Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 333–340
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Optimization of the separation of some psychotropic drugs and their respective metabolites by liquid chromatography

P. Cutroneo [a], M. Beljean [b], R. Phan Tan Luu [c], A.-M. Siouffi [c,*]

[a] *Universita degli Studi, Messina, Italy*
[b] *C.H.S. Bon-Sauveur, Caen, France*
[c] *Université P. Cezanne, Aix-Marseille III, Faculté des Sciences St. Jérôme, Marseille, France*

Received 1 April 2005; received in revised form 26 October 2005; accepted 29 October 2005
Available online 6 January 2006

Abstract

A chemometric procedure is described to optimize the separation of some drugs used in the treatment of psychotic disorders: haloperidol, levomepromazine, risperidone, venlafaxine, carbamazepine and their main metabolites: reduced haloperidol, 9-hydroxy risperidone, desmethyl levomepromazine, desmethyl venlafaxine. The purpose of the procedure is the unambiguous identification and detection in biological fluids. Isocratic reversed-phase liquid chromatography with diode array detection was utilized. An experimental design methodology was carried out in which the experimental response is selectivity. In this way the designs for mixture compounds and for process variables (five variables) was performed which produced 36 experiments to carry out. The desirability function was used to select optimum separation conditions. The procedure provides a chromatogram of well separated solutes.

Keywords: Psychotropic drugs; Liquid chromatography; Chemometrics; Desirability

1. Introduction

The major problem in routine therapeutic drug monitoring at a psychiatric hospital is that only a small percentage of the patients are in monotherapy. A relevant percentage of the patients are comedicated with other neuroleptics or tricyclic antidepressants. Drugs such as haloperidol, risperidone, clozapine, olanzapine ("atypical antipsychotics") have been introduced for the treatment of psychotic disorders. They seem to be more effective against negative symptoms and show less extrapyramidal effects [1].

In some cases even these new drugs do not completely fulfill the demands on the treatment of psychotic disorders. Anticonvulsants such as carbamazepine can be associated with antipsychotics for the treatment of behavioral disorders [2].

Rapid and reliable analytical assays are required to detect and identify drugs of toxicological importance. Measurements of serum concentrations of psychotropic drugs and their metabolites may be useful to disclose abnormal levels in patients with atypical metabolic rates or in forensic practice.

Several techniques have been proposed for the determination of psychotropic drugs in biological fluids. Reversed-phase liquid chromatography has interesting features in routine therapeutic drug monitoring since most of the drugs are water soluble and thermally labile. A survey of available literature is rather deceptive since most papers deal with one single drug and its main metabolite [3–8] and in many cases only retention times or retention factors are given. Chromatograms displaying the separation of several drugs with some metabolites are scarce.

The analyst is faced with the problem of manipulating experimental conditions, such as stationary phase type, mobile phase composition and pH and at a lower extent, temperature to obtain a chromatogram in which no co-elution of solutes would occur. HPLC methods are limited in identifying the different molecules by their retention time and more information is needed either from diode array detection [9,10] or MS spectra. In many reports UV detection is used since metabolic transformations of the molecules do not affect UV spectra characteristics, and compounds belonging to the same chemical class often display similar absorbance patterns. In such cases retention time values are important for peak identification [11,12].

* Corresponding author at: UMR 6180, Case A62, Campus de St. Jerôme, 13397 Marseille cedex 20, France. Tel.: +33 491288584; fax: +33 491289146.
E-mail address: Antoine-Michel.Siouffi@univ.u-3mrs.fr (A.-M. Siouffi).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.10.050

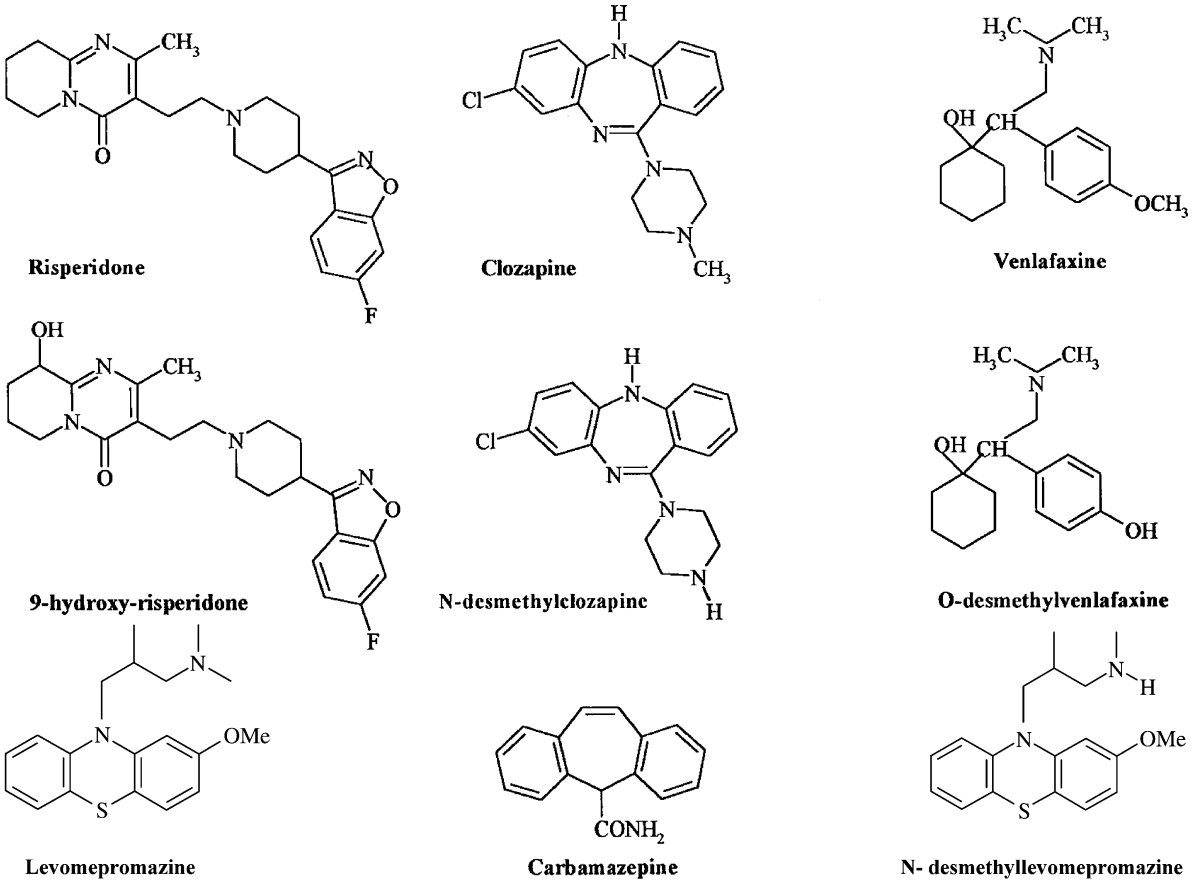

Fig. 1. Chemical structures of the selected psychotropic drugs and their metabolites.

UV spectroscopy is less selective that MS. LC–MS is very efficient for detection but in many cases MS/MS devices or FAB/MS are necessary for further analyses of the internal structures of target compounds (e.g. phenothiazines) [13]. Furthermore LC–MS or LC–MS–MS may not be truly quantitative and not yet popular in routine control at hospitals. For these reasons an optimized separation is required.

The purpose of this paper is to use a chemometric approach to handle many variables and optimize isocratic separation by reversed-phase liquid chromatography and diode array detection of a complex mixture of some selected drugs (haloperidol, risperidone, levomepromazine, venlafaxine, carbamazepine) and some active metabolites, (reduced haloperidol, 9-hydroxy risperidone, desmethylated levomepromazine, desmethylated venlafaxine), which were selected as model compounds. In Fig. 1 the chemical structures of these compounds are displayed. The method may be applied to any mixture of solutes.

An experimental design methodology was carried out to evaluate the effects of some chromatographic parameters (pH, mobile phase, ionic strength) on the separation of the analytes. A mathematical model that describes selectivity as a function of pH, buffer concentration and ternary solvent systems (buffer, acetonitrile, methanol) has been devised. A combined experimental design, in which the process variables are incorporated into the mixture experiments, was carried out. This experimental design was obtained by crossing a three-component mixture design with a classical pentagonal arrangement for the two process parameters. The experimental region for the mixture components was constrained by setting lower and upper boundaries on the component proportions. The final fitted model was used to generate contour plots of the mixture surfaces at the different settings of the other two factors.

In order to find the best compromise between selectivity and analysis time, a multicriteria decision-making approach has been used. In our study, an innovative application of the desirability function to combined experimental design was performed. This procedure has allowed us to determine an optimal zone for solute separation.

2. Experimental

Chromatographic measurements were performed on a TSP (Thermo Separation Products, San Jose, CA) instrument equipped with a Rheodyne 7125 injection valve (20 μl loop) (Touzart et Matignon, Courtaboeuf, France). Detection was performed with a diode-array (UV) (Spectrafocus, Thermo Separation Products) at the selected wavelength 220 nm. A PC 1000 connected to an M86 B × 2 Getek (Thermo Separation Products) was used for data acquisition.

Chromatography was performed with a 150 mm × 4.6 mm column packed with CycloHexyl bonded silica, called a CH column (Varian Associates, Palo Alto, CA). The average particle diameter was 5 μm. No information was available on the number of bonded moieties in μmol/m^2 from the manufacturer. The

column was thermostated at 30 °C in an oven (Cluzeau, France). The retention time of the unretained solute (t_0) was measured by the injection of either uracil solution or sodium nitrate solution.

Samples were kindly supplied by manufacturers and were as follows: haloperidol (Haldol™), reduced haloperidol, risperidone (Risperdal™) and its main metabolite 9-hydroxy-risperidone were provided by Janssen-Cilag (Issy les Moulineaux, France); carbamazepine (Tegretol™) was purchased from Novartis Farma; venlafaxine (Effexor™) and *O*-desmethyl-venlafaxine were kindly supplied by Wyeth-Lederle (Puteaux, France); levomepromazine (Nozinan™) and its desmethylated derivative were provided by Specia Rhône Poulenc Rorer (Aventis, Paris, France). Stock solutions were prepared in order to achieve 20 mg/l concentrations.

Solvents (acetonitrile and methanol) were HPLC-grade from Merck (Darmstadt, Germany), and HPLC water from Baxter (Versailles, France). Buffers were prepared according to European Pharmacopeia (III ed.) to obtain the desired pH (range 2.6–6.4). Buffers were stored in a tank with gentle helium bubbling. Mixing was performed by the pump system.

3. Stages in the procedure

3.1. The experimental design

3.1.1. The choice of experimental factors

To set the standardized chromatographic conditions, which are likely to be employed, selection of the variables must be made.

Controlled factors, which in preliminary studies have been shown to have the strongest influence on selectivity are: aqueous phosphate buffer vol.% (X_1), acetonitrile (ACN) vol.% (X_2), methanol (MeOH) vol.% (X_3), buffer concentration (X_4) and pH (X_5). Mixture components (X_1–X_3) are related in such a way that the sum of their percentages is 100%. Buffer concentration and pH are process variables since they can be varied independently.

3.1.2. The choice of experimental responses

The experimental responses will vary with the objectives fixed. Two cases will be studied in the present work as selected by the analyst.

Case 1 The goal of the analysis is simultaneous quantitation of all considered peaks, within acceptable time. Accordingly, the following optimization criteria will be fixed:

- α_{min}, or minimal selectivity factor between the two most difficult peaks to separate (critical pair) for each chromatographic condition;
- k_{max}, or retention of the last eluted peak, which is a measure of the analysis time.

An optimal separation for all the drugs can be obtained for $\alpha_{min} \geq 1.1$ values and $k_{max} \leq 11.0$ values. Once this goal has been achieved, attribution is made through the retention factors, thus involving a perfect reproducibility of chromatographic data. Temperature was therefore kept constant and retention factors were calibrated with standard compounds. Peaks were attributed by comparison to the standard compounds and by absorbance ratios measurement.

Case 2 The analytical purpose is to perform the best possible separation of all peaks, regularly and uniformly spaced in the resulting chromatogram (that could be useful in a toxicological analysis where identification of each peak is equally important). In such a case, the selectivity criterion is the arithmetic average of the α_{ji} values, calculated according to the elution order for each chromatographic condition, called $\alpha_{average}$. In order to maximize this value and to obtain a uniform distribution of the peaks, the $\alpha_{average}$/S.D. (standard deviation) ratio is utilized. In that case, analysis time should be minimized by fixing a $k_{max} \leq 11.0$ value as well.

Selectivity was preferred to resolution as optimization parameter since the peak shapes of those basic solutes are often asymmetric depending on the column, which may exhibit different performances from one manufacturer to another.

3.2. Selection of experiments: the informative approach

Due to a possible change of the elution order of the analytes, it is useful to explore the behavior of the experimental responses to a reference mixture. The selected mixture corresponds to the following mobile phase: 55% buffer, 35% acetonitrile, 10% methanol (v/v). It is not better than another but it provides information on the behavior of the solutes. The aim is to reduce the number of experiments by choosing "informative" eluent systems in such a way as to obtain information spread over the experimental field. The results obtained on these selected points enable the qualities of all the eluent systems be known by using predictive mathematical models. The validity of this forecast depends on the form of the postulated model and the choice of experiments alone. It is independent of experimental results.

3.3. Design for mixture compounds

The purpose is to investigate the three solvents' (buffer, acetonitrile, methanol) effects on selectivity and retention. Proportions of the mixture components are indicated as follows (see Section 3.1.1): X_1 = percentage of buffer, X_2 = percentage of ACN, X_3 = percentage of MeOH.

The domain of interest has a spherical shape. In order to obtain a high-quality forecast, an equiradial matrix, with a pentagonal arrangement of experimental points around the chosen reference mixture, was selected. Previous experience suggested the following experimental domain for solvent percentages (Table 1).

Table 1
Experimental domain for solvent percentages

Factor	Mixture component	Lower limit (v/v, %)	Upper limit (v/v, %)
X_1	Buffer	50	60
X_2	ACN	19	50
X_3	MeOH	0	20

Table 2
Selected experimental matrix

No.	X_1	X_2	X_3
1	0.50	0.40	0.10
2	0.53	0.29	0.18
3	0.58	0.27	0.15
4	0.59	0.36	0.05
5	0.54	0.44	0.02
6	0.55	0.35	0.10

Each experimental response, Y_i, is a function of the three variables (the solvents) in the investigated region. To detect any curvature within the domain studied, the model postulated for the percentages of the solvents is a Scheffè quadratic polynomial function:

$$Y_\mathrm{i} = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \varepsilon$$

where: Y_i is the analysis time ($k_{\max}$) or selectivity ($\alpha_{\min}$, α_{average}/S.D., α_{ji}); ε the experimental error. All other chromatographic parameters (flow rate, temperature, etc.) have been kept constant during the experiments. The selected experimental matrix consists of six experiments (Table 2).

The design space can be expressed as an equilateral triangle whose apexes are the three points representing the pure solvents. Each of the mixture design points (defined by the coordinates X_1, X_2, X_3) represents a ternary eluent system.

To facilitate the interpretation of the regression coefficients, the mixture coordinates are reported after transformation of the original variables (X_i) to pseudocomponents (x_i'), obtained through the following linear transformation:

$$x_i' = \frac{(x_i - a_i)}{R_a}$$

where a_i is the lower limit of the component i ($i = 1, 2, \ldots, q$) and $R_a = 1 - \sum_1^q a_i$.

The obtained sub-region can be described by an equilateral triangle of smaller dimensions, whose apexes are defined as *pseudocomponents* x_i' (Fig. 2).

The apexes x_1', x_2', x_3', correspond to the mixtures: buffer:ACN 81:19 (v/v); buffer:ACN 50:50 (v/v), buffer:ACN:MeOH 50:19/31 (v/v) respectively. The experimental matrix, defined in pseudocomponents, is shown below (Table 3).

Table 3
Experimental matrix in pseudocomponents

Exp. no.	x_1'	x_2'	x_3'
1	0.074	0.670	0.323
2	0.090	0.323	0.586
3	0.271	0.243	0.485
4	0.300	0.540	0.160
5	0.137	0.804	0.059
6	0.161	0.516	0.323

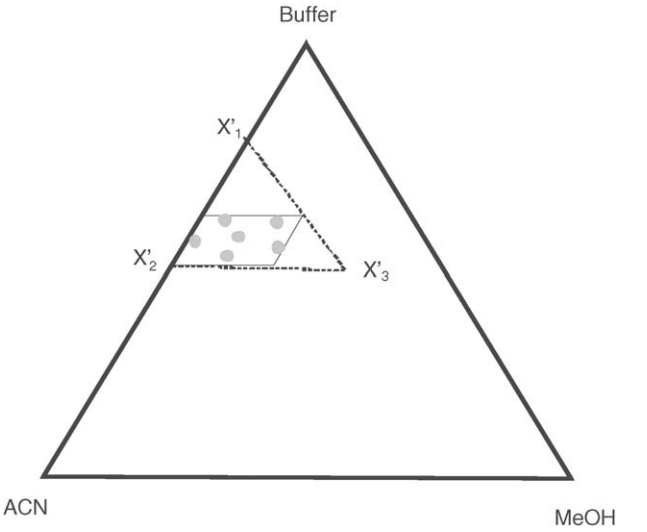

Fig. 2. Graphical display of pseudocomponents in the sub region mixture domain.

3.4. Design for process variables

Process variables (buffer molarity and pH) are factors whose levels can be varied independently one from the other. Although they do not represent mixture components, the change of their levels may affect the properties of eluent systems. Temperature is another process variable that has not been considered here.

A classical equiradial design, was selected to study the influence of the process variables. Each experimental response, Y_i, can be represented by a quadratic polynomial equation:

$$Y_i = \beta_0 + \beta_4 X_4 + \beta_5 X_5 + \beta_{44} X_4^2 + \beta_{55} X_5^2 + \beta_{45} X_4 X_5 + \varepsilon$$

where X_4 represents the buffer concentration, X_5 corresponds to pH values, Y_i is the experimental response, analysis time ($k_{\max}$) or selectivity ($\alpha_{\min}, \alpha_{\text{average}}$/S.D., α_{ji}), ε is the experimental error.

The experimental domain can be defined as follows:

- buffer concentration can vary between 0.01 and 0.05 M;
- pH range can be set between 2.5 and 6.5.

The experimental domain of spherical shape is described by natural variables, as follows:

Process variable	Centre	Variation step
U4		
Buffer concentration (M)	0.03	0.02
U5		
pH	4.5	2

An equiradial design with a classical pentagonal arrangement was selected. It consists of six experiments (Table 4, Fig. 3).

This design presents a uniform distribution of experimental points and the two variables X_4 and X_5 are examined at 4 and 5 different levels, respectively.

Table 4
Experiments with process variables

No.	X_4	X_5
1	1.000	0.000
2	0.309	0.951
3	−0.809	0.588
4	−0.809	−0.588
5	0.309	−0.951
6	0.000	0.000

3.5. Combined experimental design

To study the effects of all variables (solvent proportions, pH and ionic strength) on the method selectivity, the process variables were incorporated into the mixture experiments. The resulting combined design was obtained by crossing the three-component mixture design with a classical pentagonal arrangement for the two process parameters. The graphical representation of the experiments is displayed in Fig. 4. This type of experimental design seems to be the best choice to detect the interaction existing between solvent systems and pH.

3.6. Combined mathematical model

Data generated in the experiments are fitted by a single model incorporating the blending properties of the mixture components and the effects of the other factors. In order to fit a mathematical model to the description of the response variables as a function of process variables and mixture components, Scheffè quadratic polynomial for a three-component mixture (Eq. (1)) was multiplied by the second-order equation for the two process factors (Eq. (2)); the result of the procedure is a single complete combined model with 36 coefficients thus yielding 36 eluent systems.

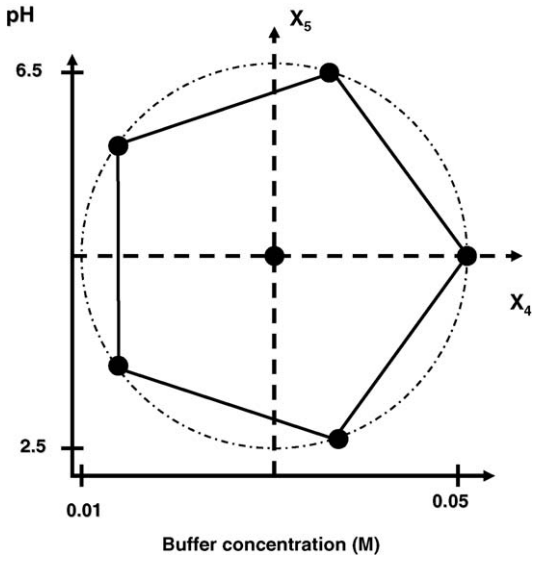

Fig. 3. Design for process variables, graphical representation of the experimental points in natural variables.

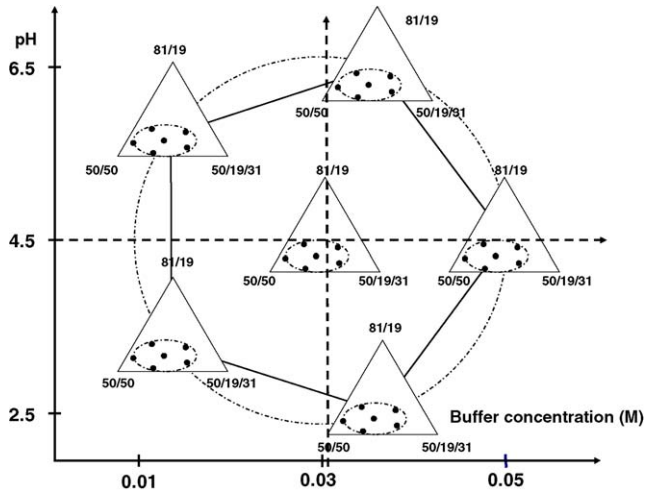

Fig. 4. Graphical display of combined experimental design.

$$
\begin{aligned}
Y_i = {} & b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 \\
& + b_{23}X_2X_3 + b_{41}X_4X_1 + b_{42}X_4X_2 + b_{43}X_4X_3 \\
& + b_{412}X_4X_1X_2 + b_{413}X_4X_1X_3 + b_{423}X_4X_2X_3 \\
& + b_{51}X_5X_1 + b_{52}X_5X_2 + b_{53}X_5X_3 + b_{512}X_5X_1X_2 \\
& + b_{513}X_5X_1X_3 + b_{523}X_5X_2X_3 + b_{441}X_4X_4X_1 \\
& + b_{442}X_4X_4X_2 + b_{443}X_4X_4X_3 + b_{4412}X_4X_4X_1X_2 \\
& + b_{4413}X_4X_4X_1X_3 + b_{4423}X_4X_4X_2X_3 + b_{551}X_5X_5X_1 \\
& + b_{552}X_5X_5X_2 + b_{553}X_5X_5X_3 + b_{5512}X_5X_5X_1X_2 \\
& + b_{5513}X_5X_5X_1X_3 + b_{5523}X_5X_5X_2X_3 + b_{451}X_4X_5X_1 \\
& + b_{452}X_4X_5X_2 + b_{453}X_4X_5X_3 + b_{4512}X_4X_5X_1X_2 \\
& + b_{4513}X_4X_5X_1X_3 + b_{4523}X_4X_5X_2X_3 \qquad (1)
\end{aligned}
$$

where:

$b_i = \beta_i + \varepsilon$

Y_i is the analysis time (k_{max}) or selectivity (α_{min}, $\alpha_{average}$/S.D., α_{ji})

3.7. Application of desirability function in a combined experimental design

In our study, an innovative chemometric approach has been applied for the selection of the optimum separation conditions by using Derringer's desirability function in a combined experimental design. The basic idea of the desirability function approach is to transform a multiple response matter into a single response matter by means of mathematical transformations.

The measured properties of each response Y_i, ($i = 1, 2, \ldots, m$), are transformed to a dimensionless desirability scale (d_i), defined as *partial desirability function*. It is thus possible to combine the results obtained for responses measured on different scales. The range of the desirability function lies between $d = 0$, for a com-

plete undesirable response, and $d = 1$, if the response is at the target value. The target values for the experimental responses of this study were the following: $\alpha_{min} > 1.1$ and $k_{max} < 11$. The responses were transformed into appropriate desirability scales d_1 and d_2, with the requirement that analysis time must be minimized, while selectivity must be maximized.

Once the function d_i is defined for each of the m responses of interest, an overall objective function (D), representing the *global desirability function*, is calculated by determining the geometric mean of the individual desirabilities.

Therefore, the function D over the experimental domain is calculated as follows:

$$D = \left(\prod_{i=1}^{m} d_i\right)^{1/m}$$

Taking into account all requirements for m responses, we can select the design variable conditions that maximize D.

A D value different from zero implies that all responses are in a desirable range simultaneously, while a D value close to 1 indicates that the combination of the different criteria is globally optimal, so that the response values are near target values.

4. Results and discussion

As was pointed out in a previous paper [14], a potassium phosphate buffer was selected as aqueous mobile phase. Preliminary experiments demonstrated the difference between sodium and potassium ions in phosphate salts. With the same salt concentration, the retention factor of haloperidol was divided by two (4.34–2.38) and the retention factor of levomepromazine experienced a 1/3 decrease (6.47–4.09) by using a potassium ion. In the same paper, we observed some shifts in retention when ternary mixtures were used as eluent. In preliminary experiments with acetonitrile/buffer binary mixtures, we observed that all solutes behave similarly which precludes any change in selectivity from varying a binary eluent composition. The variation of the retention factor in binary solvent systems (acetonitrile:potassium phosphate buffer) followed a quadratic equation:

$$\log k = a\varphi^2 + b\varphi + c$$

where φ is the percentage of acetonitrile and the c term corresponds to $\log k_w$ (the hypothetical retention in pure water).

Since the observed coefficients a, b, and c, are very close, resolution is often poor and co-elution of some solutes occurs with such binary mixtures. Following our previous experience it is worth investigating the addition of methanol. In particular, by increasing the methanol content the desmethylated derivatives of phenothiazines do not follow the same trend as their parent compounds. Furthermore in another paper [15], we observed that desmethylated derivatives of phenothiazines are eluted earlier than the parent compound when acetonitrile is the organic modifier and later than the parent compound when methanol is the organic modifier. With a ternary mixture of solvents (acetonitrile, methanol, phosphate buffer) a fine selectivity tuning may be obtained.

Isocratic elution was only considered and not gradient elution. Both techniques provide reproducible retention times. However, optimization with a gradient should include the dwell volume of the instrument [16]. From the literature, gradients with ternary mixture of solvents are not very popular and chemometric approaches to such gradients have not yet been reported. Furthermore, such gradients with ternary mixture including a phosphate buffer would require a re-equilibration time of the column that would make the analysis time rather long.

Mixture design and data processing were obtained using Nemrod software. Experimental runs (from the 36 experiments, Section 3.6) have been performed in a completely random order according to the combined design. Experimental data corresponding to the retention factor values for each drug injected alone (k_i) were recorded.

Table 5 lists the selectivity (consecutive peaks) and the highest retention factor observed with every experiment.

Validation of the model through ANOVA demonstrated that the 36-term model could be reduced to 33 terms. The F-ratio

Table 5
Experimental selectivity values (α_{min}) and analysis time (k_{max}) calculated for each chromatographic condition

Exp. no.	%Buffer	%ACN	%MeOH	Buffer molarity	pH	α_{min}	k_{max}
1	0.50	0.40	0.10	0.05	4.5	1.04	6.00
2	0.50	0.40	0.10	0.036	6.4	1.00	31.14
3	0.50	0.40	0.10	0.01	5.7	1.06	27.60
4	0.50	0.40	0.10	0.01	3.3	1.06	5.62
5	0.50	0.40	0.10	0.036	2.6	1.00	3.12
6	0.50	0.40	0.10	0.03	4.5	1.00	7.75
7	0.53	0.29	0.18	0.05	4.5	1.06	8.17
8	0.53	0.29	0.18	0.036	6.4	1.13	39.75
9	0.53	0.29	0.18	0.01	5.7	1.02	41.57
10	0.53	0.29	0.18	0.01	3.3	1.04	8.62
11	0.53	0.29	0.18	0.036	2.6	1.00	4.78
12	0.53	0.29	0.18	0.03	4.5	1.09	10.68
13	0.58	0.27	0.15	0.05	4.5	1.11	13.94
14	0.58	0.27	0.15	0.036	6.4	1.08	62.86
15	0.58	0.27	0.15	0.01	5.7	1.05	60.14
16	0.58	0.27	0.15	0.01	3.3	1.12	13.00
17	0.58	0.27	0.15	0.036	2.6	1.00	7.50
18	0.58	0.27	0.15	0.03	4.5	1.02	16.25
19	0.59	0.36	0.05	0.05	4.5	1.00	10.81
20	0.59	0.36	0.05	0.036	6.4	1.00	43.42
21	0.59	0.36	0.05	0.01	5.7	1.12	57.00
22	0.59	0.36	0.05	0.01	3.3	1.08	10.86
23	0.59	0.36	0.05	0.036	2.6	1.06	6.89
24	0.59	0.36	0.05	0.03	4.5	1.15	15.66
25	0.54	0.44	0.02	0.05	4.5	1.04	7.60
26	0.54	0.44	0.02	0.036	6.4	1.10	36.71
27	0.54	0.44	0.02	0.01	5.7	1.01	35.57
28	0.54	0.44	0.02	0.01	3.3	1.11	7.26
29	0.54	0.44	0.02	0.036	2.6	1.03	4.06
30	0.54	0.44	0.02	0.03	4.5	1.00	10.70
31	0.55	0.35	0.10	0.05	4.5	1.04	9.25
32	0.55	0.35	0.10	0.036	6.4	1.09	41.80
33	0.55	0.35	0.10	0.01	5.7	1.06	42.14
34	0.55	0.35	0.10	0.01	3.3	1.08	8.12
35	0.55	0.35	0.10	0.036	2.6	1.02	5.11
36	0.55	0.35	0.10	0.03	4.5	1.00	12.46

Table 6
Analysis of the experimental response: (a) α_{min} and (b) k_{max} variance

Source of variation	Sum of squares (SS)	Degrees of freedom	Mean square (MS)	*F*-ratio	Significance
α_{min} variance					
Regression	0.0690	32	0.0022	8.8064	4.94*
Residual	0.0007	3	0.0002		
Total	0.0698	35			
k_{max} variance					
Regression	1.102E+0004	32	3.444E+0002	10.750	3.77*
Residual	9.610E+0001	3	3.203E+0001		
Total	1.111E+0004	35			

* $\alpha < 5\%$ (significance of the test alpha < 5%).

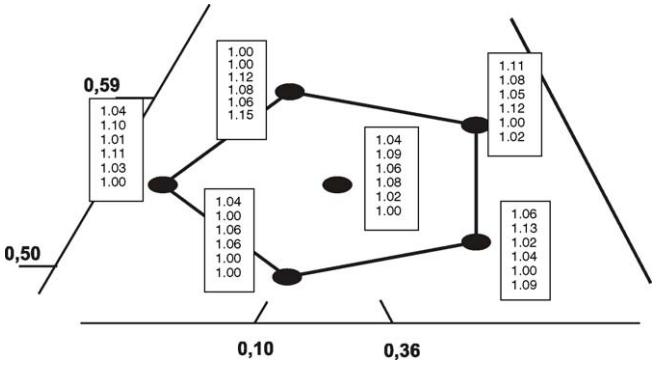

Fig. 5. Description of the response Y_i (as α_{min}) at the six buffer concentration (U1) and pH (U2) combinations. The six values at each point refer to the six ternary mobile phases from Table 2.

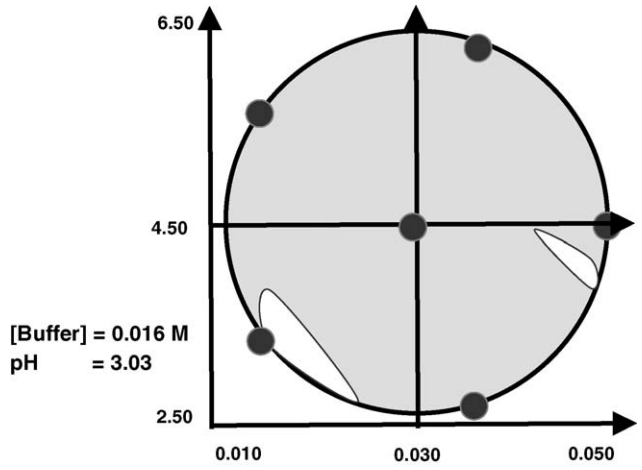

Fig. 6. Desirability function. Selection of optimal conditions: (a) buffer concentration 0.016 M/pH 3.03 and (b) mobile phase:buffer:acetonitrile:methanol: 58:23:19 (v/v/v).

relative to the regression model is significant for the two experimental responses as can be seen in Table 6.

For the sake of simplicity, estimated b'coefficients are not reported. Fig. 5 describes the response (α_{min}) in the six buffer concentrations and pH and in mixture domain respectively. It must be kept in mind that α_{min} does not represent the selectivity between the same peaks. In the cases of combined matrixes, the multicriteria optimization method used is the graphical analysis of the isoresponse graphs. In our case it will be tedious to deal with several responses and a large number of graphics. We overcame the bottleneck with the desirability function.

The coefficients of the model were calculated on the basis of the experimental responses by least squares regression. The optimum separation conditions were predicted using the desirability function as reported in Table 6. As shown in Table 7, the optimum conditions were obtained with a global degree of satisfaction of D for the three responses equal to 45.6%.

To find useful chromatographic conditions with a higher global degree of satisfaction, the desirability behavior for the selected ternary mixture as a function of buffer molarity and pH was investigated. A graphical representation is shown in Fig. 6 that exhibits two possible conditions. An optimal zone for selectivity can be found at pH values near 4.5 and for high buffer concentrations. These analytical conditions, experimentally validated, are not very useful since analysis time is longer than 40 min. For this reason the analytical setting corresponding

Table 7
Coordinates of the optimum

Variable	Factor	Optimal value
X_1	Phosphate buffer (v/v, %)	58
X_2	ACN (v/v, %)	23
X_3	MeOH (v/v, %)	19
X_4	Buffer concentration (M)	0.016
X_5	pH	3.03

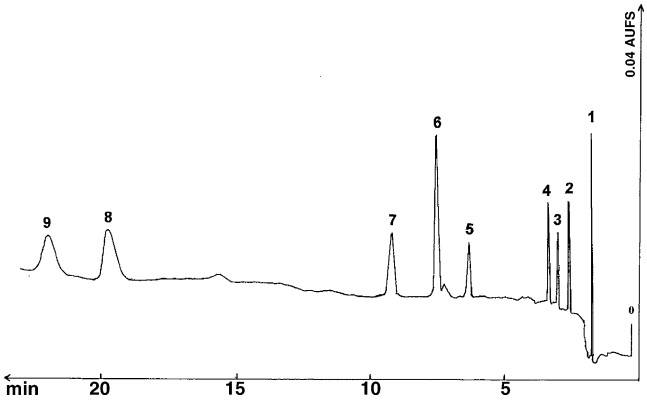

Fig. 7. Chromatogram of the mixture with optimal conditions. Column CH 250 mm × 4.6 mm flow rate 0.9 ml/min, temperature 30 °C. Order of retention: *O*-desmethyl venlafaxine; 9-hydroxy-risperidone; venlafaxine; risperidone; carbamazepine; reduced haloperidol; haloperidol; N-desmethyl levomepromazine; levomepromazine.

to 3.03 pH value and 0.016 M buffer concentration, was preferred despite to its lower robustness.

The obtained optimal conditions were experimentally verified and are displayed in Fig. 7. The determined analytical method enabled a satisfactory separation for haloperidol, levomepromazine, risperidone, venlafaxine, carbamazepine and their corresponding metabolites to be carried out in an acceptable analysis time (less than 30 min). This analysis time can be reduced with an increase in temperature, which produces an increase in diffusion coefficients, an increase in the sorption-desorption kinetics and a decrease in eluent viscosity.

5. Conclusion

It is always tedious to handle as many variables as those in mobile phase and buffer composition to find the best chromatographic conditions. Two routes are possible: either to use a predictive model based on sound theoretical background or to use a chemometric approach. The analyst does not at first know the elution order of the solutes. Furthermore, this order may change. In the first route, some parameters must be determined and preliminary experiments should be carried out to determine the variations in k from the equations. To our knowledge there are no published reports of any optimization procedure involving a ternary mixture of solvents together with a buffer based on a model. In this chemometric approach two types of variables were selected: the mixture components and the process variables. The informative approach selects experiments, which give real information. There is no need for a good separation, the only requirement is peak identification. From the informative experiment an experimental design is set up which yields two graphical descriptions of the selected response. Use of desirability function in a combined experimental design simplifies the data interpretation and allows the determination of proper conditions for separation. The shortcoming of the chemometric approach is the number of experiments and the time required.

References

[1] A.J. Wagstaff, H.M. Bryson, CNS Drugs 4 (1995) 370–387.
[2] M. Berck, Eur. Neuropsychopharmacol. Suppl. 4 (1999) 119–127.
[3] M. Aravagiri, S.R. Marder, T. Van Putten, B.D. Marshall, J. Chromatogr. B 656 (1994) 373–381.
[4] H. Hoja, P. Marquet, B. Verneuil, H. Lotfi, J.L. Dupuy, B. Penicaut, G. Lachatre, J. Chromatogr. B 688 (1997) 275–280.
[5] A.L. Lerena, R. Berecz, P. Dorado, C. Sanz de la Garza, M.J. Norberto, M. Cáceres, J.R. Gutiérrez, J. Chromatogr. B 783 (2003) 213–219.
[6] A. Avenoso, G. Facciola, M. Salemi, E. Spina, J. Chromatogr. B 746 (2000) 173–181.
[7] O.V. Olesen, K. Linnet, J. Chromatogr. B 698 (1997) 209–216.
[8] G. Aymard, P. Livi, Y.T. Pham, B. Diquet, J. Chromatogr. B 700 (1997) 183–189.
[9] M. Rittner, F. Pragst, W.R. Bork, J. Neumann, J. Anal. Toxicol. 25 (2001) 115–123.
[10] C. Frahnert, M.L. Rao, K. Grasmäder, J. Chromatogr. B 794 (2003) 35–47.
[11] Y. Gaillard, G. Pepin, J. Chromatogr. A 763 (1997) 149–163.
[12] E.M. Koves, J. Chromatogr. A 692 (1995) 103–119.
[13] Y. Mizuno, K. Sato, T. Sano, R. Kurihara, T. Kojima, Y. Yamakawa, A. Ishii, Y. Katsumata, Legal Med. 4 (2002) 207–216.
[14] M. Beljean, A.M. Siouffi, J. Chromatogr. Sci. 39 (2001) 229–234.
[15] L.D. Chi, M. Beljean, A.M. Siouffi, J. Chromatogr. Sci., in press.
[16] A.P. Schelinger, P.W. Carr, J. Chromatogr A 1077 (2005) 110–119.