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Taguchi design experiments for optimizing the gas chromatographic analysis of residual solvents in bulk pharmaceuticals

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ABSTRACT

Taguchi design experiments were conducted in order to identify and optimize the parameters that yield a maximum separation of 26 solvents commonly found in bulk pharmaceuticals. Wide-bore columns with chemically cross-linked stationary phases of different polarities were retained owing to their sensitivity and specificity in optimizing the analysis via a simple response function based on information theory.

INTRODUCTION

For several years, the search for a general method applicable to the evaluation of solvent residues in bulk pharmaceuticals has received great attention. Several procedures based on packed-column or wide-bore column separation have been published [1–4]. The US and European Pharmacopoeias propose standard procedures employing porous polymer packings or silicone phases [5,6] with direct injection or headspace sampling.

These techniques require a high-boiling solvent such as octanol or benzyl alcohol to dissolve the sample. The solubility of pharmaceuticals in these solvents is poor, $\leqslant 1\%$, thus adversely affecting the detection limits. After each analysis it is necessary to maintain the column at its highest temperature limit to eliminate the dissolving solvent and its impurities. Benzyl alcohol contains by-products such as methanol, toluene, oxidation products and benzene generated during injection. Dimethylformamide, which has been termed the universal organic solvent, seems to be a better choice.

Many strategies for the optimization of gas chromatography have been used, such as the sequential simplex method [7,8], window diagrams [9], response surface method [10] and computer-simulation techniques [11–14]. They all suffer from the poor number of parameters tested or from a difficulty in calculating the response function, often imprecise owing to uncertainties in the measurement of data.

For any optimization strategy, a mathematical function must be defined to reflect the quality of a chromatogram as a single number. Optimization then becomes a process of maximizing (or possibly minimizing) the numerical value of this function. It is a challenge to capture adequately the chromato-

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grapher's perception of quality with a mathematical expression and, consequently, considerable effort has been devoted to the development of the response function.

Response functions such as [15]

$$Ct = \sum R_i - b(t_w - t_{max}) - d(t_{min} - t_\alpha)$$

$$\prod R_s = \prod R_i$$

where

R_i = resolution between two adjacent peaks
t_{min} = minimum time required for analysis
t_{max} = maximum time acceptable
t_α = retention time of the first peak
t_w = retention time of the last peak
b and d = weighing factors

give numbers without dimensions increasing with the quality of the chromatogram. In fact, the increase is not necessarily correlated with an improvement of the separation between two adjacent peaks, $\sum R_i$ and $\prod R_i$ being strongly dependent on the chromatograms to be compared (retention times of the same order are necessary to have R_i of the same type). The interval of variation of the response function, *i.e.*, minimum and maximum, is often not known, particularly the maximum. Some workers [16] have introduced functions such as *CRF* and *COF* that remove this last drawback:

$$CRF = \sum_{i=1}^{k} \ln P_i$$

where P_i is a measure of separation between adjacent peaks

$$COF = \sum_{i=1}^{k} A_i \ln(R_i/R_{id}) + B(t_M - t_L)$$

where R_i is the resolution of the ith pair, R_{id} is the desired resolution and A_i and B are weighing factors.

All these disadvantages, mainly the uncertainties in the measurement of the resolution, the long time necessary to analyse the chromatogram and the unknown maximum of the response functions already used, convinced us to use a response function based on information theory [15,17,18], in conjunction with Taguchi design experiments.

Orthogonal array designs are used to assign factors, *i.e.*, the analytical parameters (in our case, column, temperature, flow-rate, ...) to a series of experimental combinations whose results then can be treated by a common mathematical procedure to extract independently the main effects of these factors. Emphasis is placed on identifying controlling factors and quantifying the magnitude of effects rather than just identifying statistically significant effects.

Taguchi has simplified the application of design experiments by using a standardized library of basic designs [19,20] called orthogonal arrays along with some simple methods to modify these layouts to fit individual situations.

THEORY

Optimization criteria

Let a mixture of n components be separated by gas–liquid chromatography (GLC). The resulting chromatogram is composed of k_1 singlets, k_2 doublets, ..., k_p p-uplets with $\sum_p pk_p = n$. The quantity of specific information brought by the identification of one component is

$$Q_1 = \log_2(n/1)$$

The appearance frequency is

$$F_1 = k_1/n$$

F_1 is also the probability of one component being present as a singlet, postulating that each component could be present anywhere in the chromatogram.

The contribution of the singlets to the quantity of information is given by

$$I_1 = F_1 Q_1 = (k_1/n)\log_2(n/1)$$

In the same way, the contribution of the doublets is

$$I_2 = F_2 Q_2 = (2k_2/n)\log_2(n/2)$$

The total information brought by the chromatogram is the sum of the individual contributions of each peak groups:

$$IC = \sum_p (pk_p/n)\log_2(n/p)$$

where n is the total number of components to be separated. This function varies between zero, all the peaks together ($p = n$, $k_p = 1$) and $\log_2(n)$, all the peaks separated ($p = 1$, $k_p = n$).

For instance, let a mixture of eight components be

separated. If the resulting chromatogram is composed of four singlets and two doublets, then

$$IC = 4 \times 1/8 \times \log_2(8/1) + 2 \times 2/8 \times \log_2(8/2) = 3.5$$

EXPERIMENTAL

Materials

Samples of solvents were obtained from Merck (Nogent sur Marne, France), Prolabo (Paris, France), Riedel-de Haën (Seelze, Germany). Dimethylformamide was of high-purity grade from Merck (>99.8%)

Apparatus and conditions

The method was developed on a Varian 3400 gas chromatograph equipped with a flame ionization detector, a Varian Model 8035 autosampler and Model OCA-4/90 insert from Scientific Glass Engineering (Villeneuve-Saint-Georges, France). Chromatograms were recorded on a Spectra-Physics Chromjet system. Hydrogen and air flow-rates were 37 and 260 ml/min, respectively, and the carrier gas was nitrogen with a make-up of 30 ml/min.

The columns were 50 m × 0.53 mm I.D. fused-silica columns (Chrompack, Les Ulis, France) with the thickest chemically cross-linked stationary phases available: CP Sil 5CB and CP Sil 8CB with a 5-μm film thickness and CP Sil 13CB and CP Wax 52CB with a 2-μm film thickness.

Procedure

The standard solution used in the optimization step was prepared by dissolution in dimethylformamide at 0.02% (v/v) for each solvent. Volumes of 0.4 μl were injected. The injector and detector temperature were 130 and 250°C, respectively.

RESULTS AND DISCUSSION

The experiment is designed to determine the effect of operating conditions on the separation of 26 different solvents commonly found in bulk pharmaceuticals. A number of analytical variables influence the chromatographic results obtained for a mixture of solvents. Six main injection variables affect the solvent peak shape and resolution when using 0.53 mm I.D. columns in a packed-column injection port: injector temperature, injector volume, solvent molecular weight, injection rate, sample size and flow-rate. The column parameters length, diameter, stationary phase film thickness and type of the stationary phase coating and the oven temperature programme must also be considered.

A large number of parameters can be optimized but previous experimental results [21] have indicated that the column flow and the oven temperature programme, namely the initial oven temperature, initial time, programming rate and final oven temperature, are particularly likely to affect the quality of the chromatogram. Parameters such as polarity are difficult to introduce in design experiments because one cannot assign a significant continuous number to a polarity, thus preventing the optimization step.

In order to test these five factors and the four interactions with the flow-rate, a screening experiment was conducting using an L16 (2^{15}) Taguchi orthogonal array. This fractional factorial design, at two levels, labelled (1) and (2), (Table I), is time saving in this initial step for screening five potentially important factors. A factor at two levels corresponds to one degree of freedom (degree of freedom equals the number of levels minus one). Each interaction between two factors at two levels corresponds to one times one degree of freedom. Therefore, five factors at two levels and four interactions equal nine degrees of freedom. Consequently, the L16 orthogonal array (fifteen degrees of freedom) is the minimum fractional array to choose. Each factor is assigned to a column according to the linear graph for this Taguchi orthogonal array (Fig. 1). Circles are for the main factors and the lines between them permit the estimation of the interactions (*i.e.*, if flow-rate and initial temperature are assigned to columns 1 and 2, respectively, then column 3 contains the interaction between flow-rate and initial temperature).

The sixteen experiments were done following Table II, where flow-rate is assigned to column 1, initial temperature to column 2, initial time to column 8, programming rate to column 12 and final temperature to column 6. For instance, trial 10 corresponds to flow-rate 5.3 ml/min, initial temperature 25°C, initial time 17 mn, programming rate 15°C/min and final temperature 90°C.

In such experiments a direct estimate of the

TABLE I

L16 TAGUCHI ORTHOGONAL ARRAY

Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
3	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2
4	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1
5	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2
6	1	2	2	1	1	2	2	2	2	1	1	2	2	1	1
7	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1
8	1	2	2	2	2	1	1	2	2	1	1	1	1	2	2
9	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
10	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1
11	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1
12	2	1	2	2	1	2	1	2	1	2	1	1	2	1	2
13	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1
14	2	2	1	1	2	2	1	2	1	1	2	2	1	1	2
15	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2
16	2	2	1	2	1	1	2	2	1	1	2	1	2	2	1

experimental error is not calculable because for each trial only one experiment has been done. Therefore, the residual variance was calculated with the sum of squares of columns that have not been assigned (columns 4, 5, 10, 11, 14, 15). These columns correspond to interactions with a very low probability of occurrence.

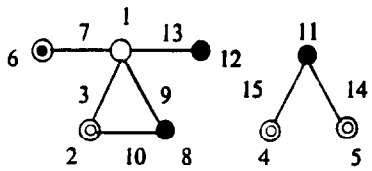

Fig. 1. Linear graph.

An analysis of variance table with pooled errors was constructed from individual contribution (*IC*) data, and it indicated that the factors initial time and final oven temperature are statistically significant at the 99.5% confidence level and initial oven temperature and programming rate at the 95% level. Column flow and the interactions between flow and the other parameters have no influence on the separation of the 26 solvents (Table III).

The most significant effects contributing to the output signal were the initial time (31.3%) and the

TABLE II

L16 TAGUCHI ORTHOGONAL ARRAY

Trial	1[a]	2[a]	3	4	5	6[a]	7	8[a]	9	10	11	12[a]	13	14	15	*IC*
1	1	1				1		1				1				4.3347
2	1	1				1		2				2				4.4406
3	1	1				2		1				2				4.0270
4	1	1				2		2				1				4.2868
5	1	2				2		1				1				4.1039
6	1	2				2		2				2				4.1039
7	1	2				1		1				2				4.1808
8	1	2				1		2				1				4.3347
9	2	1				1		1				1				4.2578
10	2	1				1		2				2				4.2578
11	2	1				2		1				2				3.9980
12	2	1				2		2				1				4.2868
13	2	2				2		1				1				4.0749
14	2	2				2		2				2				4.1808
15	2	2				1		1				2				4.0749
16	2	2				1		2				1				4.2578

[a] Column 1: Flow-rate — level 1: 2 ml/min — level 2: 5.3 ml/min
Column 2: Initial temperature — level 1: 25°C — level 2: 60°C
Column 8: Initial time — level 1: 6 min — level 2: 17 min
Column 12: Programming rate — level 1: 4°C/min — level 2: 15°C/min
Column 6: Final temperature — level 1: 90°C — level 2: 140°C

TABLE III

VARIANCE ANALYSIS

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio[a]	Contribution (%)[b]
Flow-rate	$1.123 \cdot 10^{-2}$	1	$1.123 \cdot 10^{-2}$	3.93	4.7
Initial temperature	$2.086 \cdot 10^{-2}$	1	$2.086 \cdot 10^{-2}$	7.30	8.7
Initial time	$7.525 \cdot 10^{-2}$	1	$7.525 \cdot 10^{-2}$	26.34	31.3
Final temperature	$7.249 \cdot 10^{-2}$	1	$7.249 \cdot 10^{-2}$	25.37	30.1
Programming rate	$2.835 \cdot 10^{-2}$	1	$2.835 \cdot 10^{-2}$	9.92	11.8
Flow-rate/final temperature	$1.331 \cdot 10^{-2}$	1	$1.331 \cdot 10^{-2}$	4.66	5.5
Flow-rate/initial temperature	$1.479 \cdot 10^{-3}$	1	$1.479 \cdot 10^{-3}$	0.52	0.6
Flow-rate/programming rate	$2.108 \cdot 10^{-4}$	1	$2.108 \cdot 10^{-4}$	0.07	0.1
Flow-rate/initial time	$2.108 \cdot 10^{-4}$	1	$2.108 \cdot 10^{-4}$	0.07	0.1
Pooled errors (columns 4,5,10, 11,14,15)	$1.714 \cdot 10^{-2}$	6	$2.857 \cdot 10^{-3}$		

[a] Critical variance ratio is 5.99 (95% confidence), 18.6 (99.5% confidence).
[b] Contribution is sum of squares / total sum of squares.

final oven temperature (30.1%). The next most significant factors were the programming rate (11.8%) and the initial oven temperature (8.7%).

Fig. 2 shows the effect of significant factors on the response function. A low initial oven temperature, programming rate and final oven temperature will improve the response function the most. Changing the initial time from a low to a high level will also increase the response function.

The L16 orthogonal table enabled us to choose which factors are to be included in the method for determining the optimum combination of factor levels. With the important factors (initial temperature, initial time, programming rate, final oven temperature) a full factorial design at two levels (Table IV) is used to optimize the experimental conditions. Column flow-rate was left at the low level corresponding to the best HETP value. A model can be developed that relates the design variables to the measurement of experimental behaviour. This can be done by using regression methods.

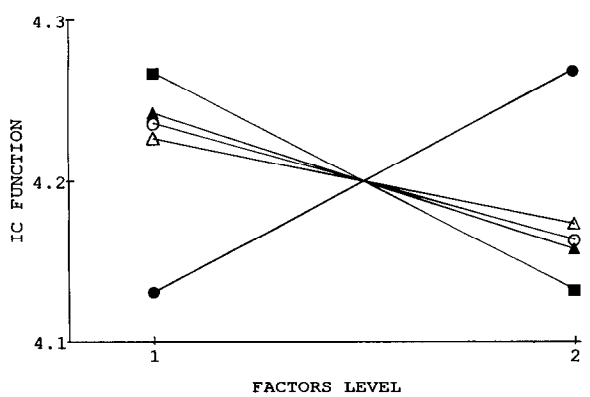

Fig. 2. Test of means (L16 Taguchi table). △ = Flow-rate (2 and 5.3 ml/min); ○ = initial temperature (25 and 60°C); ■ = final temperature (90 and 140°C); ● = initial time (6 and 17 min); ▲ = programming rate (4 and 15°C/min).

The method of steepest ascent was proposed by Box and Wilson [22]. The maximum is located by means of a series of experiments, each planned from the results of the preceding ones. First, a 2^4 full factorial design is chosen to fit a linear equation as an approximation to IC in the vicinity of the starting point.

The fitted linear equation in the coded scale is

$$IC = \overline{IC} + h_1(X_1) + h_2(X_2) + h_3(X_3) + h_4(X_4)$$

where $\overline{IC}$ is the mean of all the IC values, h_1 is the mean effect of the initial temperature, h_2 is the mean effect of the initial time, k_3 is the mean effect of the programming rate, h_4 is the mean effect of the final temperature and X_1–X_4 are the four factors being considered. The mean effect is calculated by dividing the main effect by 16, *i.e.*, the number of trials. The main effect is obtained as follows: for each trial, to the resulting IC is assigned the row sign (+ or −) corresponding to the column that permits the factor

TABLE IV

2^4 FULL FACTORIAL DESIGN

Trial	1[a]	2[a]	3[a]	4[a]	*IC*
1	−	−	−	−	4.33468
2	+	−	−	−	4.18083
3	−	+	−	−	4.44636
4	+	+	−	−	4.33468
5	−	−	+	−	4.18083
6	+	−	+	−	4.18083
7	−	+	+	−	4.44064
8	+	+	+	−	4.33468
9	−	−	−	+	4.25776
10	+	−	−	+	4.10391
11	−	+	−	+	4.28679
12	+	+	−	+	4.25776
13	−	−	+	+	4.02699
14	+	−	+	+	4.02699
15	−	+	+	+	4.10391
16	+	+	+	+	4.10391
Mean effect	$-5.486 \cdot 10^{-1}$	$1.010 \cdot 10^{-1}$	$-7.984 \cdot 10^{-1}$	$-1.260 \cdot 10^{-1}$	
Mean effect	$-3.429\ 10^{-2}$	$6.314 \cdot 10^{-2}$	$-4.990 \cdot 10^{-2}$	$-7.873 \cdot 10^{-2}$	

[a] Column1: initial temperature level −: 25°C level +: 60°C
Column 2: initial time level −: 6 min level +: 17 min
Column 3: programming rate level −: 4°C/min level +: 15°C/min
Column 4: final temperature level −: 90°C level +: 140°C

estimation and the sum is obtained. The equation becomes

$$IC = 4.225 - 3.429 \cdot 10^{-2}(X_1) + 6.314 \cdot 10^{-2}(X_2) - 4.990 \cdot 10^{-2}(X_3) - 7.873 \cdot 10^{-2}(X_4)$$

where

X_1	Initial oven temperature	X_2	Initial time
−1	25°C	−1	6 min
+1	60°C	+1	17 min
X_3	Programming rate	X_4	Final oven temperature
−1	4°C/min	−1	90°C
+1	15°C/min	+1	140°C

Then the direction of the steepest ascent is calculated for each factor by the formula $h(X^+ - X^-)/2$, where h is the mean effect obtained from the 2^4 design and X^+ and X^- are high and low levels of a factor X, respectively.

The progression steps δ are chosen in such a way that the final temperature step is 5°C. The different trials are realised from the centre of the domain studied (Table V). The *IC* value is calculated for each trial until an inversion of the function is found. This occurs for the conditions corresponding to the centre plus seven times the progression steps. The resulting chromatogram is presented in Fig. 3.

Three other columns were tested in the same way and the chromatograms obtained are presented in Figs. 4–6.

The best separation is obtained on a CP-Sil 13CB column with 24 solvents separated and two co-eluted, isopropanol and diethyl ether. On the CP-Sil 8CB column the 26 solvents are visible with two groups of three peaks, acetone, isopropanol and acetonitrile and hexane, methyl ethyl ketone and diisopropyl ether. The CP-Wax 52CB column shows two groups of co-eluting solvents, 1,1,2-trichlorotrifluoroethane and diethyl ether and methanol and ethyl acetate. These three columns allowed a good separation within an analysis time between 60 and 90 min. On the other hand, the CP-Sil 5CB column shows a short analysis time of 30 min, but with a

TABLE V

OPTIMUM SEARCHING

Trial[a]	Initial temperature (°C) (δ = −1.5)[b]	Initial time (min) (δ = 0.9)[b]	Programming rate (°C/min) (δ = −0.7)[b]	Final temperature (°C) (δ = −5)[b]	*IC*
Centre	42.5	11.5	9.5	115	
Centre + 1Δ	41.0	12.4	8.8	110	4.10
Centre + 5Δ	35.0	16.0	6.0	90	4.33
Centre + 7Δ	32.0	17.7	4.6	80	4.44
Centre + 7.5Δ	31.3	18.3	4.3	77.5	4.36
Centre + 8Δ	30.5	18.7	3.9	75	4.36

[a] Centre = $(X^+ + X^-)/2$, where X^+ and X^- are high and low levels of a factor, X.
[b] δ (Final temperature) fixed at −5°C. $\delta = [-5h(X^+ - X^-)/2]/[-7.873 \cdot 10^{-2}\ (140\text{-}90)/2]$ for other factors.

poorer separation; ethyl acetate and diisopropyl ether co-elute in a group of four solvents and toluene co-elutes with dimethylformamide.

An interesting feature extracted from the analysis tables of the Taguchi arrays is the different behaviour of a strictly apolar phase compared with more or less polar phases. All the phases require low initial and final temperatures and a long initial isothermal time, but a non-polar phase, unlike polar phases, needs a rapid programming rate. This could be attributed to the fact that polar interactions (dipole–dipole or hydrogen bonding) decrease in strength with increasing temperature.

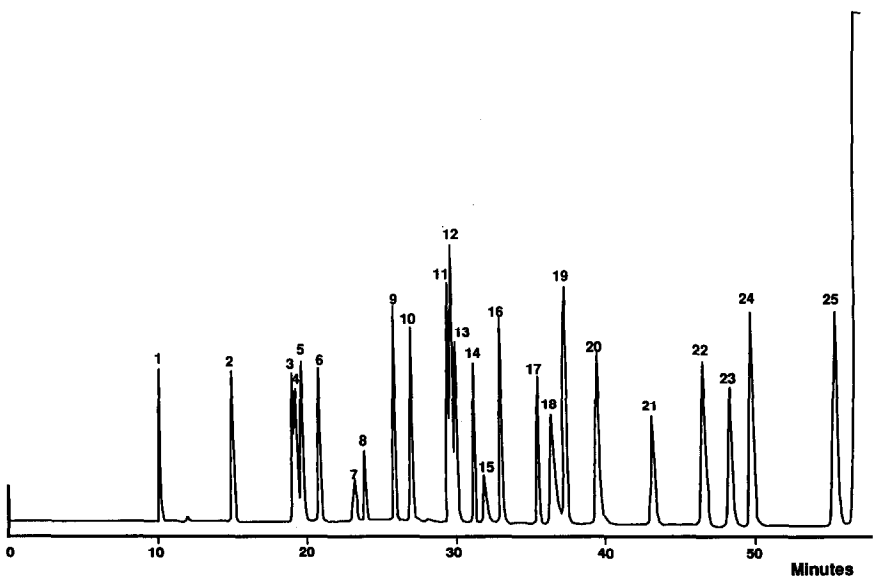

Fig. 3. Chromatogram obtained on a CP-Sil 8 CB column. Initial temperature, 32°C; initial time, 17.7 min; programming rate, 4.6°C/min; final temperature, 80°C; flow-rate, 2 ml/min. Peaks: 1 = methanol; 2 = ethanol; 3 = acetonitrile; 4 = acetone; 5 = isopropanol; 6 = diethyl ether; 7 = 1,1,2-trichlorotrifluoroethane; 8 = methylene chloride; 9 = *n*-propanol; 10 = ethyl acetate; 11 = methyl *tert.*-butyl ether; 12 = hexane; 13 = methyl ethyl ketone; 14 = diisopropyl ether; 15 = chloroform; 16 = tetrahydrofuran; 17 = methyl isobutyl ketone; 18 = *n*-butanol; 19 = cyclohexane; 20 = triethylamine; 21 = dioxane; 22 = methylcyclohexane; 23 = 1,2-dichloroethane; 24 = pyridine.

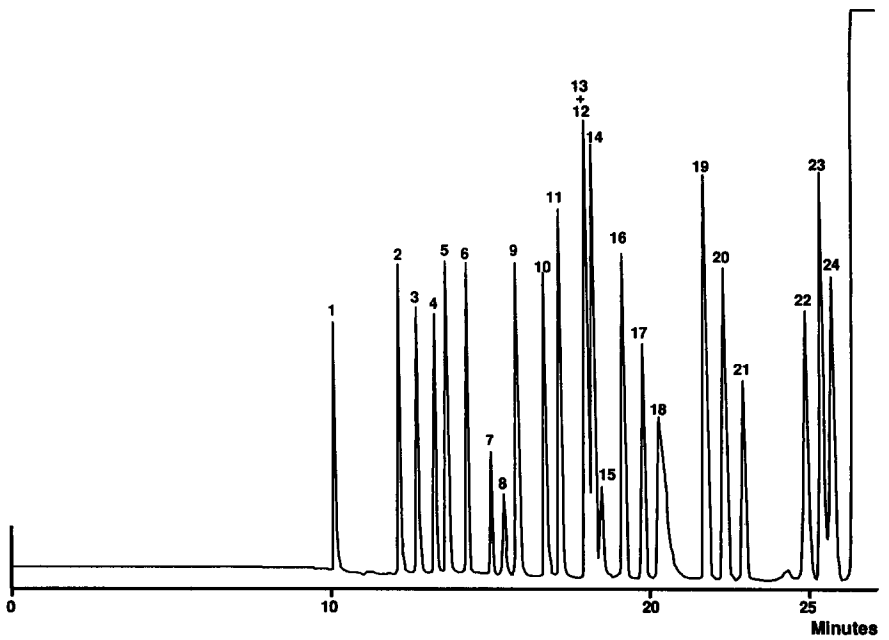

Fig. 4. Chromatogram obtained on a CP-Sil 5CB column. Initial temperature, 30°C; initial time, 5.7 min; programming rate, 12.8°C/min; final temperature, 110°C; flow-rate, 2 ml/min. Peaks: 1 = methanol; 2 = ethanol; 3 = acetonitrile; 4 = acetone; 5 = isopropanol; 6 = diethyl ether; 7 = methylene chloride; 8 = 1,1,2-trichlorotrifluoroethane; 9 = *n*-propanol; 10 = methyl *tert.*-butyl ether; 11 = methyl ethyl ketone; 12 = ethyl acetate; 13 = diisopropyl ether; 14 = hexane; 15 = chloroform; 16 = tetrahydrofuran; 17 = 1,2-dichloroethane; 18 = *n*-butanol; 19 = cyclohexane; 20 = triethylamine; 21 = dioxane; 22 = methyl isobutyl ketone; 23 = methylcyclohexane; 24 = pyridine; 25 = toluene.

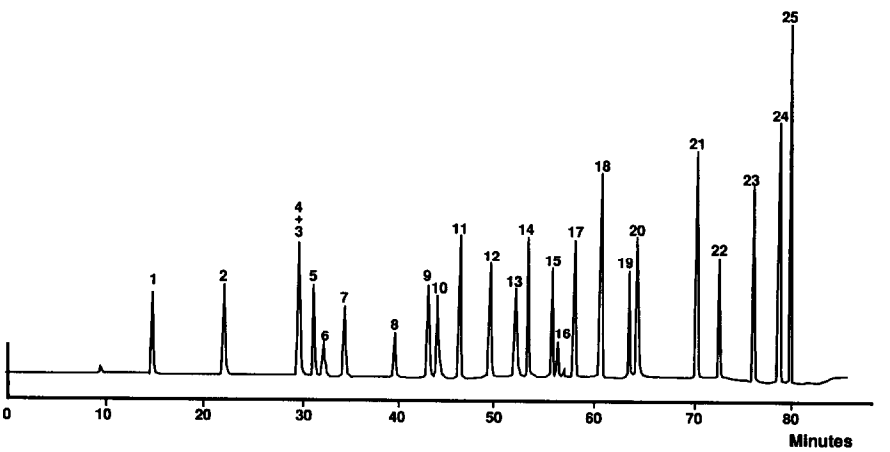

Fig. 5. Chromatogram obtained on a CP-Sil 13CB column. Initial temperature, 15°C; initial time, 30.5 min; programming rate, 1.7°C/min; final temperature, 142°C; flow-rate, 1.5 ml/min. Peaks: 1 = methanol; 2 = ethanol; 3 = diethyl ether; 4 = isopropanol; 5 = acetone; 6 = 1,1,2-trichlorotrifluoroethane; 7 = acetonitrile; 8 = methylene chloride; 9 = methyl *tert.*-butyl ether; 10 = *n*-propanol; 11 = hexane; 12 = diisopropyl ether; 13 = *n*-butanol; 14 = methyl ethyl ketone; 15 = ethyl acetate; 16 = chloroform; 17 = tetrahydrofuran; 18 = cyclohexane; 19 = 1,2-dichloroethane; 20 = triethylamine; 21 = methylcyclohexane; 22 = dioxane; 23 = methyl isobutyl ketone; 24 = pyridine; 25 = toluene.

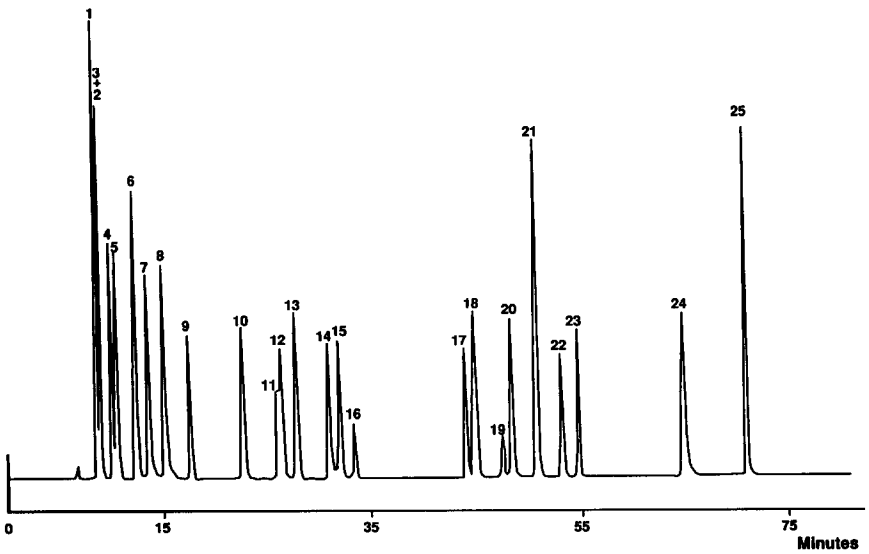

Fig. 6. Chromatogram obtained on a CP-Wax 52CB column. Initial temperature, 44°C; initial time, 23.5 min; programming rate, 1.1°C/min; final temperature, 128°C; flow-rate, 2 ml/min. Peaks: 1 = hexane; 2 = diethyl ether; 3 = 1,1,2-trichlorotrifluoroethane; 4 = diisopropyl ether; 5 = methyl *tert.*-butyl ether; 6 = cyclohexane; 7 = triethylamine; 8 = methylcyclohexane; 9 = acetone; 10 = tetrahydrofuran; 11 = methanol; 12 = ethyl acetate; 13 = methyl ethyl ketone; 14 = isopropanol; 15 = ethanol; 16 = methylene chloride; 17 = acetonitrile; 18 = methyl isobutyl ketone; 19 = chloroform; 20 = *n*-propanol; 21 = toluene; 22 = dioxane; 23 = 1,2-dichloroethane; 24 = *n*-butanol; 25 = pyridine.

CONCLUSION

The described method allowed us to optimize the separation of 26 solvents with a limited number of experiments for each column: sixteen for the L16 Taguchi table plus eight for the full factorial design. The experimental approach, unlike a traditional one (simplex method) is time saving and the simultaneous variation of all the studied factors and the study of their interactions is possible.

The best optimization could be retained as a screening method but owing to its long analysis time and low initial temperature, needing cryogenics, it could not be used as a routine analytical method.

However, a routine method could easily be extracted from the factorial design or from the modelling of *IC* to fit in with requirements such as time of analysis, detection limit or solvents for separation.

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