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Shoji Hara^a; Shigeki Ohnishi^b

^a Tokyo College of Pharmacy, Hachioji, Tokyo, Japan ^b Cosmetic Laboratory, Kanebo Ltd., Japan

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RELATIVE STRENGTH OF STRONGER SOLVENTS FOR SILICA, AMINO- AND CYANO-
ALKYL BONDED SILICA COLUMNS IN NORMAL-PHASE LIQUID CHROMATOGRAPHY

Shoji Hara¹ and Shigeki Ohnishi²

Tokyo College of Pharmacy¹, Horinouchi Hachioji, Tokyo, 192-03, Japan
and²
Cosmetic Laboratory, Kanebo Ltd., Odawara, Kanagawa, 250, Japan

ABSTRACT

The strength and selectivity of solvents such as ethyl acetate, dioxan and ethyl alcohol in n-hexane binaries were determined using steroids as solutes in normal-phase liquid chromatography of silica gel, amino- and cyano-propyl silica columns. Based on the linear relationship between the logarithm of the capacity ratio and logarithm of solvent composition, the relative strength of solvents was determined from the experimental retention data described in our earlier articles. A micro-computer data base was compiled for filing the retention behavior of the steroids. Using this computer data base, the optimization process of binary solvents for a given sample was improved. An example of the phase system design is described.

INTRODUCTION

Optimization of a phase system has been usually carried out by a trial and error manner in liquid chromatography. This often involves tedious experimental procedures in designing a suitable system for a given sample mixture. A careful consideration of the retentivity and selectivity of packings and carrier solvents is recommended as a means for avoiding this problem. We examined the relative retentivity and

TABLE I. Constant Ratios of the Linear Relationship between the Logarithms of Retention Index and Concentration of the Stronger Component in n-Hexane Binaries for a Pair of Solvents in Silica Gel and Chemically Bonded Silica Column Systems

| No | steroid | solvent column constant ratio | ethyl acetate/dioxan | | | | | |
|----|-----------------------------|-------------------------------------|----------------------|-------|-------|-------|-------|-------|
| | | | silica | | amino | | cyano | |
| | | | r_c | r_n | r_c | r_n | r_c | r_n |
| 1 | Estrone | | 1.25 | 1.28 | 1.69 | 1.75 | 1.03 | 1.16 |
| 2 | Estradiol | | 1.06 | 1.04 | 1.22 | 1.17 | 1.00 | 1.05 |
| 3 | Ethinylestradiol | | 1.05 | 1.11 | 1.27 | 1.24 | 0.94 | 1.02 |
| 4 | Estriol | | 1.06 | 0.95 | 1.23 | 1.10 | 1.01 | 0.98 |
| 5 | Testosterone | | 1.28 | 1.14 | 1.29 | 1.15 | 1.02 | 1.07 |
| 6 | Methyltestosterone | | 1.26 | 1.12 | 1.34 | 1.18 | 1.06 | 1.14 |
| 7 | Ethinyltestosterone | | 1.19 | 1.13 | 0.84 | 0.78 | 0.93 | 1.02 |
| 8 | Testosterone propionate | | 1.60 | 1.45 | 1.62 | 1.46 | 0.96 | 1.13 |
| 9 | Progesterone | | 1.36 | 1.21 | 1.23 | 1.12 | 1.07 | 1.23 |
| 10 | Deoxycorticosterone acetate | | 1.41 | 1.28 | 1.57 | 1.45 | 0.93 | 1.01 |
| 11 | Corticosterone | | 1.24 | 1.12 | 1.76 | 1.60 | 0.88 | 0.92 |
| 12 | Cortisone | | 1.23 | 1.14 | 1.49 | 1.40 | 0.82 | 0.85 |
| 13 | Cortisone acetate | | 1.20 | 1.12 | 1.12 | 1.04 | 0.84 | 0.88 |
| 14 | Prednisone | | 1.26 | 1.15 | 1.18 | 1.10 | 0.87 | 0.90 |
| 15 | Prednisolone | | 1.16 | 1.07 | 1.44 | 1.32 | 0.85 | 0.87 |
| | mean value | | 1.24 | 1.15 | 1.35 | 1.26 | 0.95 | 1.02 |
| | standard deviation | | 0.14 | 0.12 | 0.24 | 0.24 | 0.08 | 0.12 |

r_c and r_n : ratios of the intercepts and slopes in equation (1) for a pair of stronger solvents in the text.

(continued)

selectivity of various packing columns such as silica gel and chemically bonded silica columns¹⁻³). In designing a phase system, characterization of the solvent role for a given column is also necessary and consequently solvent strength and selectivity have been determined. A comparative study of the strength of stronger solvents on various column packings in normal-phase operation was made in this paper.

Two proton acceptor solvents such as ethyl acetate and dioxan, and a proton donor-acceptor solvent, ethanol, were selected as typical stronger components in a binary system containing n-hexane as the diluent. The relative ratios of the solvent strength of ethyl acetate and ethyl alcohol to dioxan as the standard solvent on silica gel, amino-

TABLE I (continued)

| solvent column | ethyl alcohol/dioxan | | | | | |
|------------------------------|----------------------|-------|-------|-------|-------|-------|
| | silica | | amino | | cyano | |
| | r_c | r_n | r_c | r_n | r_c | r_n |
| constant ratio No steroid | | | | | | |
| 1 | 0.58 | 0.69 | 0.58 | 0.69 | 1.04 | 1.12 |
| 2 | 0.58 | 0.67 | 0.65 | 0.72 | 0.84 | 0.87 |
| 3 | 0.56 | 0.64 | 0.63 | 0.67 | 0.90 | 0.91 |
| 4 | 0.49 | 0.54 | 0.49 | 0.54 | 0.57 | 0.60 |
| 5 | 0.55 | 0.62 | 0.45 | 0.55 | 0.69 | 0.75 |
| 6 | 0.55 | 0.61 | 0.48 | 0.60 | 0.73 | 0.80 |
| 7 | 0.50 | 0.58 | 0.52 | 0.59 | 0.77 | 0.84 |
| 8 | 0.45 | 0.64 | 0.28 | 0.53 | 0.64 | 0.85 |
| 9 | 0.48 | 0.55 | 0.42 | 0.64 | 0.55 | 0.57 |
| 10 | 0.47 | 0.52 | 0.35 | 0.47 | 0.53 | 0.53 |
| 11 | 0.52 | 0.56 | 0.48 | 0.52 | 0.54 | 0.56 |
| 12 | 0.49 | 0.54 | 0.40 | 0.45 | 0.48 | 0.48 |
| 13 | 0.46 | 0.51 | 0.38 | 0.44 | 0.50 | 0.50 |
| 14 | 0.47 | 0.52 | 0.39 | 0.43 | 0.48 | 0.50 |
| 15 | 0.44 | 0.48 | 0.47 | 0.53 | 0.50 | 0.54 |
| mean value | 0.51 | 0.58 | 0.46 | 0.56 | 0.65 | 0.69 |
| standard deviation | 0.05 | 0.06 | 0.10 | 0.09 | 0.17 | 0.20 |

and cyano-propylsilylated silica columns were determined using the systematic retention data of fifteen steroid hormones given in our earlier papers¹⁻³⁾.

EXPERIMENTAL

The retention data from our earlier reports¹⁻³⁾ have been used in this paper. A serial chromatographic study was carried out under strictly controlled conditions at constant temperature. The solutes are the fifteen steroids shown in Table I.

The experimental conditions for the capsaicin analogues are shown in the legend of Figure 4.

RESULTS AND DISCUSSION

The linear correlation between capacity ratio and molar concentration logarithms of the stronger solvent was confirmed in the normal-phase HPLC of binary solvents and chemically bonded silica such as amino- or cyano-propylsilylated silica and bare silica gel columns as follows: $\log k' = c - n \log X_s$ (1), where k' is the capacity ratio, X_s , the molar fraction of the stronger solvent and c and n are constants¹⁻³). On the basis of this equation and experimental retention data obtained using various steroid hormones as solutes, the solvent strengths of ethyl acetate, dioxan as typical proton acceptor solvents and ethanol as a proton donor-acceptor solvent in n-hexane-binaries were compared.

To determine relative solvent strength, the mean values of the two constants in equation (1) for the fifteen steroids were initially calculated. Correlation between retentivity and solvent composition is illustrated in Figures 1 - 3 for silica, amino- and cyano-type columns, as calculated by the mean values of slopes and intercepts in equation (1). In these figures, the average retention of the steroids is presented by the ordinate. Thus, if the solvent composition is given by the abscissa, the relative retentivity of a particular stronger component in an n-hexane binary is suggested directly by the ordinate of the figure. This is illustrated by the arrow (a) in Figure 1.

The correlation lines were distributed in a fan shape and the increment in the relative strength of a pair of stronger solvents increased as the amount of solvent decreased. The relative strength of a solvent in the binary system varied with composition. For example, when the composition of the stronger solvent was ten percent, the relative solvent strengths of ethyl acetate and ethanol to dioxan as the standard were determined as follows: 0.7, 2.4 in silica (Figure 1), 0.7, 2.8 in the amino-type column (Figure 2) and 1.2, 1.8 in cyano-type column (Figure 3), respectively.

The relative strength of these three solvents varied markedly in the silica and amino columns, but hardly at all in the cyano column. The relative strength of two proton-acceptor solvents decreased in the cyano-column, although that of the proton acceptor-donor solvent remained at the same level for the three columns.

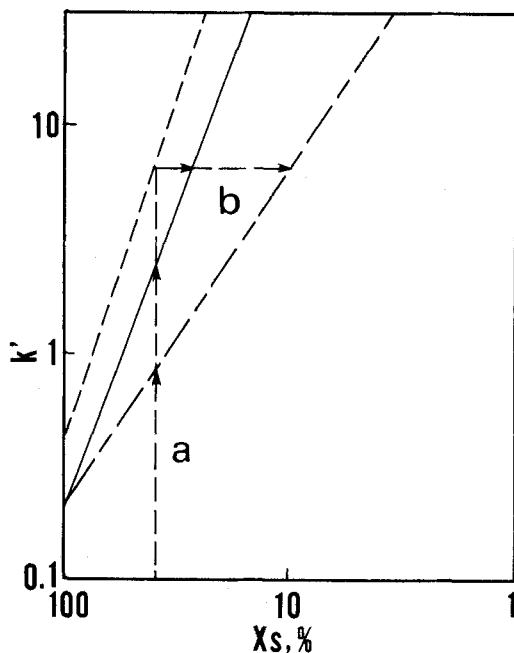


Figure 1 - 3. Logarithm of the Mean Values of the Capacity Ratios for Fifteen Steroids on Silica Gel and Chemically Bonded Silica Columns as a Function of the Logarithm of the Stronger Solvent-Concentration in n-Hexane-Binaries

Packing: silica gel (Figure 1); amino bonded silica (Figure 2); cyano bonded silica (Figure 3).

In Figures 1-3, the equi-elutropic solvent composition is represented by the abscissa; for example, intercepts on horizontal dotted lines (b) in Figure 1 show the calculated values for the composition of three solvents which should have about the same retentivity.

These facts suggest that the above mathematical relationship between the average retention index and the solvent composition would greatly facilitate the systematic optimization of a mobile phase for a given solute mixture and column.

An attempt was made to evaluate the solvent selectivity in three columns using the retention indices of various steroids. To compare the characteristics of ethyl acetate and ethyl alcohol with dioxan as the standard solvent, the quotients of the two constants c and n in equation

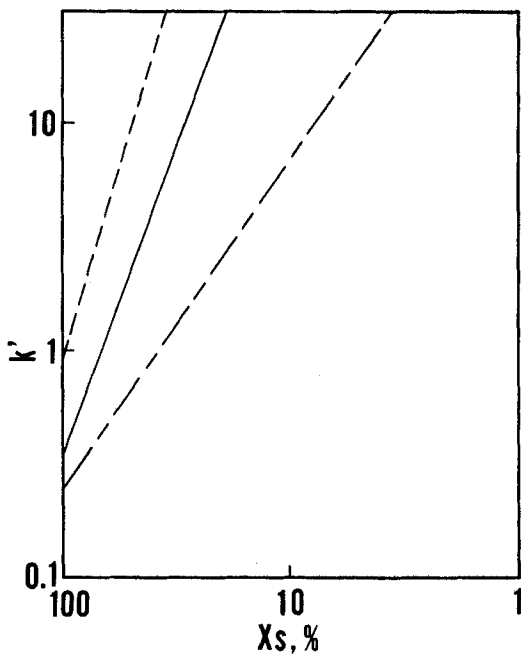


Figure 2

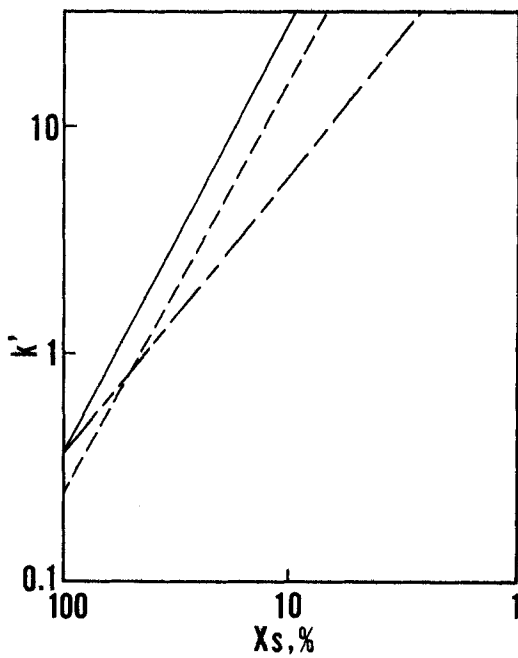


Figure 3

(1) for particular solutes were calculated. Table I shows the quotients of the constants (r_c and r_n) for silica and the two chemically bonded columns along with the mean values and the standard deviation.

In the case of the ratio for ethyl acetate-dioxan, the selectivity of solvents for steroids is suggested as follows: the strength of ethyl acetate decreases for acyl derivatives (8 - 10) as evident from larger constant values for c and n in the silica gel column and increases for corticosteroids (11 - 15) as indicated by smaller constants c and n in cyano column. However, in general, standard deviations of constant ratios in silica and cyano columns are smaller than that in the amino column and thus it is clear that selectivity resulting from changing the solvent from dioxan to ethyl acetate would be more effectively enhanced in the amino column than in the other two columns.

In the case of the ratios for ethyl alcohol-dioxan, solvent selectivity for steroids is suggested as follows: the strength of ethyl alcohol decreases for phenolic estrogens (1 - 3) by giving larger constant values of c and n in the amino and cyano bonded columns. The standard deviation of the constant ratios was least in silica gel column indicating that solvent change from dioxan to ethyl alcohol would possibly result in better selectivity of steroid retention in the other two chemically bonded columns.

From the results described above, selectivity through change can be expected to have the following order: amino $>$ cyano \approx non-bonded silica.

At our laboratory, the filing of retention data has been extensively examined by a micro-computer. The results obtained in this article as well as our earlier experimental data were compiled by a micro-computer data base. Using this data base, selection of a phase system for given samples can be carried out systematically. An example of micro-computer assisted optimization is shown below.

In order to identify the constituents of capsicum anuum tincture and related additives in cosmetic preparations, the resolution of capsaicin analogues was examined ⁴). At first, we obtained the two retention data using a phase system consisting of a silica gel column and n -hexane-dioxan binaries. According to equation (1), the solvent composition was calculated for a given capacity ratio. Using the micro-computer data base described above, the equi-elutotropic composition of a stronger solvent in n -hexane for silica, amino and cyano columns was ob-

tainable straightforward. For example, the calculated values of an equi-elutropic solvent composition, $X_{s(\text{calc})}$ were obtained by incorporating the ratios of the two constants (mean values) in equation (1) for a pair of columns as well as the quotients of the constants for a pair of stronger solvents (mean values) as follows:

$$\log X_{s(\text{calc})} = 1/n_{(\text{calc})} \times (c_{(\text{calc})} - \log k')$$
 (2)

$$n \text{ or } c_{(\text{calc-silica-ethyl alcohol})} = n \text{ or } c_{(\text{exp-silica-dioxan})} \times r_n \text{ or } r_c_{(\text{silica-ethyl alcohol/dioxan})}$$

$$n \text{ or } c_{(\text{calc-cyano-ethyl alcohol})} = n \text{ or } c_{(\text{calc-silica-ethyl alcohol})} \times n\text{- or } c\text{-ratio}_{(\text{cyano/silica-ethyl alcohol})}$$
 (3)

where calc and exp are the calculated and experimental values, respectively and the subscripts in parenthesis are phase systems. The constant ratios of r_n and r_c are from Table I and the n- or c-ratios for a pair of two columns are from our earlier paper ³⁾. The predicted values were found to be closely related to the experimental values and were thus helpful in quickly designing an optimum phase system. By both experiment and the use of the computer control of solvent composition and the design of a satisfactory phase system for the resolution of capsaicin homologues were possible. The calculated values and experimental results are shown in Table II. A mixture of solutes having closely re-

TABLE II. An Example of Micro-Computer Assisted-Phase System Optimixation for Capsaicin

| column | stronger component | predicted value [*] | | | experimental value | | |
|--------|--------------------|------------------------------|------|-----------|--------------------|------|-----------|
| | | c | n | Xs (k'=8) | c | n | Xs (k'=8) |
| silica | dioxan | | | | 4.51 | 2.54 | 26.3 % |
| cyano | dioxan | 3.20 | 1.78 | 19.5 % | 3.68 | 2.05 | 22.6 % |
| cyano | ethyl alcohol | 2.08 | 1.23 | 9.1 % | 2.03 | 1.24 | 8.1 % |

* calculated from the experimental data obtained with the silica n-hexane-dioxan phase system and equations (2) and (3) in the text.

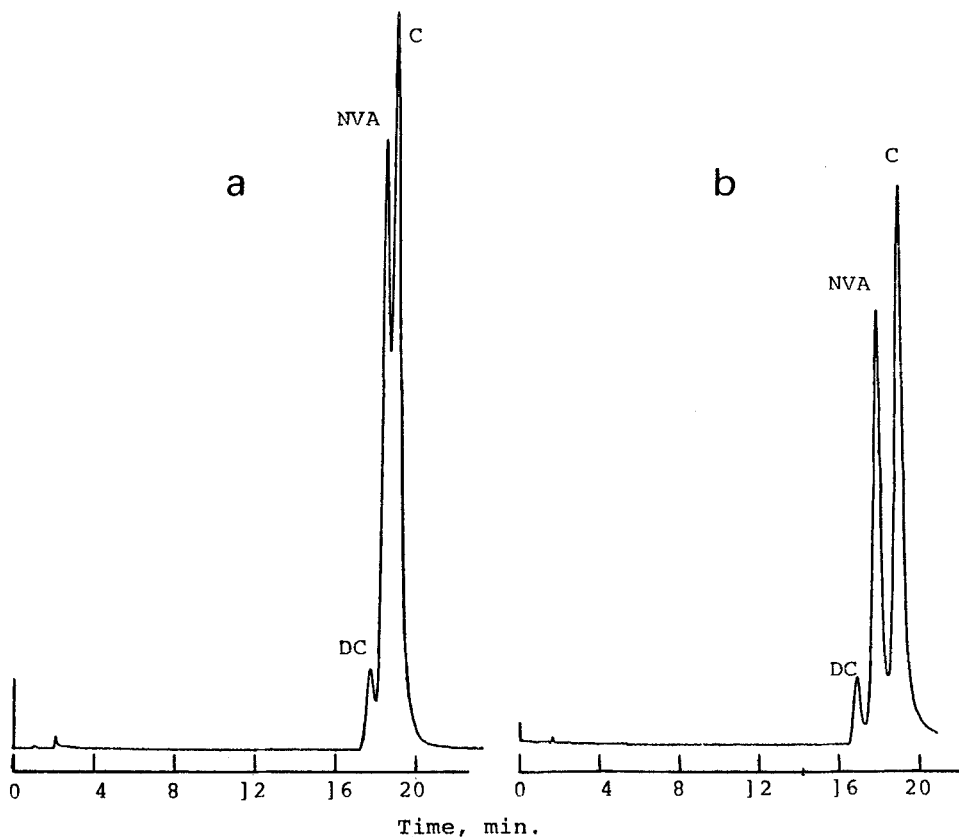


Figure 4. Chromatograms of Capsaicin Analogues

Samples: C, capsaicin; DC, dihydrocapsaicin; NVA, nonanoic acid vanillylamide. a: Column, Nucleosil 100-5; eluent, n-hexane-ethyl alcohol (23 : 2); flow rate, 1.0 ml/min; temp., 0°C. b: Column, Nucleosil 5CN (cyano-type); eluent, n-hexane-dioxan (4 : 1); flow rate, 1.2 ml/min; temp., 0°C.

lated structures was finally resolved by maintaining the capacity ratio at approximately eight and choosing a phase system with an n-hexane-dioxan binary solvent on a silica or cyano column. The chromatograms in Figure 4 show examples of incomplete (a) and an optimized (b) separations of analogous compounds.

Characterization of the solvent described in this text should be useful for finding an optimum solvent system and a suitable column in normal-phase liquid chromatography separation of given solute mixtures.

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