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Design of experiments for capillary electrophoretic enantioresolution of salbutamol using dermatan sulfate

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Abstract

Statistical experimental design was used for the optimization and for robustness evaluation of a capillary electrophoretic method developed for the enantioresolution of salbutamol. Dermatan sulfate was used as chiral selector. The goal of the study was to obtain an efficient and fast separation. An eight-run Plackett–Burman matrix was used during the optimization process for the screening of the factors and to adjust the experimental domain under study. Response surface methodology was adopted after the screening phase to obtain information about how the factors percentage of chiral selector, pH and voltage affected the considered responses resolution and analysis time. The Derringer desirability function, which makes it possible to combine results obtained for properties measured on different scales, was used to simultaneously optimize the two responses. Robustness testing was carried out using a Plackett–Burman matrix. The method was found robust as regards the response resolution while voltage and chiral selector were found to be critical factors for the robustness of analysis time response. The proposed CE method permitted the complete enantioseparation of racemic salbutamol and was applied to its chiral resolution in spiked urine samples.

Keywords: Experimental design; Enantiomer separation; Optimization; Salbutamol; Dermatan sulfate

1. Introduction

Analytical enantioresolution of racemic drugs is one of the most attractive topics in the field of pharmaceutical analysis: optical purity control and pharmacokinetic studies require rapid and highly efficient enantioseparations.

Salbutamol, 2-*tert*.-butylamino-1-(4-hydroxy-3-hydroxymethyl)phenyl-ethanol is a well known β_2-adrenoceptor agonist administered in the treatment of asthma and has also been employed for the management of preterm labor during pregnancy [1]. The enantioselective disposition in humans of salbutamol and β_2 agonists in general, has been revealed by chiral studies [2,3]. In particular, the pharmacological action of salbutamol resides in the *R*-(−) enantiomer, which moreover was found to suffer a faster metabolism in man than the *S*-(+) enantiomer [4]. On the other hand, no information is currently available on the toxicity of the inactive *S*-(+) enantiomer and the drug is still administered as a racemic mixture. Anyway, on account of the high levels found of the diasteromer, more careful studies of the distortion in the enantiomeric ratio should be performed.

Several high-performance liquid chromatography (HPLC) [3,5–9] and capillary electrophoresis (CE) [10–13] methods have been proposed for the enantioresolution of racemic salbutamol through the so-called direct approach. In particular, in CE appropriate chiral selectors are dissolved in the running

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buffer; when these addictives are able to establish interactions (e.g., electrostatic, inclusion complexations, hydrogen bond) of different strength with the two enantiomers of the racemic analyte, selective different mobilities of the solutes can be obtained [14].

Non-cyclic polysaccharides have been used as chiral additives in CE, including the family of glycosaminoglycans related to heparin which possess anionic character due to the presence of carboxy and sulfate groups. Their high aqueous solubility and low absorbance in the UV region, allow the compounds to be dissolved at relatively high concentrations in the running buffer. Labile diastereoisomeric pairs could be formed between enantiomers and glycosaminoglycans because of the combination of ionic, hydrogen bonding and hydrophobic interactions which selectively affect the mobility of the stereoisomers [15–20]. The separation mechanism involved in this electrophoretic system was defined as affinity electrokinetic chromatography similarly to that using protein as pseudostationary phase [21].

In a previous study, the mucopolysaccharide dermatan sulfate (DS), was proposed as chiral selector displaying a general enantiorecognition ability towards basic drugs [22]. In the evaluation of the potential of DS as chiral selector, different running buffer pH values (3–6.5) and different DS concentrations were explored, nevertheless concerning salbutamol a complete enantioseparation was not reached. In this study, with the aim of obtaining an efficient and fast baseline enantioresolution of this drug, a statistical experimental design was applied.

In general the optimization step requires one to explore a space defined by the independent parameters that are changed during the experimentation in order to produce desirable values of the response. To this aim the experimental design selects the best location of experimental points in the predictor space and collects these points in a design matrix [23,24]. In particular, the statistical experimental design is a way of choosing experiments efficiently and systematically to give reliable and coherent information. However, it is important to underline that experimental design does not always allow one to find an individual result more quickly than univariate optimization methods but it will generally do so with a far greater degree of certainty. If the number of variables is high, a complete response surface would be a complicated multidimensional structure requiring a strong experimental effort to be fully determined. Thus a screening phase that allows the key factors to be established is advisable.

The optimization process for the enantioresolution of salbutamol, concerning the maximization of the response resolution and the minimization of the analysis time, measured as migration time of the second enantiomer, involved a screening phase carried out by means of a Plackett–Burman matrix, followed by the response surface study with a Doehlert design.

Having to study simultaneously two responses, it was necessary to find the best compromise between the responses by a multicriteria decision-making approach. The desirability function, allowing the two responses to be simultaneously optimized, was used [25,26].

Statistical experimental design was also used to carry out robustness testing that is an important part of method validation, showing the reliability of an analysis with respect to deliberate small variations in method parameters [27–30].

The developed electrophoretic method was applied to the analysis of salbutamol in spiked human urine samples, in order to demonstrate the suitability of the enantioresolving system for the analysis of real samples.

2. Materials and methods

2.1. Materials

Dermatan sulfate (molecular mass 29 000) was a kind gift from Opocrin (Corlo, Italy). Sodium phosphate, phosphoric acid, Tris buffer and citric acid were purchased from Carlo Erba (Milan, Italy). The running buffer solutions were prepared by dissolving the Tris base in the concentration range of 0.01–0.05 *M* and the desired pH values (3.0–6.5) were obtained by adjusting the solutions with citric acid. When methanol (Carlo Erba) was employed as organic modifier of the running buffer, an appropriate volume was added to the aqueous solution to obtain mixtures in the range 0–10% (v/v). Salbutamol hemisulfate was obtained from Sigma (St.

Louis, MO, USA); aqueous solutions of 0.1 mg/ml were used as sample. The pure salbutamol sulfate enantiomers *S*-(+) and *R*-(−) were a gift from Glaxo Wellcome (Verona, Italy).

Solid-phase extraction (SPE) of urine samples was performed using Bond Elut C_{18} (500 mg) cartridges (Varian, CA, USA).

Purified water from a TKA ROS 300 system was used.

2.2. Apparatus

The electrophoretic runs were performed with a ^{3D}CE system (Hewlett-Packard, Palo Alto, CA, USA) equipped with a diode array detector. Data acquisition processing were done by HP vectra 486/100 XMZ computer. Fused-silica capillaries of 48.5 cm length (40 cm to the detector)×50 μm I.D. (Supelco, Milan, Italy) were used for the electrophoretic separations. The samples were injected by pressure (5 kPa, 10 s) and were monitored at a wavelength of 220 nm; the voltage was applied in the range of 10–30 kV and the temperature was held constant at 15°C.

The experimental design was produced, and statistical analysis of the data was performed, by NEMROD-W software [31].

2.3. CE procedure

Running buffer solutions were prepared at the desired pH and methanol percentage values. Dermatan sulfate was then dissolved at the appropriate concentration (0.5–3%) and the resulting solutions were filtered through 0.45-μm Millex-HV filter units (Millipore, Milford, MA, USA) prior to use. The capillary was rinsed between the electrophoretic runs for 10 min with each new running buffer.

2.4. Solid-phase extraction of urine samples

Spiked urine (containing salbutamol in the concentration range 1–5 μg/ml) was adjusted to pH 7.2 with 100 m*M* phosphate buffer. A 5-ml volume of the urine sample was applied to a SPE C_{18} cartridge, previously conditioned with 6 ml of methanol followed by 6 ml of 100 m*M* phosphate buffer, pH 7.2. The SPE column was subjected to a subsequent wash step with 1 ml of a mixture (3 m*M* Tris buffer–6 m*M* citric acid, pH 3.0)–methanol (80:20, v/v). Salbutamol retained on the SPE column was then eluted with 2 ml of the same solution used in the washing step.

3. Results and discussion

The effectiveness of glycosaminoglycans as chiral selector in CE is well documented [15–22] and their enantiorecognition ability was attributed to the combination of ionic, hydrogen bonding and hydrophobic interactions between the analytes and various sites of the mucopolysaccharides. The lower ionic character of chondroitin sulfates compared to that of heparin, makes the former more useful over a wide pH range, including strong acidic conditions. Dermatan sulfate, also knows as chondroitin sulfate B, differs from the other chondroitins (A and C) for the presence of iduronic acid residue instead of uronic acid (Fig. 1). In a previous work [22] DS showed good enantioselectivity toward basic racemates bearing hydroxy groups on the aromatic ring. Salbutamol was only partially resolved although the enantioresolution study was conducted in a wide pH range (3–6.5).

Since the enantiomers of salbutamol possess different pharmacokinetic properties, it was of paramount importance to develop a method able to give good enantioresolution of the drug. To this end, a CE method, based on DS, was optimized by means of a simultaneous multivariate approach, which implies the use of experimental statistical design.

3.1. Method optimization by experimental design

In the optimization of the conditions to enhance the resolution with DS, several parameters have to be considered. pH of the running buffer plays an important role since it affects the charge of both selector and selectand as well as the electroosmotic flow; moreover organic solvents, either pure or as a mixture with water, are used in CE as modifying agents of the background electrolyte (BGE) since they could change the selectivity, their electroosmotic mobility being strongly influenced by the viscosity of the electrophoretic media. Thus, the presence and

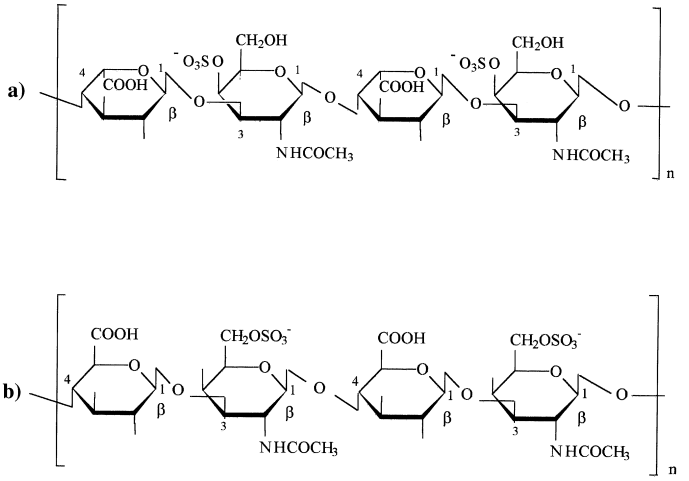

Fig. 1. Unit structure of: (a) dermatan sulfate, (b) chondroitin sulfate C.

percentage of the organic modifier (methanol) was tested in order to improve the enantioresolution of salbutamol enantiomers. Other important factors have to be considered: voltage, temperature and concentration of BGE.

The considered responses were the resolution, R (to be maximized) and the analysis time, T (to be minimized), calculated on the basis of the second enantiomer migration time.

3.1.1. Screening phase: Plackett–Burman matrix

The considered factors affecting the responses using DS as chiral selector in the background electrolyte (citrate buffer), included percentage of methanol (U_1), percentage of the chiral additive (U_2), applied field strength (U_3), pH (U_4), BGE concentration (U_5). The appropriate selection of experimental domain for each factor was made from prior experience and knowledge of the assay system (Table 1).

An eight-run Plackett–Burman design was employed for the screening of the above factors. The experimental matrix also included three experiments at the central level of each factor in order to test the model linearity and to obtain an estimate of experimental variance. The salbutamol concentration was 0.1 mg/ml and the experiments were carried out in a randomized order.

Table 1
Factors and experimental domain during the screening phase

Factor	Low level	High level
MeOH (%)	0	10
Chiral selector (%)	0.5	3.0
Voltage (kV)	10	30
pH	3.0	6.5
BGE concentration (M)	0.01	0.05

The estimate of experimental error variance allowed the significance of the coefficients to be evaluated and the analysis of variance (ANOVA) to be carried out [32]. The large differences in response values of R and T made it difficult to adequately fit a response model to the data using multi-linear regression. To alleviate this problem, R and T were converted to log (LR, LT) before the multiple linear regression analysis. The ANOVA (Table 2) pointed out that the regression models assumed were significant, thus indicating that the change in the observed response was due to the level change of factors. As regard the model adequacy, the analysis of residuals showed that the linear model was not valid.

Examination of residuals is an important part of any ANOVA. If the model is adequate, the residual should be structureless: that is, they should contain no obvious patterns. Through a study of residuals, many types of model inadequacies can be discovered [33]. In this case the residuals of the center point were bigger than the residual of other points, thus indicating that a curvature was present.

In order to identify the active factors, that is the

Table 2
Analysis of variance for the response LR and LT

Source of variation	Sum of squares	Degrees of freedom	Mean square	*F*-ratio
LR				
Regression	25.82190	5	5.16438	$5.16 \cdot 10^{5}$ [a]
Residuals	7.54457	4	1.88614	
Total	$1.76 \cdot 10^{4}$	9		
LT				
Regression	0.92388	5	0.18478	$1.54 \cdot 10^{3}$ [b]
Residuals	0.00842	4	0.00211	
Total	0.93230	9		

[a] $5.16 \cdot 10^{5} > F_{crit.} = 15.52$ (with 5 and 4 degrees of freedom and $\alpha = 0.01$).
[b] $1.54 \cdot 10^{3} > F_{crit.} = 15.52$ (with 5 and 4 degrees of freedom and $\alpha = 0.01$).

factors the change in level of which determined a statistically significant variation of the response, the statistic analysis of coefficients was considered (Table 3). The statistically significant coefficient were those the absolute value of which was greater than zero with a probability of 95%. The confidence interval was calculated starting from the estimate of coefficients, the estimate of standard error for each coefficient b_i and the value of Student's t for a 95% probability and $n-1$ degrees of freedom, where n was the number of replications. The estimate of standard error for each coefficient was given by $\mathrm{sd}/\sqrt{k}$ where k was the number of experiments of the design and sd was an estimate of standard deviation σ of the experimental response y [32].

In particular in this case all factors were statistically significant for both responses and having to optimize the two responses simultaneously, the graphic analysis of effects was used (Fig. 2) [34].

Table 3
Statistical analysis of coefficients for the response LR and LT

LR	
b_0	1.9596
b_1	0.0179[a]
b_2	1.0518[a]
b_3	−1.0179[a]
b_4	1.0404[a]
b_5	−0.0518[a]
LT	
B_0	1.097
B_1	0.0673[a]
B_2	0.0993[a]
B_3	−0.3150[a]
B_4	−0.0361[a]
B_5	−0.0246[a]

[a] Significant effect.

The advantage of this plot is that the numerical values of the effects are displayed. This analysis requires the construction of a bar graph in which the length of each bar is proportional to the absolute effect value. The effects that exceed the reference lines, corresponding to the 95% confidence interval, are those significant for the response. From Fig. 2 it is also possible to decide the new experimental domain to be explored in order to obtain the optimization of the considered response. In the right panel of Fig. 2 the positive effects are represented, while the left panel reports the negative effects. It is easy to see that the trend of the responses is similar, but, if we consider that LR has to be maximized and LT has to be minimized, it is clear that a compromise has to be found. In particular methanol percentage (U_1) and BGE concentration (U_5) were fixed at their central level, that is at 5% and 0.03 *M*, respectively. The experimental domain for the other variables was adjusted according to the sign of the respective coefficient, as follows: chiral selector percentage 0.5–3%, pH 3.0–6.5, voltage 16–24 kV.

3.1.2. Response surface study: Doehlert design

Response surface mapping is an effective way of locating the optimum if the data fit to the chosen polynomial model. In this case the response surface was approximated by second-order polynomial function and a Doehlert design was used to estimate the coefficients. The points of Doehlert matrix correspond to the vertices of the solid generated from a regular simplex by taking differences among its

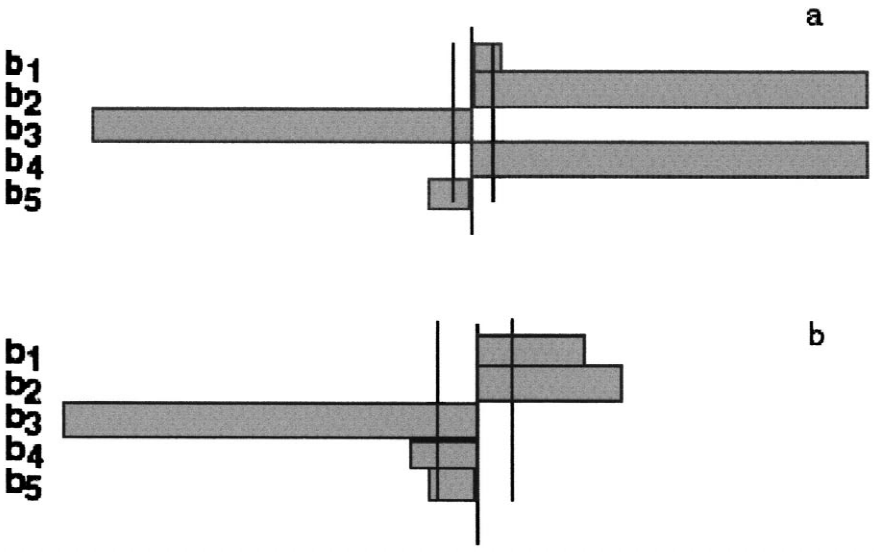

Fig. 2. Graphic analysis of effects. The lines define the 95% confidence interval. Right panel: positive effects; left panel: negative effects; (a) log resolution response (LR); (b) log analysis time response (LT).

vertices and in general are equal to k^2+k+n, where k is the number of the factors and n the number of central points. Replicates at the central level of the variables are performed in order to validate the model by means an estimate of experimental variance. An important properties of Doehlert design regards the number of levels that each variable takes. For three variables the number of levels are 5, 7, 3 [32].

In this case chiral selector concentration (U_1) was studied at three levels, pH (U_2) at five levels and voltage (U_3) at seven levels. Salbutamol concentration was 0.1 mg/ml and the experiments were carried out in a randomized order according to the experimental matrix reported in Table 4. During the response surface study the response T was transformed in the log (LT), while for the response R this transformation was not necessary. The ANOVA pointed out that the regression model was significant and valid for both responses R and LT. The response surfaces for R and LT maintaining chiral selector percentage at its central level 1.75% are shown in Fig. 3a and b. In order to maximize R it is important that the pH has a value from the center to the high level, while at these pH values, voltage seems to be not important; for LT minimization it is important a high voltage and a low or high pH. However, in order to find the best compromise between the two responses, a multicriteria decision making was considered and a total desirability function D that weights the responses together, with one single criterion, was used to optimize the two responses simultaneously. This function is a measure of overall quality and provides convenient means to compare

Table 4
Doehlert experimental matrix used during the response surface study

	x_1	x_2	x_3
1	1.000	0.000	0.000
2	−1.000	0.000	0.000
3	0.500	0.866	0.000
4	−0.500	−0.866	0.000
5	0.500	−0.866	0.000
6	−0.500	0.866	0.000
7	0.500	0.289	0.816
8	−0.500	−0.289	−0.816
9	0.500	−0.289	−0.816
10	0.000	0.577	−0.816
11	−0.500	0.289	0.816
12	0.000	−0.577	0.816
13	0.000	0.000	0.000
14	0.000	0.000	0.000
15	0.000	0.000	0.000
16	0.000	0.000	0.000
17	0.000	0.000	0.000

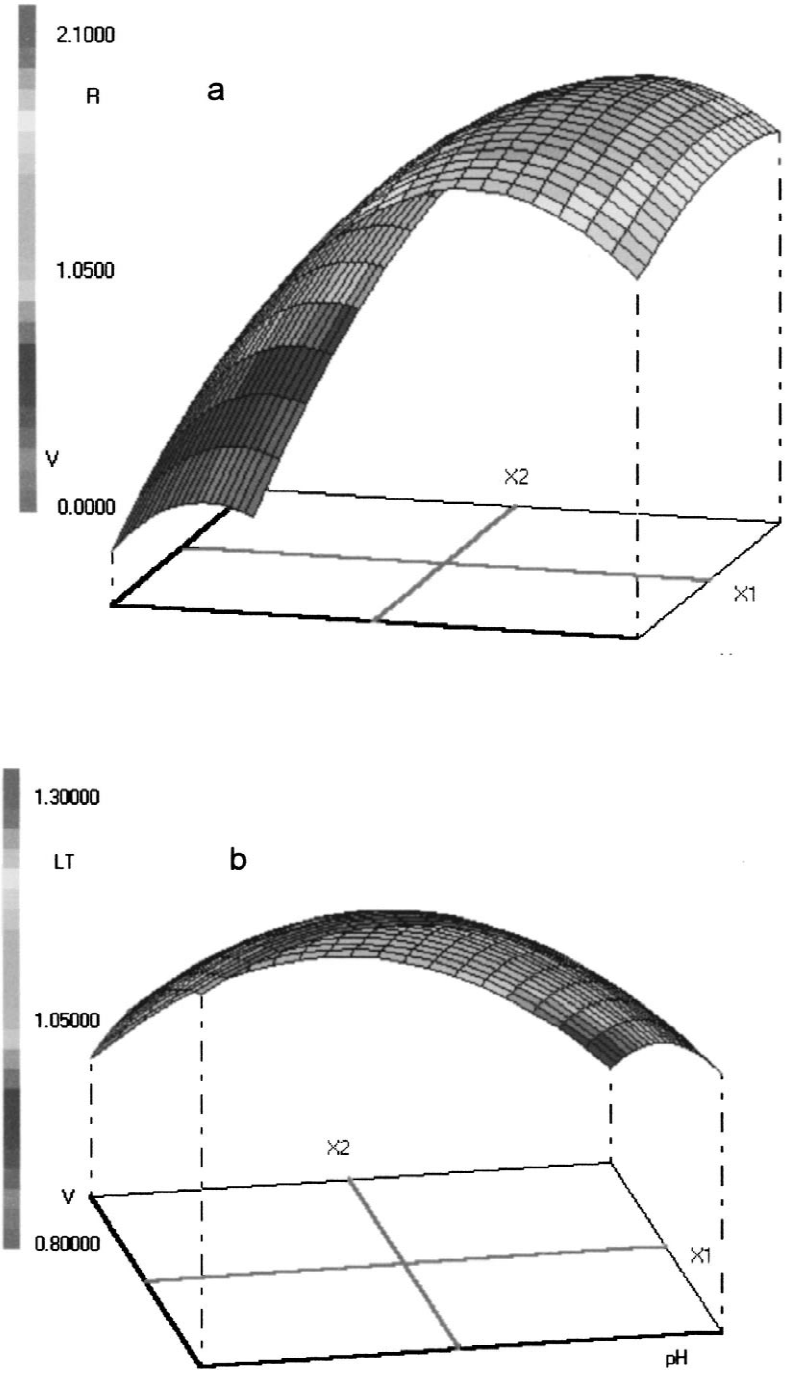

Fig. 3. Three-dimensional plot of pH against voltage, maintaining chiral selector concentration at the central level, 1.75%; (a) resolution response (R); (b) log analysis time response (LT).

several responses and to select the optimum with the most desirable properties. Briefly, the measured responses are transformed to a dimensionless desirability (d) scale. The scale of the desirability function ranges between $d=0$, for a completely undesired response, to $d=1$ for a fully desired response, above which further improvements would have no importance. In the second step the overall quality D is calculated combining the desirability values obtained for the different criteria by means the geometric mean: $D=(d_1\times d_2\times \ldots d_m)^{1/m}$ [35].

Depending on the importance attributed to a response, the individual d_i functions can be weighed and the total D function assumes the form: $D=(d_1^{w_1}\times d_2^{w_2}\times \ldots d_m{}^{w_m})^{1/(w_1+w_2\ldots+w_m)}$. An algorithm of calculation is then applied to the D function in order to determine the set of variable values that maximizes it. The value of D is the highest at conditions where combination of the different criteria is globally optimal [35].

The responses R and LT were transformed into appropriate desirability scale d_i, d_1 and d_2, respectively, according to the fact that R had to be maximized and LT minimized (Fig. 4a and b). Giving the same weight to the responses, the overall function D had the form: $D=(d_1\times d_2)^{1/2}$. The three-dimensional plot of D, maintaining chiral selector percentage at its central level 1.75%, is shown in Fig. 5. The D value is maximum for high value of voltage and pH about 5, while in the other regions of experimental domain the D value decreases until 0. In particular a good point was: pH, 5.3; voltage, 24 kV; chiral selector concentration, 1.75%. The D value for these conditions was 0.80. Another good point seemed to be pH, 6; voltage, 24 kV; chiral selector concentration, 2.2% with a $D=0.85$; however, an increase of chiral selector concentration decreased peak height. Reducing the chiral selector concentration, a higher peak height and a lower cost per analysis were obtained; thus, the first point was chosen as optimum.

A typical electropherogram obtained applying the optimized conditions (pH, 5.3; voltage, 24 kV; chiral selector concentration, 1.75%; methanol percentage, 5%; BGE concentration, 0.03 M) is presented in Fig. 6; by injection of the single pure enantiomers, the migration order proved to be S-(+) followed by R-(−).

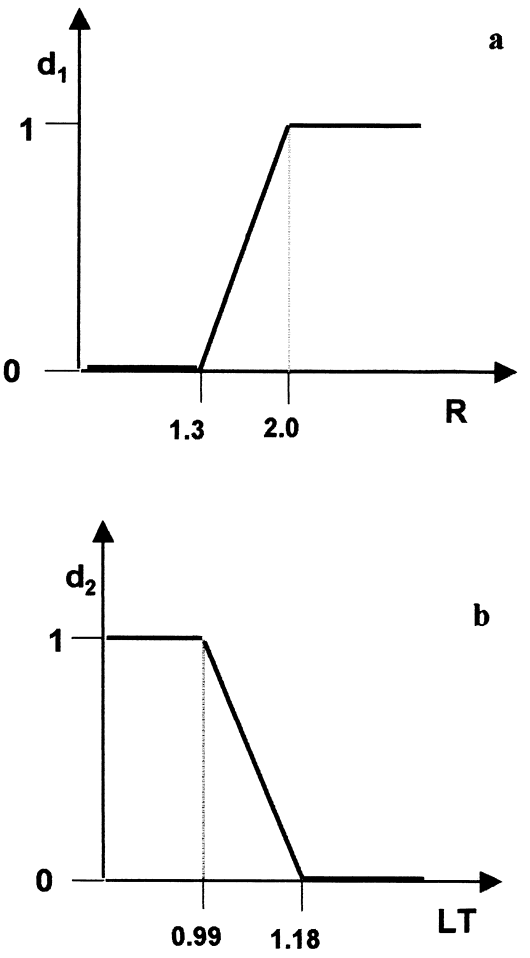

Fig. 4. Transformations of response y_i into desirability values d_j. The undesirable responses and the fully desired responses are reported. (a) Resolution response (R); (b) log analysis time response (LT).

3.2. Robustness testing

Robustness testing was performed in order to obtain information about those critical parameters affecting the responses (R, T). The robustness of the method was tested using experimental design in order to study the simultaneous variation of the factors. As a result of the data analysis, one is able to indicate which of the tested factors are not robust for the considered response. When factors that are not robust are detected one can decide to change the method or to control the factor in question more strictly.

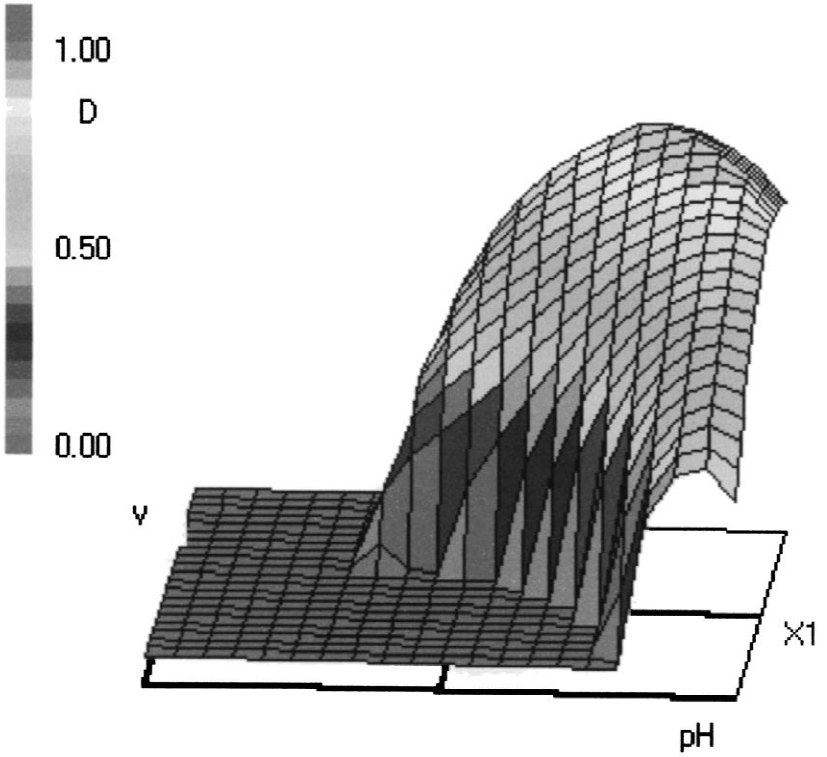

Fig. 5. Graphical representation of the overall desirability function *D*. pH, (x_1) is plotted against voltage, *V* (x_2) maintaining chiral selector concentration at the central level, 1.75%.

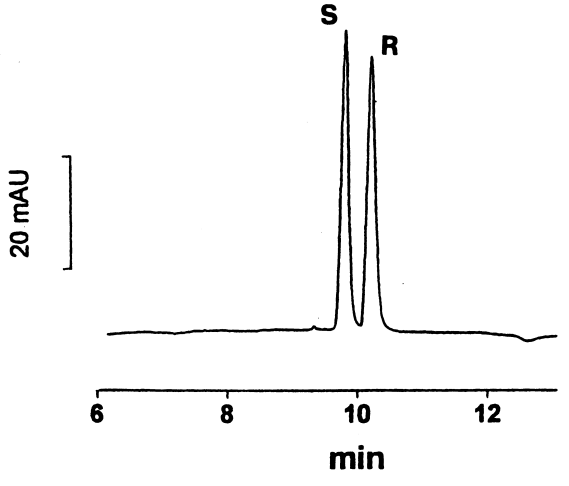

Fig. 6. Separation of enantiomers of salbutamol under the optimized conditions: buffer Tris base (0.03 *M*) adjusted to pH 5.3 with citric acid and containing 1.75% dermatan sulfate, 5% methanol; fused-silica capillary 48.5 cm (40 cm effective length)×50 μm I.D.; applied voltage 24 kV; detection at 220 nm; temperature 15°C; injection time 10 s.

To carry out robustness testing with experimental design tools requires the selection of the factors and the levels at which to test them, followed by the selection of a suitable experimental design. The choice of the design depends on the number of the factors to test and on the postulated model. In general linear models are usually sufficient because of the small experimental domain and for the reduction in the number of experiments. For each controlled factor it is necessary to know its optimized value in order to define the interval within which it can be controlled. In this study the ranges examined were small deviations from the method settings (not more than ±10%) (Table 5) which would normally occur and a linear model was postulated. An eight-run Plackett–Burman design was chosen to evaluate if a change in factor value produced a statistically significant variation of the observed responses. To test the model linearity and to have an estimation of experimental variance, four experiments with the

Table 5
Factors and experimental domain during robustness testing

Factor	Low level	High level
MeOH (%)	4.8	5.2
BGE concentration (*M*)	0.028	0.032
pH	5.1	5.5
Voltage (kV)	22	26
Chiral selector (%)	1.5	2.0

optimized conditions, corresponding to the center of experimental domain, were also carried out. ANOVA showed that as regards the response *R* the model assumed was not significant, indicating that no factor influenced the response. Thus the method could be considered robust for this response.

As regards the response analysis time, measured as migration time of the second enantiomer, the model was found valid and significant. In particular, statistical analysis of effects, pointed out that changes in concentration of chiral selector and voltage, were critical for the response. Thus, a precautionary statement should be included in the procedure for these factors.

3.3. Analysis of spiked urine samples

Enantioseparation of salbutamol obtained in the previous study using DS as chiral selector [22], led to a partial resolution ($R_s = 0.9$) obtained spending about 15 min; the effect of varying DS concentration suggested that no improvement in the resolution was shown over 1% DS under the screened conditions using an univariate optimization approach.

By means of application of statistical experimental design, a baseline separation of salbutamol enantiomers was achieved with a significant reduction of the migration time (about 10 min) compared to the previous described conditions [22]; in general, the analysis time of the proposed system was in the order of the more rapid analytical CE and HPLC methods described in literature for the same drug [7,9,12,15,36].

The optimized CE method was, therefore, applied to the analysis of salbutamol in spiked urine samples in order to offer a method useful for studies on the drug enantioselective disposition in humans.

Relatively higher levels (in the order of μg/ml) of the analyte are present in urine compared to that of plasma levels (1–2 ng/ml) after intravenous, rectal and oral administration of racemic salbutamol; a cumulative urinary effect is displayed due to the active renal excretion [3,6]. Anyway, a SPE procedure was performed on the urine samples spiked with known quantities of racemic salbutamol in order to have both clean-up and concentration effect. The percentage analytical recovery was measured by comparing the correct peaks area (of both the resolved enantiomers) obtained from injection of a known amount of the pure racemic compound with those measured from the analysis of extracted urine samples spiked with (±)-salbutamol hemisulfate to give a final concentration ranging from 2 to 5 μg/ml. At the concentration level of 4 μg/ml, a 84.5% mean recovery (RSD 1.8%, $n = 5$), evaluated on the second migrating enantiomer [*R*-(−)] within-day, was obtained; the inter-day reproducibility was RSD 4.5%, ($n = 7$). This result can be considered satisfactory even if in the literature higher recovery are reported [7,36]. Given the very good repeatability obtained, further attempts to increase the recovery were not performed; moreover using the chosen elution mixture [(3 m*M* Tris buffer–6 m*M* citric acid, pH 3.0)–methanol, 80:20, v/v] in the SPE step an higher detection response was observed compared to that reached when more concentrate buffers were applied. This increase, evaluable around a 20% of the detection response, could be ascribed to a stacking effect.

Linearity of response was evaluated in the concentration range of 1–5 μg/ml for salbutamol spiked urine; under the optimized electrophoretic conditions, linear calibration graphs were obtained reporting the correct peak area of the singles salbutamol enantiomers (y) versus the concentration of racemic salbutamol hemisulfate (c). The equations describing the lines were $y = (0.175 \pm 0.02598)c + (0.09 \pm 0.08078)$ and $y = (0.1835 \pm 0.00555)c + (0.04 \pm 0.02037)$ for the first [*S*-(+)] and the second [*R*-(−)] migrating enantiomer, respectively. In both cases high correlation coefficient was reached ($r = 0.999$, $n = 5$). These data support the effectiveness of the obtained enantioresolution, allowing the selective quantitation of both the single enantiomers. The limit of detection (expressed as signal-to-noise ratio of 3) evaluated for the injected racemic salbutamol hemisulfate at 200 nm was found to be 0.2 μg/ml.

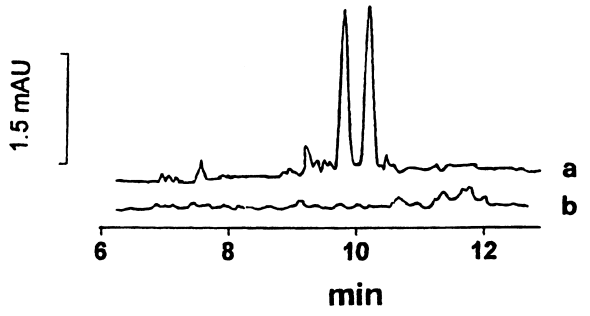

Fig. 7. Electropherograms of urine samples. (a) Urine spiked with salbutamol hemisulfate (3 μg/ml) after SPE procedure; (b) blank urine after SPE procedure. Conditions as in Fig. 6.

Fig. 7 shows the salbutamol enantioresolution from the urine matrix applying the optimized conditions.

4. Conclusions

This paper shows how the study of a new chiral selector combined with the optimization by means of experimental design, can be advantageous for the enantioresolution of chiral drugs. In particular the use of experimental design strategies for the optimization and robustness testing allowed a fast and efficient salbutamol enantioresolution to be obtained using dermatan sulfate as chiral selector.

As the selector is a heterogeneous polymer the influence of the source of dermatan sulfate was also evaluated. Two different batches of the used glycosaminoglycan (molecular masses 29 000 and 30 200) from the same manufacturer were used without differences of responses (migration time and resolution) under the optimized conditions. When dermatan sulfate provided by another company (molecular mass 23 000) was used, the enantioresolution was only slightly reduced.

The possibility of dealing with several responses at a time, obtaining the best compromise between different goals, was a very important aspect, since separative techniques usually required a simultaneous optimization of several responses. The method was found to be robust for the responses resolution and analysis time, apart from, for the latter, the critical factors voltage and chiral selector concentration for which a precautionary statement should be included in the procedure.

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