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## Note

### Formulation of multicomponent mobile solvents for liquid chromatography

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One approach to the optimization of the chromatography of a multicomponent mixture is to select the strength of the mobile solvent to elute the components of the mixture in a suitable retention-time range, and then to adjust resolution by changing the selectivity of the mobile solvent. Snyder<sup>1,2</sup> has devised a set of solvent characterization parameters, based on the work of Rohrschneider<sup>3</sup>, and has proposed the use of these parameters for formulating binary mixtures of different selectivity, but the same overall solvent strength.

The use of three or more components in the mobile solvent offers the possibility of improving the effectiveness of this approach<sup>4,5</sup>, since the selectivity of a multicomponent mobile solvent can be varied continuously throughout a range. We present here a scheme for efficiently and systematically varying the composition of a multicomponent mobile solvent, while keeping the overall solvent strength constant; this scheme may be generally useful for optimizing separations in high-performance liquid chromatography (HPLC). We used this method to optimize the reversed-phase HPLC separation of a mixture of four closely related corticosteroids (cortisone, hydrocortisone, prednisolone and prednisone) by means of a three-component mobile solvent consisting of water, methanol and tetrahydrofuran.

#### THEORY

We assumed that the overall eluting strength of a mixture of several miscible liquids is simply the volume-weighted mean of the strengths of the components<sup>1</sup> and derived (see Appendix) a set of formulae that gives the proportions in which the components are to be combined to yield a mixture with a given eluting strength. If the eluting-strength parameters of a set of pure liquids are  $P_1, P_2, \dots, P_n$  (where  $P_1 > P_2 > \dots > P_n$ ), then the respective volume fractions,  $X_1, X_2, \dots, X_n$ , of these liquids to be combined to give a mixture of eluting strength,  $P$ , (where  $P_1 \geq P \geq P_n$ ) can be deduced as set out below.

#### *Three-component mixtures*

A mixture ( $X_1 + X_2 + X_3 = 1$ ) has the required eluting strength if, and only if,  $X_1$  is non-negative and in the range

$$\frac{P - P_3}{P_1 - P_3} \geq X_1 \geq \frac{P - P_2}{P_1 - P_2} \quad (1)$$

and

$$X_2 = \frac{P - P_3}{P_2 - P_3} - \left( \frac{P_1 - P_3}{P_2 - P_3} \right) X_1 \quad (2)$$

and

$$X_3 = 1 - X_1 - X_2 \quad (3)$$

The entire selectivity range of the mobile solvent can be spanned without repetition by varying  $X_1$  through its range expressed in eqn. 1. One arrives at the optimum solvent composition by trial and error, but this procedure eliminates all those compositions that would cause large shifts to longer or shorter retention times.

There is an alternative method for formulating these three-component mixtures, which is equivalent to the above method (see Appendix) if the volume changes on mixing are neglected. Further, this alternative method may be simpler in practice because it can be used without performing calculations with eluting-strength parameters, and it allows the use of a two-channel solvent programmer for the actual mixing (Fig. 1).

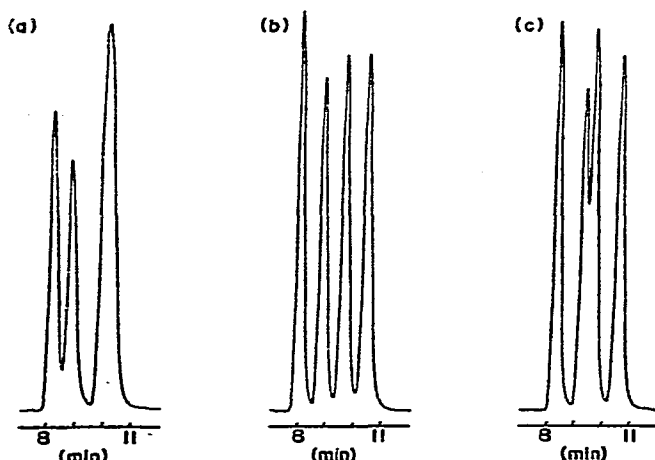


Fig. 1. Optimization of the selectivity of the ternary mobile phase water-methanol-tetrahydrofuran by combining the binary mixtures A (water-methanol, 42:58) and B (water-tetrahydrofuran, 73:27). (a) Mobile phase A; (b) mobile phase 0.14 A + 0.86 B (water-methanol-tetrahydrofuran, *ca.* 69:8:23); (c) mobile phase B. The order of elution is prednisone, cortisone, prednisolone, hydrocortisone. Mobile phase B was formulated by trial and error to have the same average eluting strength as phase A.

One formulates the two binary mixtures A (components 1 and 3) and B (either components 1 and 2 or components 2 and 3) that have the desired eluting strength,  $P$ ; this can be done by calculation from a set of solvent-strength parameters or by experimentation. In the former instance, one calculates the unique mixtures of A and B that each simultaneously satisfy the equations  $P = P_i X_i + P_j X_j$  and  $X_i + X_j = 1$ . In the latter instance, one arrives by trial and error at the compositions of A and B that give the same desired retention time for either a single chosen peak in the chro-

matogram or the average retention time of all the peaks. In either instance, combining A and B in varying proportions from 100% A to 100% B yields, without repetition, all and only those three-component mixtures that have the given eluting strength. Therefore, if no combination of A and B produces the required resolution, then no ternary mixture of these solvents at this strength will be effective.

#### Four-component mixtures

A mixture ( $X_1 + X_2 + X_3 + X_4 = 1$ ) has the required eluting strength if, and only if,  $X_1$  is non-negative and in the range

$$\frac{P - P_4}{P_1 - P_4} \geq X_1 \geq \frac{P - P_2}{P_1 - P_2} \quad (4)$$

and  $X_2$  is non-negative and in the range

$$\frac{P - P_4}{P_2 - P_4} - \left(\frac{P_1 - P_4}{P_2 - P_4}\right) X_1 \geq X_2 \geq \frac{P - P_3}{P_2 - P_3} - \left(\frac{P_1 - P_3}{P_2 - P_3}\right) X_1 \quad (5)$$

and

$$X_3 = \frac{P - P_4}{P_3 - P_4} - \left(\frac{P_1 - P_4}{P_3 - P_4}\right) X_1 - \left(\frac{P_2 - P_4}{P_3 - P_4}\right) X_2 \quad (6)$$

and

$$X_4 = 1 - X_1 - X_2 - X_3 \quad (7)$$

These four-component mixtures can also be obtained by combining three binary mixtures (see Appendix) —A, B and C— that each have the required eluting strength,  $P$ . If  $P > P_2$ , which is usual, then these binary mixtures are A (components 1 and 4), B (1 and 3), and C (1 and 2). If  $P_2 > P > P_3$ , then the binary mixtures are A (1 and 4), B (2 and 3), and C (either one of 1 and 3 or 2 and 4). If  $P_3 > P$ , then the binary mixtures are A (1 and 4), B (2 and 4), and C (3 and 4).

#### EXPERIMENTAL

Chromatography was carried out with a DuPont Zorbax ODS column ( $4.9 \times 250$  mm) at ambient temperature, using a Waters Model 204 liquid chromatograph with the absorbance detector at a wavelength of 254 nm and a sensitivity of 0.05 ABS. The flow-rate was 1.0 ml/min, and the pressure was *ca.* 1800 p.s.i., depending on the mobile-solvent composition. Injections, all 15  $\mu$ l, were made with a Waters WISP 710A sample processor. The absorbance was recorded with a Varian A-25 10-inch strip-chart recorder set at a sensitivity of 10 mV full-scale and a chart speed of 50 in./h. Retention times and peak widths (full width at half maximum) were determined from the chart.

Mobile solvents were formulated by volume using house-distilled water, methanol (Burdick and Jackson, Muskegon, MI, U.S.A.; suitable for liquid chromatography) and tetrahydrofuran (Burdick and Jackson, UV grade). Tetrahydrofuran was purified just before mixing by passing it through a column ( $2 \times 15$  cm) of alumina (ICN, Irwine, CA, U.S.A.; alumina acid, activity grade I). The solvent mixtures were filtered (Millipore, type LS, 5.0  $\mu$ m) under vacuum before use.

Cortisone (Research Plus Steroid Laboratories), hydrocortisone, prednisolone and prednisone (all USP Reference Standard) were used as received.

#### RESULTS AND DISCUSSION

Mixtures of cortisone (22  $\mu\text{g/ml}$ ) and hydrocortisone (17  $\mu\text{g/ml}$ ), and of prednisolone (20  $\mu\text{g/ml}$ ) and prednisone (15  $\mu\text{g/ml}$ ), each dissolved in water-methanol (42:58), were separately chromatographed with each different mobile solvent. These solutions were first chromatographed using a series of water-methanol mixtures of different solvent strengths to determine a solvent-strength parameter,  $P$ , for calculating water-methanol-tetrahydrofuran mixtures of equal strength; water-methanol-tetrahydrofuran mixtures of strength  $P = 7.61$  were formulated because this strength was judged to give a good balance between resolution and elution time.

The retention times and peak widths determined from these chromatograms are given in Table I. The average retention time of the chromatograms is insensitive to changes in solvent composition in the middle of the range (from 48 to 66 parts of water). The variation of the average retention time is larger in the extremes of the range (from 42 to 48 and from 66 to 71 parts of water), but even there the variation is smaller than that resulting from changing the overall solvent strength. Drift in retention times during data collection was less than 3%, determined by chromatography using water-methanol (42:58) as mobile solvent at intervals during data collection. Thus, Snyder's solvent-strength parameters were useful for controlling the eluting strength of the solvent in the system studied here.

TABLE I

RETENTION TIMES AND PEAK WIDTHS DETERMINED FROM CHROMATOGRAMS  
THF = Tetrahydrofuran;  $t_R$  = retention time (min);  $t_W$  = full width at half maximum (min).

| Mobile phase composition (%) |                    |     | Elution strength $P^*$ | Prednisone |       | Cortisone |       | Prednisolone |       | Hydrocortisone |       |
|------------------------------|--------------------|-----|------------------------|------------|-------|-----------|-------|--------------|-------|----------------|-------|
| H <sub>2</sub> O             | CH <sub>3</sub> OH | THF |                        | $t_r$      | $t_w$ | $t_r$     | $t_w$ | $t_r$        | $t_w$ | $t_r$          | $t_w$ |
| 38                           | 62                 | 0   | 7.51                   | 6.47       | 0.26  | 6.90      | 0.27  | 7.78         | 0.29  | 7.94           | 0.28  |
| 40                           | 60                 | 0   | 7.56                   | 7.62       | 0.30  | 8.26      | 0.33  | 9.33         | 0.35  | 9.62           | 0.35  |
| 42                           | 58                 | 0   | 7.61                   | 8.90       | 0.37  | 9.73      | 0.38  | 11.08        | 0.40  | 11.40          | 0.42  |
| 44                           | 56                 | 0   | 7.66                   | 10.00      | 0.38  | 10.82     | 0.40  | 12.47        | 0.43  | 12.63          | 0.43  |
| 43                           | 56                 | 1   | 7.61                   | 8.15       | 0.29  | 8.82      | 0.33  | 10.36        | 0.37  | 10.60          | 0.36  |
| 45                           | 52                 | 3   | 7.61                   | 7.84       | 0.27  | 8.45      | 0.29  | 10.22        | 0.34  | 10.51          | 0.34  |
| 48                           | 46                 | 6   | 7.61                   | 7.30       | 0.26  | 7.86      | 0.27  | 9.67         | 0.33  | 9.97           | 0.33  |
| 54                           | 34                 | 12  | 7.61                   | 7.19       | 0.25  | 7.73      | 0.26  | 9.46         | 0.33  | 9.81           | 0.32  |
| 60                           | 22                 | 18  | 7.61                   | 7.30       | 0.25  | 8.07      | 0.26  | 9.25         | 0.29  | 10.05          | 0.32  |
| 66                           | 10                 | 24  | 7.61                   | 7.54       | 0.24  | 8.34      | 0.26  | 9.11         | 0.29  | 9.89           | 0.31  |
| 71                           | 0                  | 29  | 7.61                   | 8.07       | 0.26  | 9.01      | 0.28  | 9.35         | 0.29  | 10.23          | 0.29  |

$$* P = \sum X_i P_i; P_{\text{H}_2\text{O}} = 9; P_{\text{CH}_3\text{OH}} = 6.6; P_{\text{THF}} = 4.2.$$

The resolution, capacity factors, and separation factors calculated from the data in Table I are shown in Table II. The resolution values reveal an optimum in the overall resolution in the composition region 60 to 66 parts of water. The values of the separation factors show that the changes in resolution are due mainly to changes in the selectivity of the mobile solvent, rather than to changes in efficiency.

TABLE II  
PARAMETERS CALCULATED FROM RESULTS IN TABLE I

| Mobile phase composition (%) |                    |     | Resolution*     |                 |                 | Capacity factors** |                 |                 |                 | Separation factors               |                                  |                                  |
|------------------------------|--------------------|-----|-----------------|-----------------|-----------------|--------------------|-----------------|-----------------|-----------------|----------------------------------|----------------------------------|----------------------------------|
| H <sub>2</sub> O             | CH <sub>3</sub> OH | THF | R <sub>12</sub> | R <sub>23</sub> | R <sub>34</sub> | k' <sub>1</sub>    | k' <sub>2</sub> | k' <sub>3</sub> | k' <sub>4</sub> | k' <sub>2</sub> /k' <sub>1</sub> | k' <sub>3</sub> /k' <sub>2</sub> | k' <sub>4</sub> /k' <sub>3</sub> |
| 38                           | 62                 | 0   | 1.0             | 1.9             | 0.3             | 1.61               | 1.78            | 2.14            | 2.20            | 1.11                             | 1.20                             | 1.03                             |
| 40                           | 60                 | 0   | 1.2             | 1.9             | 0.5             | 2.07               | 2.33            | 2.76            | 2.88            | 1.13                             | 1.18                             | 1.04                             |
| 42                           | 58                 | 0   | 1.3             | 2.1             | 0.5             | 2.59               | 2.92            | 3.47            | 3.60            | 1.13                             | 1.19                             | 1.04                             |
| 44                           | 56                 | 0   | 1.3             | 2.4             | 0.2             | 3.03               | 3.36            | 4.03            | 4.09            | 1.11                             | 1.20                             | 1.01                             |
| 43                           | 56                 | 1   | 1.3             | 2.6             | 0.4             | 2.29               | 2.56            | 3.18            | 3.27            | 1.12                             | 1.24                             | 1.03                             |
| 45                           | 52                 | 3   | 1.3             | 3.4             | 0.5             | 2.16               | 2.41            | 3.12            | 3.24            | 1.12                             | 1.29                             | 1.04                             |
| 48                           | 46                 | 6   | 1.3             | 3.6             | 0.5             | 1.94               | 2.17            | 2.90            | 3.02            | 1.12                             | 1.34                             | 1.04                             |
| 54                           | 34                 | 12  | 1.3             | 3.5             | 0.6             | 1.90               | 2.12            | 2.81            | 2.96            | 1.12                             | 1.33                             | 1.05                             |
| 60                           | 22                 | 18  | 1.8             | 2.6             | 1.6             | 1.94               | 2.25            | 2.73            | 3.05            | 1.16                             | 1.21                             | 1.12                             |
| 66                           | 10                 | 24  | 1.9             | 1.7             | 1.6             | 2.04               | 2.36            | 2.67            | 2.99            | 1.16                             | 1.13                             | 1.12                             |
| 71                           | 0                  | 29  | 2.1             | 0.7             | 1.8             | 2.25               | 2.63            | 2.77            | 3.13            | 1.17                             | 1.05                             | 1.13                             |

$$* R = \frac{2(t_r - t'_r)}{1.67(t_w + t'_w)}$$

$$** k' = \frac{t_r - t_0}{t_0}; t_0 = 2.48 \text{ min (determined by injection of water).}$$

## APPENDIX

*Derivation of formulae*

Eqns. 1-7 are obtained as follows. Given that

$$P = \sum_{i=1}^n X_i P_i \quad (8)$$

$$1 = \sum_{i=1}^n X_i \quad (9)$$

$$1 \geq X_i \geq 0 \quad i = 1, 2, \dots, n \quad (10)$$

$$P_1 > P_2 > \dots > P_n \quad (11)$$

$$P_1 \geq P \geq P_n \quad (12)$$

then eqns. 2 and 6 are obtained by combining eqns. 8 and 9. Expression 1 is obtained by combining expressions 2, 3, 9, 10, 11 and 12. First, the left-hand side of expression 10 is discarded as redundant using eqn. 9. Then eqns. 2 and 3 are combined in turn with expression 10 to yield a pair of inequalities, which are rearranged into the form of expression 1 using expressions 11 and 12. Expressions 4 and 5 are obtained in similar fashion. Eqns. 3 and 7 are simply rearrangements of eqn. 9. None of these manipulations is of the kind that introduces extraneous solutions; therefore, all the compositions consistent with expressions 1-3 and 4-7 are consistent with eqns. 8 and 9.

The validity of obtaining all the mixtures consistent with expressions 8-12 by combining various binary mixtures is demonstrated as follows<sup>6</sup>. Consider the  $n$ -dimensional vector space over the real field in which the vectors are the ordered sets  $(X_1, X_2, \dots, X_n)$ . The  $n-1$  dimensional sub-space of this space, for which  $\sum_{i=1}^n (P - P_i) \cdot X_i = 0$ , contains a unique vector representing each solvent composition consistent with expressions 8-12. The sets of vectors corresponding to the binary mixtures A and B for  $n = 3$  (and A, B and C for  $n = 4$ ) are linearly independent and hence are bases of the sub-space. Further, these basis sets are the ones of practical usefulness, since these sets (and only these sets) insure the existence of positive coefficients for all vectors for which  $X_i \geq 0, i = 1, 2, \dots, n$ .

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