Planar Chromatographic Method Development Using the PRISMA Optimization System and Flow Charts

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Abstract

This study presents a modern planar chromatographic method-development procedure, based on the "PRISMA" optimization system, in which the optimum separation is achieved systematically and the structures and properties of the substances to be separated are not known. The procedure consists of three stages. In the first of these the basic conditions the stationary phase, vapor phase, and individual solvents are selected with a TLC procedure (generally in nonsaturated chromatographic chambers). In the second stage, the optimum combination of the selected solvents is determined with the PRISMA model. The third part of the procedure includes the selection of the development mode (circular, linear, or anticircular); the selection of an appropriate forced-flow chromatographic technique (over-pressured layer chromatography or rotation planar chromatography) with high-performance thin-layer chromatographic plates; the transfer of the optimized mobile phase to the various analytical, planar, or column preparative liquid chromatographic techniques; and the selection of the operating conditions. For practical reasons, the optimization process is presented with the help of flow charts.

Introduction

With the introduction of highly sophisticated and automated instruments for high-performance liquid chromatography (HPLC), many mobile phase optimization procedures and criteria (e.g., selectivity) have been described and summarized extensively (1–4). On the basis of a three-dimensional representation, the solvent classification of Snyder (5), and the seven-point optimization method of Glajch et al. (6), the "PRISMA" mobile phase optimization model was developed by Nyiredy et al. in 1985. The model for manual selection of solvents and optimization of the mobile phase was developed first for thin-layer chromatography (TLC) (7) and HPLC (8) and later applied to forced-flow planar chromatographic (FFPC) techniques such as overpressured layer chromatography (OPLC) (9) and rotation planar chromatography (RPC) (10). A computer-assisted HPLC mobile phase optimization procedure was published later (11,12). The applicability of the PRISMA system for planar chromatographic (PC) separations has been presented elsewhere (13). The study of the vapor phase (14), role of chamber saturation (15) in the optimization of PC, and transfer of the optimized analytical mobile phase to the various analytical and preparative forced-flow liquid chromatographic techniques provided the basis for an automatic optimization system for nonpolar compounds (16). In PC method development, the selection of the stationary and vapor phases, a suitable development mode, and the forced-flow technique established the PRISMA optimization system (17).

In the last 15 years or more the applicability of the PRISMA optimization system has been discussed in hundreds of papers. This study describes a modern PC method development process based on this optimization system. The optimum PC separation can be achieved systematically and the structures and properties of the substances to be separated do not have to be known. For practical reasons the strategy is also given in the form of flow charts.

Experimental

The PRISMA system for PC

The PRISMA system in PC (Figure 1) consists of three parts. The first is the selection of the basic conditions: the stationary phase, vapor phase, and individual solvents for the mobile phase optimization process.

In the second part, the optimum combination of the individual

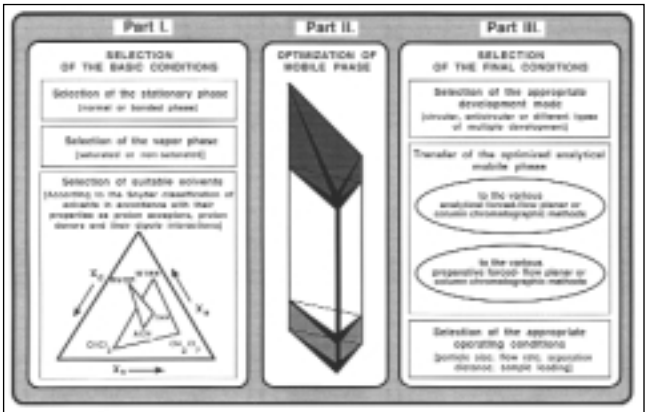


Figure 1. The PRISMA system for planar chromatography (17).

solvents selected is determined by means of the PRISMA model. By use of this three-dimensional geometric design all mobile phase combinations of between two and five solvents can be characterized. The third part of the procedure entails the selection of the development mode (circular, linear, or anticircular), chromatographic technique [high-performance thin-layer chromatography (HPTLC), OPLC, or RPC], and the transfer of the optimized TLC mobile phase to the various analytical, preparative planar, and column liquid chromatographic techniques.

Selection of the basic conditions (the first part of the PRISMA system)

Selection of the stationary phase

PC separations have been performed on unmodified, modified, and impregnated stationary phases because of differences between the chemical properties of the adsorbent material and the substances of the sample to be separated (18). Different types of the chromatographic process have been distinguished on the basis of the types of interaction involved. Because silica has excellent separating power and is the most used (90–95%) stationary phase in normal phase (NP)-PC, the PRISMA optimization process usually starts with this adsorbent.

In the event that the structures and properties of the substances to be separated are known, the decision flow chart for systematic selection of the appropriate separation technique and stationary phase are given in Figure 2.

If the sample contains compounds with a wide polarity range, the automatic multiple development (AMD) mode is considered. For aromatic hydrocarbons or isomeric compounds, either reversed-phase with aqueous buffers or ion-pair chromatography might be appropriate. If the sample to be separated is extremely polar and contains homologous compounds, reversed-phase chromatography is the best option. If the sample

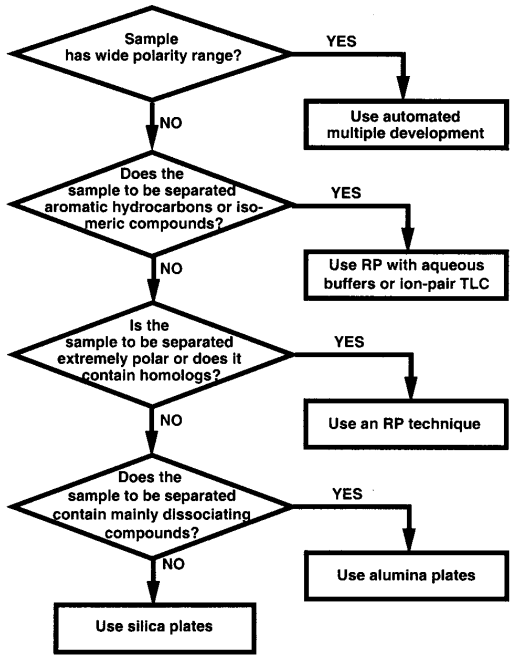


Figure 2. Flow chart for selection of the stationary phase and separation technique.

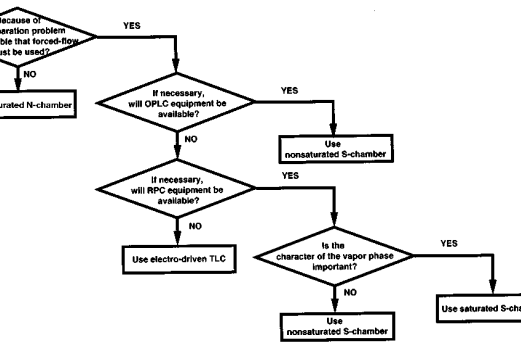


Figure 3. Flow chart for selection of chamber type and saturation (17).

Table I. Solvents for the Normal Phase TLC Optimization Procedure with Snyder's Suggested Solvent Classification and Values (5)

Group	Solvent	Solvent strength (s_i)	x_e	x_d
–	n-Hexane	0	–	–
I.	n-Butyl ether	2.1	0.44	0.18
	Diisopropyl ether	2.4	0.48	0.14
	Methyl-t-butyl ether	2.7	0.49	0.14
	Diethyl ether*	2.8	0.53	0.13
II.	i-Pentanol	3.7	0.56	0.19
	n-Butanol	3.9	0.56	0.19
	i-Propanol	3.9	0.55	0.19
	n-Propanol	4.0	0.54	0.19
	Ethanol*	4.3	0.52	0.19
	Methanol	5.1	0.48	0.22
III.	Tetrahydrofuran*	4.0	0.38	0.20
	Pyridin	5.3	0.41	0.22
	Methoxyethanol	5.5	0.38	0.24
	Methylformamide	6.0	0.41	0.23
	Dimethylformamide	6.4	0.39	0.21
	Dimethylsulfoxide	7.2	0.39	0.23
IV.	Acetic acid*	6.0	0.39	0.31
	Formamide	9.6	0.36	0.23
V.	Dichloromethane*	3.1	0.29	0.18
	1,1-Dichloroethane	3.5	0.30	0.21
	Benzylalcohol	5.7	0.40	0.30
VI.	Ethyl acetate*	4.4	0.34	0.23
	Methyl ethyl ketone	4.7	0.35	0.22
	Dioxane*	4.8	0.36	0.24
	Acetone	5.1	0.35	0.23
	Acetonitrile	5.8	0.31	0.27
VII.	Toluene*	2.4	0.25	0.28
	Benzene	2.7	0.23	0.32
	Nitrobenzene	4.4	0.26	0.30
	Nitromethane	6.0	0.28	0.31
VIII.	Chloroform*	4.1	0.25	0.41
	Dodecafluoroheptanol	8.8	0.33	0.40
	Water	10.2	0.37	0.37

* something here???????????

contains mainly dissociated compounds, alumina is the most suitable stationary phase (17).

Selection of the vapor phase

Selection of the chamber type and vapor space is possible with PC only; in practice, little attention is devoted to this (14).

In PC, two basic types of chromatographic chambers must be distinguished. In the generally used normal (N)-chamber, the distance between the plate and the wall of the chromatographic tank is more than 3 mm. If this distance is smaller, the chamber is said to have the sandwich (S) configuration (15). Both types of chamber can be used for saturated or nonsaturated systems. As a rule of thumb, if the sample contains more than seven substances or the separation is very difficult, nonsaturated S-chambers should be selected because this enables subsequent transfer of the optimized TLC mobile phase to a forced-flow separation technique. If the sample contains fewer than seven compounds to be determined quantitatively, saturated N-chambers can be selected (15).

Often the separation problem cannot be solved by use of capillary-driven TLC because of the relatively modest separating power of this mode. If so, the use of different forced-flow techniques (OPLC or RPC) is necessary; this must be considered during selection of the vapor phase. Selection of the vapor phase therefore depends on whether or not forced-flow techniques are available for the final separation. The chambers used for forced-flow planar separations can also be assigned to the noted two categories. The chambers used for OPLC are nonsaturated S-chambers that are devoid of any vapor space. This must be considered when selecting the appropriate solvents and during optimization of the mobile phase. In RPC, the size and therefore the extent of saturation (10) of the vapor phase can be varied; the micro (M)- and ultramicro (UM)-chambers are S-type chambers. In M-chamber RPC, the plate rotates with the small chromatographic chamber and the vapor space is saturated rapidly. In UM-chamber RPC, there is almost no vapor space, similar to OPLC (9).

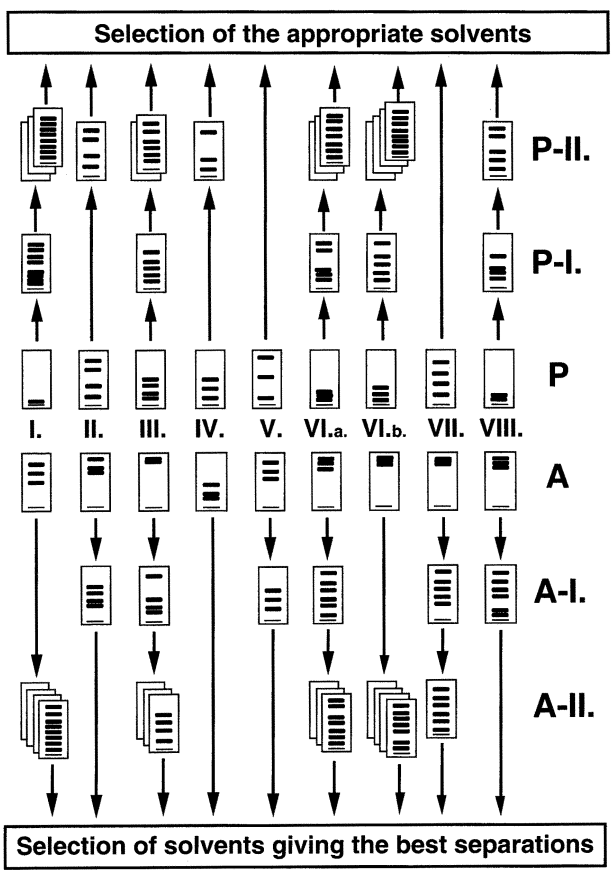


Figure 4. Selection of the individual solvents with the first part of the system (13).

Among the forced-flow methods OPLC has the highest separating power because of the possibility of using the optimum mobile phase velocity on the HPTLC plate and the long separation distance. If the separation distance is an important factor in the final separation, OPLC should be selected and the preassay for mobile phase selection should be performed in a nonsaturated S-chamber. If RPC equipment is available to improve the efficiency of the final separation, the chamber of choice for the preassay depends on the type of compound to be separated. If a vapor phase of an acidic or basic character is important for the separation, a saturated S-chamber (if available) is the right choice for TLC preassay. If a saturated S-chamber is not available, a saturated N-chamber is useful. Otherwise, a nonsaturated S-chamber should be chosen for transposition of the mobile phase to an RPC separation. The decision flow chart for selection of the vapor phase is given in Figure 3.

Selection of the individual solvents

The third step in the first part of the PRISMA strategy is solvent selection. This is based on the solvent classification of Snyder (5), who classified more than 80 solvents into 8 groups for NP chromatography according to their properties as proton acceptors (xa) or proton donors (xd) and their dipole interactions (xn). From these 8 groups, 27 solvents commonly used in PC have been listed in Table I.

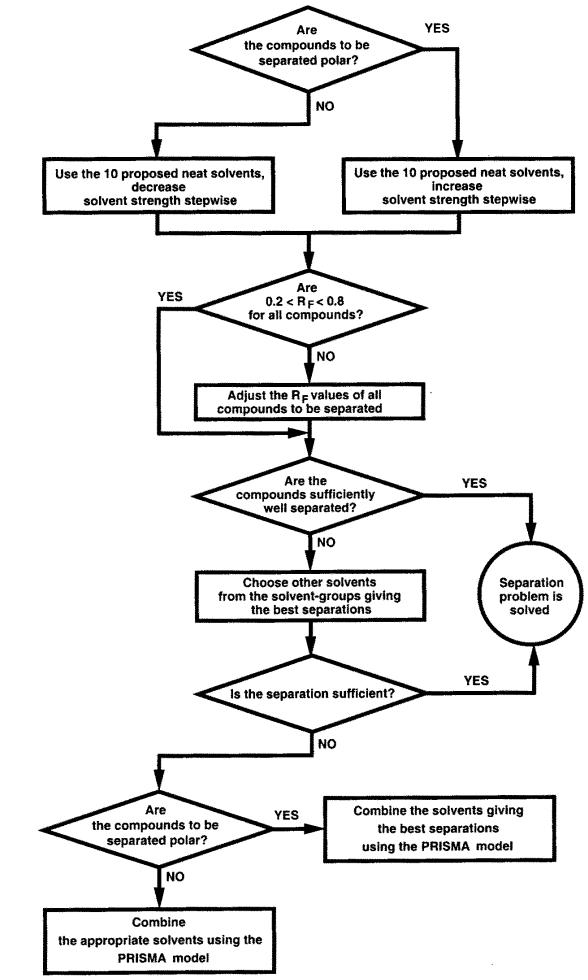
For the selection of suitable solvents, the first experiments are conducted on silica TLC plates in nonsaturated chromatographic chambers with the ten solvents indicated by stars in Table I. After these first TLC experiments with the neat solvents, the solvent strength must either be reduced or increased so that the substance zones are distributed between RF 0.2 and 0.8. The two theoretical situations are depicted as "A" and "P" in Figure 4. If the compounds to be separated migrate in the upper third of the plate (A-I in Figure 4), the solvent strength must be reduced by dilution with hexane [solvent strength (si) = 0]. If the substances do not migrate with the neat solvents, the solvent strength must be increased (P-I in Figure 4) by the addition of a small amount of water. In both circumstances the solvent strength should be varied so that better distribution of the substance zones is obtained. Consequently, the structures and properties of the compounds to be separated need not be known. Their classification as nonpolar (A) or polar (P) compounds (13) can be made in accordance with their behavior in these TLC experiments.

If a good separation is obtained with particular solvents, homologs or other solvents of the same group can also be tested, as indicated by A-II and P-II in Figure 4. After these experiments, the solvents giving the best separations are chosen for further optimization for nonpolar compounds. For optimization of the mobile phase for polar compounds, the solvents selected should

include one in which the compounds do not migrate—necessary for the subsequent transfer of the mobile phase to certain forced-flow techniques—by use of the third part of the PRISMA system (13). Occasionally suitable separation can be achieved with the first part of the system. The individual steps of solvent selection by use of the PRISMA system are summarized in a flow chart in Figure 5.

The PRISMA model (the second part of the system)

The PRISMA model, the pivotal component of the PRISMA system (Figure 6), can be visualized as a graphic spatial representation of si and the proportions of the components that determine the selectivity. Between two and five solvents can usually be selected for construction of the PRISMA model.



FFigure 5. Flow chart for selection of the individual solvents.

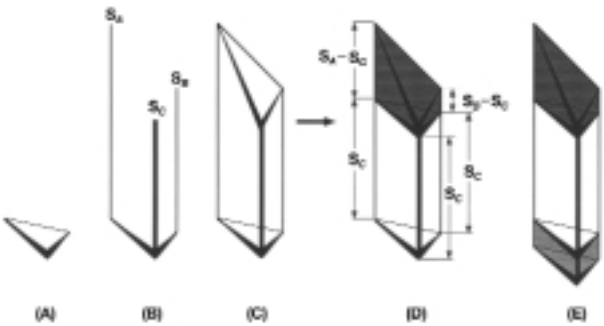


Figure 6. Graphic representation of the PRISMA model.

If the si values of the neat solvents are plotted vertically above an equilateral triangle (Figure 6A) and if the two-dimensional representation of the solvent concentrations, which primarily influence the selectivity, is plotted on the horizontal plane, a prism is obtained with an equilateral triangle as its base. The lengths of the edges of the prism correspond to the solvent strengths of the neat solvents (S_A, S_B, and S_C) in question (Figure 6B). Because different solvents are of different strengths, the lengths of the edges of the prism are generally unequal and the top plane of the prism will not be parallel and congruent with its base (Figure 6C).

If the prism is theoretically cut parallel to the base at the height of the lowest edge (determined by the solvent with the lowest solvent strength in the system) the lower part gives a regular prism (Figure 6D) that has top and bottom planes that are parallel equilateral triangles. The PRISMA model thus consists of three parts: the regular part of the prism (white in Figure 6) with congruent base and upper surfaces; the irregular truncated top prism (dark gray in Figure 6); and the base, symbolizing the modifier (light gray in Figure 6). The solvent strength values of the modifiers are treated as an additive by the PRISMA model (13). For the sake of simplicity, the solvent strength values of the modifiers (e.g., acids and ion pairs) are neglected because they are usually present at low, constant concentrations (generally 0.1% and 3%).

In NP chromatography, the upper irregular part of the model is used for characterization of mobile phases for the separation of polar compounds, whereas the regular part is used to characterize mobile phases for the separation of nonpolar substances. In typical reversed-phase chromatography the regular part is used for the separation, irrespective of the polarity of the compounds to be separated.

The irregular part of the model

The three corners of the top cover (irregular) triangle of the

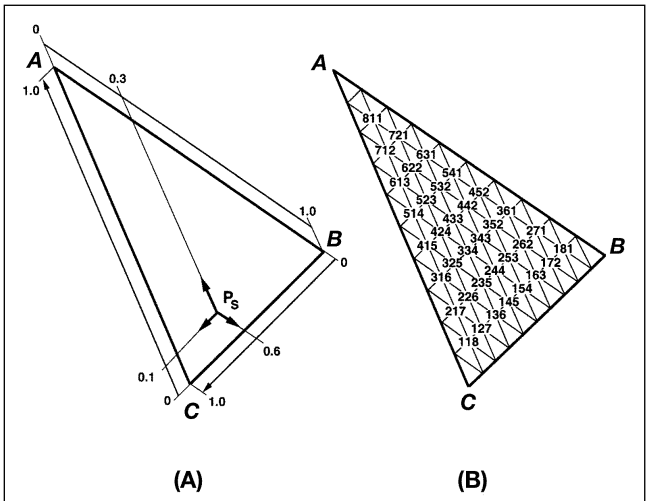


Figure 7. Combination of three neat solvents (A, B, C) on the irregular top triangle of the PRISMA model. (A) represents the volume fractions of the solvents A, B, and C at point PS. and (B) is the selectivity points in the top triangle representing the combinations of the three solvents by three-digit numbers

prism represent the three undiluted neat solvents (Figure 7). The corner corresponding to the longest edge of the prism is equivalent to solvent A (the solvent with the highest solvent strength), whereas solvent *C* (the solvent with the lowest solvent strength) corresponds to the corner of the shortest edge of the prism. In the triangle shown on the left of Figure 7, a particular solvent composition (P_S) can be characterized by the volume fractions of the corners. Here the volume fraction of solvent *A* is 0.1, solvent *B* is 0.3, and solvent *C* is 0.6 (this means that for the mobile phase characterized by point P_S, the concentration (%, v/v) of solvent *A* is 10%, solvent *B* is 30%, and solvent *C* is 60%).

This point of the triangle where the tenfold values (P_A, P_B, and P_C) of all three characteristic volume fractions are integers can be defined by a three-digit number. This number, in which the sum of the digits is 10, can be obtained by multiplying the volume fractions by 10 and arranging them in order of diminishing solvent strength. The solvent composition shown in Figure 7A can therefore be defined by point 136 (which means a mixture of 10% solvent *A*, 30% solvent *B*, and 60% solvent *C*). The triangle on the right of Figure 7 shows all compositions of the three solvents on the cover plate of the prism. They are characterized by integer three-digit numbers and defined as selectivity points (P_S). Because the center of the triangle cannot be described by use of the system already defined, it is characterized by definition as P_S = 333, because this composition is obtained if equal amounts are taken from all three (*A*, *B*, and *C*) solvents (Figure 7B). Because the three solvents selected usually differ in solvent strength, all selectivity points on the surface of the cover plate of the irregular part of the prism represent different solvent strengths and selectivity.

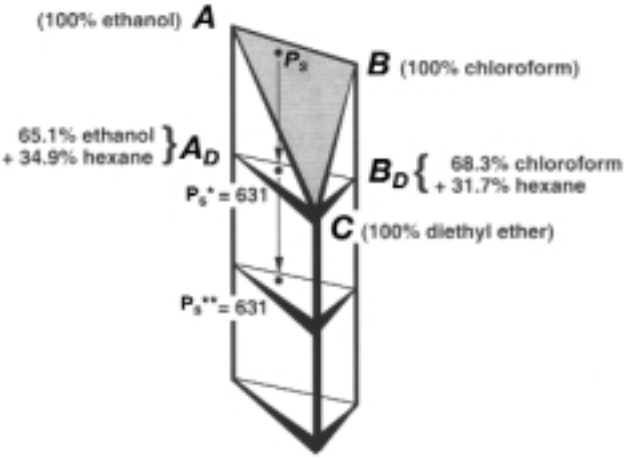


Figure 8. Example of the calculation of mobile phase composition with the PRISMA model in NP chromatography

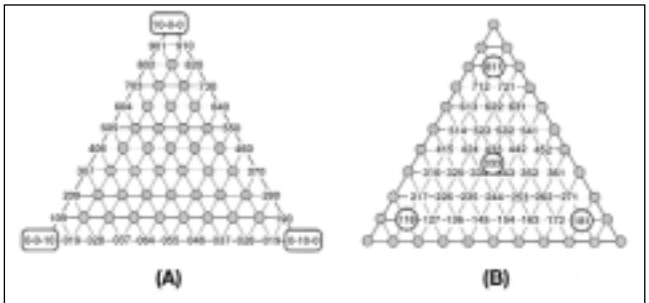


Figure 9. The selectivity points in the regular part of the PRISMA model. (A) selectivity points in the corners represent two solvent combinations; those along the edges of the triangle represent three solvent combinations. (B) selectivity points in the triangle represent four solvent combinations (the three neat solvents and the solvent-strength regulator).

The points along the edges of the cover plate represent mixtures of two solvents ("*A* and *B*", "*B* and *C*", and "*A* and *C*"). Inside the irregular triangle the selectivity points represent mixtures of the three solvents (*A*, *B*, and *C*). Dilution of a mobile phase mixture with a solvent of zero solvent strength (hexane in NP chromatography, water in reversed-phase chromatography) gives mixtures of four solvents, which are characterized by the same selectivity point but have lower solvent strengths. These solvents are represented by the inside points of the upper, irregular part of the prism. Of course, mobile phase mixtures with a solvent strength lower than that of solvent *C* are excluded.

Because in the irregular top triangle the solvent strength differs at each selectivity point, which greatly influences the separation, the steps between two selectivity points are often very large (9). The solvent mixtures between these selectivity points can then be described by three two-digit numbers (e.g., P_S = 57 – 18 – 25). Although even finer adjustments can be made (e.g., P_S = 56.7 – 18.4 –24.9), 1% accuracy is usually sufficient.

The regular part of the model

The base and top plane of the regular part of the prism are congruent equilateral triangles. The height of this part of the prism corresponds to the solvent strength of the weakest solvent. Because of the original selection of a decreasing order of solvent strength for solvents *A*, *B*, and *C*, this is solvent 'C'. This means that corner 'C' of the regular prism represents undiluted solvent *C*. The solvent mixtures represented by corners A_D and B_D (Figure 8) can be obtained by diluting solvents *A* and *B* to the solvent strength of *C* with the use of a solvent of zero strength.

Mobile phases characterized by other points on the top cover plate can be obtained by mixing the solvents represented by the corners of the top cover plate in the volume proportions that correspond to the point in question. The selectivity points representing the two- and three-solvent compositions (the second and third solvent is the solvent strength regulator) are given on the left of Figure 9. The selectivity points symbolizing four solvent compositions frequently used in optimization are characterized—similarly to the points on the top irregular part—by three-digit numbers (on the right in Figure 9). The 4 basic selectivity points within the prism (P_S = 333, 811, 181, and 118) for 4 solvent mixtures are also shown in Figure 9.

The selectivity points on the vertical planes of the regular part of the prism can be obtained by diluting the solvent mixtures with a solvent of zero strength. The solvent strength values decrease from top to bottom; at the base of the prism the solvent strength value is zero. Hexane is used to reduce the mobile phase strength in NP chromatography and water in RP chromatography. If sections are prepared from the regular prism parallel to the base, triangles with different solvent strengths are obtained. Therefore, all points on one of these triangles represent the same solvent strength, whereas all points on a vertical straight line correspond to the same selectivity (13).

Calculation of mobile phase composition

Construction of the irregular and regular parts of the prism and of the solvent composition corresponding to individual points is demonstrated in Figure 9 for use of ethanol (S_A = 4.3), chloroform (S_B = 4.1), and diethyl ether (S_C = 2.8) in NP chromatography; hexane (S_D = 0) is used to adjust solvent strength. Point *C* on the top of the regular prism corresponds to undiluted diethyl ether and the other two corners (A_D and B_D) are obtained by diluting ethanol and chloroform with the zero-solvent-strength hexane to a solvent strength of 2.8. These correspond to mixtures of 65.1% ethanol and 34.9% hexane (corner A_D) and 68.3% chloroform and 31.7% hexane (B_D), as can be seen in Figure 8.

The total solvent strength of a four-solvent (*A*, *B*, *C*, and hexane, the solvent strength regulator) mobile phase is the sum of the solvent strengths of the volume fractions (f) of the single solvents (17): $S_T = f_A \times S_A + f_B \times S_B + f_C \times S_C$, which gives a solvent strength of 4.09 for P_S = 631 on the top irregular triangle (S_T = 0.6 x 4.3 + 0.3 x 4.1 + 0.1 x 2.8 = 4.09). The same relationship between the concentrations of *A*, *B*, and *C* is valid for dilution of this solvent mixture to S_T = 2.8.

For the calculation of mobile phase composition it must be considered that in the regular part of the prism a minimum of two corners of the triangles represent solvents containing the solvent strength regulator. For solvent strength 2.8 and P_S^* = 631 (Figure 8), the f of the solvent mixture represented by corner A_D (65.1% ethanol and 34.9% hexane) is 0.6, the volume fraction of the solvent mixture represented by corner B_D (68.3% chloroform and 31.7% hexane) is 0.3, whereas that of corner *C* (100% diethyl ether and 0% hexane) is 0.1. Accordingly, the concentration of ethanol in the final mixture (in P_S^* = 631 at S_T = 2.8) is 30.45% (0.6 x 65.1), that of chloroform is 20.49% (0.3 x 68.3), that of diethyl ether is 10% (0.1 x 100), and that of hexane is 30.95% (100 – 39.06 – 20.49 – 10).

Dilution of the solvent characterized by the point P_S^* (S_T = 2.8; PS = 631) with hexane in different proportions gives mixtures with the same selectivity ($P_S^* = P_S^{**}$) but lower solvent strengths [e.g., S_T = 1.4; P_S = 631 (15.23% [30.45:2] ethanol + 10.25% [20.49:2] chloroform + 5% [10:2] diethyl ether + 69.52% [30.95:2 + 50] hexane)]. Obviously the same solvent composition can also be obtained by first calculating the compositions at the edges of the prism, corresponding to the solvent strength desired, and subsequently mixing these in the proportions given by the volume fractions (selectivity point).

Relationship between the irregular and regular parts of the model

As can be deduced from the example calculation above, the relationship between the volume fractions of the top irregular and the regular parts of the model are not identical, because, as is apparent from Figure 8, the regular triangle with the highest solvent strength is geometrically a projection of the top irregular triangle. Only point C is identical in both triangles, all other points characterize other solvent compositions.

The selectivity points in the top irregular triangle can be described as PS = PA; PB; PC. In the highest regular triangle, point C represents a neat solvent (PC = PCD), where the corners consist of PAD and PBD instead of PA and PB. So the selectivity points on the top regular triangle can be described as PS* = PAD; PBD; PCD, where

$$PAD = (S_A \times P_A)/ST;\ P_{BD} = (S_B \times P_B)/S_T;\ \text{and}$$
$$P_{CD} = (S_C \times P_C)/S_T = P_C \qquad \text{Eq. 1}$$

The conversion of the selectivity points between the irregular and regular triangle can be described as follows:

$$P_S^* = (S_A \times P_A)/S_T;\ (S_B \times P_B)/S_T;\ (S_C \times P_C)/S_T \qquad \text{Eq. 2}$$

The projection of ethanol, chloroform, and diethyl ether on the top irregular triangle (S_T = 4.09) in P_S = 631, will be at:

$$S_T = 2.8,\ P_S^* = (4.3 \times 60)/4.09;\ (4.1 \times 30)/4.09;$$
$$(2.8 \times 10)/4.09 \qquad \text{Eq. 3}$$

which gives the selectivity point P_S^* = 63.08 – 30.07 – 6.85 rounded off as P_S^* = 63.1 – 30.1 – 6.9. This means that the relationship between the different solvents (c_A: c_B: c_C) is constant within the top irregular triangle for the different solvent strengths (in sections parallel to the top irregular triangle) and is also constant within the regular part at horizontal sections (11). Therefore, if P_S is defined as a selectivity point in the top irregular triangle and P_S^* and P_S^{**} in the regular part, P_S $P_S^* = P_S^{**}$; the mobile phase must therefore always be characterized by the sol-

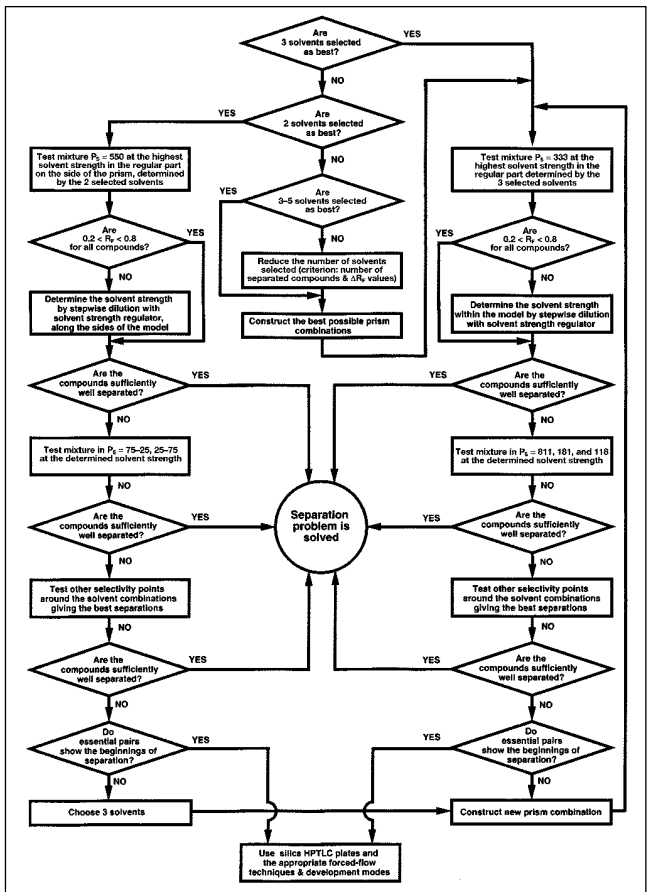


Figure 10. Flow chart for mobile-phase optimization for non-polar substances (13).

vent strength and the selectivity point.

Optimization strategy

The strategy for optimizing the solvent combination for nonpolar compounds is depicted in a flow chart in Figure 10.

If three solvents were selected in the first part of the system, optimization is achieved within the regular part of the model with the help of the four basic selectivity points. If two solvents were selected, optimization is achieved along a side of the prism. In both instances the solvent strength is adjusted first, then different selectivity points are tested. If 3–5 solvents are selected as best, the number of solvents is reduced after the criteria number of separated substances and R_F values have been met. If the solvent combinations tested with the equivalent strategy did not result in sufficient separation, or if the beginning of a separation of the essential substance pairs could not be observed, other solvents must be selected and the process must be repeated, as indicated in the flow chart.

The flow chart for optimization of the solvent combination for polar compounds is shown in Figure 11. For polar compounds optimization is always started on the top irregular triangle of the model, either within the triangle (if three solvents were selected) or along one side (if two solvents were selected). Water is usually used as modifier to increase the solvent strength and reduce tailing; if it is used as one of the selected solvents, several selectivity points cannot be tested because of miscibility problems

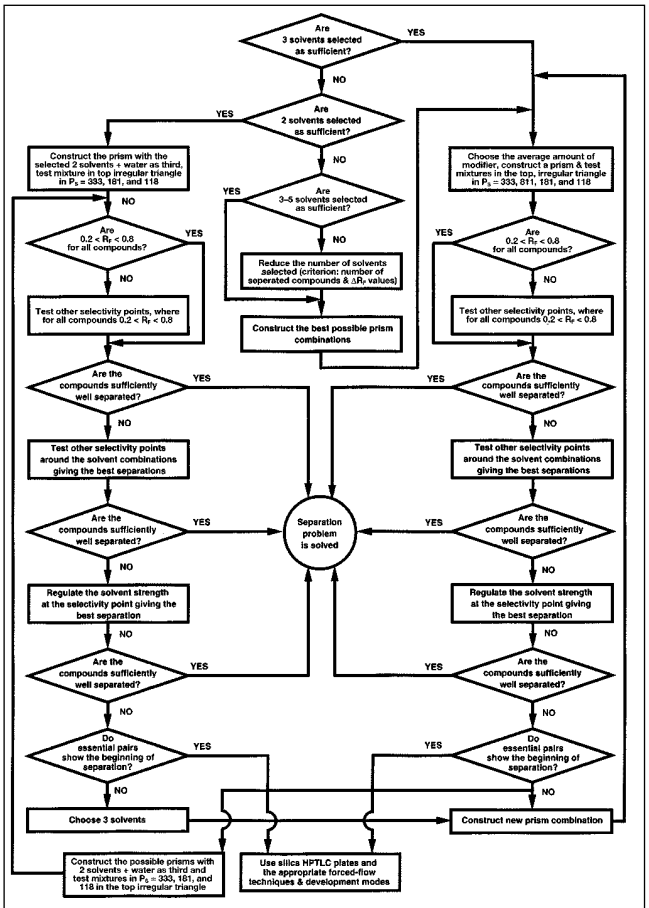


Figure 11. Flow chart for mobile-phase optimization for polar substances (13).

(especially near P_S = 811).

When the selectivity points on the top triangle are changed the solvent strength is also changed; a small change in the selectivity point might therefore result in a large difference in resolution, especially when the solvent strength of the selected solvents differs substantially. The subsequent procedure is similar to that for the nonpolar substances but the solvent strength must be adjusted after suitable selectivity has been obtained (19).

Optimization of the solvent strength and selectivity points must be performed until the beginnings of a separation of the compounds, at least, are obtained. This can usually be achieved with the first PRISMA combination, if the individual solvents were selected correctly.

Automatic mobile-phase optimization

For the separation of nonpolar compounds by use of an NP stationary phase and saturated TLC systems at a constant solvent strength the correlation between the selectivity points is described (16) by the function:

$$hRF = a(P_S)2 + b(P_S) + c \text{ (horizontal function)} \quad \text{Eq. 4}$$

The hRF values were always measured along the edges of the triangle through two basic selectivity points. The vertical correlation at constant selectivity points between a variety of solvent strengths can be described by:

$$S_T = d \ln hRF + e \text{ (vertical function)} \quad \text{Eq. 5}$$

Measurements on three solvent-strength levels are needed for calculation of hRF values for all selectivity points in the spatial design, because the vertical correlation can be linearized. These correlations are also relevant when constant amounts of modifiers are used. From these correlations the chromatographic behavior of substances to be separated can be predicted at all selectivity points within the PRISMA model in saturated chromatographic chambers. The separation quality of predicted chromatograms is assessed by use of the chromatographic response function (CRF) (2). Twelve measurements are needed to find a local optimum, and fifteen for the global optimum. Pelander et al. (20) studied the application of these relationships to the irregular part of the model. They found that for saturated chambers there was a linear correlation between RF and the solvent strength at a constant PS value. Again 12 measurements were needed to find a local optimum and 15 for the global optimum.

For nonsaturated chromatographic systems using mobile phases containing *n* solvents (multicomponent mobile phases), *n* fronts can occur. Because the compounds to be separated can be located between the different fronts, these correlations are not valid.

The Third Part of the System

Selection of the mode of development

When the best possible mobile phase composition has been determined on silica, a suitable development mode and separation technique must be selected. If the beginnings of a separation of the compounds are observed, use of linear development mode

and OPLC are indicated. If the separation problem is in the upper R_F range the anticircular mode should be used; if the problem is in the lower R_F range the circular development mode should be chosen (21). For both, the development is first performed on a TLC plate; if the separation is insufficient HPTLC plates with a suitable forced-flow technique can be employed (as summarized in Figure 12).

If the separation problem cannot be solved by use of this strategy and silica, other stationary phases must be tested and the optimization process must be started again with the first part of the system.

Transfer of the optimized mobile phase

The main point of the strategy for transfer of the optimized mobile phase is that separation of samples is generally begun in a nonsaturated TLC chamber, in which the compounds to be separated are placed between the hRF values of 25 and 80. In fully off-line separations the mobile phase optimized in an nonsaturated TLC chamber can be transferred without change—by use of an appropriate prerun—to analytical OPLC (22). For the separation of nonpolar compounds this prerun can be performed with hexane (si = 0); for separation of polar substances the prerun can be performed with any component of the mobile phase in which the components do not migrate (selection of this solvent should be considered during optimization of the mobile phase) (9). By use of ultramicro chamber RPC (U-RPC) the optimized TLC mobile phase can be used directly; no prerun is necessary because, compared with OPLC, U-RPC is not a completely closed chamber and some vapor phase is present. Both methods enable the possibility of "scaling-up" to the corresponding preparative techniques and the use of column rotation planar chromatography (23).

For fully off-line separation techniques dry filled preparative columns (flash chromatography, low pressure liquid chromatography, and medium pressure liquid chromatography) can be equilibrated with the solvent used for the prerun in analytical OPLC, whereas for slurry packing the slurry must be prepared using the same solvent as was used for the prerun of OPLC or U-RPC. In both techniques air bubbles can be eliminated by pumping of the appropriate quantity of the solvent used for the prerun; the preparative separation can then be started with the optimized nonsaturated TLC mobile phase (24).

Figure 13A illustrates the possibilities of direct transfer after optimization of the TLC procedure in nonsaturated chromatographic chambers (24). Different lines show the transfers applicable for different methods. Dotted lines and thin lines denote transfers between off-line and on-line methods, respectively. Thick lines indicate that the optimized mobile phase can be transferred without change between different solid–liquid planar and column chromatographic techniques, whether off-line or online.

If the TLC mobile phase was optimized in saturated chambers to improve the separations the M-RPC technique can be used,

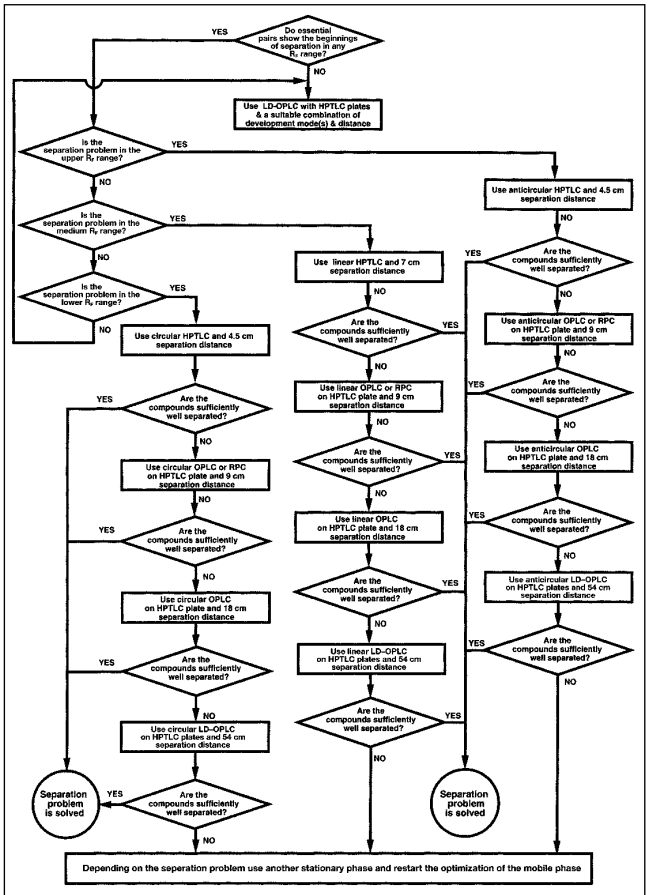


Figure 12. Flow chart for selection of the mode of development and the forced-flow technique (17).

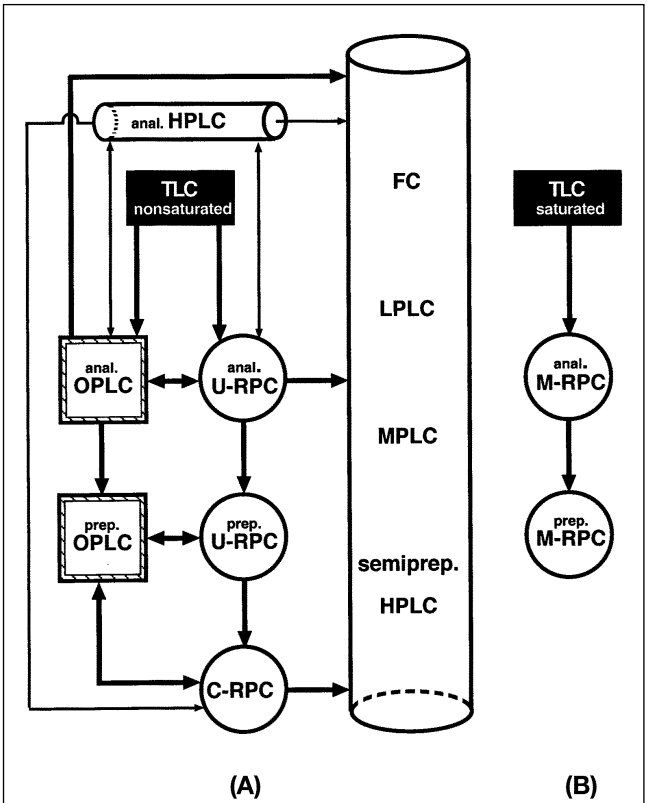


Figure 13. Transfer of the optimized TLC mobile phase for analytical and preparative forced-flow column and planar chromatographic techniques. (A) Procedure starting with nonsaturated chromatographic chambers and (B) starting with saturated chromatographic chambers

without altering the TLC mobile phase (Figure 13B), irrespective of whether off-line or line techniques are used (24).

Discussion

Application of the PRISMA method

Several hundred papers have reported the use of the PRISMA system for method development in planar chromatography, for up-scaling procedures, and for physicochemical studies. The system has also been used for biomedical, environmental, and toxicological analysis. Dozens of papers cover the separation of different classes of substance, some of them (25–58) are listed in Table II.

Table II. Selection of Publications Using the PRISMA Optimization System for Planar Chromatographic Separation

Substance classes	Authors	References
Alkaloids	L. Botz, L.Gy. Szabó	(25)
	M. Waksmundzka-Hajnos, A. Petruczynik	(26)
Amino Acids	Sz. Nyiredy et al.	(27)
	M. Remelli et al.	(28)
Amphetamine derivatives	Zs. Fatér et al.	(29)
Anilines	M. Waksmundzka-Hajnos, A. Petruczynik	(30)
Anthraquinones	K. Danielson, G.W. Francis	(31)
Barbiturates	Zs. Fatér et al.	(32)
	T. Aman et al.	(33)
Benzodiazepines	C. Cimpoiu et al.	(34)
Coumarins	P. Bruno et al.	(35)
	P. Vuorela et al.	(36)
Dyes	S. Gocan et al.	(37)
Essential oils	I. Wassmuth-Wagner, H. Jork	(38)
	P. Dugo et al.	(39)
Estrogens	S.K. Poole et al.	(40)
Fatty Acids	A. Pyka, K. Bober	(41)
Flavonoids	K. Dallenbach-Tölke	(42)
	M.A. Hawryl, E. Soczewinski	(43)
Hepatotoxins	A. Pelander et al.	(44)
Herbicides	J. Tekel	(45)
Indole derivatives	T. Yrjönen et al.	(46)
Insecticides	W. Funk et al.	(47)
Iridoids	K. Dallenbach-Toelke	(48)
	P. Junior	(49)
Pharmaceuticals	H.D. Ahmed, C.F. Poole	(50)
	H.E.M. Salomies, P.K. Salo	(51)
Phenols	J. Bladek et al.	(52)
	M. Waksmundzka-Hajnos, A. Petruczynik	(30)
Pigments	Q.M. Andersen, G.W. Francis	(53)
	K. Morita et al.	(54)
Prostaglandins	P. Bruno	(55)
Saponins	G. Bader et al.	(56)
	K. Dallenbach-Toelke et al.	(57)
Synthetic polymers	L.S. Litvinova	(58)

Conclusion

For both nonpolar and polar substances the optimization strategy proposed is always started on silica. The individual solvents can be selected by performing parallel experiments in ten chambers. If a solvent was not characterized by Snyder (11), an approximate value is chosen from one of its listed homologs. We recommend the use of a nonsaturated chamber because this facilitates mobile phase transfer to OPLC (5); this is also the reason for selection of a so-called suitable solvent in the first part of the system for polar compounds.

For the separation of nonpolar compounds optimization with the PRISMA model is generally a rapid process because a few experiments are sufficient to enable determination of the optimum mobile phase composition. In contrast, optimization with the second part of the system is a longer process for polar compounds because of the simultaneous change of solvent strength and selectivity. When water in particular is one of the solvents selected for construction of the triangle, a small change in selectivity results in a large change in resolution. More chromatographic experience is therefore necessary if the separation problem is to be solved rapidly (17).

Modern FFPC techniques can be employed systematically to improve the separation efficiency on fine particle-size HPTLC plates. The PRISMA system combines the appropriate development mode with the appropriate forced-flow technique and use of a mobile phase of optimized composition. This offers special possibilities for solving difficult planar chromatographic separation problems.

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