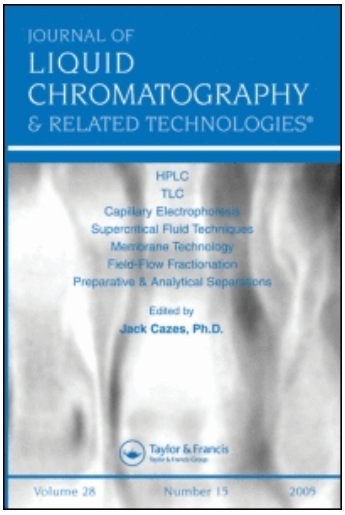

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SINGLE-PEAK RESOLUTION CRITERIA FOR OPTIMIZATION OF MOBILE PHASE COMPOSITION IN LIQUID CHROMATOGRAPHY

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SINGLE-PEAK RESOLUTION CRITERIA FOR OPTIMIZATION OF MOBILE PHASE COMPOSITION IN LIQUID CHROMATOGRAPHY

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ABSTRACT

Three criteria that evaluate the single peak resolution (peak purity) in chromatography, the free height fraction, free area fraction, and valley ratio, are examined. The main advantages of these criteria against other criteria based on peak pair separation, are that the measurements are not affected by the identity of neighboring peaks and are normalized, which make them very intuitive. The methodology is illustrated through the isocratic separation of mixtures of several sulphonamides (sulphacetamide, sulphadiazine, sulphadimethoxine, sulphaguanidine, sulphamerazine, sulphamethazine, sulphamethoxazole, sulphamethizole, sulphamonomethoxine, sulphanilamide, sulphapyridine, sulphaquinoxaline, and sulphathiazole).

Among the three proposed criteria, the measurement of free area fractions gave the best description of the resolution, indepen-

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dent of the overlapping degree of the chromatographic peaks. The usefulness of this criterion for the resolution of individual peaks in a complex mixture is shown. The best separation conditions are reached easily for the individual compounds, and take into account peak shape and size.

INTRODUCTION

In the chromatographic analysis of complex samples, the objective is often not the total separation of the sample, but rather the adequate separation of one, or a few, compounds of interest. This objective can be reached by the systematic observation of the separation at varying mobile phase composition. The best conditions can also be achieved with the aid of optimization strategies, which use the information from a small number of experimental runs.[1] These strategies are based on the application of resolution criteria that measure the separation of the peaks, and can be very effective. The problem is, however, how reliable the applied criteria are to describe peak resolution.

Several elementary resolution measurements of diverse complexity have been reported for liquid chromatography.[2-6] Most of them associate a numerical value to each pair of peaks in a chromatogram. A resolution criterion has been developed in our laboratory that associates, in contrast, a value to each compound in a mixture, differentiating the contributions of the different peaks in a chromatogram.[7] This criterion measures the peak purity of individual peaks through the calculation of free area fractions in predicted chromatograms. Several advantages are achieved:

(i) the measurements are not affected by the identity of the neighboring peaks that overlap the considered peak;

(ii) free area fractions are normalized values and have a straightforward meaning, which is useful to understand the information obtained throughout the optimization process;

(iii) the measurements can easily be combined into a single global value (such as the product of elementary resolutions), and further combined with other quality criteria (i.e., to penalize longer retention times, or larger asymmetry factors);

(iv) problems related to peak crossing are avoided; and (v) operations, such as weighting or exclusion of peaks, are facilitated.

A realistic picture of the changes of peak resolution with mobile phase composition requires not only a good description of the position of the peak, but also the prediction of its shape. This is especially desirable to predict asymmetrical and low-efficiency peaks, or symmetrical peaks which are very close to each other. For the success of the free area fractions strategy, we developed a peak

shape model, which provides accurate measurements of peak areas even for highly asymmetrical peaks.[8]

In this work, the performance of the free area fractions criterion is compared with two other resolution criteria that evaluate the single peak resolution. The two latter criteria are based on the position of the peak maximum or the valleys formed between neighboring peaks. The usefulness of single peak strategies for the resolution of individual peaks in a complex mixture is also shown. The methodology is illustrated through the isocratic separation of mixtures of eight and twelve sulphonamides. These drugs are commonly used as anti-bacterial and anti-infective agents in medicine and veterinary practice. Sulphonamide residues present in minute concentrations in foods of animal origin may pose a health threat to consumers. For this reason, their separation and monitoring have drawn much attention.[9,10]

The sulphonamides were chromatographed with mobile phases of sodium dodecyl sulphate (SDS) and acetonitrile, which have shown to be a good choice to analyze complex mixtures of these drugs.[11] The proposed methodology is, however, valid for other chromatographic modes described by a retention model, which is the only step that should be adapted to consider other techniques.

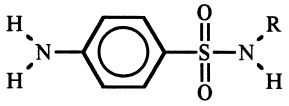
EXPERIMENTAL

Reagents

The probe compounds were the sulphonamides (Figure 1) sulphacetamide, sulphadiazine, sulphadimethoxine, sulphaguanidine, sulphamerazine, sulphamethoxazole, sulphamethizole, sulphamonomethoxine, sulphanilamide, sulphapyridine, sulphaquinoxaline, and sulphathiazole, purchased from Sigma (St. Louis, MO), and sulphamethazine from Aldrich (Milwaukee, WI). Stock standard solutions of 100 μg/mL were prepared for all compounds by dissolving the solid reagents in a few milliliters of ethanol, and making up to the mark with 0.10 M sodium dodecyl sulphate (99% purity, Merck, Darmstadt, Germany).

An ultrasonic bath (Selecta, Model 617, Barcelona, Spain) was used to facilitate dissolution. After dilution to 3 μg/mL with 0.10 M SDS, 20 μL of the drug solutions were injected into the chromatograph.

The mobile phases contained SDS at concentrations above its critical micellar concentration (8.13×10^{-3} M), and acetonitrile (Scharlau, Barcelona). The pH was buffered at 3 with 0.01 M citric acid (Sigma) and sodium hydroxide (Panreac, Barcelona). The sulphonamide solutions and mobile phases were filtered through Nylon membranes (0.45 μm, Micron Separations, Westboro, MA). Nanopure water (Barnstead, Sybron, Boston, MA) was used throughout.



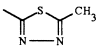
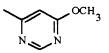
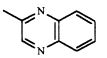
		R			R
Sulphacetamide	(A)	—$COCH_3$	Sulphamethoxazole	(G)	
Sulphadiazine	(B)		Sulphamethizole	(H)	
Sulphadimethoxine	(C)		Sulphamonomethoxine	(I)	
Sulphaguanidine	(D)		Sulphanilamide	(J)	H
			Sulphapyridine	(K)	
Sulphamerazine	(E)		Sulphaquinoxaline	(L)	
Sulphamethazine	(F)		Sulphathiazole	(M)	

Figure 1. Structures of the sulphonamides.

Apparatus

The liquid chromatograph (Agilent, Model HP 1050, Palo Alto, CA) was equipped with an isocratic pump, an autosampler (Model HP 1100), and a UV-visible detector set at 275 nm. The signal was acquired by a PC computer connected to the chromatograph through an integrator (Model HP 3396A), using the *PEAK-96* software (Agilent, Avondale, PA).

An ODS-Hypersil column (5 μm particle size, 100 mm x 4.6 mm i.d., Hewlett-Packard, Waldbronn, Germany) was placed after a C_{18} Nucleosil guard column (30 mm x 4.0 mm i.d., Scharlau). The flow-rate was 1.0 mL/min. The chromatographic separations were made at 22 ± 2°C. The dead time was taken as the mean value of the first deviation of the baseline in the chromatograms obtained by injection of the micellar solutions of the probe compounds (t_0 = 0.80°min).

MATHEMATICAL TREATMENT

Prediction of Retention Factors and Peak Shapes

The retention factors were predicted using a mechanistic model, which is based on the classical equation reported by Arunyanart and Cline-Love[13] for pure micellar mobile phases (without modifier).[12]

$$k = \frac{k_{AS}\dfrac{1}{1+K_{AD}\varphi}}{1+K_{AM}\dfrac{1+K_{MD}\varphi}{1+K_{AD}\varphi}[M]} \qquad (1)$$

where k is the retention factor, *[M]* the molar concentration of surfactant forming micelles, φ the volumetric fraction of organic modifier, K_{AS} the partition coefficient between water and stationary phase multiplied by the phase ratio, K_{AM} the solute-micelle binding constant, and K_{MD} and K_{AD} are constants that describe the modification of water-micelle equilibrium in the presence of an organic solvent. The parameters of the model were obtained using the k data from five mobile phases, four located in the corners of a rectangular experimental design and one at its center.

The description of peak shape, needed for the simulation of chromatograms and calculation of the resolution, was made using a linearly-modified Gaussian model.[8]

$$h(t) = H\,\mathrm{epx}\left(-\frac{1}{2}\frac{(t-t_R)^2}{\left[s_0 + s_1(t-t_R)^2\right]}\right) \qquad (2)$$

where H is peak height, t_R the retention time, s_0 a measurement of peak width at the maximum, and s_1 a distortion factor.

Measurement of Peak Resolution

The quality of the separation of a compound from other compounds in a mixture (i.e., elementary peak resolution) was measured using the following three criteria that yield normalized values.

Free Height Fraction

$$r_i = 1 - \frac{h_i}{h_i} \qquad (3)$$

where h_i is the peak height at the retention time of compound i, and h'_i the height of the chromatogram formed by the remaining compounds at that time (Figure 2a).

Valley Ratio

The resolution is measured using Eq. 3, but h'_i is now an interpolated height at the retention time of compound i, which is obtained drawing a straight-line between the two valleys formed by the considered peak and the preceding and following peaks (Figure 2b). For the first and last peaks in the chromatogram, h'_i is the height of the single valley.

Free Area Fraction

$$r_i = 1 - \frac{w_i}{w_i} \qquad (4)$$

where w_i is the total peak area of compound i, and w'_i the area under that peak, overlapped by the chromatogram formed by the remaining compounds (Figure 2c).

The elementary resolution values for each mobile phase were reduced to a single value (the product of elementary resolutions) in order to describe the global resolution (R) in the chromatogram.[14] This product varies between 0 (when at least one peak is fully overlapped) to 1 (when all peaks are base-line resolved).

Treatment of the data was made with *CHROM*, a set of MSDOS programs developed in our laboratory to assist chromatographic optimization. A previous reduced version for micellar liquid chromatography has been commercialized as *MICHROM*.[15]

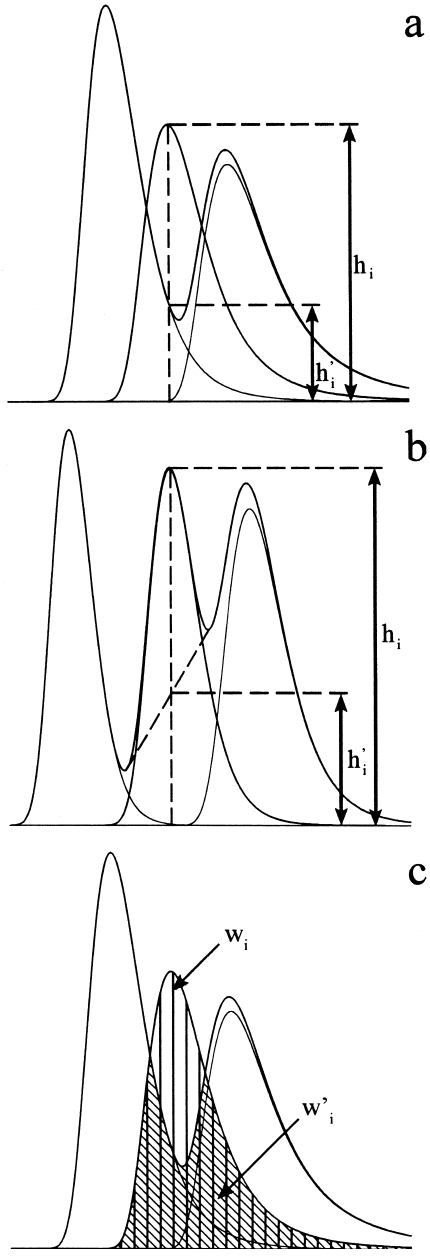


Figure 2. Meaning of the measured parameters for: (a) free height fraction, (b) valley ratio, and (c) free area fraction.

RESULTS AND DISCUSSION

Comparison of the Elementary Resolution Criteria

Three elementary resolution criteria that associate a measurement to each peak in a chromatogram were studied. These criteria require an accurate measurement of peak shape, especially in the case of the calculation of free area fractions. The reliability of each criterion to describe the relative separation of a set of compounds was examined by comparison of the global resolution values, at varying mobile phase composition, with the resolution observed directly on the chromatograms. For this study, a set of eight sulphonamides eluted with mobile phases of SDS and acetonitrile at pH 3 was selected. The mixture of these compounds was found to be adequate, since all chromatographic peaks were base-line resolved in an extensive region, and some peaks overlapped partially or completely in other regions depending on the composition of the mobile phase. At higher pH the resolution was poorer.

The compounds, ordered according to their retention times, were: sulphacetamide, sulphadiazine, sulphamerazine, sulphathiazole, sulphamethizole, sulphamonomethoxine, sulphapyridine, and sulphadimethoxine. The same elution order was followed in the whole experimental domain.

In order to know the chromatographic behavior of the sulphonamides, chromatograms of the individual compounds were obtained for five mobile phases: 0.025 M SDS, 0.125 M SDS, 0.025 M SDS-6% (v/v) acetonitrile, 0.125 M SDS-6% (v/v) acetonitrile, and 0.075 M SDS-3% (v/v) acetonitrile. The measured retention data, efficiencies (plate counts), and asymmetry factors, were used to predict chromatograms at other mobile phase compositions inside the ranges 0.025-0.125 M SDS, and 0-6% (v/v) acetonitrile. The calculations of peak position and shape were made with the software *CHROM* using Eqs. 1 and 2. The global errors in the prediction of retention factors (considering the five mobile phases of the experimental design) were: sulphacetamide (0.5%), sulphadiazine (0.6%), sulphamerazine (0.3%), sulphathiazole (1.4%), sulphamethizole (1.3%), sulphamonomethoxine (0.7%), sulphapyridine (2.4%), and sulphadimethoxine (1.5%).

The simulated chromatograms corresponded to 441 mobile phases, arranged in a regular distribution containing 21 levels in surfactant and 21 levels in organic solvent. In this study, normalized peak areas were considered. The effect of peak area in the resolution is examined in the next section.

The retention factors of the least and most retained sulphonamides were k = 1.0 and 5.7 for the strongest mobile phase (0.125 M SDS-6% acetonitrile), and k = 3.2 and 61.2 for the weakest (0.025 M SDS), respectively. It was observed that the separation of the eight drugs was good in the ranges 0.025-0.06 M SDS and 3-6% acetonitrile. However, at lower volume fraction of acetonitrile, the peaks of

sulphapyridine and sulphadimethoxine became closer and overlapped, whereas at higher concentration of SDS, the peaks of sulphathiazole and sulphamethizole overlapped, and in a lower extent those of sulphadiazine-sulphamerazine, and sulphamonomethoxine-sulphapyridine-sulphadimethoxine.

The product of elementary resolution values at varying mobile phase composition was calculated according to each criteria. The corresponding regular matrices were used to draw the contour maps depicted in Figure 3. The three diagrams show a region of maximal resolution in the upper left corner of the experimental domain, and a slow decrease in the resolution at increasing concentration of SDS and decreasing volume fraction of acetonitrile. Although the general shape of the diagrams is similar, the extent of the region of maximal resolution is different from one another. The plateau is rather large for the free height fractions (Figure 3a), and small for the valley ratio criterion (Figure 3b).

In a chromatographic optimization, an optimum initially selected is often discarded and other regions of poorer resolution but lower analysis time, or greater robustness, selected. A good description of the resolution in the whole experimental domain, or at least in a wide region around the maximal resolution, is, thus, desirable.

Figure 4 illustrates chromatograms at three mobile phase compositions. The first chromatogram (0.050 M SDS-3.3% acetonitrile) shows a quite good but not complete separation for all sulphonamides, the peaks of sulphathiazole (M)-sulphamethizole (H) and sulphapyridine (K)-sulphadimethoxine (C) exhibit a small overlapping. Most peaks are partially overlapped in the second chromatogram (0.080 M SDS-4% acetonitrile). In the third chromatogram (0.035 M SDS-1.5% acetonitrile), although the resolution is good for most peaks, the peaks of sulphapyridine and sulphadimethoxine overlap largely. Ideally, the different situations should be outlined by the values of global resolution, which are given in Table 1.

The free height areas criterion yields high resolution values for the three chromatograms, which evidently do not agree with the observed relative separation. The valley ratio seems to be a better criterion but it is perhaps too strict. It penalizes excessively small overlappings, which makes the resolution values decay rapidly when the separation between peaks decreases.

The three elementary criteria also yielded different compositions for the mobile phase of maximal resolution (Figure 5). It was, however, similar for the valley ratios (0.025 M SDS-5.4% acetonitrile, Figure 5b) and free area fractions (0.030 M SDS-4.8% acetonitrile, Figure 5c), although for the former criterion, the analysis time was somewhat larger. For these mobile phases, the global resolution was $R < 1$ (Table 1), but base-line separation was almost reached. Again, the result obtained with the free height criterion was very unsatisfactory. The resolution for the optimal mobile phase composition (0.050 M SDS-5.7% acetonitrile, Figure 5a) was not complete and the calculated resolution was too high:

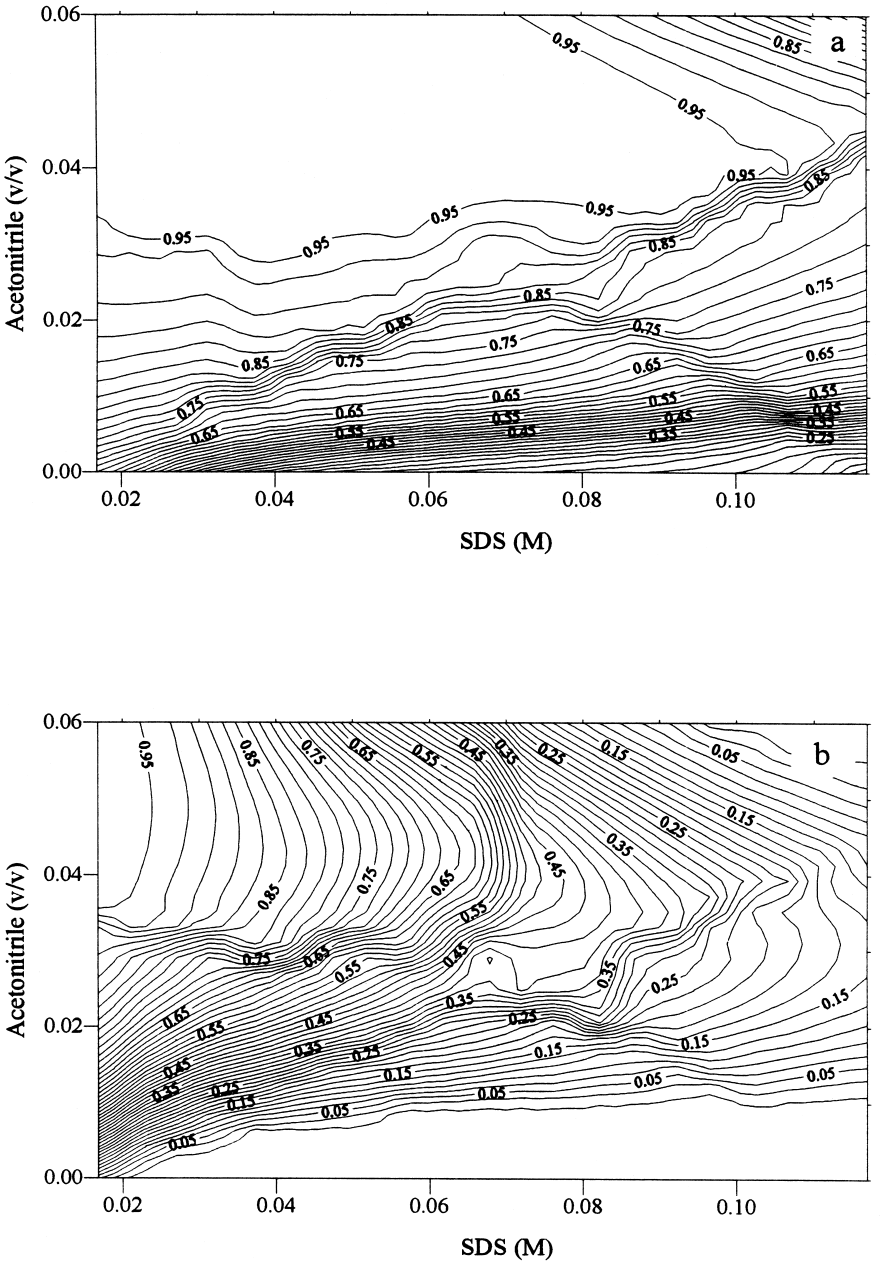


Figure 3. Contour maps of resolution according to the single peak criteria: (a) free height fraction, (b) valley ratio, and (c) free area fraction. Set of eight sulphonamides.

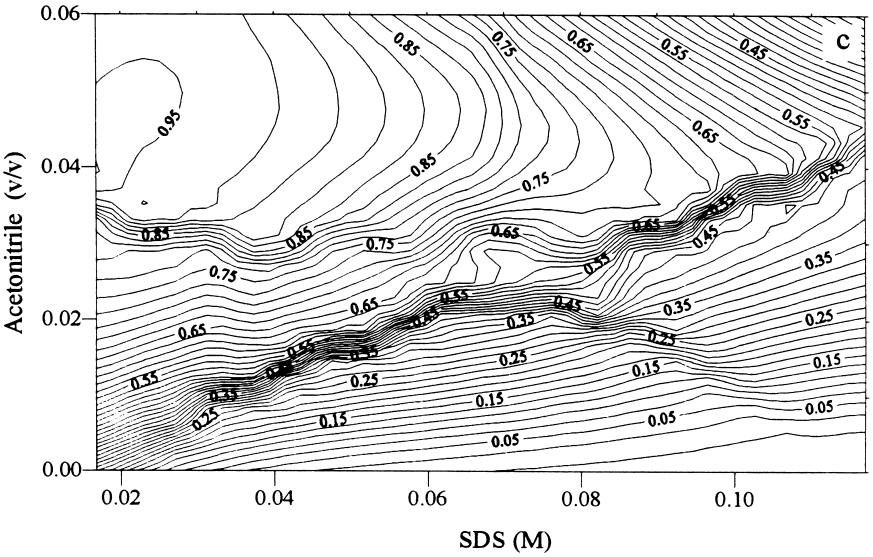


Figure 3. Continued.

0.990. The reason for this result is that this criterion does not sufficiently take into account the shape of the chromatographic peaks, which for the given example are broad and asymmetrical.

Usefulness of Single-Peak Resolution Criteria

The following studies were performed using the free areas criterion, which seems to adequately evaluate the resolution independent of the overlapping degree of chromatographic peaks. The set of compounds was extended to 12, the previous eight sulphonamides with the exception of sulphathiazole, and the following compounds (global prediction errors for the retention factors using Eq. 1 are given in parenthesis): sulphanilamide (0.2%), sulphamethazine (1.1%), sulphamethoxazole (0.9%), sulphaguanidine (1.3%), and sulphaquinoxaline (3.5%).

Figure 6a shows the optimal predicted chromatogram (0.025 M SDS-5.7% acetonitrile, R = 0.402) for the set of 12 sulphonamides. The corresponding experimental chromatogram is illustrated in Figure 6b. A good agreement between both chromatograms is observed, the separation is however not com-

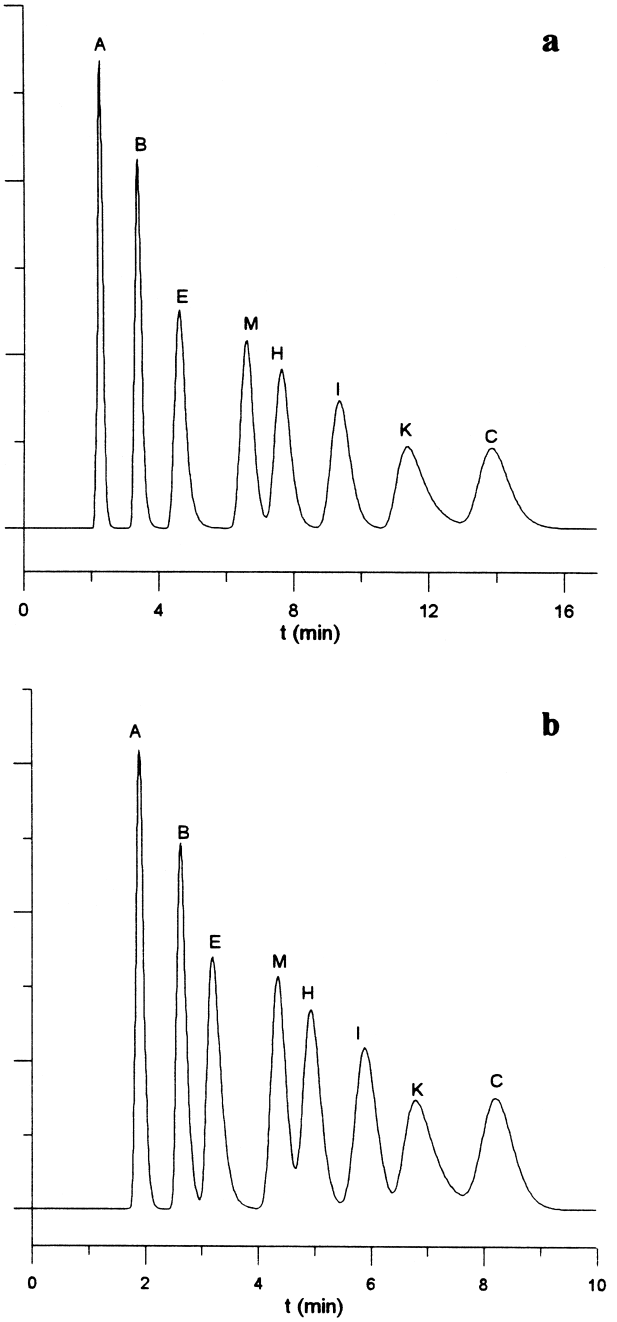


Figure 4. Predicted chromatograms for: (a) 0.050 M SDS-3.3% acetonitrile, (b) 0.080 M SDS-4.0% acetonitrile, and (c) 0.035 M SDS-1.5% acetonitrile. See Figure 1 for compound identification.

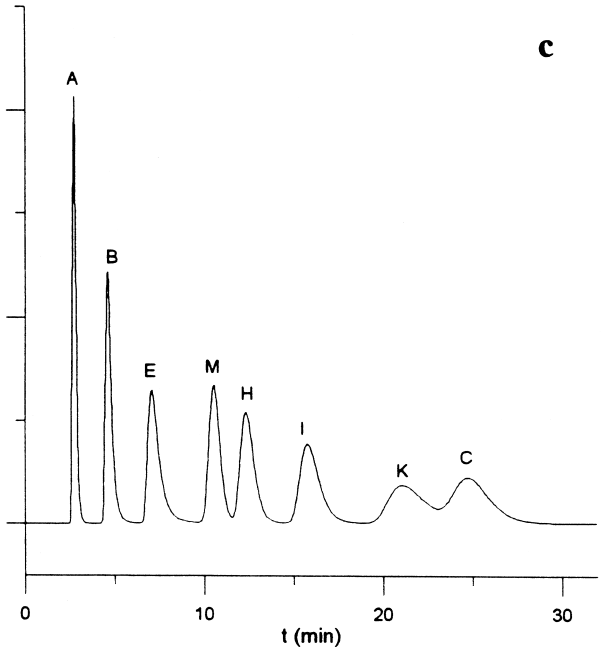


Figure 4. Continued.

Table 1. Global Resolution Values Obtained in the Separation of Eight Sulphonamides Eluted with Micellar Mobile Phases, According to Several Single Peak Criteria

Criteria	Free Height	Valley Ratio	Free Area
Optimal composition			
SDS (M)	0.050	0.025	0.030
Acetonitrile (%)	5.7	5.4	4.8
Global resolution	0.990	0.970	0.952
SDS-Acetonitrile		Global Resolution	
0.050 M-3.3%	0.976	0.803	0.886
0.080 M-4.0%	0.963	0.497	0.768
0.035 M-1.5%	0.860	0.474	0.597

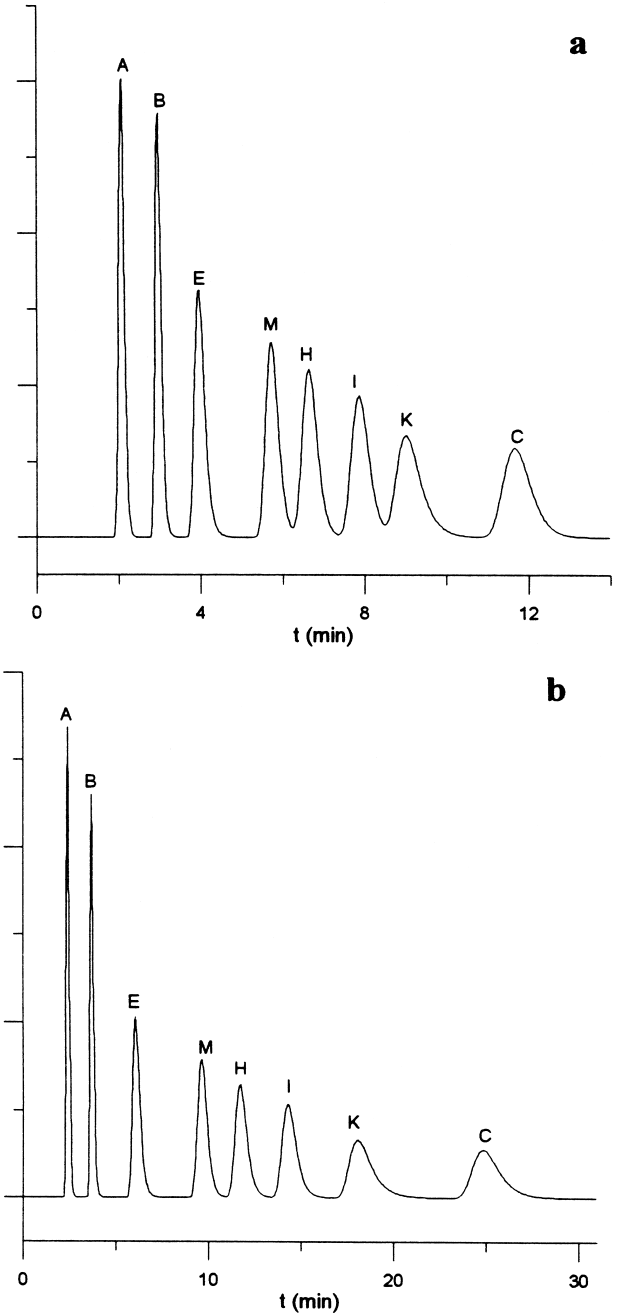


Figure 5. Predicted chromatograms for the optimal mobile phase composition according to: (a) free height fraction (0.050 M SDS-5.7% acetonitrile), (b) valley ratio (0.025 M SDS-5.4% acetonitrile), and (c) free area fraction (0.030 M SDS-4.8% acetonitrile). See Figure 1 for compound identification.

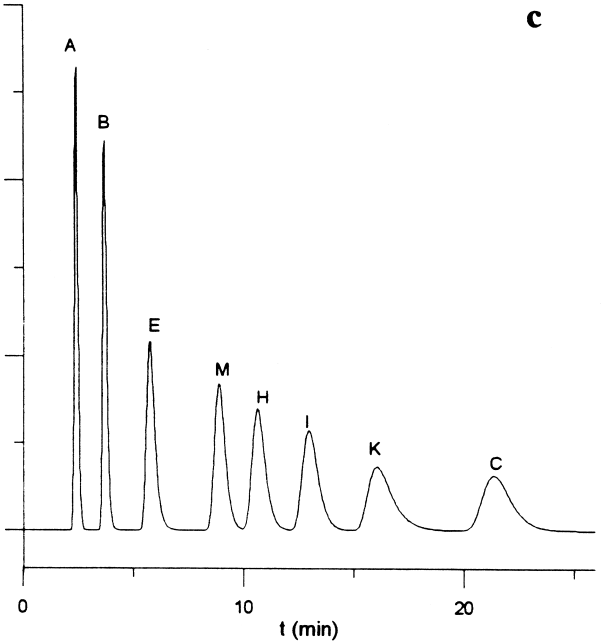


Figure 5. Continued.

plete. The overlapping of sulphacetamide (A)-sulphanilamide (J), on the one side, and sulphamethizole (H)-sulphamethoxazole (G)-sulphamonomethoxine (I)-sulphaguanidine (D), on the other, is important. Also, sulphamethazine (F)-sulphamethizole (H), sulphaguanidine (D)-sulphapyridine (K), and sulphadimethoxine (C)-sulphaquinoxaline (L), overlap slightly. Elementary peak purities, measured according to Eq. 4, are summarized in Table 2 for the whole set of compounds chromatographed with the optimal mobile phase. Resolution values $r < 0.98$ indicate a non-negligible overlapping.

Figure 7 depicts the surfaces of elementary resolution of some sulphonamides (in the mixture of 12 compounds), drawn for the whole experimental domain. Each response surface describes the separation of a given compound from the remaining in the mixture, at varying mobile phase composition. Sulphadiazine (Figure 7a), sulphamethazine (Figure 7b), and sulphamerazine exhibit base-line resolution in a large region of the examined concentration ranges of surfactant and organic solvent. The elementary resolution of sulphacetamide (Figure 7c), sulphamonomethoxine (Figure 7d), sulphamethoxazole, sulphamethizole, and sulphanilamide is maximal (but far from being complete) at the lowest SDS concentration and highest acetonitrile volume fraction.

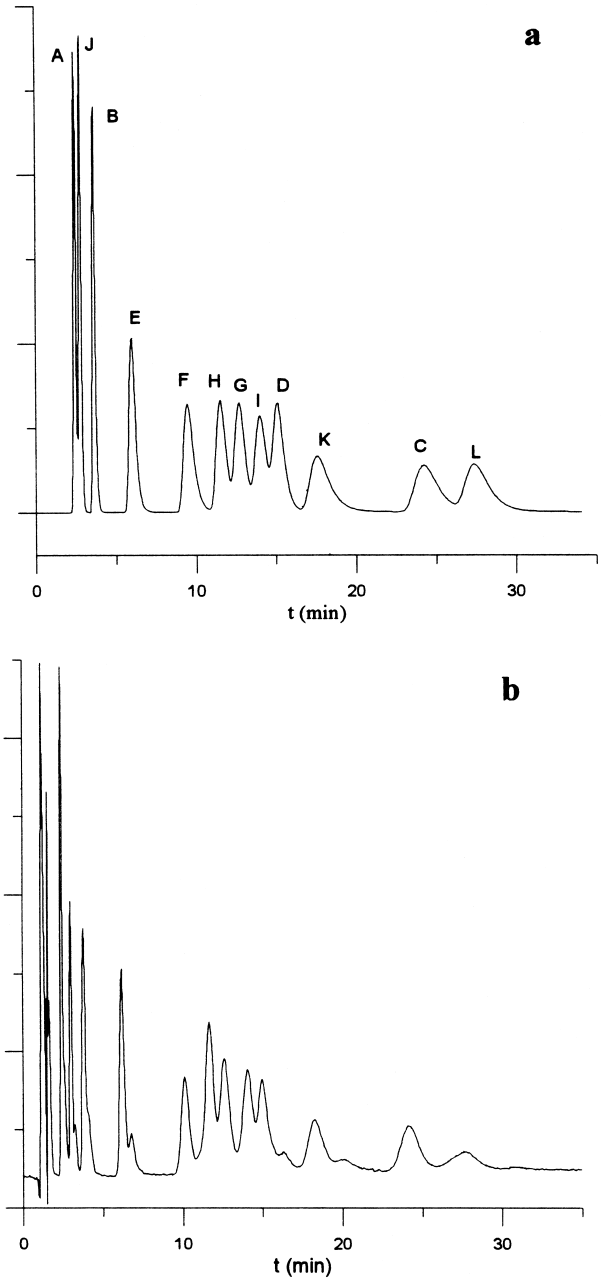


Figure 6. Predicted (a) and experimental (b) chromatograms for 12 sulphonamides eluted with the optimal mobile phase (0.025 M SDS-5.7% acetonitrile) according to the free area fraction criterion. See Figure 1 for compound identification.

Table 2. Optimal and Limiting Resolution for the Individual Compounds in the Mixture of 12 Sulphonamides

Compound	Optimal Resolution[a]	Limiting Resolution	SDS-Acetonitrile[b]
Sulphacetamide	0.974	0.976	0.025 M-6.0%
Sulphadiazine	1.000	1.000	0.040 M-5.1%
Sulphadimethoxine	0.924	0.937	0.025 M-6.0%
Sulphaguanidine	0.851	0.943	0.025 M-3.3%
Sulphamerazine	0.996	0.998	0.050 M-6.0%
Sulphamethazine	0.985	0.992	0.045 M-6.0%
Sulphamethoxazole	0.862	0.863	0.025 M-6.0%
Sulphamethizole	0.916	0.974	0.060 M-4.2%
Sulphamonomethoxine	0.796	0.864	0.025 M-3.6%
Sulphanilamide	0.973	0.977	0.025 M-6.0%
Sulphapyridine	0.963	0.966	0.025 M-6.0%
Sulphaquinoxaline	0.908	0.920	0.025 M-6.0%

[a]Mobile phase: 0.025 M SDS-5.7% acetonitrile.
[b]Mobile phase composition to reach the limiting resolution.

At other concentrations, the resolution decays rapidly. Finally, the behavior of sulphaguanidine (Figure 7e), sulphapyridine (Figure 7f), sulphadimethoxine, and sulphaquinoxaline is very irregular, and the resolution often low. The valley observed in Figure 7f is produced by the reversal of the peaks of sulphapyridine and sulphaguanidine, whereas the valleys in Figure 7e correspond to the reversals of sulphapyridine-sulphaguanidine, and sulphaquinoxaline-sulphadimethoxine.

Although complete resolution is not feasible for the whole mixture of 12 sulphonamides, the resolution criterion may find better separation conditions for the individual compounds. The maximal free area fractions that can be achieved for each compound in the mixture (limiting resolutions)[16] are given in Table 2. These values indicate that base-line resolution can be only reached for sulphadiazine, sulphamerazine, and sulphamethazine. Enough good resolution is also possible for sulphacetamide, sulphamethizole, sulphanilamide, and sulphapyridine. The resolution of sulphamethoxazole and sulphamonomethoxine is always poor with the SDS-acetonitrile system.

It is more interesting to point out the improvement of resolution obtained for sulphaguanidine (from $r = 0.851$ to 0.943), sulphamethizole (from $r = 0.916$ to 0.974), and sulphamonomethoxine (from $r = 0.796$ to 0.864), when the individual resolution of each compound is maximized instead of the global resolution for the 12 sulphonamides (Table 2). The former and latter compounds require a lower amount of acetonitrile with respect to the global optimal mobile phase (3.3% and 3.6% against 5.7%), to reach their maximal resolution. Both concen-

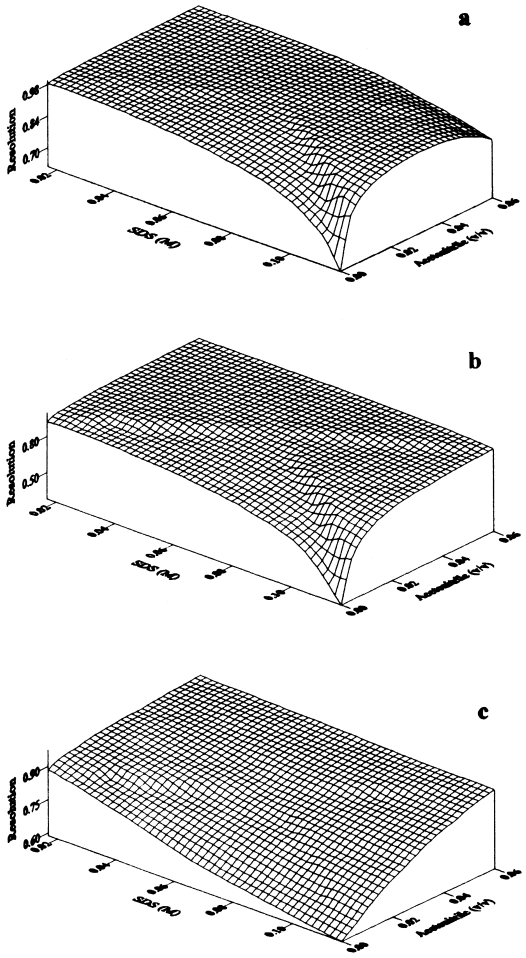


Figure 7. Surfaces of individual resolution (free area fractions) for several components in the mixture of 12 sulphonamides: (a) sulphadiazine, (b) sulphamethazine, (c) sulphacetamide, (d) sulphamonomethoxine, (e) sulphaguanidine, and (f) sulphapyridine.

trations of surfactant and organic modifier should be changed for sulphamethizole (0.060 M SDS-4.2% acetonitrile instead of 0.025 M-5.7% acetonitrile) to get almost complete resolution. It should be noted, that the resolution values for sulphaquinoxaline are rather low ($r = 0.920$), considering the overlapping degree observed in the chromatogram of Figure 6a. This low value can be due to a loss of accuracy in peak simulation using Eq. 2, because of the large asymmetry of this peak.

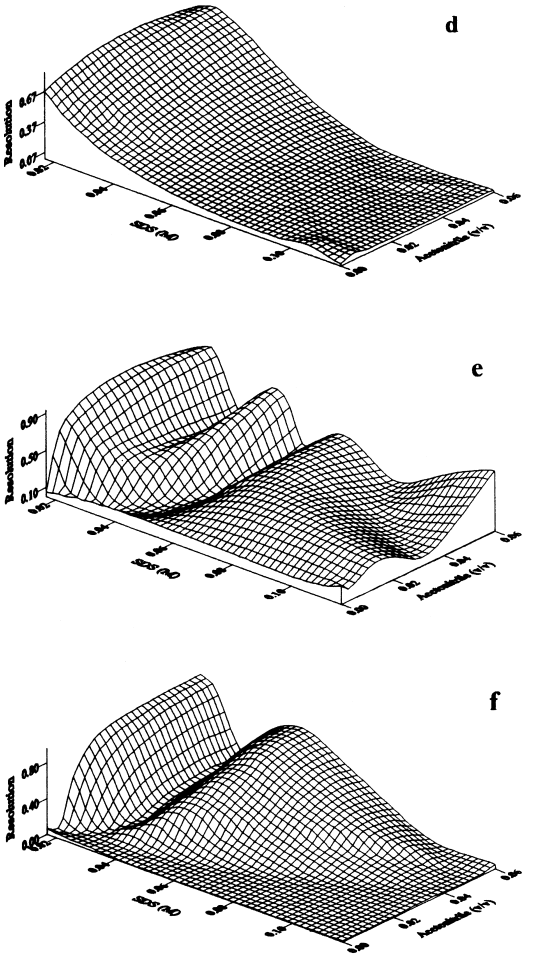


Figure 7. Continued.

Figure 8 shows the chromatograms obtained for the mobile phases that gave maximal resolution for sulphaguanidine (D) and sulphamethizole (H): 0.025 M SDS-3.3% acetonitrile and 0.060 M SDS-4.2% acetonitrile, respectively. The chromatograms should be compared with that obtained for 0.025 M SDS-5.7% acetonitrile in Figure 6a, where the drugs were appreciably overlapped by a preceding and a following peak, respectively. The separation from neighboring peaks was almost complete in the new conditions (Figure 8). Figures 8a and 8c depict the predicted chromatograms, whereas the chromatograms in Figures 8b

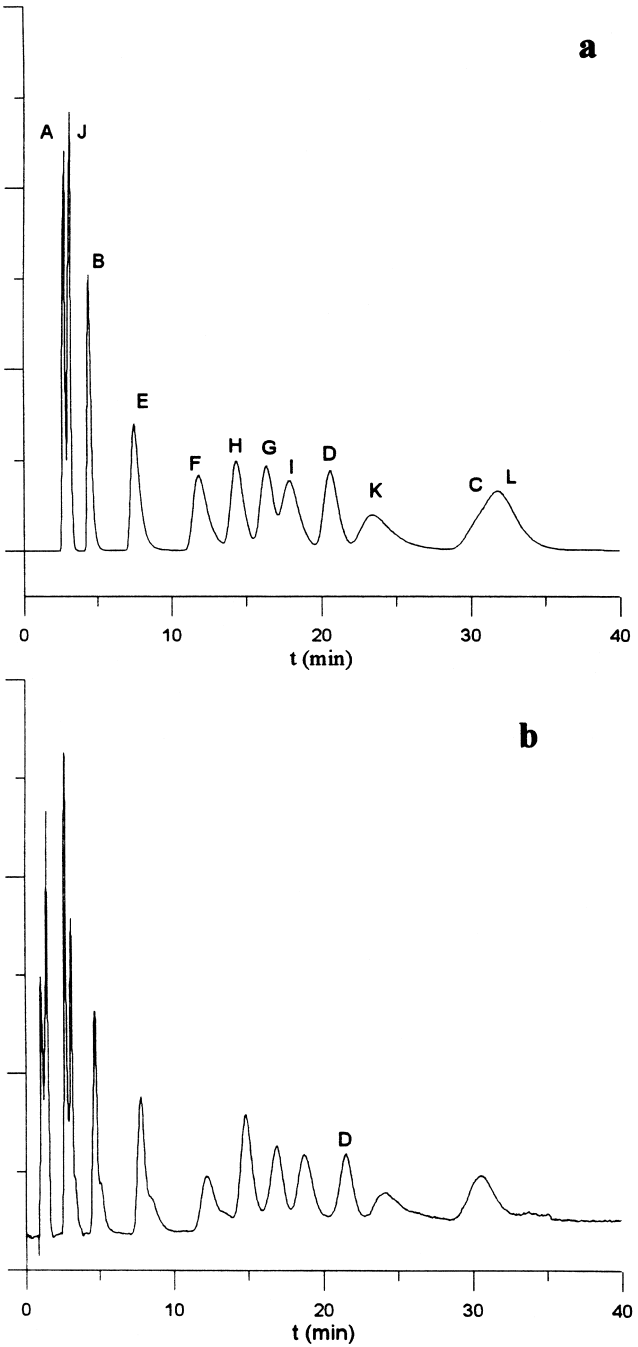


Figure 8. Predicted (a,c) and experimental (b,d) chromatograms for the optimal separation of individual compounds in the mixture of 12 sulphonamides: (a,b) sulphaguanidine (D, 0.025 M SDS-3.3% acetonitrile), and (c,d) sulphamethizole (H, 0.060 M SDS-4.2% acetonitrile).

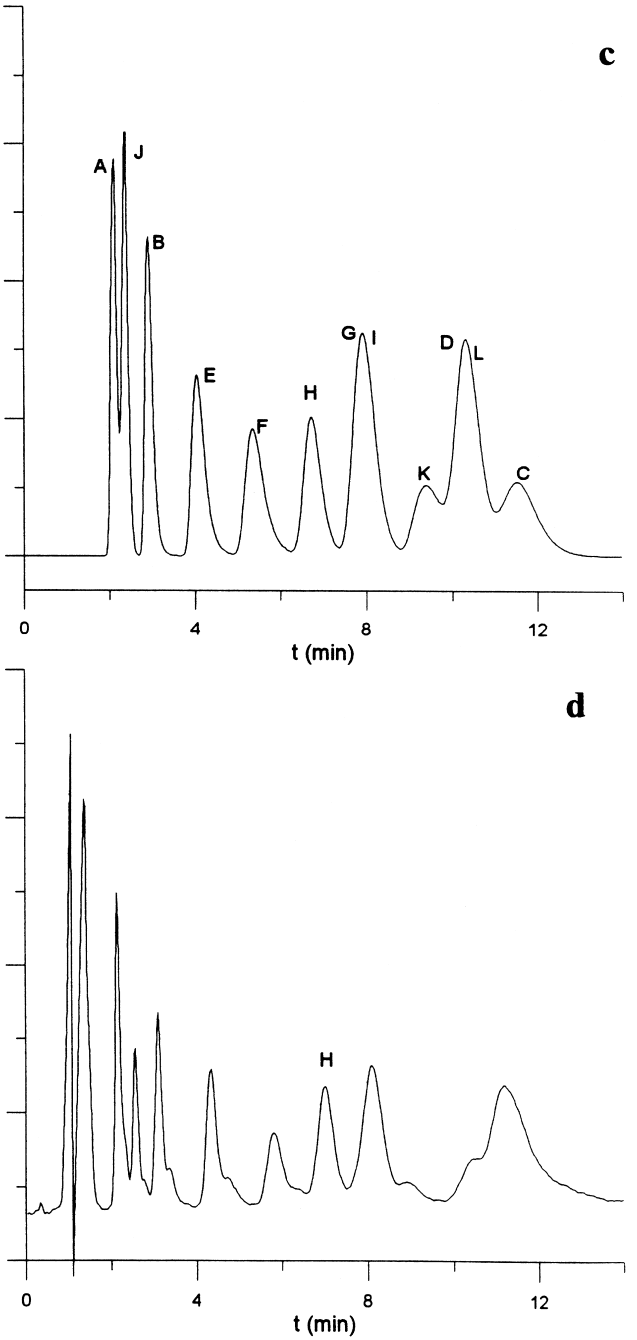


Figure 8. Continued.

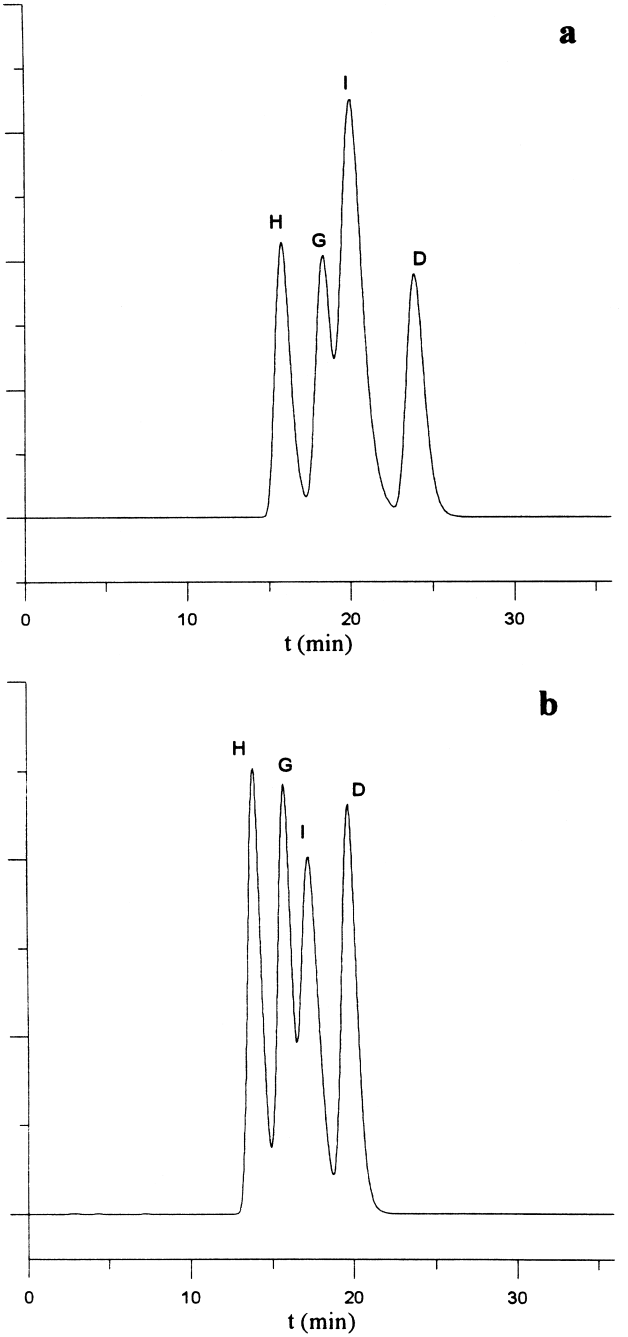


Figure 9. Dependence of the resolution of sulphamonomethoxine (I) with peak area. Relative area of the drug with respect to the normalized area of sulphamethizole (H), sulphamethoxale (G), and sulphaguanidine (D): (a) 2 ($r_I = 0.0936$), (b) 1 ($r_I = 0.0929$), (c) 0.5 ($r_I = 0.0900$), and (d) 0.1 ($r_I = 0.0788$). Mobile phase: 0.025 M SDS-3.6% acetonitrile.

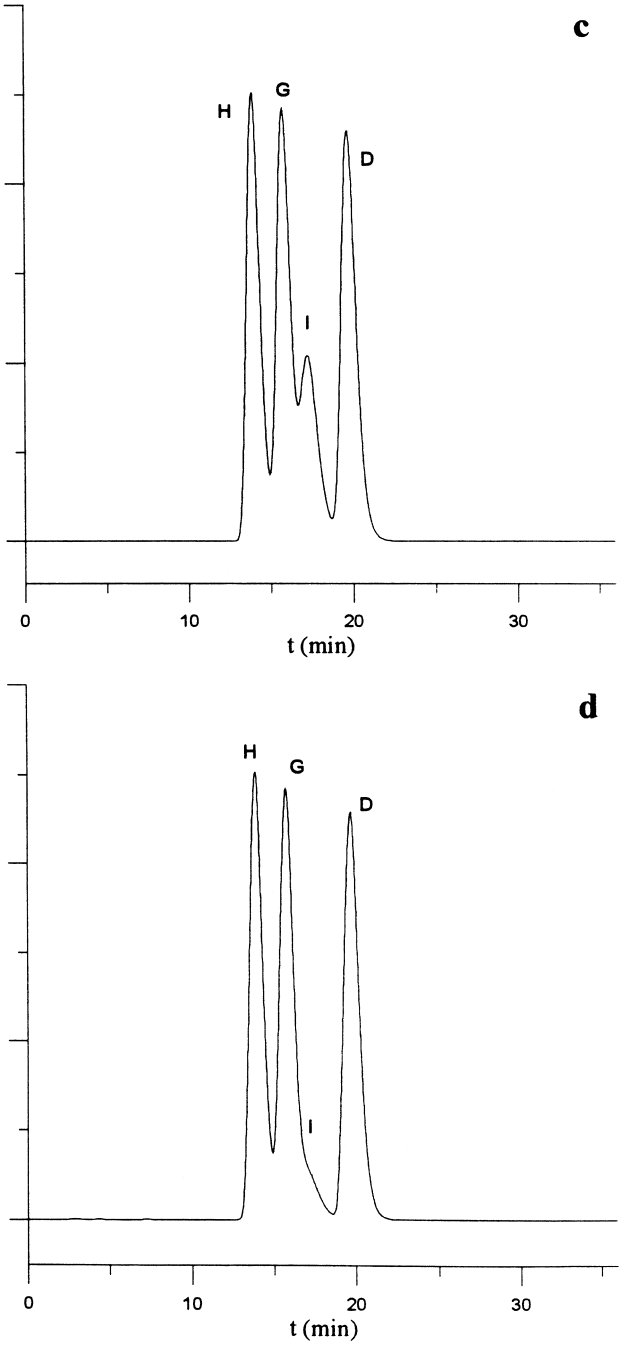


Figure 9. Continued.

and 8d were obtained by injecting into the chromatograph a solution containing the 12 sulphonamides. The agreement between predicted and experimental chromatograms is excellent, which indicates again the reliability of the proposed strategy.

Finally, one of the advantages of the single peak criteria is the possibility of considering peak areas. In fact, peak purities depend on the size of the overlapping peaks, and this should not be ignored. The inclusion of peak areas allows, in a realistic way, the optimization of the separation of a mixture where the relative concentrations are known. Using normalized areas, the actual separation can be somewhat different from that predicted, especially in the case of complex separations. An example is given in Figure 9, where the separation of sulphamonomethoxine (I) from sulphamethizole (H), sulphamethoxale (G), and sulphaguanidine (D) is examined. The optimal mobile phase for the former compound was used: 0.025 M SDS-3.6% acetonitrile. The areas of the peaks of the three accompanying compounds were normalized, whereas the area of sulphamonomethoxine was varied.

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