Experimental design for the optimization of the separation of aliphatic amines by ion chromatography

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ABSTRACT

The analysis of aliphatic amines in industrial solutions by reversed-phase chromatography presents some drawbacks owing to interferences with system peaks and other compounds in the sample. This technique is applicable only to simple mixtures. Ion chromatography, commonly used for inorganic ions, has been applied to the separation of aliphatic amines and offers the advantage of specificity for ionizable compounds and can be associated with conductivity detectors. The use of alkali metal ions as eluting ions improves the separation. The addition of an organic solvent to the aqueous mobile phase improves the efficiency for long-chain amines. Optimization using chemometric methodology is presented for the separation of alcoholamines and primary, secondary and tertiary aliphatic amines using an ion-exchange silica-based column. The influence of the various components of the mobile phase was studied.

INTRODUCTION

Industrial solutions used as descalers, corrosion inhibitors and surfactants often contain primary, secondary and tertiary amines and there is a need to know and monitor the composition of these solutions, but few analytical methods are fully satisfactory. Many high-performance liquid chromatographic (HPLC) systems used for separations without derivatization of organic compounds of an ionic character employ reversed-phase (RP) HPLC in the form of either ion-suppression or ion-pair chromatography. Both techniques are successful with acidic compounds, but their application to the separation of cationic molecules encounters many difficulties. The high-pH mobile phases necessary for ion suppression of basic compounds are incompatible with silica bonded packings, and the multiple equilibrium which governs the ion-pairing mechanism on reversed-phase packings gives rise to system peaks which often interfere with the separated peaks. Moreover, RP-HPLC is not a specific

means of separating amines and interferences from non-ionic species can occur when analysing complex samples. Nevertheless, some examples of separations of basic compounds using these techniques have been published, particularly in the biochemistry field for catecholamines [1] and to a lesser exent amino acids [2,3].

For aliphatic amines, there are numerous examples of separations of non-ionic derivatized compounds by RP-HPLC [4–7], but in contrast very few examples of suitable separations without derivatization have been reported [8]. Very often in these instances only a small number of compounds are considered and poor efficiencies and low resolutions are obtained. Depending on the origin of the reversed-phase column packing, differences in the end-capping procedure applied to the various bonded silicas can cause variations in the quality of the separation from one column to another.

Another approach to the chromatographic analysis of amines is ion-exchange chromatography (IEC). Its use in the separation of amino acids and biogenic amines has been reported for many years using commercial devices [9–11]. However, despite reasonable analytical results, these methods were complex and tedious, the analysis time was often long and the separation was not efficient. Since the late 1970s, the performance of ion-exchange columns has greatly increased with the advent of ion chromatography (IC) for the separation of inorganic ions, but so far applications to organic ions have not been frequent.

Among the numerous HPLC methods that have been developed for the separation and determination of amino compounds, some are very well adapted for application to particular compounds, but most of the more recent ones involve a separation after precolumn derivatization. Indeed, there has always been a lack of a more general HPLC procedure for the separation of amines whatever the organic moiety in these cationic molecules. With aliphatic amines another problem is the poor detectability of these compounds owing to the absence of any chromophoric groups in the molecule.

This paper deals with the separation of primary, secondary and tertiary aliphatic amines. Amines are often present in samples containing many other organic components that can interfere with their separation. The method presented here is based on IEC as this can be a specific separation mode for these compounds. For the same reasons, conductivity is used as a specific detection mode. The influence of the various components of the mobile phase that can affect the separation of the amines was studied using a chemometric approach.

EXPERIMENTAL

Reagents

All amines and solvents were of high-purity grade from Prolabo (Paris, France) and Fluka (Mulhouse, France). Water was deionized using an Elgastat (High Wycombe, UK) Spectrum apparatus. The injected solutions were prepared by dissolving the amines in pure water if possible or in water plus an organic solvent when necessary to effect solubility.

As the mobile phase is a mixture of water and an organic solvent, any reference to pH corresponds to that of the aqueous part of the mobile phase adjusted with nitric acid. To this aqueous solution was then added the organic solvent containing nitric

TABLE I

MIXTURES ANALYSED

No.	Amine	Mixture M_1: amount (g) in 10 ml water solution	Mixture M_2: amount in 10 ml acetonitrile–water (25:75) solution
1	Ethanolamine	0.010	—
2	Methylamine	0.020	—
3	Diethanolamine	0.022	—
4	Ethylamine	0.028	—
5	Triethanolamine	0.036	0.0365
6	n-Propylamine	0.036	—
7	N-Methyldiethanolamine	0.042	0.042
8	n-Butylamine	0.044	—
9	Diethylamine	0.049	—
10	Trimethylamine	0.080	0.080
11	Cyclohexylamine	0.078	—
12	Triethylamine	0.073	0.073
13	1-Aminopropanolamine	—	0.057
14	Benzylamine	—	0.088
15	Dipropylamine	—	0.111

acid at the same concentration as in the acidified water. When necessary, alkali metal salts were then directly dissolved in the mobile phase and the amount of cation added is expressed as the volume of a 1 mol/l solution added to 1 l of mobile phase.

Amine solutions

The separation of amines was performed by studying the optimization of the separation of two different mixtures, M_1 and M_2, listed in Table I. These mixtures, which contained some amines in common, were chosen from among fifteen different compounds and they were made up so as to contain alcoholamines, primary, secondary and tertiary amines. Long-chain amines such as octylamine could not be included in these mixtures because they were poorly soluble in the mobile phases.

Choice of column

As regards organic cations, the separation of some short-chain aliphatic amines has been described using conditions similar to that used for metal cations [12], but there are few examples of amine separations depending on the length of the organic moiety [13]. Amino compounds are charged species with dimensions and a lipophilic character that make them analogous to metal complexes. Therefore, the separation of amines was performed on the same ion-exchange column as for the complex species [14,15], that is, on a high-capacity silica-based exchanger.

Mobile phase

As amines have both ionic and lipophilic character, their retention depends on two distinct mechanisms: ion exchange and lipophilic interaction with the alkyl chain bearing the ionic sites. Therefore, the mobile phase is made up in such a way as to use the two mechanisms to control the elution. As regards the ion-exchange mechanism, the strength of a simple acidic solution, even at low pH, is not sufficient to elute

amines with a suitable efficiency (see Table II, experiment 1). It is then necessary to use more strongly eluting cations than the proton and therefore alkali metal salts were added to the mobile phase. Lipophilic interactions are controlled by adding an organic solvent to the mobile phase, and methanol, tetrahydrofuran (THF) and acetonitrile were tested. It must be noted that, as conductivity detection is used, the higher the efficiency of the solvent, the less will be its amount in the mobile phase and the better will be the detection sensitivity.

Instrumentation

The ion-exchange column was a Nucleosil 5A (Macherey, Nagel & Co., Düren, Germany) column (25 cm long × 0.46 cm I.D.) of 5-μm particle size. Mobile phase was pumped at a flow-rate of 1 ml/mn by a Gilson (Villiers le Bel, France) Model 302 reciprocating pump and samples were injected via a Rheodyne Model 7010 valve equipped with a 20-μl loop. To prevent bubbling, the mobile phase was continuously degassed with helium during all the experiments.

The mobile phase conductance was measured with a Wescan 213 A conductivity detector (Techmation, Paris, France). The conductivity cell and the column were placed in a oven regulated at 30°C.

Chemometric methodology

To optimize the separation of a number of compounds, the traditional approach would consist in studying separately each factor which influences the separation. For each retained factor, the capacity factor and the selectivity would be studied for each pair of compounds. On such a basis, a recent and exhaustive study of the separation of four isomeric compounds taking into account three experimental factors led to the measurement of nearly 200 chromatograms in order to achieve a perfect routine separation of the four isomers [16].

A chemometric approach to such a problem is based on the use of an optimum (in a statistical sense) matrix of experiments which allows the simultaneous variation of all the experimental factors studied. This, associated with a methodological approach, gives rise to a number of experiments which can be drastically reduced in comparison with the traditional methodology, while providing the optimum information. Chemometric methods are based on the explicit use of a mathematical model linking the observed response Y and the influencing factors X_i. Variables are usually "coded" to have a range of variation from -1 to 1. Generally, a polynomial relationship is satisfactory. For example, the simplest model is $Y = b_0 + \sum b_i X_i$, where the b_i coefficients represent the linear effects of the factors X_i on the response Y.

In this work, the qualitative or quantitative experimental factors which are likely to control the separation of amines include the nature and amount of the solvent added to the aqueous mobile phase, the nature and concentration of the eluting cation, the nature of the anions bound to this cation and the pH. The study consisted of three parts: an exploratory study in order to define a likely experimental domain, a screening of the influential factors, including qualitative factors, and an optimization of the factors that have a quantitative effect on the separation.

RESULTS AND DISCUSSION

Preliminary study

Preliminary information on the influence of an alkali metal and on the impor-
tance of the addition of organic solvents on the retention of various amino com-
pounds is shown in Figs. 1 and 2. However, in order to adopt a chemometric process
rather than a traditional approach, a series of experiments were carried out as dis-
played in Table II. The factors studied were the nature and the amount of the organic
solvent, the nature of the eluting cation and of the corresponding co-ion and their
concentrations. As it had been found that the lower the pH, the faster is the sep-
aration (a decrease in pH from 2.6 to 2.0 give a 25% decrease in the retention time),
the pH was kept at the lowest value compatible with the column, *i.e.*, 1.5.

The responses of interest in this study were the retention time of three particular
amines: ethanolamine [$Y^{(a)}$], ethylamine [$Y^{(b)}$] and diethylamine [$Y^{(c)}$]. The experi-
mental conditions and the values $Y_j^{(u)}$ (*j*th experiment; u = a, b or c) obtained for
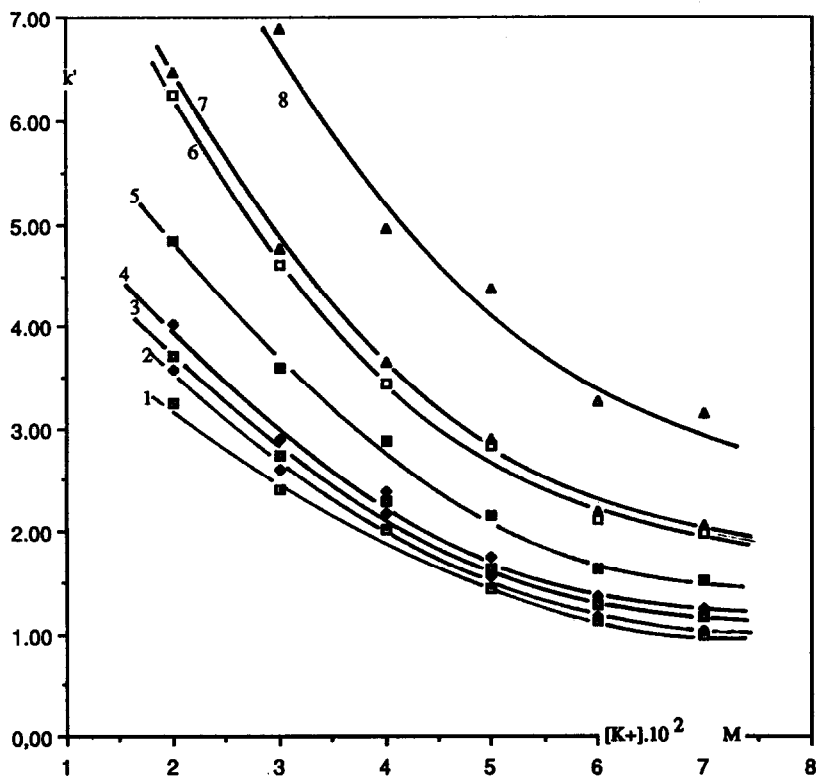these preliminary experiments are shown in Table II.

Fig. 1. Influence of K$^+$ concentration on the retention of amines. Mobile phase, water–methanol (64:36);
KNO$_3$ solution pH, 1.5; column, Nucleosil SA (250 × 4.6 mm I.D.). The numbers refer to the amines in
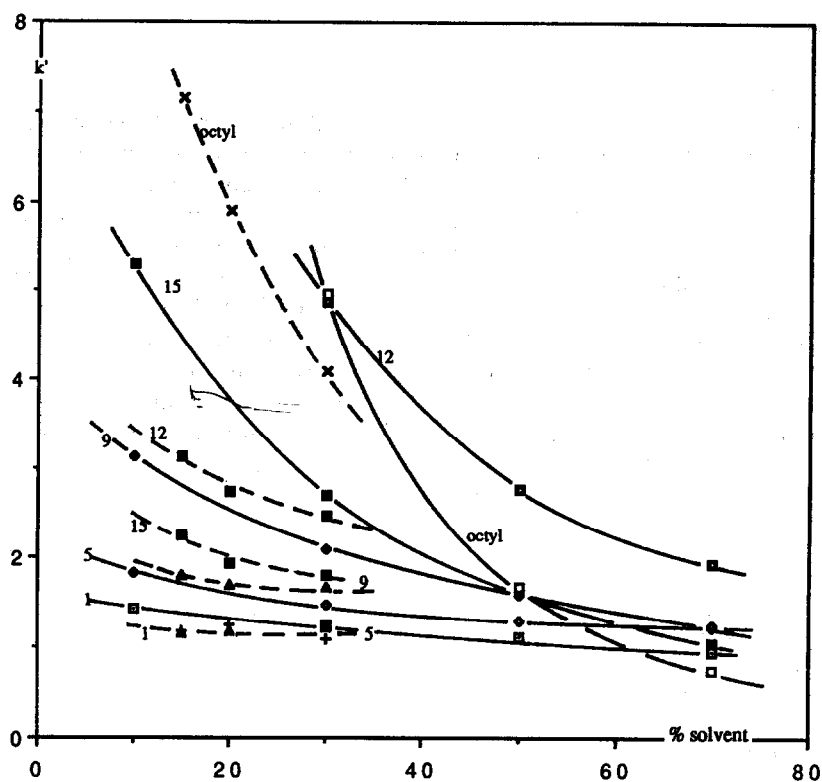Table I.

Fig. 2. Dependence of the retention of amines on the amount of methanol (solid lines) or THF (dashed lines). Mobile phase, $[KNO_3] = 5 \cdot 10^{-2}$ M, pH = 1.5; column, Nucleosil SA (250 × 4.6 mm I.D.). The numbers refer to the amines in Table I.

TABLE II

PRELIMINARY EXPERIMENTS

Expt. No.	Solvent	[solvent] (%)	Cation	Anion	[Eluted cation] ($\times 10^{-2}$ M)	Retention time (min)		
						$Y^{(a)}$	$Y^{(b)}$	$Y^{(c)}$
1	CH_3OH	10	H^+	NO_3^-	a	21	28	—
2	CH_3OH	10	Na^+	Ac^-	1	15.5	20.8	25
3	CH_3OH	10	Na^+	Ac^-	5	9.4	9.7	19
4	CH_3OH	30	Na^+	Ac^-	5	7.4	8.5	12.4
5	CH_3OH	50	Na^+	Ac^-	5	7.2	8	10.2
6	CH_3OH	50	Na^+	NO_3^-	5	6.9	7.7	9.8
7	CH_3OH	50	K^+	NO_3^-	5	6.1	6.5	8
8	CH_3OH	10	K^+	NO_3^-	5	6.3	7.5	12.8
9	CH_3OH	30	K^+	NO_3^-	5	6.2	7	9.6
10	THF	30	K^+	NO_3^-	5	6.1	6.5	8

a HNO_3: amount necessary for pH 1.5.

The interpretation of these results is as follows:

the comparison between runs 1 and 2 shows the necessity to use an eluting cation to avoid an excessive retention time;

the comparison between experiments 2 and 3 shows the effect of the concentration of eluting cation: the higher is the concentration, the shorter is the retention time, as displayed in Fig. 1;

the set of experiments 3–5 shows that increasing the methanol concentration decreases the retention time, as seen in Fig. 2;

the comparison between experiments 5 and 6 shows the influence of the nature of the co-ion: nitrate gives lower retention times than acetate;

the comparison between experiments 6 and 7 shows the influence of the nature of the eluting cation: K^+ is a stronger eluent than Na^+;

experiments 7–9 show again the effect of an increase in the methanol concentration but, with potassium nitrate, this effect is much less than with sodium acetate;

the comparison between experiments 9 and 10 shows the effect of organic solvent, THF producing shorter retention times than methanol.

Screening design

From the above results a choice of the studied factors was made: nature of the solvent, amount of solvent, amount of eluting cation and pH.

For a given response $Y^{(u)}$, the model

$$Y^{(u)} = b_0^{(u)} + b_1^{(u)} X_1 + b_2^{(u)} X_2 + b_3^{(u)} X_3 + b_4^{(u)} X_4$$

will allow the estimation of the effect $b_i^{(u)}$ in which we are interested: $b_1^{(u)}$ will be the effect of the type of solvent, $b_2^{(u)}$ the effect of the amount of solvent, $b_3^{(u)}$ the effect of the potassium nitrate (eluting cation) and $b_4^{(u)}$ the effect of pH.

A two-level fractional factorial design [17] of eight experiments would be appropriate for this problem. A 2^{4-1} fractional factorial design with the defining relation $I = -1234$ was used:

Run No.	X_1	X_2	X_3	X_4	$Y^{(u)}$ (observed response)
1	−1	−1	−1	+1	$Y_1^{(u)}$
2	+1	−1	−1	−1	$Y_2^{(u)}$
3	−1	+1	−1	−1	$Y_3^{(u)}$
4	+1	+1	−1	+1	$Y_4^{(u)}$
5	−1	−1	+1	−1	$Y_5^{(u)}$
6	+1	−1	+1	+1	$Y_6^{(u)}$
7	−1	+1	+1	+1	$Y_7^{(u)}$
8	+1	+1	+1	−1	$Y_8^{(u)}$

From this experimental matrix, an effect matrix of eight columns corresponding to the different combinations of columns 1, 2 and 3 was built:

Column 0 (−1234)	1	2	3	4 (−123)	5 (12)	6 (13)	7 (23)	$Y^{(u)}$ (response)
+1	−1	−1	−1	+1	+1	+1	+1	$Y_1^{(u)}$
+1	+1	−1	−1	−1	−1	−1	+1	$Y_2^{(u)}$
+1	−1	+1	−1	−1	−1	+1	−1	$Y_3^{(u)}$
+1	+1	+1	−1	+1	+1	−1	−1	$Y_4^{(u)}$
+1	−1	−1	+1	−1	+1	−1	−1	$Y_5^{(u)}$
+1	+1	−1	+1	+1	−1	+1	−1	$Y_6^{(u)}$
+1	−1	+1	+1	+1	−1	−1	+1	$Y_7^{(u)}$
+1	+1	+1	+1	−1	+1	+1	+1	$Y_8^{(u)}$

Our model involves only first-order terms but it is possible to compute eight various linear contrasts l_i obtained by adding together all the response values with plus signs in columns, 0, 1, 2, etc., subtracting all those with minus signs and dividing by the appropriate column divisor 8. For example,

$$l_1^{(u)} = [- Y_1^{(u)} + Y_2^{(u)} - Y_3^{(u)} + Y_4^{(u)} - Y_5^{(u)} + Y_6^{(u)} - Y_7^{(u)} + Y_8^{(u)}]/8$$

$$l_1^{(1)} = (-0.598 + 0.724 - 0.486 + 0.548 - 0.676 + 0.693 - 0.338 + \\ + 0.561)/8 = 0.428/8 = 0.0535$$

The confouding scheme is given by:

$$l_0^{(u)} = b_0^{(u)} - b_{1234}^{(u)}$$
$$l_1^{(u)} = b_1^{(u)} - b_{234}^{(u)} \qquad l_2^{(u)} = b_2^{(u)} - b_{134}^{(u)}$$
$$l_3^{(u)} = b_3^{(u)} - b_{124}^{(u)} \qquad l_4^{(u)} = b_4^{(u)} - b_{123}^{(u)}$$
$$l_5^{(u)} = b_{12}^{(u)} - b_{34}^{(u)} \qquad l_6^{(u)} = b_{13}^{(u)} - b_{24}^{(u)} \qquad l_7^{(u)} = b_{23}^{(u)} - b_{14}^{(u)}$$

where $b_i^{(u)}$ are main effects for the studied response $Y^{(u)}$, $b_{ij}^{(u)}$ are two-factor interaction effects, $b_{ijk}^{(u)}$ are three-factor interaction effects, etc.

Assuming that interactions between more than two factors are negligible, it is possible to estimate separately main effects which are confounded with three-factor interactions, and to estimate only the sum of two-factor interactions effects.

Experimental domain (factors and levels)

THF and acetonitrile (ACN) were chosen as solvents because they are better solvents than methanol for amines. The amount of solvent was varied from 5% to a upper limit of 25% to retain satisfactory mobile phase conductivity.

Potassium nitrate was chosen as the eluting species as K^+ is a stronger eluent than Na^+, and as the eluting strength effect of nitrate is higher than that of acetate. Its concentration range in the mobile phase varied from $2 \cdot 10^{-2}$ to $6 \cdot 10^{-2}$ M.

The influence of pH was studied between pH 2 and 5.

The experimental domain (factors and levels) in the screening experiments is given in Table III. The amounts of solvent and potassium nitrate solution added to the mobile phase are expressed as the volume added to obtain 1 l of mobile phase.

TABLE III

FACTORS AND LEVELS FOR THE FACTORIAL STUDY

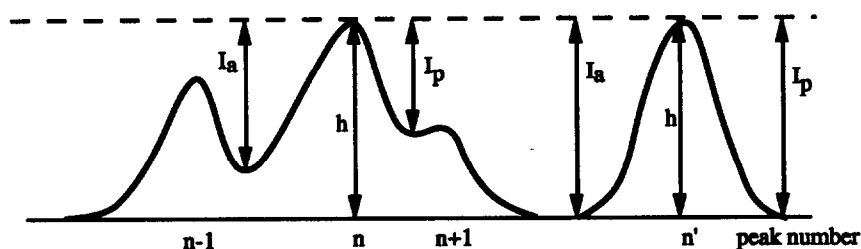| Factor		Level	
		-1	$+1$
X_1	Nature of solvent	THF	ACN
X_2	Amount of solvent (ml)	50	250
X_3	Amount of KNO_3 solution (ml)	20	60
X_4	pH	2	5

Fig. 3. Graph giving the directions for the separation index calculation.

Observed response

Chromatographic response functions (CRF) have been studied by numerous workers as reported by Lu and Huang [18]. We propose a very simple one, consistent with a limited number of experiments.

The studied response, *i.e.*, the evaluation of the quality of the separation, is made through an "overall separation index, I_s", calculated according to the directions in Fig. 3.

Each peak n is related to $y_n = 1/2\ (I_a/h + I_p/h)$, which gives $y_n = 1$ for each completely separated peak, since $I_a = I_p = h$ in that case.

To take into account the time limit of the analysis, y_n is set to 0 if the retention time is over 60 min.

For the whole set of separated peak $Y_n = \sum y_n$, and if there are N components in the analysed mixture, the overall separation index is

$$I_s = Y_n/N = \sum y_n/N = \text{the response function } Y^{(u)}.$$

According to this CRF, the nearer to 1 is the response, the better is the separation.

Analysis of results

The experimental matrices for both mixtures M_1 [vector $Y^{(1)}$] and M_2 [vector $Y^{(2)}$], in natural (experimental) variables U_i or coded variables X_i, are shown in Table IV. For each response vector $Y^{(1)}$ or $Y^{(2)}$ the calculated coefficients are given in Table V.

TABLE IV

TWO-LEVEL FRACTIONAL FACTORIAL DESIGN

Run No.	U_1	U_2	U_3	U_4	X_1	X_2	X_3	X_4	$Y^{(1)}$	$Y^{(2)}$
1	THF	50	20	5	-1	-1	-1	1	0.598	0.798
2	ACN	50	20	2	1	-1	-1	-1	0.724	0.744
3	THF	250	20	2	-1	1	-1	-1	0.486	0.621
4	ACN	250	20	5	1.	1	-1	1	0.548	0.670
5	THF	50	60	2	-1	-1	1	-1	0.676	0.942
6	ACN	50	60	5	1	-1	1	1	0.693	0.703
7	THF	250	60	5	-1	1	1	1	0.338	0.556
8	ACN	250	60	2	1	1	1	-1	0.561	0.856

TABLE V

COEFFICIENTS OF THE MODEL FOR SEPARATION INDEX

Effect	$Y^{(1)}$	$Y^{(2)}$
b_0	0.578	0.736
b_1	0.053	0.007
b_2	−0.095	−0.060
b_3	−0.011	0.028
b_4	−0.034	−0.054
Confounding:		
$(b_{12}-b_{34})$	0.018	0.080
$(b_{13}-b_{24})$	0.006	0.008
$(b_{14}-b_{23})$	0.023	−0.002

Mixture M_1 [response $Y^{(1)}$]. The amount of solvent (X_2) is the factor that has the major effect $[b_2^{(1)} = -0.095]$, followed by, to a lesser extent, the nature of the solvent (X_1) and the pH (X_4), while the influence of K^+ concentration (X_3) seems to be low. From the results it can be concluded that:

on average, acetonitrile gives a better separation than THF $[X_1 = 1$ with $b_1^{(1)} = 0.053]$;

the lower the amount of solvent, the better is the separation $[X_2 = -1$ with $b_2^{(1)} = -0.095]$;

it is better to work at pH 2 than at pH 5 $[X_4 = -1$ with $b_4^{(1)} = -0.034]$;

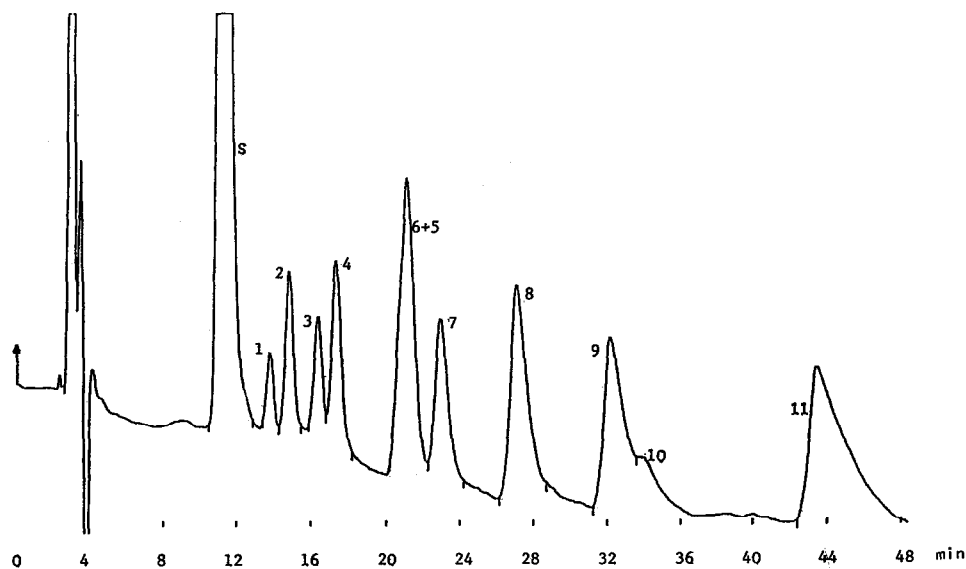$[K^+]$ has little effect as b_3 is small $[b_3^{(1)} = -0.011]$.

Fig. 4. Separation of mixture M_1 according to the conditions of experiment 2 (factorial design), Table IV. Flow-rate, 1 ml/min. Other conditions as mentioned in the text. The numbers refer to the amines in Table I.

Experiment 2, which fulfills the best compromise according to these constraints ($X_1 = 1$, $X_2 = X_3 = X_4 = -1$), actually gives the best separation among the eight experiments performed. Fig. 4 shows the chromatogram obtained under these conditions. The first detected peaks correspond to unretained solutes, $i.e.$, the sample solvent and the anionic solutes. The system peak which correspond to the eluting ions is then eluted as a significant peak before the analysed compounds.

It should be noted that the effect of X_3 seems to contradict the theoretical knowledge of the influence of K^+ in cation-exchange chromatography. However, it can be considered that although the effect of $[K^+]$ is low as a main effect, it can play a significant role in the interaction effects, but to estimate the b_{ij} terms without confounding, eight additional experiments would have been necessary. Nevertheless, although it is not possible to have an unconfounded estimate of all b_{ij} coefficients, an X_iX_j interaction can be discussed using the following type of diagram where the plane is divided into four quadrants by two axes X_i and X_j:
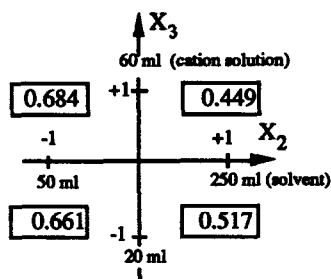
The right and upper value represents the average of the experimental response when $X_i = 1$ and $X_j = 1$ {in our example $X_2 = 1$ and $X_3 = 1$ for runs 7 and 8, and the average is $[Y_7^{(1)} + Y_8^{(1)}]/2 = (0.338 + 0.561)/2 = 0.449$}. The other three values are calculated in an analogous manner using the corresponding combinations ($X_i = -1$ and $X_j = 1$, $X_i = -1$ and $X_j = -1$, $X_i = 1$ and $X_j = -1$).

From the X_2X_3 diagram corresponding to the interaction between the percentage of organic solvent in the mobile phase (X_2) and the K^+ concentration (X_3), it can be seen that for $X_2 = -1$ (5% solvent), the response varies from 0.661 to 0.684 when X_3 goes from -1 to 1 ($[K^+]$ goes from $2 \cdot 10^{-2}$ to $6 \cdot 10^{-2}$ M). When X_2 is at the level -1 (25% solvent), the separation index decreases from 0.517 to 0.449 for the same $[K^+]$ variation. This shows that the effect of $[K^+]$ is a function of the solvent amount and $vice$ $versa$.

It should be noted that such an observation, which shows the dependence of the influence of one factor on the levels of the other factors, could not be evidenced using the traditional optimization approach (sequential single factor at a time), despite the large number of experiments performed.

$Mixture$ M_2 $[response$ $Y^{(2)}]$. The conclusions are the same as for the mixture M_1 regarding the effects of X_2 and X_4, but the effects of X_1 and X_3 are different:

the influence of the nature of the solvent is of little importance [$b_1^{(2)} = +0.007$];

it is better to use a small amount of solvent [$X_2 = -1$ with $b_2^{(2)} = -0.060$];

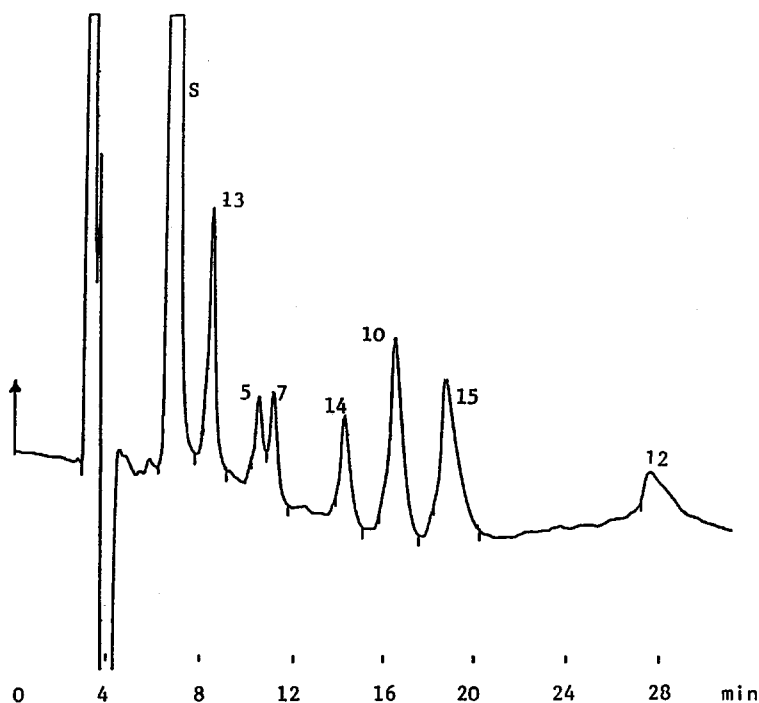the higher the K^+ concentration, the better is the separation [$X_3 = +1$ with $b_3^{(2)} = 0.028$];

Fig. 5. Separation of mixture M_2 according to the conditions of experiment 5 (factorial design), Table IV. Flow-rate, 1 ml/min. Other conditions as mentioned in the text. The number refer to the amines in Table I.

it is better to use pH 2 than pH 5 [X_4 = 1 with $b_4^{(2)}$ = −0.054].

Experiment 5, which fulfills the best compromise among these constraints (X_1 = −1, X_2 = −1, X_3 = 1, X_4 = 1), actually gives the best separation index from the eight experiments performed. The corresponding separation is shown in Fig. 5.

As above, the examination of the interaction diagrams brings additional information to the previous conclusions:
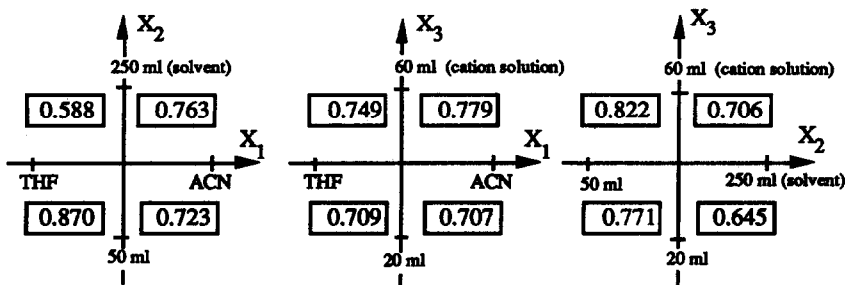
Examination of the interaction between X_1 and X_2 shows that on average the best separation is obtained either for a small amount of THF or to a lesser extent for a large amount of acetonitrile.

TABLE VI

SEQUENTIAL SIMPLEX DESIGN FOR MIXTURE M_1

Run No.	K^+ (ml)	CH_3CN (ml)	I_s	$t_R{}^a$ (min)
1	20	30	0.705	45
2	60	30	0.767	44
3	40	90	0.924	52
4	80	90	0.875	27
5	60	150	0.793	25
6	20	150	0.829	70

^{a} t_R = Retention time of last peak.

From the interaction between X_1 and X_3 it appears that the best separations are obtained with $[K^+] = 6 \cdot 10^{-2} M$, whatever the solvent. The interaction between X_2 and X_3 shows that the best separations are obtained for $[K^+] = 6 \cdot 10^{-2} M$ and for a small amount of solvent near 5%.

Simplex optimization for the twelve-amines mixture M_1

In order to simplify the problem of the separation of this mixture, we can reasonably make two final choices: use acetonitrile as the solvent, and work at pH 2, as a pH lower than 2 would risk damage to the stationary phase. As there is a high interaction level between the amount of acetonitrile and the K^+ concentration, the simplex method [19–23] is used to find the optimum combination of these two factors, with the following coded parameters:

factors	centre of the domain	variation step
ml KNO_3 (1 M solution)	40	23
ml CH_3CN	50	40

These results are given in Table VI. Runs 1, 2 and 3 correspond to the initial simplex (Fig. 6). Vertex 1 (run 1) has the worst response and vertex 3 (run 3) has the best response. Reflection is accomplished to generate the new vertex 4 (run 4). Then
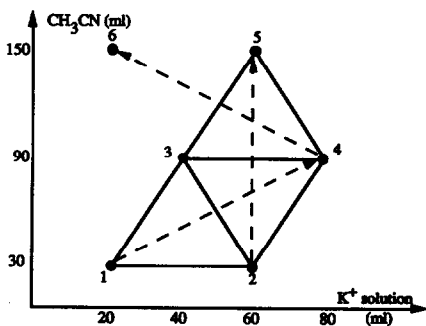

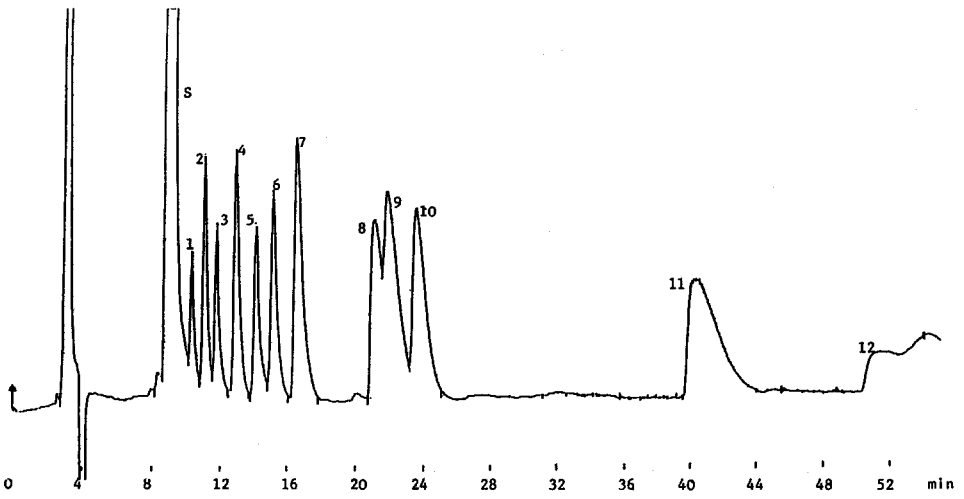Fig. 6. Evolution of sequential simplex design.

Fig. 7. Separation of mixture M_1 according to conditions of experiment 3 (simplex design), Table VI. The numbers refer the amines in Table I.
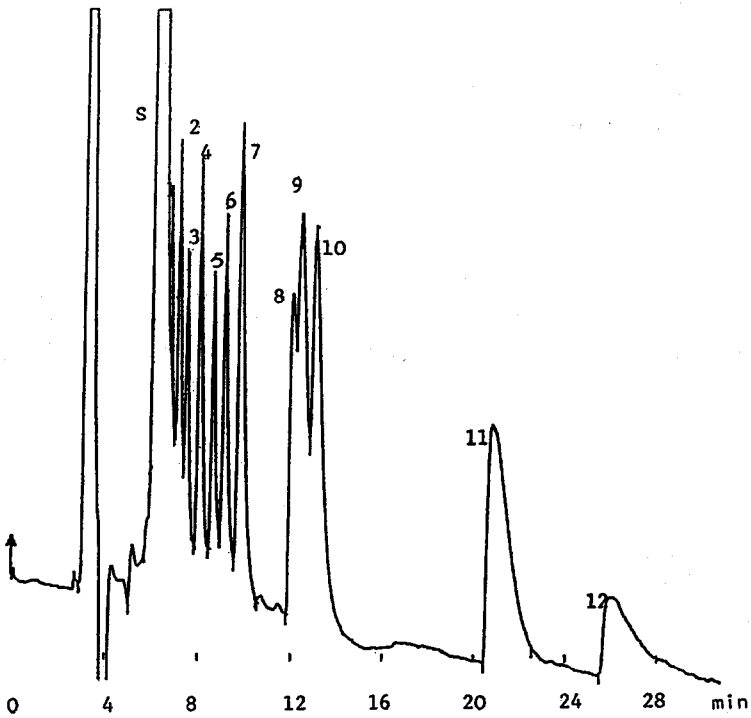

Fig. 8. Separation of mixture M_1 according to conditions of experiment 4 (simplex design), Table VI. The numbers refer to the amines in Table I.

evolution brings us to vertex 5 (run 5) and, if we apply the reflection rule, we obtain vertex 2. It is necessary to prevent oscillation about a ridge; a new vertex is obtained by rejecting the next-to-worst vertex instead of the worst vertex: vertex 6 (run 6). It seems that in this part of the domain with the elution system used, the optimum separation index is obtained with run 3. The best I_s value is 0.924. This run corresponds to an almost complete separation of the twelve compounds to be separated, as shown in Fig. 7. Only the separation between peaks 8, 9 and 10 is not fully achieved, although peak 10 is almost separated, as $y_8 = 0.639$, $y_9 = 0.613$ and $y_{10} = 0.913$.

If the analysis time is taken into account (the retention time for the last peak is t_R in Table VI), the conditions of experiment 4 can be reasonable as the separation index is 0.875 and the whole analysis time is 27 min instead of 52 min for experiment 3. This also implies that it is accepted that the first peaks be eluted on the system peak tail and that the resolution between peak 8, 9 and 10 be decreased further: $y_8 = 0.55$, $y_9 = 0.484$, $y_{10} = 0.781$. The chromatogram of experiment 4 is presented in Fig. 8.

CONCLUSIONS

A chemometric approach to the separation of aliphatic amines has allowed us to find optimum separation conditions with a limited number of experiments: for mixture of twelve amines, taking into account four elution factors, it was shown that only 24 experiments allow the study of the simultaneous variation of all the studied experimental factors and information on the interactions between the mobile phase components has been obtained that could not be obtained with a traditional approach, *i.e.*, the variation of one factor at a time.

A chromatographic system based on both an ion-exchange mechanism and a lipophilic interaction mechanism proved very efficient for separating amino compounds. However, the sensitivity of the detection of the separated compounds has not been studied, and the large amounts of organic solvent in the mobile phase necessary to elute the more lipophilic amines cause a decrease in the dissociation of the ionizable solutes, a decrease in the mobile phase conductivity and a poor detection limit for these compounds. Other detection modes such as spectrophotometry coupled with a post-column derivatization device or light-scattering detection might be preferable as such detection could allow elution gradient and perhaps further improvements of the separation of amines by this HPLC method.

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