfy the goals, the Derringer's desirability function was used, thus converting a multi-response problem into a single-response one.

It would not be enough to optimize only the magnitude of the Sa/N for the total of analytes. Given the satisfactory resolution and selectivity of the employed analytical system as a result of the proper selection of chromatographic conditions, the variability of Sa/N should be of concern, especially when noisy signals is the case, under assorted experimental conditions [26,27]. Taking into account, at the same time, the aspects of maximizing the Sa/N and reducing the variability of this ELSD response, the overall desirability D was obtained by combining single desirability functions, according to the equation

$$D = \sqrt[n]{d_1^{p1} \times d_2^{p2} \times \cdots \times d_8^{p8}}$$

where the individual desirability functions d_{1-4} represent the magnitudes of Sa/N and d_{4-8} their respective relative standard deviations (RSD). The partial desirabilities d of the responses are drawn with $d = 0$, $d = 1$, $0 < d < 1$ for undesirable, desirable and acceptable values of the response i, respectively [28]. Linear desirability functions were chosen for the responses. It must be considered that the lowest and highest relative standard deviations were given $d = 1$ and $d = 0$, respectively, while, on the other hand, lowest and highest calculated Sa/N were given $d = 0$, $d = 1$, respectively. In other words, the global optimum refers to those experimental conditions where maximum Sa/N and lowest RSD values were obtained, at the same time, for all studied analytes. Equal weights were given to the responses, i.e. $p^1 = p^2 = \cdots = p^8 = 1$, since both Sa/N and RSDs were considered equally important in the overall desirability function, in the above equation. When $0 < D < 1$, an acceptable compromise among the different responses was found.

The main effect plots of independent factors on global multicriteria desirability are shown in Fig. 5. The zones, where the set of requirements are satisfied, were revealed and corresponded to the following conditions: ACN in mobile phase, 73% (v/v); drift-tube temperature, 42 °C; mobile phase flow rate, 1.25 mL/min; formate content, 16 mM; nitrogen flow rate, 3.23 L/min and photomultiplier gain, 10. It is interesting to notice in the desirability graphs that the optimum conditions can be attained in a fairly broad range of values of the variables signifying robustness of the studied system on fluctuations of the selected factors. The resulting optimal conditions were proved to be highly acceptable for the determination of all four analytes using HILIC–ELSD. Desirability functions seem to be an effective tool due to their wide versatility for transforming and optimizing individual responses separately by way of an overall desirability function, thus obtaining global optimal chromatographic conditions and detector settings.

5. Conclusions

The analytes under consideration, which can readily be separated under typical HILIC conditions, display distinct behavior. The chromatographic factors in conjunction with the complex mechanism of ELSD demonstrate that knowledge of analyte characteristics and proper selection of the chromatographic conditions are critical in maximizing the chromatographic response and establishing an analytical method towards accommodating the need for improving the chromatographic analysis.

The study of chromatographic factors and ELSD settings by the experimental design, under HILIC conditions, highlight the diverse behavioral manner that different analytes may display. Caffeine, which sublimes differentiates from the thermosensitive pantothenic acid, as well as from inositol and taurine, which are thermostable, they do not sublime and have high melting points. The instrumental settings of an ELSD and separation conditions in HILIC, as evidenced by the generated results, play multiple role on response maximization. This fact inevitably renders the selection of global optimum conditions a tough task, particularly with classical optimization approaches. When looking for the working conditions of an analytical procedure, it is normal to consider various criteria to be optimized. The results of this study showed that Derringer's desirability function in combination with response surface mapping can successfully be applied to the HILIC area for modeling and process optimization. Whether the compromise solution is satisfactory or not, depends on the specific analytical problem and on the requirements for the analysis. Non-volatile analytes are very frequently the focus of HPLC–ELSD method development and, from the practical method performance standpoint, this study can be a helpful point of departure to develop, evaluate and interpret similar or even more complex cases.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.06.050.

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