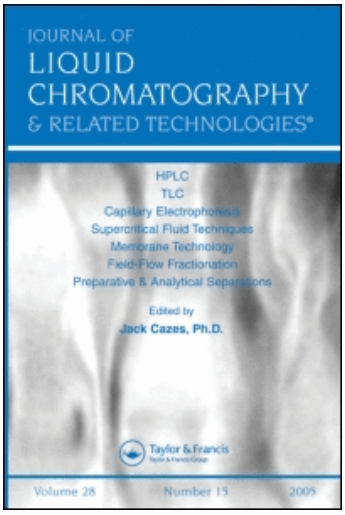

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UTILIZATION OF A SYSTEMATIC SOLVENT SELECTION METHOD FOR THE HPLC DETERMINATION OF A TRACE ISOMERIC CONTAMINANT

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ABSTRACT

A systematic solvent selection method is used for the development of HPLC conditions for the separation and detection of trace amounts of threo isomer in samples of the bronchodilating drug procaterol, (±)-(R*,S*)-8-hydroxy-5-[1-hydroxy-2-[(1-methylethyl)amino]butyl]-2(1H)-quinoline (erythro isomer). The method involves the chromatography of a mixture of the two isomers using seven different mixtures of three base solvents as mobile phases and the mapping of resolutions using a computer-generated mathematical model. The optimized mobile phase predicted by the model gives excellent resolution between the two isomers at levels as low as 1% of the threo compound.

INTRODUCTION

Maximizing the HPLC separation of the components of a binary mixture is especially important when the later-eluting material is present in a very small amount. In such cases, loss of detection or quantitation of the minor component can occur when the chromatographic peaks overlap even slightly. Often a trial-and-error

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process is employed for optimization of experimental conditions in such separations. Recently, however, a number of systematic optimization methods have been developed (1-7) which combine fundamental chromatographic principles with existing statistical processes to achieve maximum HPLC resolution.

Of particular utility in optimizing liquid chromatographic separations of complex mixtures is a method developed by Glajch and coworkers (7), based upon the mapping of resolutions between peak pairs using seven different mobile phase mixtures. This "overlapping resolution mapping" (ORM) technique has been used in the development of both normal and reverse phase liquid chromatographic conditions for the separation of a variety of chemical mixtures, including those of substituted naphthalenes (8), isomeric phenols (9) and aflatoxins (10).

Because it has as its basis the optimization of separations between pairs of chromatographic peaks, the ORM method is particularly well-suited for use in the systematic selection of HPLC conditions for maximum separation of a binary mixture. We have explored this potential by applying the ORM method in the development of an optimum solvent system for the separation of the bronchodilating drug procaterol, (±)-(R*,S*)-8-hydroxy-5-[1-hydroxy-2-[(1-methylethyl)amino]butyl]-2(1H)-quinoline (erythro isomer) from its threo isomer, a potential minor contaminant. In this paper, we discuss the details of the optimization of this separation and describe general schemes for the application of the ORM method for similar separations.

EXPERIMENTAL

Materials: All solvents were glass distilled (Burdick and Jackson Laboratories). Samples of procaterol and its threo isomer were synthesized in these laboratories using published procedures (11).

Apparatus: The HPLC system consisted of a Perkin-Elmer Series 4 quaternary solvent delivery system, a Rheodyne injector (20 µl loop) and an octadecylsilane column (Altex Ultrasphere ODS, 5 µm particle size, 250 mm x 4.6 mm). The system was fitted with a Perkin-Elmer Model 85B spectrophotometric detector operated at a fixed wavelength of 254 nm, and a Perkin-Elmer Model ISS-100 chromatographic autosampler. Chromatographic data were recorded and processed on an IBM Model 9000 data system.

Procedure: The optimization procedure consisted of chromatographing 20 µl aliquots of an aqueous solution containing 2 mg/ml of procaterol and 1 mg/ml of its threo isomer, in seven different mobile phases (see Results and Discussion). Final comparisons of optimized and unoptimized conditions were made by chromatographing an aqueous solution of 1 mg/ml of procaterol and 0.01 mg/ml of its threo isomer, using the mobile phases specified in Figure 2. Throughout the entire study, the flow rate of the mobile phase was set at a constant 1 ml/minute.

Equations: Chromatographic capacity factors, k', were calculated by equation I:

$$k' = \frac{t - t_o}{t_o} \qquad (I)$$

where t is the retention time of the component of interest, and t_o is the retention time of an unretained substance, determined by injection of an aqueous solution of sodium nitrite.

Chromatographic resolutions, R, between procaterol and its threo isomer were calculated by equation II:

$$R = \frac{1.18\ \Delta t_r}{W_1 + W_2} \qquad \text{(II)}$$

where Δt_r is the difference in retention times between the two chromatographic peaks and W_1 and W_2 are the widths of the two peaks at their half-heights (12,13).

Predicted acetonitrile-water and tetrahydrofuran-water mixtures of equal strength to the initially-used 30% methanol - 70% water mobile phase (Table I) were calculated using equation III:

$$S_t = \sum_i S_i \Theta_i \qquad \text{(III)}$$

where S_t is the total solvent strength of the initially-used solvent mixture (in this case, 78), S_i is the "solvent strength weighting factor" (water: 0; methanol: 2.6, acetonitrile: 3.2, tetrahydrofuran: 4.5), and Θ_i is the volume fraction of solvent "i" in the mixture (14). For the purpose of these calculations only, the presence of acetic acid in each of the mobile phases was ignored.

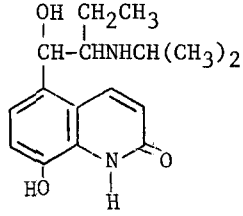
For the quantitative mapping of resolutions (R), data for each of seven mobile phase mixtures was fit to a cubic model (15), based upon equation IV:

$$R = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \qquad \text{(IV)}$$

where the X_i's are the volume fractions of each base solvent (see Table 2) and the B_i's are constants. Equation IV was solved and the resolution map was produced by a BASIC computer program (available on request) written for an Apple II+ computer equipped with an Epson MX-80 dot-matrix printer. This program is based on an earlier FORTRAN program developed by Hare and Brown (16).

RESULTS AND DISCUSSION

The development of an HPLC method for the separation of procaterol (I) from its threo isomer was prompted by the potential for threo isomer contamination in bulk lots of the drug from



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partial inversion of configuration at one of the compound's two chiral centers during its synthesis or storage. While their physical properties are similar, the two isomers differ widely in their bronchodilating activities (11), and thus the quantitation of the amount of threo isomer contamination in samples of procaterol is highly desirable. The two isomers can be distinguished from each other by proton NMR spectroscopy (11,17), but until now no quantitative chromatographic separation has been reported in the literature.

While selecting parameters for a routine HPLC separation of a binary mixture may often be accomplished through methods much simpler than that described here, developing an HPLC system for either trace analysis or preparative separations requires maximization of resolution to reduce peak overlap caused by column overload or any slight tailing of the chromatographic peaks. For trace analysis, this is especially true when the minor component consistently elutes after the main component, which is the case in the present separation. In such situations, a systematic scheme can ensure the development of optimized conditions which may eliminate later uncertainties.

The method described by Glajch and coworkers is designed for the systematic development of the optimum mobile phase for the analysis of a mixture by reverse-phase HPLC (8). Basically, the method consists of four steps:

1. A methanol-water mobile phase, X_1, is chosen which allows for the elution of all components of interest within a given k' range (usually $1 \leq k' \leq 10$).

2. Two other aqueous mobile phases, X_2 and X_3, of different selectivity but equivalent solvent strength (i.e., giving similar k' values) are chosen. The volume fraction of water in each of these iso-eluotropic base-solvents is usually calculated through Snyder's solvent-strength equation III (14) and then refined experimentally if necessary. Generally, these two mobile phases consist of mixtures of acetonitrile and water and tetrahydrofuran and water.

3. The chromatograms of the sample of interest are obtained in each of the three base-solvents as the mobile phase, as well as every possible 1:1 mixture of each base solvent. The chromatogram of the sample is then obtained using a final 1:1:1 ternary mixture of each base solvent.

4. The resolutions between each peak pair in the chromatograms of the sample of interest are either qualitatively or quantitatively compared, and the mixture of base solvents giving the best resolution is deduced and used as the optimized mobile phase.

This method, which is based upon the principles of Snyder's well-known solvent-selectivity triangle (18), seeks to maximize resolution between the components of a mixture by combining the unique selectivities of three base solvents.

Application of the ORM method to the separation of a binary mixture is in many ways simpler than for a multicomponent mixture. Loss of peak identity due to possible inversion of elution order can be eliminated by the development of the method on an unequal mixture of standards, in this case, a 2:1 mixture. Moreover, since only one peak pair is being considered, quantitative mapping and modelling of the resolutions is simplified. For this quantitative mapping, we have written a BASIC computer program, which fits resolutions obtained from the seven solvent mixtures to a cubic model (15), and then predicts resolutions for any other possible mixture. The results are displayed in a triangular

response surface diagram (Figure 1) from which the solvent composition giving the highest resolution may be read (16).

While the general applicability of the quantitative cubic model in the mapping of HPLC resolutions has been demonstrated in several cases (7,8,9,10) its use in the present situation requires a few considerations. Firstly, the sheer number of resolutions needed to be calculated makes it advantageous for these calculations to be carried out by a programmable integrator or data system at the time the data is acquired (19). Since the data system we used (as well as most systems) is capable of determining peak widths at half-heights, we have calculated chromatographic resolutions using equation II, which is based upon this "width at half-height" parameter (12,13). Although such an equation is easily incorporated into most integrators or data systems, the assumption of completely Gaussian chromatographic peaks upon which it is based (13) is not rigorously correct, especially in the present case, where some band broadening and assymetry is expected as the amount of sample injected or the chromatographic scale is increased to look for evidence of a trace contaminant. For this reason, the resolutions calculated by equation II can be considered only in a relative manner in this study. Aside from the inapplicability of the normal assumption of a resolution of 1.5 giving baseline peak separation (13,14), however, this does not limit the applicability or usefulness of the calculated resolutions to a great degree in the optimization process. The

quantitative model is simply used to predict a solvent system giving the maximum resolution, without regard to the magnitude of the actual value obtained.

The composition of the three base-solvent systems used for the present method are displayed in Table 1, along with k' values obtained for the procaterol isomers using these solvents. Each of these solvent systems gave good peak shapes for the two compounds, as well as k' values between 7 and 9. Apparently the addition of 1% acetic acid to the aqueous portion of the base solvents (to improve peak shape) rendered inapplicable the prediction of acetonitrile-water and tetrahydrofuran-water mixtures of equal strength to the methanol-water mixture on the basis of the solvent-strength equation III (14). Both predicted iso-eluotropic solvent mixtures, listed in parentheses in Table 1, were clearly too strong, and needed to be modified somewhat.

Table 2 lists the experimentally-determined resolutions between the procaterol isomers using each of seven mixtures of the three base solvents. Of the seven mixtures used, highest resolution was achieved with the mixture consisting of 50% X_1 and 50% X_2, or 15 parts methanol, 7 parts acetonitrile, and 78 parts aqueous acetic acid. The resolution achieved by mixing the tetrahydrofuran solvent (X_3) with the methanol solvent (X_1) was significantly lower than that obtained with the methanol solvent alone, while mixing the tetrahydrofuran solvent (X_3) with the acetonitrile solvent (X_2) resulted in only slight increases in resolution over that obtained with the acetonitrile solvent alone.

TABLE 1

Base Solvent Compositions and Chromatographic Capacity Factors

Solvent	First Component	Θ_1	Second Component	Θ_2	k'_e	k'_t
X_1	MeOH	.30	HOAc	.70	7.6	8.8
X_2	ACN	.14 (.24)*	HOAc	.86 (.76)*	7.5	8.7
X_3	THF	.03 (.17)*	HOAc	.97 (.83)*	8.0	8.9

MeOH = methanol, ACN = acetonitrile, THF = tetrahydrofuran, HOAc = 1% aqueous acetic acid, Θ_1 = volume fraction of first component, Θ_2 = volume fraction of second component, k'_e = capacity factor of procaterol, k'_t = capacity factor of threo isomer, * Number in parentheses is the volume fraction predicted by equation II to give k' values similar to solvent X_1.

TABLE 2

Experimentally-Determined Chromatographic Resolutions Between Erythro and Threo Isomers

Mixture	$\%X_1$	$\%X_2$	$\%X_3$	Resolution
1	100	0	0	2.41
2	0	100	0	1.77
3	0	0	100	1.87
4	50	50	0	2.66
5	50	0	50	1.29
6	0	50	50	1.90
7	33.3	33.3	33.3	0.98

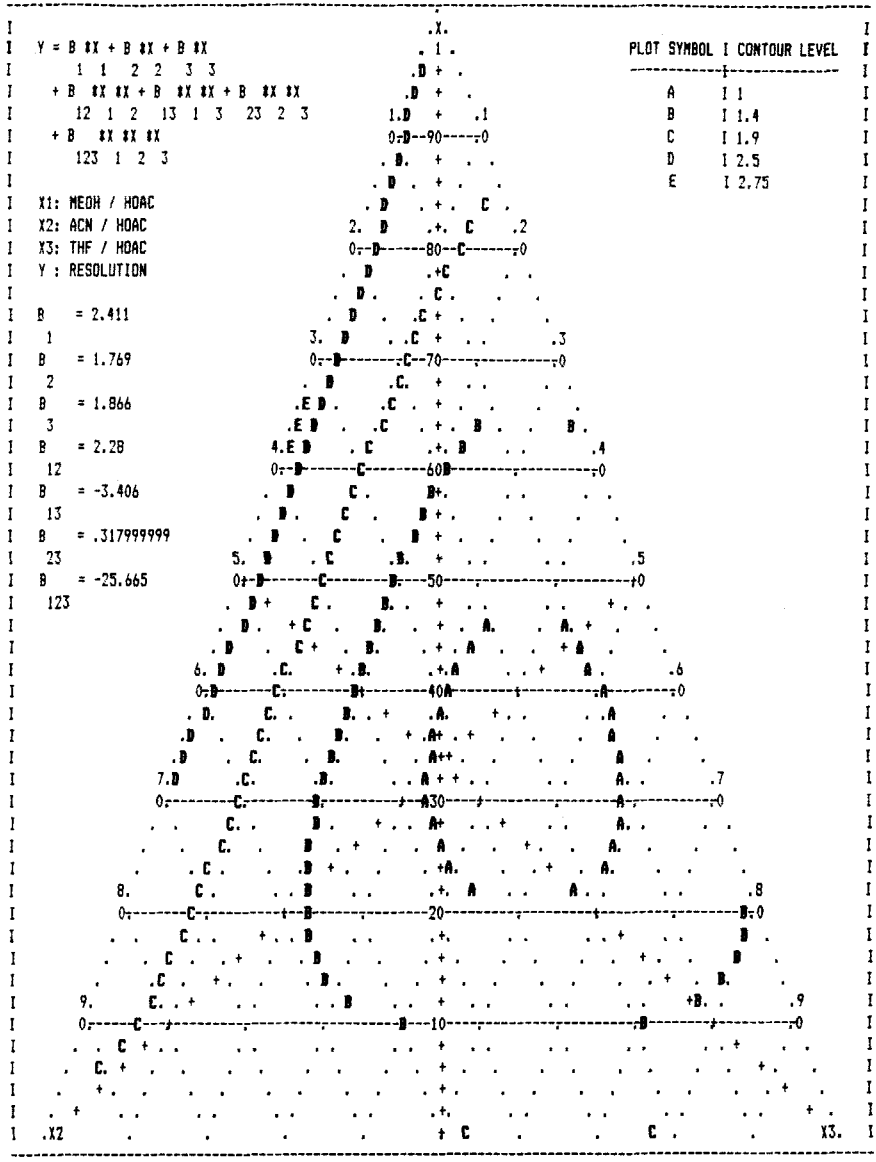

FIGURE 1. Computer-generated resolution map for the separation of procaterol and its threo isomer.

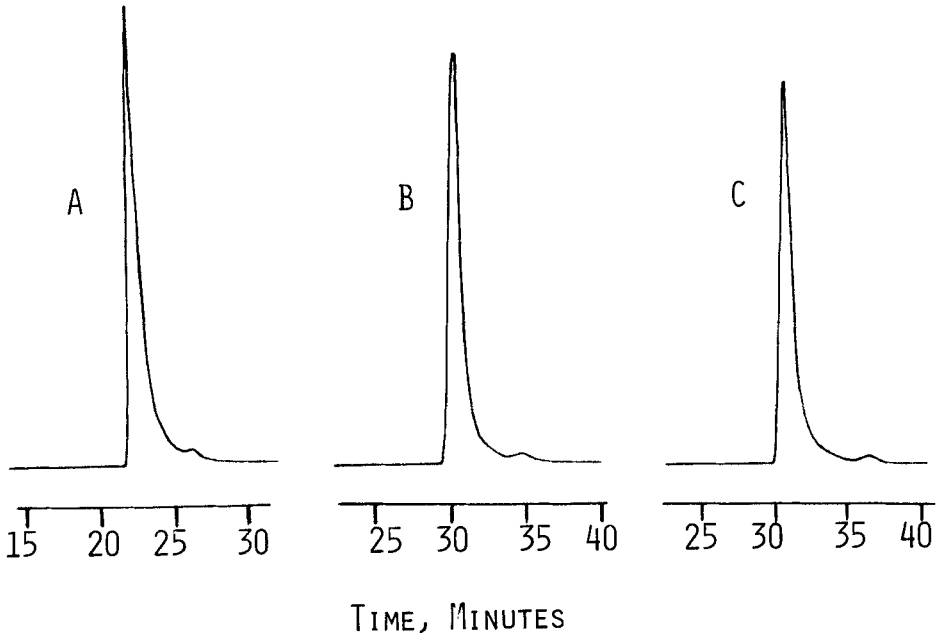

FIGURE 2. HPLC Chromatograms of procaterol containing 1% threo isomer using mobile phases consisting of: A - 33% X_1, 20% X_2 and 47% X_3; B - 100% X_2; C - 63% X_1 and 37% X_2.

The computer-generated resolution map for the overall X_1-X_2-X_3 solvent system is shown in Figure 1. As expected from the trends in the experimental data, the computer model predicts that highest resolutions are achieved for the two isomers using mixtures of the methanol and acetonitrile base solvents. The predicted optimum mixture consists of 63% X_1 and 37% X_2, or 18.9% methanol, 5.2% acetonitrile, and 75.9% aqueous 1% acetic acid.

Application of the predicted optimum mobile phase to the determination of trace amounts of threo isomer in procaterol produces consistent and usable results. Figure 2 displays the

chromatograms of a sample of procaterol which is contaminated with 1% threo isomer, using the predicted optimum solvent as well as two other mixtures as mobile phases. While resolution is not complete in any of the chromatograms, owing possibly to column overload, the best resolution is obtained with the predicted optimum solvent (Figure 2C). Also consistent with the computer model is the poor separation of erythro and threo isomers (Figure 2A) with a mobile phase consisting of 9.9% methanol, 2.8% acetonitrile, 1.4% tetrahydrofuran and 85.9% aqueous acetic acid (i.e., 33% X_1, 20% X_2 and 47% X_3). Clearly, the high degree of separation achieved with the use of the predicted optimum solvent reduces overlap to a minimum, and results in a desirable HPLC system for determination of threo isomer contaminant.

CONCLUSIONS

In this study, the systematic solvent selection method developed by Glajch and coworkers was used for the optimization of the mobile phase for the HPLC separation of a drug and a trace contaminant. While three base solvent systems were initially used in the method development, a mixture of two of them was ultimately found to give the best separation. Through the use of this method, the precise composition of the solvent giving the optimum separation was predicted, and one of the three solvents could be confidently eliminated from consideration. Thus, this quantitative, systematic method of solvent selection has been demonstrated to be a highly useful technique for the maximization of

conditions for the HPLC determination of trace contaminants, even when only one such material is known to be present.

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