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Optimization of chromatographic selectivity of twelve sulphonamides in reversed-phase high-performance liquid chromatography using mixture designs and multi-criteria decision making

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ABSTRACT

For the optimization of the reversed-phase high-performance liquid chromatographic separation of twelve sulphonamides using a quaternary mobile phase, quadratic regression models were calculated. The three pseudo-components were buffer–methanol, buffer–acetonitrile and buffer–tetrahydrofuran and had identical solvent strengths. The capacity factors of the sulphonamides were determined at ten mobile phase compositions. The calculated regression models were used to optimize the resolution of the mobile phase and to simulate a chromatogram under optimum mobile phase conditions. The simulated optimum separation showed great similarity with a chromatogram measured under optimum conditions.

INTRODUCTION

Reversed-phase high-performance liquid chromatography (RP-HPLC) is probably the most frequently utilized method in chromatography. Several structurally related compounds in a sample may be separated by the high selectivity of RP-HPLC. The most difficult part of the separation of multi-solute samples is finding the optimum experimental conditions for the separation. Mobile phase optimization is the most commonly utilized method.

By application of chemometric techniques, optimization by trial and error can be avoided and optima will (often) be reached faster. Criteria to be optimized are mostly the capacity factor of the last-eluting solute and the separation power (resolution or selectivity) of the system. The capacity factor of the last-eluting solute (k'_{max}) should be minimized and the separation power (here expressed by the resolution $R_{i,j}$ [1]) should be maximized ($R_{s_{min}}$). $R_{s_{min}}$ is the resolution of the worst-separated pair of peaks in a chromatogram. Similarly, it is possible to calculate the minimum selectivity α_{min} of a chromatogram. However, when calculating the selectivity $\alpha_{i,j}$ of two solutes i and j, the influence of the capacity factor on the separation is not taken into account: higher capacity factors of two solutes i and j may result in equal $\alpha_{i,j}$ values (*i.e.*, the quality of the chromatogram due to this criterion remains constant), whereas the resolution $R_{i,j}$

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decreases (*i.e.*, the quality of the chromatogram decreases owing to this criterion, which is actually the case, as bands broaden as a result of increasing capacity factors). We prefer minimum resolution $R_{s_{min}}$ to the minimum selectivity α_{min}, as the former corrects for band broadening.

Schoenmakers [2] introduced the "calibrated normalized resolution product" (CNRP), which is the product of the separation factor of each peak pair ($2R_{i,j}$ for one plate) divided by the mean separation factor. Chromatograms in which all peaks are equally spaced give optimum values for CNRP. This criterion has the disadvantage of not being specific; it is a relative criterion (relative to the mean of the separation factors), whereas the resolution is an absolute criterion.

Glajch *et al.* [3] combined a mixture design statistical technique and the solvent strength and selectivity theory of Rohrschneider [4] and Snyder [5,6] to obtain a systematic method for optimizing the mobile phase composition in RP-HPLC, which they called "overlapping resolution mapping" (ORM). This ORM technique has been modified since then and adapted to new approaches of mobile phase optimization [7,8].

A recent paper [9] discussed the theory of application of mixture experimental design techniques and criteria used in mobile phase optimizations. Previous papers showed a quadratic effect of mobile phase composition on retention behaviour [9–11]. An equation was given to describe the retention behaviour of one solute in a quaternary system. The quadratic equation for one solute in a ternary system is

$$\ln k' = a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + e \qquad (1)$$

In special cubic models an additional term is used ($a_{123}x_1x_2x_3$). Three variables represent the composition of a mobile phase; they are introduced here as x_1, x_2 and x_3 and represent the fractions of the (pseudo-)components in the mobile phase. a_1–a_{123} are the regression coefficients to be estimated. The residual error is given by e. For estimation of the quadratic model at least seven (six coefficients plus an error term) experiments (design points) are needed; the special cubic model requires eight experiments.

The experiments to be carried out are planned previously according to some experimental design. The experimental results are collected and then any response criterion selected can be modelled. These methods of data processing are called simultaneous optimization methods. The experiments of such simultaneous methods have to be carried out at random.

For future research concerning studies on liquid–liquid extraction of sulphonamides with determination by HPLC, a mobile phase composition had to be selected that separates the sulphonamides within an acceptable period of time and with acceptable resolution. This paper discusses the simultaneous optimization of the separation of a mixture of twelve sulphonamides using mobile phase selectivity optimization in an isoeluotropic RP-HPLC system with mixture design statistical techniques and "multi-criteria decision making" (MCDM). Criteria used for selecting an optimum mobile phase are the capacity factor and the resolution.

EXPERIMENTAL

Instruments and instrumental conditions

The assay was performed with an HPLC system consisting of a Spectra-Physics (San Jose, CA, U.S.A.) Model SP8700 solvent delivery system used at a flow-rate of 1.0 ml min^{-1} and a Kratos (Ramsey, NJ, U.S.A.) Model 757 UV detector, wavelength 260 nm, range 0.005 a.u.f.s., rise time 1 s.

Injections of sulphonamide standard solutions into a Zymark (Hopkinton, MA, U.S.A.) Z 310 HPLC injection station, equipped with an electrically controlled Rheodyne valve and a 20-μl sample loop, were performed by a Zymate II robot system. A Zymark Z 310 analytical instrument interface was used to control the HPLC injection station. The analytical column was a 100 × 4.6 mm I.D. Microsphere 3-μm C_{18} cartridge system (Chrompack, Middelburg, The Netherlands). Data analysis was performed by means of a Spectra-Physics Chromjet SP4400 computing integrator.

Calculations were performed on an IBM PS/2 Model 60 computer using the POEM (predicting optimum eluent mixtures) software package written in Pascal [9]. This package calculates mixture models using multiple linear regression, performs validation of the models by ANOVA for judging descriptive capability, and cross-validation ("leave one out method", LOOM) to give a mean predicted error sum of squares (mPRESS [12]):

$$\text{mPRESS} = \frac{1}{n}\sum_{i}^{n}(y_i - \hat{y}_{(i)})^2$$

where n is the number of observations, $\hat{y}_{(i)}$ represents the predicted value of the ith observation with the use of a model in which the ith observation is not incorporated and y_i is the experimental value of the ith observation. With mPRESS the predictive power of a model can be judged.

Chemicals and reagents

Twelve sulphonamides were supplied by Sigma (St. Louis, MO, U.S.A.): sulphacetamide (AC), sulphamethoxazole (OX), sulphamethizole (MT), phthalylsulphacetamide (PT), sulphisomidine (SO), sulphathiazole (TH), sulphapyridine (PY), sulphamerazine (ME), sulphamethoxypyridazine (MP), sulphachloropyridazine (CP), sulphaguanidine (GU) and sulphanilamide (AN). Acetonitrile (ACN), tetrahydrofuran (THF) and methanol were supplied by Labscan (Dublin, Ireland) and were of HPLC grade. Acetic acid (100%) (HA_c), triethylamine (TEA), phosphoric acid (85%) (H_3PO_4) and potassium dihydrogenphosphate (KH_2PO_4) were all of analytical-reagent grade and supplied by Merck (Darmstadt, Germany). Water was purified by using Milli-RO-15 and Milli-Q water purification systems (Millipore, Bedford, MA, U.S.A.). Unless stated otherwise, water of Milli-Q quality was used.

A phosphate buffer (pH 3.0; 0.05 *M*) was prepared by dissolving 6.80 g of KH_2PO_4 in 1000 ml of water. The pH was adjusted at 3.0 using concentrated phosphoric acid. To this buffer 4.15 ml of TEA and 10 ml of acetic acid were added. This buffer was used to prepare binary mobile phases with an organic modifier. Before use, the mobile phases were filtered through a Millipore Type HVLP filter (0.45 μm) and degassed before use by ultrasonification for 15 min.

Stock solutions of sulphonamides were prepared by dissolving 100 mg of the compounds in 100 ml of methanol to give concentrations of 1000.0 mg l^{-1}. These solutions were stored at +4°C.

A test solution was prepared containing all twelve sulphonamides. The concentration of each sulphonamide was 500 $\mu g\ l^{-1}$. This solution was used to select solvent strength and to compare predicted and measured chromatograms. The solution was stored at +4°C.

Peak identification

As different mobile phases with different organic modifiers may cause changes in elution order, peak identification was necessary. Separate injection of all sulphonamide standard solutions in each mobile phase is very time consuming. A method was selected for the separate identification of the components after injection of a mixture of solutes.

Snyder *et al.* [13] recommended a number of methods of peak tracking. Some of these methods require diode-array detectors or detection at two wavelengths. Other techniques use two samples in which each solute has a different concentration. Peak-height or peak-area ratios for a given compound are then predictable. They are equal to the concentration ratio in each sample.

We divided the twelve sulphonamides into four groups. Within each group, concentrations of sulphonamides in the standard solutions were made such that peak areas had a ratio of 1:2:4:8. Even if two or more solutes overlap each other completely in the chromatogram, a unique new peak area is measured. Table I gives the different sulphonamide mixtures and their concentrations.

Optimization of solvent strength

The suitable solvent strength was determined by eluting a test mixture containing all solutes in mobile phases with different fractions of ACN in phosphate

TABLE I

TEST MIXTURES FOR PEAK IDENTIFICATION

Test mixture	Component	Peak area	Stock solution in 100 ml (μl)
1	Sulphisomide	15 000	22
	Sulphapyridine	60 000	85
2	Sulphaguanidine	15 000	16
	Sulphacetamide	30 000	50
	Sulphathiazole	60 000	90
3	Sulphamerazine	15 000	30
	Sulphanilamide	30 000	52
	Sulphamethizole	60 000	110
4	Sulphamethoxypyridazine	7500	10
	Sulphachloropyridazine	15 000	30
	Phthalylsulphacetamide	30 000	68
	Sulphamethoxazole	60 000	110

buffer. A suitable fraction of ACN in buffer (*i.e.*, a mixture of ACN in buffer in which the capacity factors of the solutes eluted satisfy the restriction $1 \leqslant k' \leqslant 10$) can be read from a plot of the logarithm of the capacity factor of the first- and last-eluting solute *versus* the fraction of ACN. Binary mixtures of buffer and methanol or buffer and THF with equal solvent strength to maintain roughly equal capacity factors can be calculated using the nomograph of Snyder *et al.* [13]. This nomograph is an approximation and was adapted from empirical data of Schoenmakers *et al.* [14,15]. The three pseudo-components obtained (x_1, x_2 and x_3) can be placed at the vertices in a mixture triangle (Fig. 1). All mixtures of x_1, x_2 and x_3 result in equal solvent strengths (isoeluotropic plane) and (theoretically) equal maximum analysis times.

Experimental design

Design points have to be distributed evenly in the factor space x_1, x_2 and x_3. Eight design points suffice to investigate both quadratic and special cubic models. However, we decided to create some extra degrees of freedom for an intensive evaluation of the models; in this investigation ten design points were measured. Fig. 2 illustrates the design points used. Corresponding fractions of x_1, x_2 and x_3, buffer, ACN, methanol and THF are given in Table II. Experiments 4, 5, 6, 7 and 8 were repeated to investigate system reproducibility.

RESULTS AND DISCUSSION

Optimization of solvent strength

The first-eluting solute in the test mixture was sulphaguanidine in mixtures of 2%, 5% and 10% ACN in buffer. With mobile phases compositions of 2% and 5% ACN in buffer, the analysis time for the last-eluting solute, sulphamethoxazole, was very long. For this reason, this solute was introduced into mobile phases with 10%, 15% and 20% ACN, respectively.

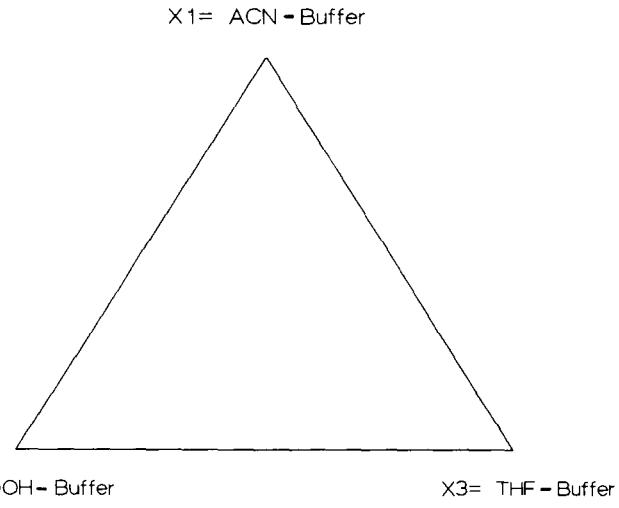

Fig. 1. Isoeluotropic mixture triangle with three pseudo-components of buffer with ACN, methanol (MeOH) and THF.

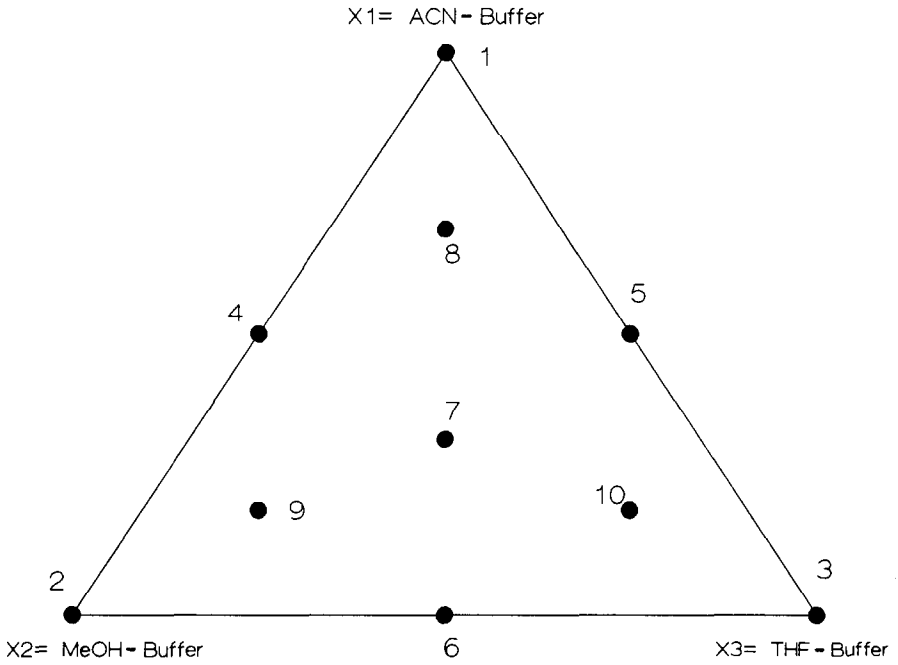

Fig. 2. Experimental design for the optimization of the separation of the twelve sulphonamides. For corresponding fractions of the solvents, see Table II.

The dead time of the HPLC system was determined to be 65 s and the plate number of the column was 6300. The results of the determination of the isoeluotropic HPLC system are given in Table III. In Fig. 3, a plot of the logarithm of the capacity factor *versus* the fraction of ACN leads to a buffer–ACN mixture where k' varies from *ca.* 1 to 10. A mixture of 10% ACN in buffer gives the best compromise for this restriction. With this mobile phase composition, the capacity factors of the sulphon-

TABLE II

EXPERIMENTAL DESIGN FOR THE SEPARATION OF TWELVE SULPHONAMIDES

No.	Pseudo-component fractions			Solvent fractions			
	x_1	x_2	x_3	Buffer	ACN	Methanol	THF
1	1.0000	0.0000	0.0000	0.9000	0.1000	0.0000	0.0000
2	0.0000	1.0000	0.0000	0.8500	0.0000	0.1500	0.0000
3	0.0000	0.0000	1.0000	0.9200	0.0000	0.0000	0.0800
4	0.5000	0.5000	0.0000	0.8750	0.0500	0.0750	0.0000
5	0.5000	0.0000	0.5000	0.9100	0.0500	0.0000	0.0400
6	0.0000	0.5000	0.5000	0.8850	0.0000	0.0750	0.0400
7	0.3333	0.3333	0.3333	0.8900	0.0333	0.0500	0.0267
8	0.6667	0.1667	0.1667	0.8950	0.0667	0.0250	0.0133
9	0.1667	0.6667	0.1667	0.8700	0.0167	0.1000	0.0133
10	0.1667	0.1667	0.6667	0.9050	0.0167	0.0250	0.0533

TABLE III

ANALYSIS TIMES AND CAPACITY FACTORS OF THE FIRST-ELUTING (SULPHAGUANIDINE) AND LAST-ELUTING (SULPHAMETHOXAZOLE) SOLUTES

Parameter	Sulphaguanidine			Sulphamethoxazole		
	2% ACN	5% ACN	10% ACN	10% ACN	15% ACN	20% ACN
t_r	127	106	86	868	565	320
k'	0.95	0.63	0.32	12.35	7.69	3.92
Log k'	−0.02	−0.20	−0.50	1.09	0.89	0.59

amides vary from 0.32 to 12.4. Corresponding proportions of methanol and THF were 15% and 8%, respectively.

Optimization of solvent selectivity

Retention times of the twelve sulphonamides were determined and capacity factors were calculated for every design point. Table IV gives the capacity factors of all solutes in the mobile phase system used. The data demonstrate that the transfer rules of the solvent strength theory do not guarantee a constant analysis time. Binary mixtures

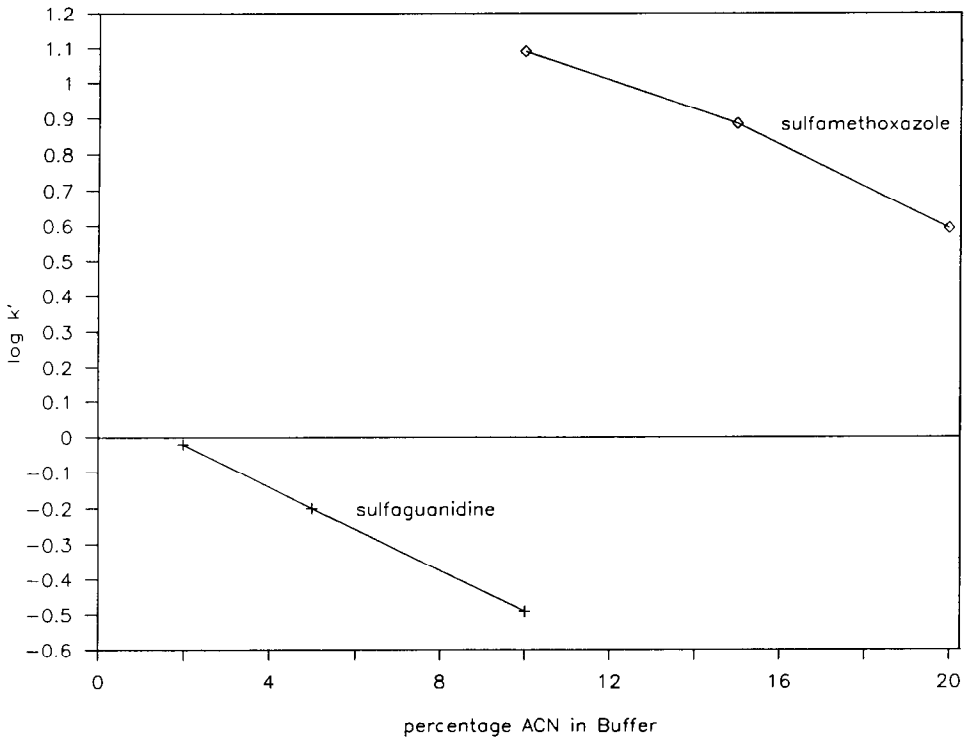

Fig. 3. Determination of the isoeluotropic HPLC system. Sulphaguanidine and sulphamethoxazole were used to determine the suitable solvent strength.

TABLE IV

MEASURED CAPACITY FACTORS OF TWELVE SULPHONAMIDES WITH TEN DIFFERENT MOBILE PHASE COMPOSITIONS

No.	Mobile phase fraction of				Capacity factor (k')[a]											
	Buffer	ACN	Methanol	THF	GU	SO	AC	TH	PY	ME	AN	MT	MP	PT	OX	CP
1	0.900	0.100	0.000	0.000	0.37	1.48	1.58	3.14	3.35	3.80	5.49	6.95	7.58	13.77	15.14	11.09
2	0.850	0.000	0.150	0.000	0.23	1.83	1.03	2.45	2.87	3.45	5.69	6.06	7.75	17.08	10.09	8.62
3	0.920	0.000	0.000	0.080	0.20	0.72	1.28	1.63	1.63	2.05	2.28	3.32	3.08	7.55	11.55	8.92
4	0.875	0.050	0.075	0.000	0.29	1.77	1.35	3.00	3.34	3.83	6.03	7.11	8.31	17.98	13.52	10.62
5	0.910	0.050	0.000	0.040	0.18	0.77	1.14	1.74	1.74	2.15	1.89	3.62	3.62	6.46	10.17	7.31
6	0.885	0.000	0.075	0.040	0.23	0.92	1.25	1.83	1.83	2.18	1.83	3.80	3.80	9.22	10.51	8.08
7	0.890	0.033	0.050	0.027	0.22	0.95	1.17	1.88	1.95	2.34	2.34	4.14	4.14	9.06	10.20	7.77
8	0.895	0.067	0.025	0.013	0.26	1.14	1.34	2.49	2.49	2.94	3.51	5.45	5.60	11.86	12.72	10.43
9	0.870	0.017	0.100	0.013	0.18	1.06	1.06	1.88	2.02	2.37	2.82	4.29	7.46	10.86	9.55	7.46
10	0.905	0.017	0.025	0.053	0.18	0.74	1.17	1.63	1.63	2.05	1.51	3.37	3.25	7.48	10.23	7.80

[a] For abbreviations, see *Chemicals and reagents*.

of methanol in buffer and THF in buffer with presumed isoeluotropic compositions as compared with 10% ACN in buffer resulted in other k' ranges: k'_{max} varies from 10.2 to 18.0. For our application this was of minor importance, as in the MCDM procedure it is possible to minimize k'_{max} simultaneously with maximizing $R_{s_{min}}$.

The reproducibility of the HPLC system under the conditions examined was acceptable. The mean relative standard deviation (R.S.D.), of the capacity factors for replicate design points was 1.4%. The logarithm of the capacity factor is modelled as a function of mobile phase compositions for both quadratic and special cubic models. Table V gives the statistical evaluation of both models. The values are averages for the twelve solutes. The R.S.D. of the difference between model-predicted and measured values ($RSD_{(\hat{y}-y)}$) was little better for the quadratic model. Allthough the special cubic model explains more variations of the data than the quadratic model, the descriptive power of the quadratic model is better with respect to the adjusted correlation coefficient. The adjusted correlation coefficient is the multiple correlation coefficient R^2 corrected for the number of model coefficients. In this way, the descriptive power of different model types can be compared. The mPRESS value of the quadratic model was approximately 63% of that of the special cubic model. This indicates that the predictive power of the quadratic model is also better. The mPRESS value of sulphamethoxypyridazine was about 50% worse than those of the other solutes.

It was concluded that the quadratic model was the best model to use for the prediction of optimum mobile phase compositions for the separation of these twelve sulphonamides. The model coefficients of the quadratic models of the twelve solutes were used to calculate a minimum resolution plot: for every mobile phase composition (in steps of 1% of the mixture components) in the factor space a minimum resolution is calculated. This is the resolution of the worst-separated solute pair. This minimum resolution plot is given in Fig. 4.

Although the maximum capacity factor for every mobile phase composition should be about constant as a result of the solvent strength optimization, a k'_{max} plot (Fig. 5) demonstrates that this assumption is not completely correct. Both measured and predicted values of k'_{max} vary from 9.5 to 18.0. This variation is a result of the fact that the solvent strength theory of Snyder is an approximation, as stated before.

Although it is not typical to optimize both the minimum (min) k'_{max} and maximum (max) $R_{s_{min}}$ in isoeluotropic ternary mobile phase systems we performed MCDM [9,16], in which min-k'_{max} and max-$R_{s_{min}}$ were weighed against each other. Fig. 6 gives an MCDM plot of $R_{s_{min}}$ *versus* k'_{max}. A mixture composition of 1% x_1, 93% x_2 and 6% x_3 corresponding to an $R_{s_{min}}$ of 1.8 and a k'_{max} of 14.8 (analysis time 17 min) was chosen as the optimum mobile phase composition. A chromatogram was simulated

TABLE V

STATISTICAL EVALUATION (MEAN VALUES) OF QUADRATIC AND SPECIAL CUBIC MODELS FOR TWELVE SULPHONAMIDES

Model	Explained variation (%)	$R^2_{adjusted}$	R.S.D. (%) $(\hat{y}-y)$	mPRESS
Quadratic	94.81	0.8915	8.29	0.0538
Special cubic	95.94	0.8781	8.50	0.0847

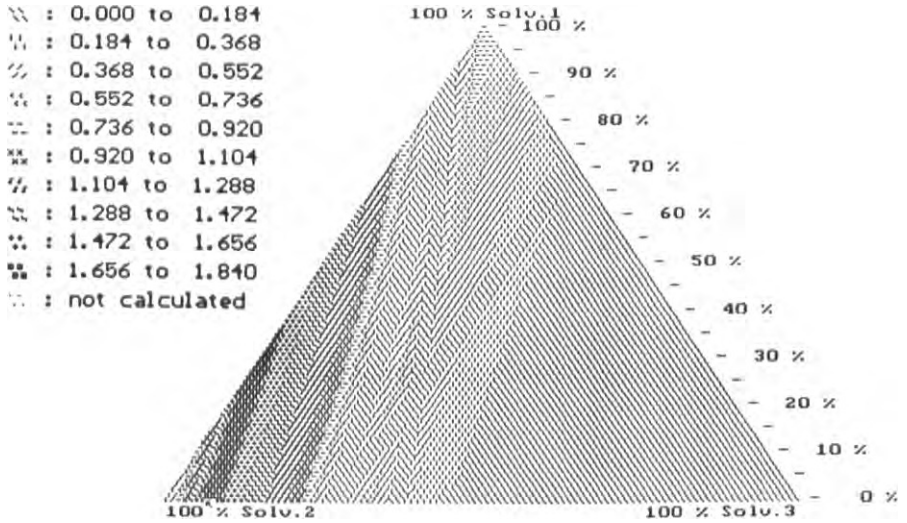

Fig. 4. Minimum resolution plot of the optimization of the separation of the twelve sulphonamides in the ternary isoeluotropic HPLC system.

with this mobile phase with the regression coefficients of the quadratic models (Fig. 7). A max-$R_{s_{min}}$ of 1.8 and a min-k'_{max} of 14.8 were predicted under these conditions. The measured chromatogram with the same mobile phase conditions (Fig. 8) showed great similarity with the predicted chromatogram. Table VI gives measured and predicted retention times for all the sulphonamides. The retention times in the measured chromatogram are slightly shorter (0–8%) compared with the simulated chromatogram, with only sulphamethoxypyridazine having a large deviation (18.4%). This

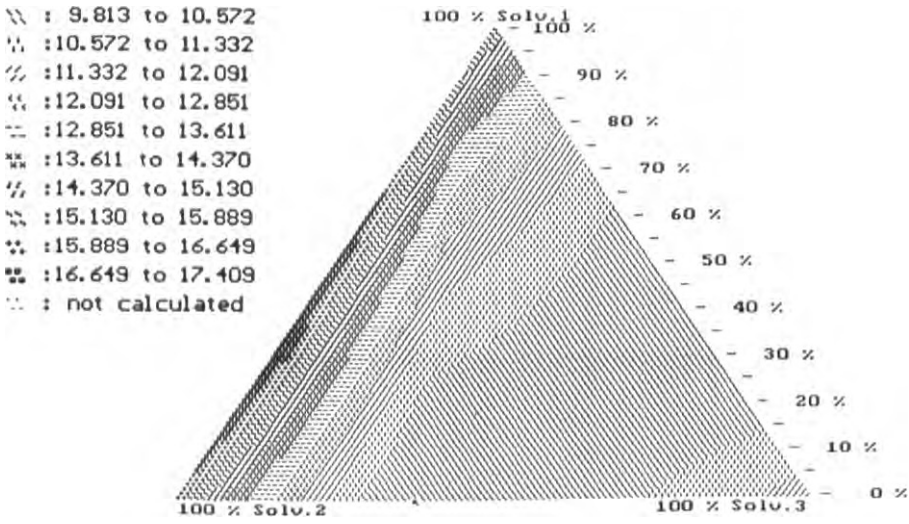

Fig. 5. Maximum capacity factor plot of the optimization of the separation of the twelve sulphonamides in the ternary isoeluotropic HPLC system.

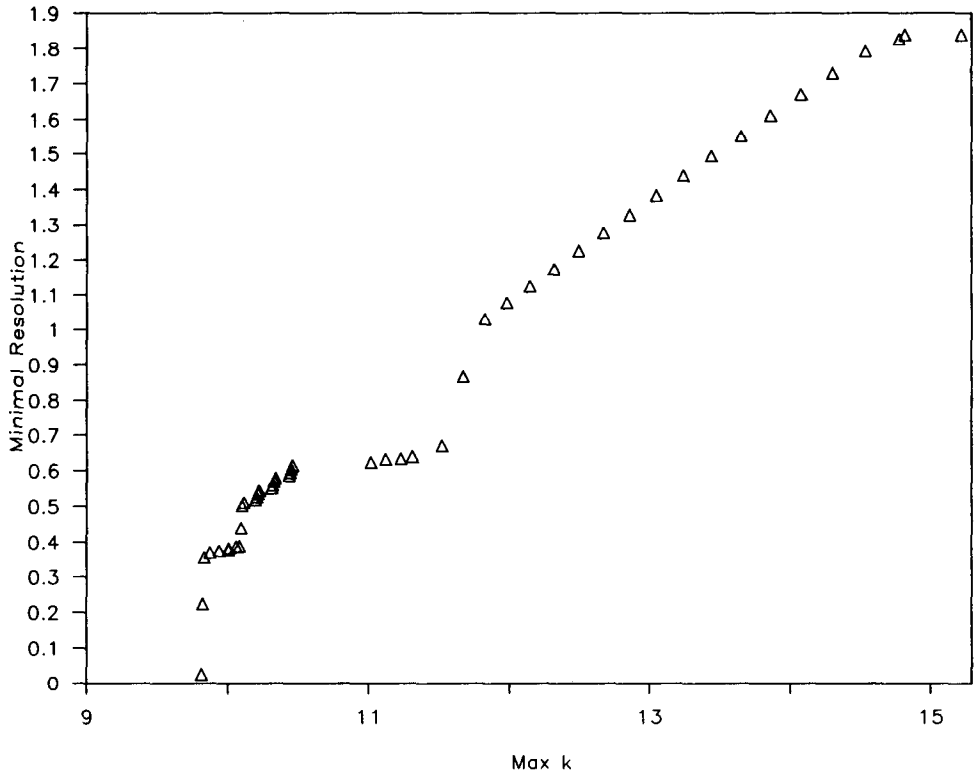

Fig. 6. MCDM plot of the minimum resolution *versus* the maximum capacity factor. Pareto-optimum points are given.

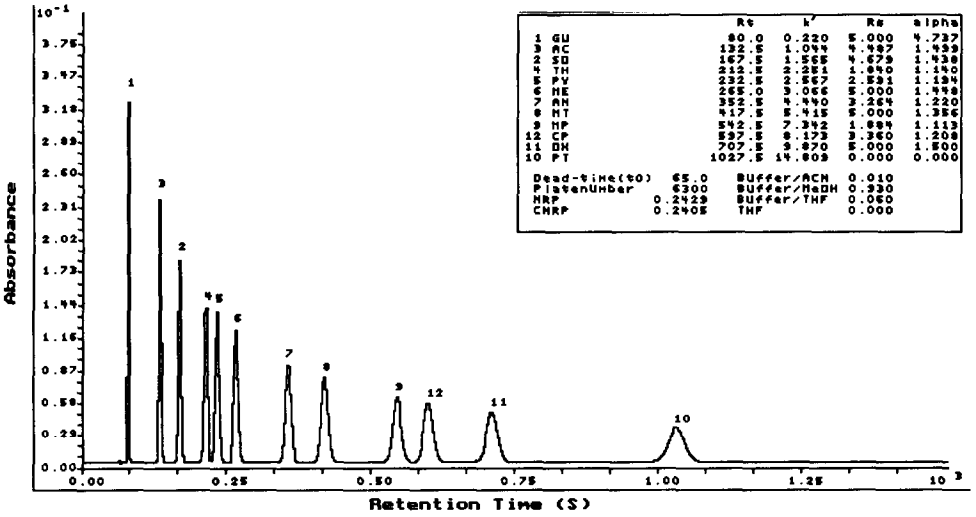

Fig. 7. Predicted chromatogram with the optimum mobile phase composition (1% x_1, 93% x_2 and 6% x_3). Data are given in Table VI. N = 6300; t_0 = 65 s. Rt = Retention time.

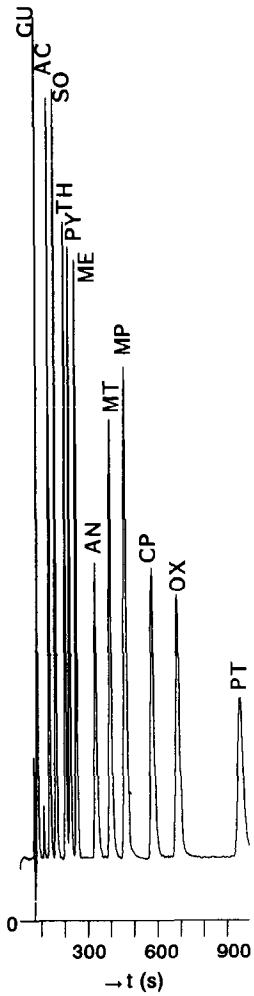

Fig. 8. Measured chromatogram with the optimum mobile phase composition (1% x_1, 93% x_2 and 6% x_3). Data are given in Table VI.

deviation may be due to the fact that the predictive power (expressed by mPRESS) of the model for sulphamethoxypyridazine is low. The peak orders in the simulated and measured chromatograms were the same.

CONCLUSIONS

Optimization of solvent strength and solvent selectivity for the separation of twelve sulphonamides using isoeluotropic eluents in mixture design optimization techniques was performed. It was concluded that the transfer rules of the solvent strength theory do not guarantee a constant analysis time. Isoeluotropic mobile phases in accordance with Snyder's theory did not result in equal maximum capacity factors.

TABLE VI

MEASURED AND PREDICTED RETENTION TIMES FOR THE TWELVE SULPHONAMIDES IN THE OPTIMUM MOBILE PHASE COMPOSITION (1% x_1, 93% x_2 AND 6% x_3)

Solute[a]	t_R (s)		Δt_R (s)	Δt_R (%)
	Measured	Predicted		
GU	79	80	1	1.3
AC	132	132.5	0.5	0.4
SO	159	167.5	8.5	5.3
TH	201	212.5	11.5	5.7
PY	220	232.5	12.5	5.7
ME	248	265	17	6.9
AN	330	352	22	6.7
MT	393	417.5	24.5	6.2
MP	458	542.5	84.5	18.4
CP	574	597.5	25.5	4.4
OX	683	707.5	24.5	3.6
PT	952	1027.5	75.5	7.9

[a] For abbreviations, see *Chemicals and reagents*.

The separation of twelve solutes using statistical mobile phase optimization techniques is readily possible from both an analytical point of view (resolution) and an economic point of view (analysis time). The logarithm of the capacity factor was modelled in a ternary pseudo-component mobile phase system. The best predictive and descriptive power can be achieved by fitting quadratic models to these retention data. The quadratic models were used to optimize both maximum capacity factor and resolution and to predict an optimum chromatogram. Predicted and measured chromatograms show great similarity. The analysis time was optimized to 17 min.

The peak orders in the simulated and measured chromatograms were the same. Although the predicted analysis time for one compound had a large deviation from the experimental analysis time, this had no influence on the quality of the final chromatogram.

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REFERENCES

1 J. R. Gant, J. W. Dolan and L. R. Snyder, *J. Chromatogr.*, 185 (1979) 153.
2 P. J. Schoenmakers, *J. Liq. Chromatogr.*, 10 (1987) 1865.
3 J. L. Glajch, J. J. Kirkland, K. M. Squire and J. M. Minor, *J. Chromatogr.*, 199 (1980) 57.
4 L. Rohrschneider, *Anal. Chem.*, 45 (1973) 1241.
5 L. R. Snyder, *J. Chromatogr.*, 92 (1974) 223.
6 L. R. Snyder, *J. Chromatogr. Sci.*, 16 (1978) 223.
7 P. J. Schoenmakers, A. J. H. C. Drouen, H. A. H. Billiet and L. de Galan, *Chromatographia*, 15 (1982) 688.

8 J. W. Weyland, C. H. P. Bruins and D. A. Doornbos, *J. Chromatogr. Sci.*, 22 (1984) 31.
9 P. M. J. Coenegracht, A. K. Smilde, H. J. Metting and D. A. Doornbos, *J. Chromatogr.*, 485 (1989) 195.
10 R. Tijssen, H. A. H. Billiet and P. Schoenmakers, *J. Chromatogr.*, 122 (1976) 185–203.
11 J. W. Weyland, *Thesis*, State University Groningen, Groningen, 1986.
12 D. W. Osten, *J. Chemometr.*, 2 (1988) 39.
13 L. R. Snyder, J. L. Glajch and J. J. Kirkland, *Practical HPLC Method Development*, Wiley, New York, 1988, p. 32.
14 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.
15 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 218 (1981) 259.
16 A. K. Smilde, A. Knevelman, P. M. J. Coenegracht, *J. Chromatogr.*, 369 (1986) 1.