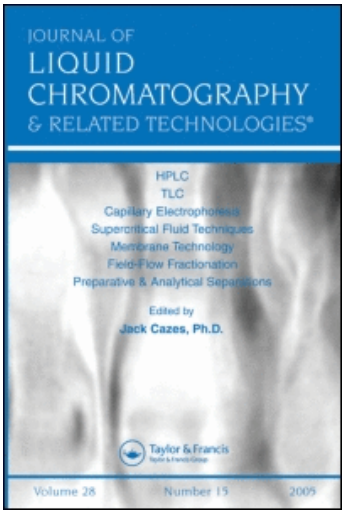

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A NEW QUATERNARY MOBILE PHASE SYSTEM FOR OPTIMIZATION OF TLC SEPARATIONS OF ALKALOIDS USING MIXTURE DESIGNS AND RESPONSE SURFACE MODELLING

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ABSTRACT

A new combination of four organic solvents is proposed for the optimization of TLC separations of basic drugs and alkaloids. The solvents are diethylamine (DEA), methanol (MeOH), chloroform ($CHCl_3$) and ethylacetate (EtAc). They were selected from a collection of ten solvents used in Normal Phase TLC mobile phases recommended for the separation of alkaloids and basic drugs in the literature. The selection was based on the classification of solvents according to selectivity and solubility parameters. Excluded were apolar and weak solvents that show no selective (polar) properties and are used only for the adjustment of the solvent strength. Polar solvents from different selectivity groups were selected to combine as many as possible selective effects in one solvent system. The final choice was made considering the displacement theory for Liquid Solid Chromatography.

The four solvents have been intended for application in an optimization procedure that uses mixture designs and response surface modelling. The factor space is a tetrahedron of which only in a part suitable experiments can be performed, i.e. the design space. Experimentation in the design space should allow the simultaneous optimization of solvent strength and solvent selectivity. The quality of TL chromatograms was characterized by two criteria: the separation of the worst separated pair of spots ($R_s$min) and the R_f value of the slowest moving spot (kmax). In this way it is possible to influence not only the separation of the spots but also their place on the TL plate. The new four solvent system has been tested successfully in the separation of the parent alkaloids of four dry plant materials: Ipecacuanha root, Cinchona bark, Belladonna leaf and Opium. The predicted mobile phases gave better or equally good separations than the mobile phases prescribed by the European or Netherlands Pharmacopoeia for these separations. Moreover a substantial reduction of the number of solvents necessary for the composition of the mobile phase was achieved as the official procedures need eight different solvents.

INTRODUCTION

Systematic optimization of the mobile phase is an important topic in liquid chromatography. However, a far greater number of articles deals with HPLC than with TLC. In TLC triangular mixture designs, first applied in HPLC (1), were used in normal (2) and reversed (3) phase TLC. Response surface modelling is the basis of this technique and the capacity factor of each solute, the response, is modelled in a four solvent mixture. One solvent is used for the adjustment of the solvent strength, e.g. hexane in NP-TLC, and by the three other solvents, e.g. methylene chloride, methyl tert-butyl ether and actonitrile, the selectivity is controlled. Three isoeluotropic binary mixtures or pseudocomponents are composed of hexane and the three "selectivity" solvents. The capacity factor is described as a function of three pseudocomponents, which constitute the vertices of a mixture triangle. The response surfaces of the individual solutes are used to construct overlapping resolution maps (1,2) or minimal resolution plots (4) to select a mixture that provides optimal separation.

The PRISMA model (5) was also applied in TLC (6). The basic concept is again to optimize firstly the solvent strength and subsequently the selectivity in a triangular mixture solvent diagram. The three selectivity adjusting solvents are selected from ten solvents used in preliminary experiments and diluted, if necessary, by the solvent strength adjusting solvent (hexane). The optimal solvent mixture is found by testing appropriate solvent mixtures located at "selectivity points".

In this paper we propose a four component solvent system for optimization of NP-TLC separations of alkaloids. The optimization procedure uses response surface modelling and mixture designs, but contrary to the above mentioned procedures solvent strength and solvent selectivity are adjusted simultaneously using a four component mixture space or tetrahedron.

In the next sections we discuss the selection of the four solvents defining the factor space, the selection of the design space and of the experimental design, the optimization criterion, and the POEM software used for modelling the response surface and locating the optimal solvent composition.

THEORY

Factor Space

The European and Netherlands Pharmacopoeia use for the TLC separation of parent alkaloids of four dry plant materials: Ipecacuanha root (7), Cinchona bark (8), Belladonna leaf (9) and Opium (10), four different mobile phases for which eight different solvents are needed.

The purpose of our investigation was the selection of at most four solvents for use in a mixture design technique. The separation of the parent alkaloids of the above mentioned dry plant materials should be accomplished by mobile phases for which only four solvents are needed. The mobile phase compositions had to be found by a systematic optimization procedure.

We decided to use maximally four solvents because:
-such a solvent system should allow sufficient variation of solvent strength and of selectivity;
-the factor space of a four component mixture is a tetrahedron. The frequently used quadratic model can be constructed from a mixture design for which only 10 experiments are necessary;
-the outcome of our selection procedure suggested the use of four solvents (see below).

Although the classification of solvents in a selectivity triangle by Snyder (11) and his selection of solvents for optimization in Liquid Solid Chromatography (LSC) (2) is generally accepted, we decided to follow a different approach. We studied which TLC systems had been used in general screening procedures for the identification of alkaloids and basic drugs.

Waldi et al. (12) introduced eight mobile phases composed of seven solvents for the identification of 54 alkaloids, and Norfalise and Mees (13) needed nine mobile phases to identify 34 alkaloids. Later different mathematical criteria were used for the selection of a limited number (4 to 8) of TLC systems from a larger collection of proven systems. Moffat et al. introduced the discriminating power (14) and selected from a collection of 37 TLC systems seven mobile phases composed of eight solvents (15). De Clerq and Massart (16) analyzed the dataset of Moffat based on hundred basic drugs and confirmed their choice of TLC systems using the information content as criterion for the selection. Schepers et al. (17) determined also the R_f values of one hundred basic drugs in eight TLC systems for which he needed eight solvents. The identification power and the mean list length were used to evaluate the systems. Musumarra et al. (18) selected four mobile phases composed of eight solvents after performing principal component analysis on a dataset consisting of the R_f values of 55 drugs in 40 eluents.

It is quite remarkable that all 25 selected mobile phases, mentioned above, can in fact be composed of only ten solvents. In Table 1 only eight of the ten solvents are given: the strong

Table 1

Properties of Selected Solvents, Data from Refs. 11, 19, 20.

solvent	S(si)	x_e	x_d	x_n	group	d_a	d_b	d_o	d_{in}
Cyclohexane	0.0	-	-	-	[b]	-	-	-	-
Toluene	0.22	.25	.28	.47	VII	-	0.6	-	-
Chloroform	0.31	.25	.41	.33	VIII[c]	6.5	0.5	3.0	0.5
Ethyl acet.	0.48	.34	.23	.43	VIa	-	2.7	4.0	1.0
Acetone	0.53	.35	.23	.42	VIa	-	3.0	5.1	1.5
Butanol	0.54[a]	.59	.19	.25	II	[d]	[d]	[d]	[d]
Diethylamine	0.55[a]	[d]	[d]	[d]	[d]	[d]	[d]	[d]	[d]
Methanol	0.70	.48	.22	.31	II	8.3	8.3	4.9	0.8

[a]: calculated with S(silica) = 0.77 S(alumina); [b]: irrelevant because of low polarity; [c]: close to group VIII; [d]: no data available. See text for explanation.

ammonia and acetic acid solutions are omitted, because we decided not to use aqueous solutions to avoid miscibility problems.

Summarized in Table 1 are: S(si), the solvent strength on silica (19); the selectivity parameters: x_e is proton acceptor, x_d is proton donor and x_n is dipole interactor (11); group is the selectivity group; the polar solubility parameters: d_a is proton donor, d_b is proton acceptor, d_o is orientation (dipole) and d_{in} is induction parameter (20). The dispersion parameter is not given; only polar interactions are considered, because these are most important in the separation of nitrogen bases on silica. The ten solvents used for composing the 25 mobile phases cover six of the eight selectivity groups of Snyders selectivity triangle. Groups II, VI, VII, and VIII are listed in Table 1; acetic acid is classified in group IV and diethylamine may be located in the basic top of the triangle above group I. The initial ten solvents of the PRISMA optimization system (21) cover all eight selectivity groups, but before the optimization of the mobile phase is started, this number of solvents is reduced to a number between 3 and 5 by a structured series of

experiments whereby the choice depends on the results of preliminary separations of the sample.

The statistical mixture design technique, however, demands an a priori decision with regard to the selection of the solvents. The solvents may be chosen from the selectivity triangle. To achieve the greatest variability of the selectivity the solvents have to be chosen from groups furthest from each other in the triangular plot. The assumption is of course that the classification in the triangle accurate, but reservations have been expressed in an critical review on strategies for optimizing the mobile phase in planar chromatography (22).

Because we wanted a solvent system suited for the separation of alkaloids, decided to look for solvents especially used for the separation of alkaloids. Moreover we decided to base our selection procedure not exclusively on the selectivity triangle but to consider also other criteria such as: the capability to prevent tailing, solubility parameters, flow velocity constants and the requirement of the displacement theory (24).

Chemisorption of basic compounds may occur because of the acidic properties of the silanol groups. This causes pronounced tailing from the point of application to the final spot. Chemisorption can be prevented by using basic mobile phases or by impregnating the silica layer with a basic solution. As we preferred not to pretreat the silica, we decided to select dietylamine (DEA) as one of the solvents for our quaternary solvent system.

Cyclohexane and toluene were ruled out because of low polarity as indicated by the solvent strength. Chloroform was selected because it is stronger and the only proton donor in Table 1. In this case the proton donor property is indicated not only by the solvent selectivity parameters, but also by the solubility parameters. The solvent properties as indicated by the selectivity parameters do not always agree with those pointed out by the solubility parameters. The discrepancies may be caused by the empirical character of the selectivity parameters, which are

based on probes not specific for only one type of interaction. On the other hand the simplifying assumptions in the solubility parameter theory may lead to different characteristics. The discrepancy is clearly illustrated by methanol in Table 1. According to the selectivity parameters strong proton acceptor properties are attributed to MeOH, but the solubility parameters indicate equally strong acid and base characteristics, because it is an assumption of the solubility parameter theory. The main strength of both concepts is the classification of solvents into groups of similar selectivity.

Having chosen DEA and $CHCl_3$ and rejected cyclohexane and toluene, we could therefore confine the remainder of the selection procedure to choices between ethyl acetate vs. acetone (group VIa) and butanol vs. methanol (group II). Ethyl acetate (EtAc) and methanol (MeOH) were chosen because their flow velocity constants (85 and 65 cm/s) differ less from the values of DEA and $CHCl_3$ (69 and 75 cm/s) than the corresponding values of acetone and butanol (118 and 13 cm/s) do (23). Equal flow velocity constants are desirable to prevent demixing of the solvent front.

The four selected solvents represent different selectivity aspects: DEA is a strong base; $CHCl_3$ is a proton donor; EtAc is a dipole interactor and MeOH has proton donor and proton acceptor properties. Accordingly the selected solvent system may provide a large variation of selectivity, but the selected solvent system was also considered in view of the displacement theory for LSC (24).

The displacement theory emphasizes the importance of hydrogen bonding and of localization effects in LSC: polar molecules (solvent or solute) tend to take fixed positions above the active adsorption sites of the adsorbent and there is one-to-one bonding between the polar molecule and the adsorption site. Less polar molecules, however, do not localize but move about freely within the absorbed layer. A varying ratio of a less polar, non-localizing solvent, i.e. $CHCl_3$, and the more polar,

localizing ones, i.e. EtAC, DEA or MeOH, changes the solvent strength and can create large changes in mobile phase selectivity due to differences in the relative localization of solvent and solute molecules; the so called solvent - solute localization. The solvent selectivity depends also to a lesser extend on the use of localizing solvents that are either basic or non-basic, i.e. DEA or EtAC, i.e. solvent specific localization.

The silanol groups of the silica surface have a basic oxygen and an acidic proton and are capable to hydrogen bonding with proton acceptors as well as proton donors, i.e. MeOH, which also affects the solvent selectivity. Another influence on the selectivity is a change in hydrogen bonding between the solute and the solvent molecules, which can occur at higher concentrations of the hydrogen bonding solvent.

As stated above DEA also interacts with the acidic proton of the silanol group and may be a necessary mobile phase component to prevent chemisorption of basic compounds.

The selection of the four solvents is almost according to the guidelines of Snyder (24): a non-localizing polar: $CHCl_3$, a basic localizing: DEA, and a non-basic localizing solvent: EtAc, are selected. MeOH, however, is chosen in stead of a non-polar solvent strength adjusting solvent like hexane because TLC of basic substances on silica usually requires fairly strong eluents. Moreover, the inclusion of MeOH in our solvent system enlarges the selective possibilities of the system due to strong hydrogen bonding interactions.

Design Space and Experimental Design

The selection of four solvents, DEA, $CHCl_3$, EtAC and MeOH, as components of a mixture defines a tetrahedron as factor space (Fig.1). The response of a TLC mixture experiment, i.e. the logarithm of the capacity factor, k, is a function of the fractions X_1, X_2, X_3 and X_4 of the components of the mixture. The

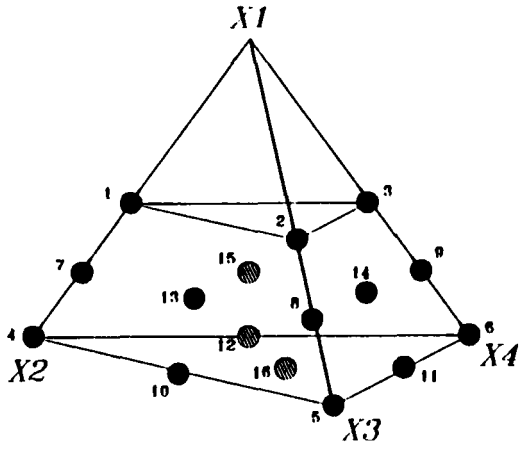

FIGURE 1. Solvent tetrahedron and truncated design space. Points refer to corresponding numbers of Table 2. X1 is diethylamine (DEA), X2 is a mixture of equal parts of methanol (MeOH) and ethylacetate (EtAc), X3 is $CHCl_3$ and X4 is EtAc.

function is a polynomial, for example,

$$\ln k = A_1X_1 + A_2X_2 + A_3X_3 + A_4X_4 + A_{12}X_1X_2 + A_{13}X_1X_3 + A_{14}X_1X_4 + A_{23}X_2X_3 + A_{24}X_2X_4 + A_{34}X_3X_4 + E \quad (1)$$

with which a ten term quadratic model can be constructed. Usually a quadratic model is sufficient, but a special cubic model may be also used:

$$\ln k = \Sigma A_iX_i + \Sigma A_{ij}X_iX_j + \Sigma A_{ijk}X_iX_jX_k + E \quad (2)$$

This model may provide a better description of the response surface, but requires at least 14 experiments, (four more than the quadratic) to estimate the 14 $A_{i(jk)}$ coefficients. If an estimate of the residual error, E, is to be obtained, then 11 and

15 experiments are necessary for the quadratic and special cubic model respectively.

If the response over the whole factor space is of interest, the design points for measuring the response can be uniformly distributed over all possible mixtures of the components. In chromatography, however, meaningful responses are usually restricted to a limited part of the factor space: the design space or feasible region. Therefore it was decided not to use pure MeOH but to use a binary mixture of equal parts of MeOH and EtAc as pseudocomponent, X_2. Moreover the factor space was constrained to 0.4 X_1 (=DEA) and to 0.95 X_3 (=$CHCl_3$). These choices are appropriate guesses based on a few introductory experiments and a study of literature data (25).

If a quadratic or special cubic model is to be constructed at least 10 or 14 design points are necessary. The location of the points was guided (Fig. 1 and Table 2) by the following considerations:

- an extreme vertices design ensures a good delimitation of the design space: points 1 to 6,
- the construction of a quadratic model requires measurement of the factor at least at 3 levels and the robustness of a design is enhanced by a regular distribution of the experiments: points 7 to 12,
- the construction of a special cubic model requires the estimation of three factor interactions: points 13 to 16,
- for a given model the variance of the regression coefficients can be minimized by a suitable choice of the design matrix. Design evaluation according to this criterion, A-optimality (26), indicates the importance of points at half height of the truncated tetrahedron. Accordingly points on the edges, which are especially important for the quadratic model, are duplicated: points 17 to 19; points on the faces are of particular significance for the special cubic model and are repeated nearby: points 20 to 22,

TABLE 2

Coordinates and Mobile Phase Compositions at the Design Points

Point number Fig.1	Coordinates X_1	X_2	X_3	X_4	Mobile Phase Composition DEA	MeOH	$CHCL_3$	EtAc
1	.40	.60	---	---	.40	.30	---	.30
2	.40	---	.60	---	.40	---	.60	---
3	.40	---	---	.60	.40	---	---	.60
4	---	1.00	---	---	---	.50	---	.50
5	---	.10	.90	---	---	.05	.90	.05
6	---	---	---	1.00	---	---	---	1.00
7	.20	.80	---	---	.20	.40	---	.40
8	.20	---	.80	---	.20	---	.80	---
9	.20	---	---	.80	.20	---	---	.80
10	---	.60	.40	---	---	.30	.40	.30
11	---	---	.50	.50	---	---	.50	.50
12	---	.50	---	.50	---	.25	---	.75
13	.20	.40	.40	---	.20	.20	.40	.20
14	.20	---	.40	.40	.20	---	.40	.40
15	.20	.40	---	.40	.20	.20	---	.60
16	---	.30	.30	.40	---	.15	.30	.55
17	.20	.80	---	---	.20	.40	---	.40
18	.20	---	.80	---	.20	---	.80	---
19	.20	---	---	.80	.20	---	---	.80
20	.20	.50	.30	---	.20	.25	.30	.25
21	.20	---	.10	.70	.20	---	.10	.70
22	.20	.50	---	.30	.20	.25	---	.55
23	.18	.28	.27	.27	.18	.14	.27	.41
24	.40	.20	.40	---	.40	.10	.40	.10
25	.40	.10	---	.50	.40	.05	---	.55
26	.40	.50	---	.10	.40	.25	---	.35
27	.05	---	.95	---	.05	---	.95	---
28	.05	---	---	.95	.05	---	---	.95
29	.05	---	.475	.475	.05	---	.475	.475

- four additional points are chosen because an exchange algorithm based on A-optimality indicates their importance: the center point 23, and points 24 to 26.

These considerations lead to more points than necessary because only 10 or 14 points are required minimally by a quadratic and a special cubic model respectively.

For this truncated design space, however, it is preferable to

define clearly the upper triangle by the points 1 to 3, although one point in the middle of the triangle should be sufficient to construct the models. This means that in our opinion 12, respectively 16 points, are a better minimum number of design points. This makes an estimation of E also possible.
We decided to use 26 points because we wanted more degrees of freedom for the comparison of different models. This number gives us sufficient degrees of freedom at the cost of a reasonable experimental effort given the speed of the TLC method. The mobile phase composition at the 26 experimental points is given in Table 2. Only points 1 to 16 are shown in Fig. 1 for reasons of clarity. The addition of three more points, 27 to 29 shall be explained in the next section.

Model Construction and Optimization Criteria

After the factor space and the experimental design have been selected, chromatograms of the alkaloids are obtained at eluent compositions corresponding with the design points and the retention factors, R_f, are measured. From the R_f values the capacity factors, k, of the components are calculated by

$$k = 1/R_f - 1 \qquad (3)$$

Then the logarithm of the capacity factor is used to construct the models because this transformation converts the proportional variance of the data to a constant variance (27). After construction of the models by multiple linear regression chromatograms are predicted at all solvent compositions of the design space to obtain a grid with a 1%, 2% or 5% interval in X_1 to X_4. Next the resolutions, R_s, are calculated for all adjacent spots at all points of the grid, where

$$R_s = 0.25\ (k_1/k_2 - 1)\ (N.R_{fm})^{0.5}\ (1 - R_{fm}) \qquad (4)$$

k_2 and k_1 are the capacity factors of the adjacent spots, N is

the plate number and R_{fm} is the mean retention factor of the adjacent spots (28). k_2 and k_1 are calculated from eqn. 3, which is not possible if the spot does not move from the start point. By replacing the design points 5, 6 and 11 by the points 27 to 29 this problem was solved in all but one case.

The minimal value of the resolution at every grid point, $R_s$min, is stored. Also the highest capacity factor at every grid point, kmax, is stored. The purpose of using two criteria, $R_s$min and kmax is to select the eluent compositions that give the "best" chromatograms. The quality of a chromatogram may be assessed by considering two aspects of a chromatogram. The most important is a good separation of the two spots that are most difficult to separate: $R_s$min has to be sufficiently high. The other aspect is the location of the spots. Spots at smaller R_f values are to be preferred because spots are smaller at low R_f values: usually higher kmax values are to be preferred. Very high values of kmax, however, (larger than about 100) are to be avoided, because this keeps the lowest spot at the start point.

Accordingly a sorting routine has been incorporated that sorts on ranges of increasing $R_s$min and kmax values. From all eluent compositions only those are selected that provide the highest resolutions. Subsquently these compositions can be searched for suitable values of kmax. This excludes eluent compositions that give values of $R_s$min that are too low and also eluent compositions that give unsuitable kmax values, whereby an important data reduction is obtained.

Data reduction is important because the optimization procedure scans all grid points of the design space and results in a long list of $R_s$min values with the corresponding eluent compositions and an equally long list of the corresponding kmax values. It is not possible to produce contour plots in the three dimensions of the factor space that would allow an easy selection of suitable ranges of $R_s$min and kmax. Graphics output of the tetrahedron can only serve an illustrative purpose to indicate where the highest $R_s$min values and suitable ranges of kmax can be

found. The proposed sorting routine gives a good selection of clusters of suitable eluent compositions through which triangular cross sections can be constructed in the tetrahedron. In these planes contour plots can be constructed for an easy selection of eluent compositions that correspond with suitable values of $R_s$min and kmax.

EXPERIMENTAL

Chloroform, ethyl acetate, methanol and alkaloids were of analytical reagent grade, dietylamine was chemically pure. All reagents were used without further purification. Solutions of the alkaloids were made at concentrations of 2 mg/ml by dissolving in methanol. With disposable micropipettes 4 micrograms were spotted on precoated, 20x20 cm, silicagel 60 F-254 TLC plates (Merck). Of the Belladonna alkaloids 8 micrograms were used.

The samples of the individual alkaloids were spotted 1 cm from the lower edge of the plate, which was developed by each of the 29 mobile phases of Table 2 in ascending mode in a pre-saturated (30 minutes) tank. The solvent front moved 100 mm in 15 to 25 minutes. Room temperatures varied from 19 to 21 °C and the relative humidity of the atmosphere varied from 39% to 54%. The spots were visualized by spraying with potassium iodobismuthate solution (29).

Software

Calculations were performed on an IBM-XT compatible personal computer using the POEMTL (Predicting Optimal Eluent Mixtures for TLC) software package written in Pascal. The POEMTL package is designed for the optimization of mobile phases consisting of two, three or four (pseudo)components using mixture designs in the separation of at most fifteen solutes.

The first two sections handle the definition of the experimental design and the data input. The model section permits the choice of different polynomials to model the (logarithm) of

the capacity factor as a function of the eluent composition. The coefficients are calculated by multiple linear regression and validated by an analysis of variance. From the models of the capacity factors response surfaces of $R_s$min and kmax are calculated. Ranges of suitable values of both criteria can be selected and depicted inside the tetrahedron. Response surfaces of a triangular cross section or a triangular face of the tetrahedron can be plotted as contour plots. The response values and the corresponding eluent compositions can also be listed. A TL chromatogram can be simulated for a given mobile phase composition.

RESULTS AND DISCUSSION

The R_f values of the 14 parent alkaloids were determined at the 29 eluent compositions of Table 2. The results are shown in Figs. 2A - D and presented in four groups according to the presence of the alkaloids in the dry plant material: Fig.2A corresponds with Ipecacuanha root, Fig.2B with Cinchona bark, Fig.2C with Belladonna leaf and Fig.3D with Opium. Solvent compositions 5, 6, and 9 are composed of $CHCl_3$ and/or EtAc and are not strong enough and most alkaloids remain at the starting point. As these R_f values can not be used for the calculation of the capacity factor, points 5, 6 and 9 were substituted by 27, 28 and 29, which contain 5 per cent DEA besides $CHCl_3$ and/or EtAc. In these mobile phases the R_f values of all alkaloids except narceine were greater than zero (Figs.2A-D). Solvent compositions for which a given alkaloid did not move from the point of application were not used for the construction of the corresponding model. Inspection of the Figs. 2A - D shows that the chosen solvent system provides good to reasonable separations for all four groups of alkaloids at one or more design points. This is obvious for the alkaloids of Ipecacuanha (Fig.2A) and Belladonna (Fig.2C) The eluent composition of design points 8 (=18) and 14 give a reasonable separation for the Cinchona

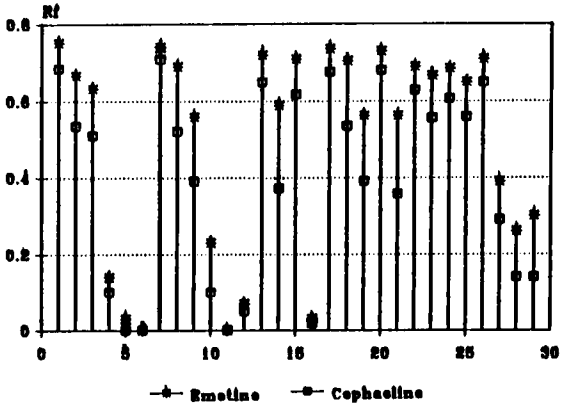

FIGURE 2A. Chromatograms of the parent alkaloids of Ipecacuanha root. Numbers refer to the eluent compositions of Table 2.

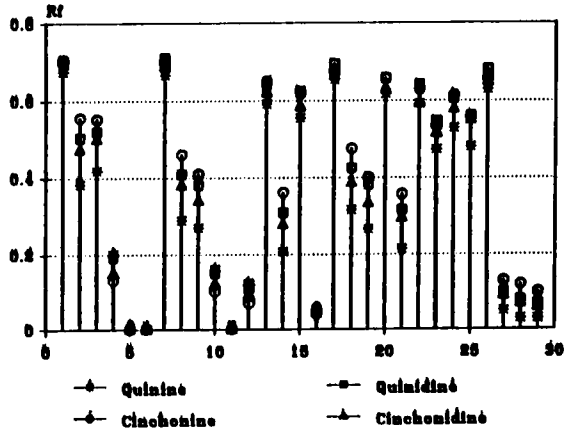

FIGURE 2B. Chromatograms of the parent alkaloids of Cinchona bark. Numbers refer to the eluent compositions of Table 2.

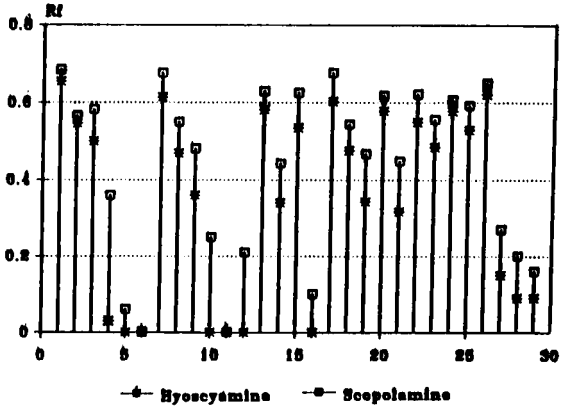

FIGURE 2C. Chromatograms of the parent alkaloids of Belladonna leaf. Numbers refer to the eluent compositions of Table 2.

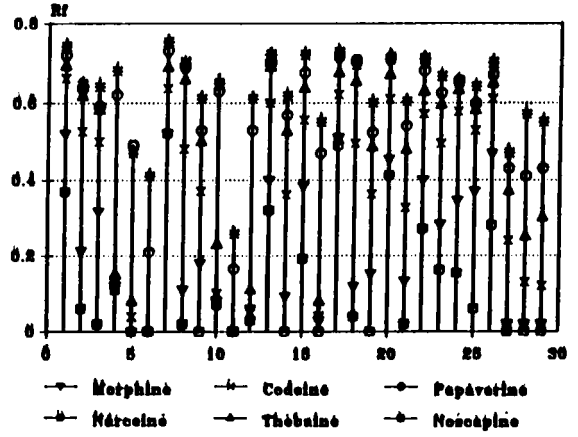

FIGURE 2D. Chromatograms of the parent alkaloids of opium. Numbers refer to the eluent compositions of Table 2.

alkaloids (Fig.2B) and a good separation is obtained for the opium alkaloids by eluent composition of point 21 (Fig.2D).

This means that a major goal has been achieved: the four solvents of this solvent system allow the selection of mobile phases for the separation of the parent alkaloids of the four plant materials. The separation of the alkaloids of Ipecacuanha, Cinchona and Belladonna can be realised by the use of only two solvents: DEA and $CHCl_3$.

For the simple separations of the alkaloids of Ipecacuanha and Belladonna it is not necessary to proceed with the optimization procedure because the compositions at quite a few design points give good separations. Nevertheless response surface modelling can give an indication of the steepness of the response surface at a given eluent composition. A small slope is to be preferred because small variations in the mobile phase composition should not lead to large variations of the R_f values. The model selection shall be discussed for Ipecacuanha root and the location of the optimum solvent composition shall be illustrated for Cinchona bark and Opium.

Ipecacuanha root

Special cubic and quadratic models were considered. The models were constructed firstly by using all available design points (1 to 29, minus points 5, 6 and 11, Fig.1) and secondly by using the minimum number of design points that was necessary in our opinion (see Theory, Design Space and Experimental Design). This minimum number implies points 1 to 4, 7 to 10, 12 and 27 to 29 (Fig.1) for a quadratic model based on 12 points and moreover points 13 to 16 (Fig.1) for a special cubic model based on 16 points. These models were estimated by multiple linear regression for cephaeline and emetine. An analysis of variance of eight models (four per compound) showed that the null hypothesis, which states that the assumed relation does not exist, could be rejected in all cases at the 2 percent level of significance or

TABLE 3

Comparison of Experimental and Predicted R_f x 100 values

Eluent comp. Nr	DEA	$CHCl_3$	Experim. Em	Experim. Ce	Spcub.26 Em	Spcub.26 Ce	Quad.26 Em	Quad.26 Ce	Spcub.16 Em	Spcub.16 Ce	Quad.12 Em	Quad.12 Ce
01	.24	.76	73	55	73	59	75	62	73	59	73	59
02	.16	.84	67	47	66	51	67	53	65	51	65	51

Spcub. is a special cubic model, Quad. is a quadratic model. The model number indicates the number of design points used for construction of the model. Predicted values under model heading.

lower. The percentage explained variance by the models varied from 93.6 to 99.7 percent.

The results did however not allow to prefer clearly one model to the others and we therefore decided to compare the measured R_f values with those predicted by the different models for 19 eluent compositions, i.e. 17 experimental points and 2 new measurements, at eluent compositions 01 and 02. The eluent compositions 01 and 02 were introduced as independent tests for the models that were based on 26 points; for the models based on 12 and 16 points the eluent compositions 20 to 26 are also independent tests. The results for eluent compositions 01 and 02 are given in Table 3 as an example. No model preference could be based on the differences between the R_f values predicted by the different models and the experimental values at the above mentioned 19 eluent compositions. The models had a tendency to deviate in the same positive or negative manner for a given experiment and some experiments seemed more difficult to predict for all models. The mean values of the absolute deviation of the eight models varied from 2.4 to 4.6 R_fx100 values and did not differ significantly at the 5 percent level. Again no clear preference for a definite model was apparent.

Therefore we decided to use all available information and to base our predictions on a quadratic model based on 26 points. A

more detailed study of the response surfaces and control experiments are necessary especially to elucidate the adequacy of models based on 12 and 16 points in comparison to those based on a larger number of experiments. This is, however, outside the scope of the present investigation.

If we restrict the number of solvents of the mobile phase to two, because of reasons of simplicity, then good separations are obtained by binary mixtures of DEA and $CHCl_3$ or of DEA and EtAc. The optimum concentration of DEA is about 15 percent (composition 02) in the first case and about 20 percent (composition 9) in the second. Both mobile phases have more simple composition than the one recommended by the European Pharmacopoeia: chloroform:methanol:strong ammonia = 95:6.5:0.5.

Cinchona bark

The mobile phase compositions at design points 8 (= 18) and 14 provided the best separations of all design points. The compounds quinidine and cinchonidine are the most difficult pair to separate properly and the $R_s$min was equal to 0.92 and 0.85, respectively. This resolution was calculated by eqn. 4 for an average plate number N = 3000. This plate number was an estimate from measured spot diameters and R_f values and corresponds reasonably well with the maximum platenumber that is obtainable for particles with a diameter of 15 micrometer (23). The absolute value of the plate number is of no importance; the main purpose of the calculation of $R_s$min is to have a measure for comparison of separations at different eluent compositions.

Again quadratic models were constructed by the use of 26 design points. The maximal predicted $R_s$min was about 0.9. Scanning of the factor space for $R_s$min between the limits of 0.8 and 1.0 revealed three clusters of eluent compositions that can provide these values of $R_s$min (Fig.3). Two clusters are located near the X1-X3 and X1-X4 edges of the tetrahedron. A plot of the response surface of the X1,X3,X4 face of the tetrahedron is shown in Fig.4.

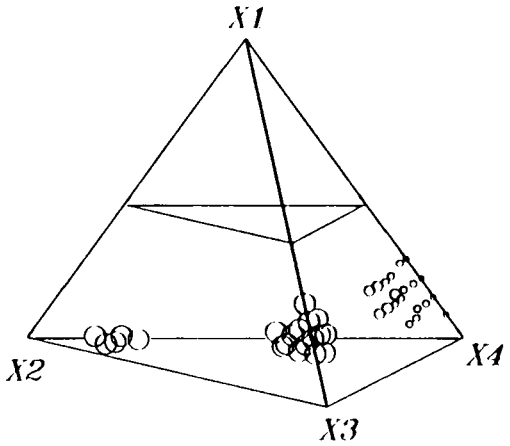

FIGURE 3. Solvent tetrahedron of figure 1 with clusters of eluent compositions that give chromatograms of the parent alkaloids of Cinchona bark for which $R_s$min values lie in the range 0.8 to 1.0.

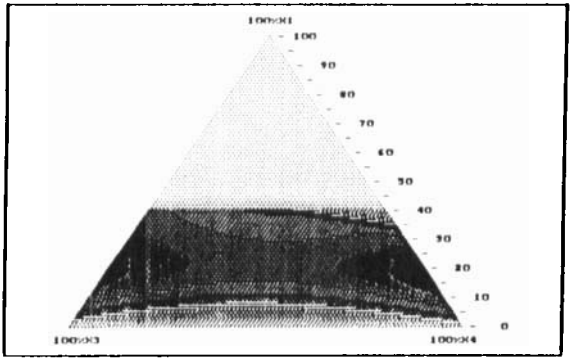

FIGURE 4. Response surface (contour plot) of $R_s$min of the X1,X3,X4 face of the tetrahedron for the separation of parent alkaloids of Cinchona bark. Different symbols correspond to ten different ranges of $R_s$min values extending from 0.0 to 1.0. The highest values (optima) of $R_s$min are indicated by the dark areas near the X1-X3 and X1-X4 axes.

Predicted to be the best binary eluents were: 18 percent DEA in $CHCl_3$ and 17 percent DEA in EtAc. The quality of the chromatograms obtained with these mobile phases was approximately the same and somewhat better than the one obtained with a mobile phase composed of 10 percent DEA in $CHCl_3$, which is recommended by the European Pharmacopoeia, but all separations were insufficient: $R_s$min was circa 0.6. The eluent composition of design point 18 (20 percent DEA in $CHCl_3$) produced a better resolution and a small increase of the percentage of DEA in $CHCl_3$ to 24 percent gave a better chromatogram with a $R_s$min value of 0.95. Complete resolution of quinidine and cinchonidine was not obtained.

Belladonna leaf

The separation of hyoscyamine and scopolamine appears to be no problem. If we consider only binary eluent compositions then design points 3, 4, 8 (=18), 9 (=19), 27 and 28 give good separations (Fig.2C). This means that binary mixtures of DEA and $CHCl_3$ with a DEA content varying from 5 to 20 percent, binary mixtures of DEA and EtAc with a DEA content varying from 5 to 40 percent and binary mixture of equal parts of MeOH and EtAC provide all good separations. A somewhat more complicated mobile phase is recommended by the European Pharmacopoeia: acetone:water:strong ammonia = 90:7:3.

Quadratic models were constructed using 23 experiments because at the design points 5, 6, 10, 11, 12 and 16 one ore more alkaloids remained at the start point. A chromatogram with a ternary mobile phase consisting of DEA:MeOH:EtAc = 10:30:60 was predicted to have R_f values 0.35 and 0.57 for hyoscyamine and scopolamine respectively; the experimental values were 0.50 and 0.62.

Opium

A quadratic model of narceine had to be constructed from only 19 design points because at ten eluent compositions narceine

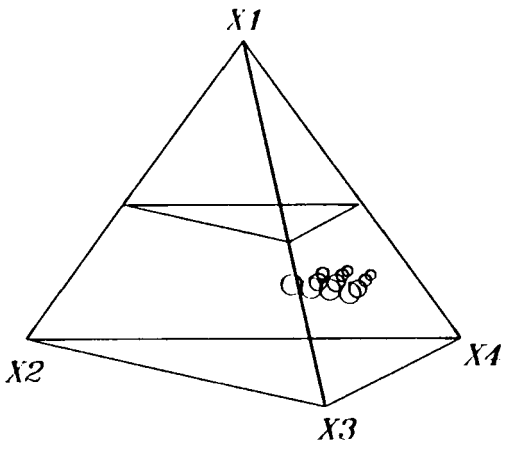

FIGURE 5. Solvent tetrahedron of figure 1 with cluster of eluent compositions that give chromatograms of the parent alkaloids of opium for which $R_s$min values are greater than 1.2.

remained the start point (Fig.2D). For the models of the other alkaloids 26 experiments were available. This means that the design space of narceine is smaller than the design space of the other alkaloids and is confined to the space between the two triangular cross sections defined by the design points 1, 2, 3, and 4, 8, 9, respectively (Fig.1). This limits also the space of the response surfaces of $R_s$min and kmax of all alkaloids to this smaller design space. To find the largest possible design space additional experiments should be performed with mixtures of DEA and $CHCl_3$ and/or EtAc of which the concentration of DEA is varied between 5 percent (points 27, 28 and 29) and 20 percent (points 8, 14 and 9). We decided not to perform additional experiments, but to investigate firstly the smaller design space.

A scan of the design space for $R_s$min values greater than 1.2 showed a cluster of eluent compositions (Fig.5) of which some were ternary mixtures located in the X1,X2,X4 face of the

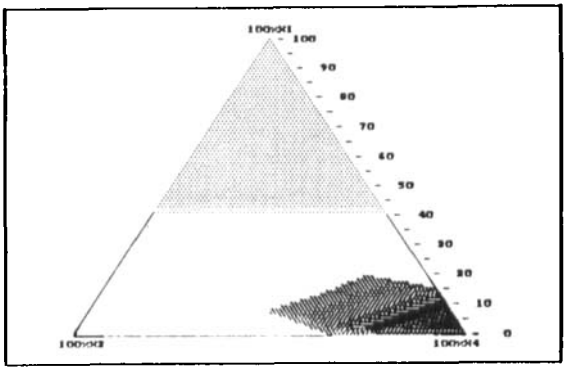

FIGURE 6A. Response surface (contour plot) of $R_s$min of the X1,X2,X4 face of the tetrahedron for the separation of the parent alkaloids of opium. Ten different ranges of $R_s$min values greater than 1.2 are shown. Lower values are not depicted. Highest values are located near X4 vertex outside design space.

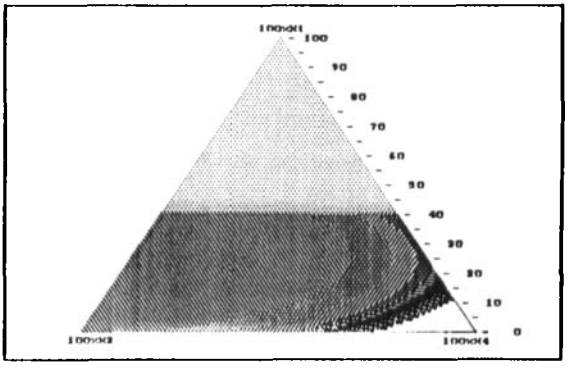

FIGURE 6B. Response surface (contour plot) of kmax of the X1,X2,X4 face of the tetrahedron for the separation of the parent alkaloids of opium. Ten different ranges of kmax values smaller than 100 are shown. Highest values near X4 vertex are not depicted.

tetrahedron. Contour plots of $R_s$min and kmax of this face are shown in Figs.6A and B. In Fig.6A only those eluent compositions are depicted that give $R_s$min values greater than 1.2. The response surface is also extrapolated outside the boundaries of the design space, which is not allowed and therefore this part of the response surface is rather unreliable. Fig.6B shows a contour plot for kmax values smaller than 100. The kmax is the capacity factor of the slowest moving compound, i.e. narceine. Therefore Fig.6B shows which eluent compositions give R_f values of narceine greater than about 0.01. The eluent compositions that can be found in Fig.6A as well as in Fig.6B should produce chromatograms that have a resolution of the worst separated pair of spots greater than 1.2 and also an R_f value of narceine greater or equal to 0.01.

The best mobile phase composition predicted inside the design space of narceine was: DEA:MeOH:EtAc = 16:7:77. The experimental value of $R_s$min was much lower than predicted: 0.6 versus 1.5 and no good separation was obtained.

If a slight extrapolation beyond the design space was accepted then an eluent composition of DEA:MeOH:EtAc = 10:7:83 should produce a chromatogram with a higher $R_s$min but a smaller R_f value of narceine. This mobile phase composition was tested and the measured values of $R_s$min and R_f were 1.7 and 0.03 respectively versus predicted values of 2.2 and 0.01. A good separation is also obtained by the eluent composition of design point 21: DEA:$CHCL_3$:EtAC = 20:10:70. The respective values of $R_s$min and of R_f of narceine were 1.2 and 0.03. Both eluents give better chromatograms than the chromatogram obtained by toluene:acetone:ethanol:strong ammonia = 20:20:3:1. This eluent recommended by the Netherlands Pharmacopoeia gives a good separation of all spots but narceine does move from the start.

CONCLUSIONS

The results show that the chosen four solvents form a promising quaternary solvent system to compose mobile phases for the TLC separation of alkaloids or basic compounds.

From the quaternary solvent system mobile phases were selected for the separation of the parent alkaloids of Ipecacuanha root, Cinchona bark and Belladonna leaf, which were better than the mobile phases prescribed by the European or Netherlands Pharmacopoeia: separations were either better for difficult samples or could be performed by eluents composed of a smaller number of solvents.

The quaternary solvent system has reduced the number of solvents used for the above mentioned separations from eight to four and therefore it may be expected that it may contribute in a general way to more efficient mobile phase composition in TLC by reducing the number of necessary solvents.

In this exploratory investigation the results are based on R_f measurements at 26 eluent compositions to obtain a good impression of the possibilities of the quaternary system. Optimization should be possible by using a smaller number of design points, but this needs further investigation.

A more detailed study of the of the accuracy of the predictive power of the response surfaces is desired, because it is our opinion that the response surface of $R_s$min can indicate indeed clusters of good eluent compositions in the factor space, but that the accuracy of quantitative predictions needs to be improved.

REFERENCES

1 J.L. Glajch, J.J.Kirkland, K.M. Squire, J.M. Minor, J. Chromatogr.,199 57 (1980).
2 J.L. Glajch, J.J. Kirkland, L.R.Snyder, J. Chromatogr., 238 269 (1982).
3 H.J. Issaq, J.R. Klose, K.L. McNitt, J.E. Haky, G.M. Muschik, J. Liq. Chromatogr., 4 2091 (1981).
4 P.M.J. Coenegracht, Nguyen Van Tuyen, H.J. Metting, P.J.M. Coenegracht-Lamers, J. Chromatogr. 389 351 (1987).
5 Sz. Nyiredy, B. Meier, C.A.J. Erdelmeier, O. Sticher, J. High Resol. Chromatogr. & Chromatogr. Commun., 8 186 (1985).
6 Sz. Nyiredy, C.A.J. Erdelmeier, B. Meier, O. Sticher, Planta Medica 241 (1985).
7 Netherlands Pharmacopoeia, ninth ed., The Hague, 1983, (94) II, p.291.

8 Netherlands Pharmacopoeia, ninth ed., The Hague, 1983, (174) III, p.37.
9 Netherlands Pharmacopoeia, ninth ed., The Hague, 1983, (221) III-209
10 Netherlands Pharmacopoeia, eighth ed., The Hague, 1978, p. 811.
11 L.R. Snyder, J. Chromatogr. Sci., 16 223 (1978).
12 D. Waldi, K. Schnakerz, F. Munter, J. Chromatogr. 6 61 (1961).
13 A. Norfalise, G. Mees, J. Chromatogr. 31 1379 (1967).
14 A.C. Moffat, K.W. Smalldon, C. Brown, J. Chromatogr. 90 1 (1974)
15 A.C. Moffat, Thin layer chromatography. In: Clarke's Isolation and Identification of Drugs, A.C. Moffat (Ed.). Pharmaceutical Society, London 1986, pp.160-170.
16 H. De Clerq, D.L. Massart, J. Chromatogr. 115 1 (1975).
17 P.G.A.M. Schepers, J.P. Franke, R.A. de Zeeuw, J. Anal. Toxicol. 7 272 (1983).
18 G. Musumarra, G. Scarlata, G. Scirma, G. Romano, S. Palazzo, S. Clementi, G. Guilietti, J. Chromatogr. 295 31 (1984).
19 L.R. Snyder, High-Performance Liquid Chromatography, Vol. 3, pp. 157-223, Cs. Horváth (Ed.), Academic Press, New York, 1983.
20 B.L. Karger, L.R. Snyder, C. Eon, J. Chromatogr., 125 71 (1976).
21 Sz. Nyiredy, K. Dallenbach-Tölke, O. Sticher, J. Planar Chromatogr. 1 336 (1988).
22 D. Nurok, Chem. Rev. 89 363 (1989).
23 F. Geiss, Fundamentals of Thin Layer Chromatography, Alfred Hüthig Verlag, Heidelberg, 1987, Chapter II.
24 L.R. Snyder, Mobile-Phase Effects in Liquid-Solid Chromatography. In : High-Performance Liquid Chromatography, Vol. 3, editor: Cs. Horváth, Academic Press, New York, 1983, pp.157-223.
25 A. Baerheim Svendsen, R. Verpoorte, Chromatography of Alkaloids, Part A: Thin Layer Chromatography, Elsevier Scientific Publishing Company, Amsterdam, 1983, Chapter 3.
26 D.M. Steinberg, W.G. Hunter, Technometrics, 26 71 (1984).
27 G.E.P. Box, N.R. Draper, Empirical Model-Building and Response Surfaces, J. Wiley & Sons, New York, 1987, Chapter 8.
28 F. Geiss, Fundamentals of Thin Layer Chromatography, Alfred Hüthig Verlag, Heidelberg, 1987, Chapter III.
29 Netherlands Pharmacopoeia, ninth ed., The Hague, 1983, I, p.423.