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Behaviour of resolution by changing solvent strength and selectivity in the 'PRISMA' model using reversed-phase HPLC for biogenic amines

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Abstract

The retention behaviour of 17 dansylated biogenic amines in 6 linear gradients at 13 solvent combinations, expressed by the selectivity points (P_s) according to the 'PRISMA' model, was investigated. The dependence between the retention times (t_r) and different gradients was examined. Three dimensional resolution (R_s) maps for each peak-pair in the different gradients at the 13 selectivity points were constructed, and the affect of the gradients on separation were investigated. The study showed that the dependence between the gradients and t_r values of dansyl amides can be expressed using quadratic functions with a high degree of accuracy. These functions are well suited for estimating the resolution in different gradients and P_s. The three dimensional R_s maps clearly demonstrated the changes between the different gradients and P_s. This was of considerable benefit when searching the optimum mobile phase by changing both the solvent strength (S_T) and selectivity. © 1997 Elsevier Science B.V.

Keywords: Resolution behaviour; Gradient elution; 'PRISMA' model; Dansylated biogenic amines

1. Introduction

When analysing new compounds by HPLC accurate optimization of the chromatographic system is crucial. Many different optimization procedures are used to optimize HPLC separations, such as overlapping resolution mapping [1–3], window diagrams [4], sequential simplex procedures [5,6] and iterative mixture design [7]. The increasing application of computers in the optimization of chromatographic systems has influenced the development of commercial optimization software [8–12].

One of the optimization methods also used in HPLC is the 'PRISMA' model developed by Nyiredy et al. (1985) [13]. The three dimensional 'PRISMA' model is an easy and efficient method for systematically optimizing the mobile phase in various liquid chromatography techniques. In the 'PRISMA' model the elution can be carried out in isocratic or gradient mode. Gradient elution can be performed in order to change solvent strength

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(S_T), selectivity or both. These three gradient possibilities are called isoselective multisolvent gradient elution (IMGE), selective multisolvent gradient elution (SMGE) and isocratic multisolvent gradient elution (ICMGE) [14,15].

Gradient elution is widely applied in liquid chromatography because it is a powerful method for separating complex mixtures with widely varying retention characteristics. It can also be used as a rapid development method for isocratic separation by HPLC [16,17]. Biogenic amines have a broad range of polarity, and gradient elution is preferred instead of isocratic. Also the polyamines spermidine and spermine require gradient elution because they are strongly retained on the column. The naturally occurring amines, especially polyamines, are normal constituents of human, animal and plant cells. They play an important role in cell division, growth, and differentiation [18]. The technique widely used for analysing biogenic amines is HPLC [19,20].

In this work, the chromatographic behaviour of 17 difficult separable dansyl amides was studied using 6 different linear gradients at 13 solvent combinations mixed according to the 'PRISMA' model. The influence of solvent strength (S_T) and different solvent combinations described by the selectivity points (P_s) on resolution was investigated. The aim of the study was to express mathematically the dependence between the retention behaviour of dansyl amides and various gradient times, and to determine the availability of the obtained relations when estimating the resolution. The aim was also to develop an easy and simple method for determining the mixture of eluents giving the best separation under the conditions used for dansyl amides.

2. Theory

2.1. The 'PRISMA' system

The 'PRISMA' system is a mixture design model and it consists of three parts. The first part includes the selection of basic parameters, e.g. the chromatographic system, stationary phase and the suitable individual solvents. The solvents are selected on the basis of the Snyder classification of solvents [21], according to their properties as proton acceptors and proton donors and their dipole interactions. In the second part the optimal conditions of the selected solvents are determined by the 'PRISMA' model. The third part concerns the transfer of the optimized mobile phase between the various column and planar chromatographic

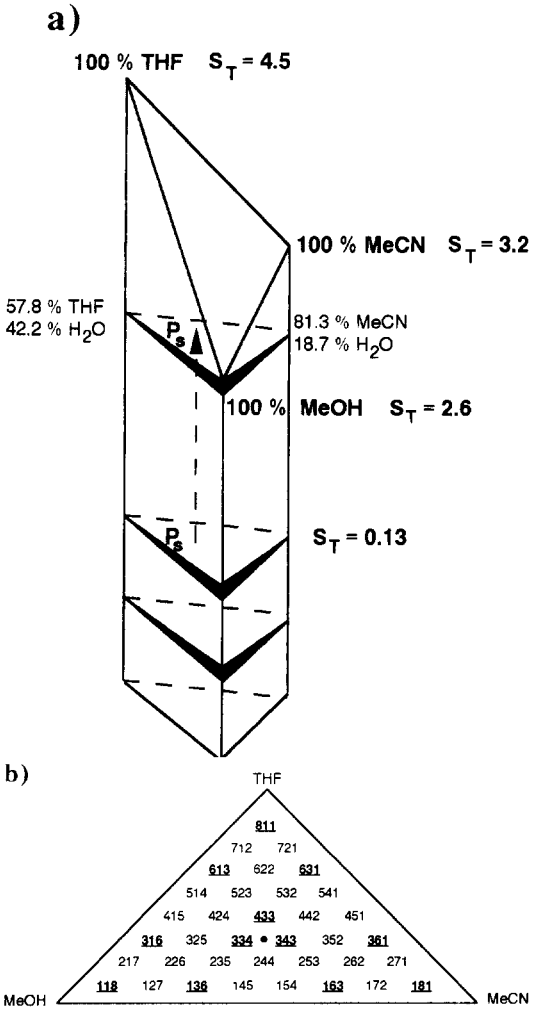


Fig. 1. (a) The three dimensional 'PRISMA' model. The solvent strength (S_T) was increased during the chromatographic run from 0.13 to 2.6 while keeping the selectivity (P_s) constant. (b) The selectivity points describing the horizontal plane in the regular part of the 'PRISMA' model. The 13 studied selectivity points are underlined and the middle point (P_s = 333) is denoted by (●).

techniques and the adjustment of necessary operating parameters, e.g. flow rate, development mode (in the case of planar chromatography) and particle size of the stationary phase.

2.2. The 'PRISMA' model

The actual optimization of separation is made by the second part of the system; the 'PRISMA' model. The model can be visualized as a three-dimensional geometrical design, which correlates the solvent strength (S_T) with the selectivity of the mobile phase. It consists three parts: an irregular top part, a regular middle part and the lower part symbolizing the modifier(s) (Fig. 1a). The lengths of the edges of the prism correspond to the solvent strengths of the used pure solvents. If these pure solvents are diluted to the same solvent strength as the solvent with the lowest S_T with hexane in normal-phase chromatography (NP-HPLC) or water in reversed-phase chromatography (RP-HPLC) the regular part is obtained. Solvent strength and/or incidental tailing can be influenced by small amounts of modifiers, symbolizing by the lower part of the prism. These are usually present in low and constant concentrations and the solvent strength values of the modifiers are neglected in the optimization process [22]. The mixtures of mobile phases, i.e. the volume fractions (f) of the diluted organic solvents, can be represented by the selectivity points (P_s), and they can be depicted as three-digit numbers (Fig. 1b). These numbers—where the sum of the three digits is 10—are obtained by multiplying the volume fractions by 10 and arranging them in order of diminishing solvent strength. The points symbolize quaternary, ternary or binary solvent mixtures. The construction of the model and the role of the solvent strength and the selectivity points are described extensively in Nyiredy et al. (1989) [23].

3. Materials and methods

The biogenic amines (see Table 1 for structures) and dansyl chloride were purchased from Fluka

Table 1
Structure of the biogenic amines

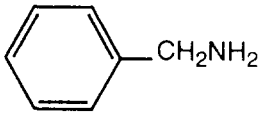
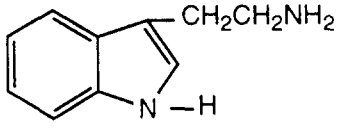
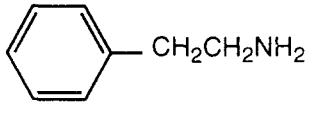
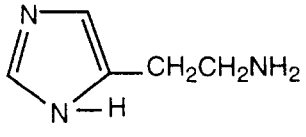
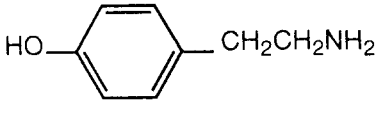
1 Ethanolamine	$HOCH_2CH_2NH_2$
2 Ethylamine	$CH_3CH_2NH_2$
3 Propylamine	$CH_3CH_2CH_2NH_2$
4 Diethylamine	$(CH_3CH_2)_2NH$
5 Butylamine	$CH_3CH_2CH_2CH_2NH_2$
6 Benzylamine	
7 Tryptamine	
8 Phenethylamine	
9 Pentylamine	$CH_3CH_2CH_2CH_2CH_2NH_2$
10 Histamine	
11 Putrescine	$NH_2CH_2CH_2CH_2CH_2NH_2$
12 Cadaverine	$NH_2CH_2CH_2CH_2CH_2CH_2NH_2$
13 Hexylamine	$CH_3CH_2CH_2CH_2CH_2CH_2NH_2$
14 1,6-Diaminohexane	$NH_2CH_2CH_2CH_2CH_2CH_2CH_2NH_2$
15 Tyramine	
16 Spermidine	$NH_2(CH_2)_4NH(CH_2)_3NH_2$
17 Spermine	$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$

Table 2
The retention data of 17 dansyl amides at $P_s = 631$ in six gradients

	15 min	20 min	25 min	30 min	35 min	40 min
Ethanolamine	8.42	9.53	10.44	11.31	12.12	12.94
Ethylamine	11.05	12.88	14.59	16.2	17.7	19.2
Propylamine	12.29	14.57	16.68	18.68	20.86	22.48
Diethylamine	12.76	15.24	17.73	19.97	22.09	24.2
Butylamine	13.42	16.08	18.53	20.91	23.2	25.5
Benzylamine	13.59	16.3	18.88	21.38	23.76	26.17
Tryptamine	13.64	16.47	19.11	21.69	24.19	26.72
Phenethylamine	14.06	16.94	19.7	22.37	24.92	27.5
Pentylamine	14.44	17.43	20.23	22.94	25.58	28.21
Histamine	14.52	17.68	20.65	23.56	26.39	29.23
Putrescine	14.32	17.38	20.32	23.19	25.95	28.74
Cadaverine	14.67	17.85	20.91	23.9	26.78	29.69
Hexylamine	15.35	18.63	21.78	24.82	27.77	30.72
1,6-diaminohexane	15.09	18.43	21.59	24.69	27.73	30.77
Tyramine	16.21	19.91	23.48	26.97	30.38	33.78
Spermidine	16.52	20.4	24.11	27.76	31.35	34.95
Spermine	18.05	22.08	26.52	30.77	34.91	39.06

Table 3
The retention data of 17 dansyl amides in 30 min gradient at thirteen selectivity points

	30 Min												
	811	631	361	181	163	136	118	316	613	333	334	343	433
Ethanolamine	13.69	11.31	11.7	11.93	12.94	13.74	14.05	13.06	12.04	12.59	12.65	12.43	12.27
Ethylamine	19.49	16.2	16.82	17.33	17.81	17.8	17.74	17.16	16.6	17.26	17.29	17.18	17.08
Propylamine	22.63	18.68	19.24	19.72	20.21	20.04	19.82	19.76	19.12	19.74	19.69	19.63	19.55
Diethylamine	23.75	19.97	21.27	22.01	22.54	22.01	21.55	21.04	20.44	21.48	21.46	21.5	21.28
Butylamine	25.56	20.91	21.57	22.04	22.52	22.18	21.81	21.66	21.48	22.05	21.99	21.97	21.91
Benzylamine	26.41	21.38	21.75	22.36	22.63	22.2	21.93	22.03	22.09	22.31	22.23	22.19	22.26
Tryptamine	26.88	21.69	22.04	22.25	22.88	22.64	22.34	22.63	22.58	22.8	22.73	22.64	22.7
Phenethylamine	27.53	22.37	23.03	23.51	24.08	23.6	23.26	23.31	23.15	23.61	23.54	23.51	23.5
Pentylamine	28.09	22.94	23.71	24.22	24.64	24.09	23.6	23.66	23.58	24.19	24.09	24.12	24.06
Histamine	28.84	23.56	24.94	26.04	26.73	26.09	25.18	25.31	24.51	25.64	25.66	25.62	25.37
Putrescine	28.52	23.19	24.21	24.94	25.47	24.69	23.97	23.96	23.78	24.89	24.48	24.57	24.4
Cadaverine	29.41	23.9	24.98	25.77	26.21	25.28	24.51	24.59	24.5	25.29	25.18	25.29	25.12
Hexylamine	30.37	24.82	25.76	26.33	26.64	25.82	25.21	25.48	25.53	26.15	26.01	26.09	26.0S
1,6-diaminohexane	30.37	24.69	25.86	26.73	27.05	26.09	25.5	25.31	25.27	26.13	25.99	26.15	25.97
Tyramine	33.1	26.97	28.45	29.57	29.53	28.18	27.32	27.79	27.77	28.65	28.52	28.68	28.52
Spermidine	34.61	27.76	29.32	30.66	30.43	29.38	27.76	28.17	28.31	29.29	28.62	28.89	29.17
Spermine	39 99	30 77	32 87	34 92	33 62	31 25	30 06	30.82	31.22	32.3	32.01	32.51	32.27

AG (Switzerland). Ammonia, sodium hydroxide and sodium bicarbonate were purchased from Merck (Germany). The water was distilled and deionised. Methanol (Baker, Holland), acetonitrile and tetrahydrofuran (Merck, Germany) were of HPLC quality.

The HPLC system consisted of a Beckman System Gold programmable solvent module 126, autosampler 502, programmable detector module 166 (Fullerton, USA) coupled to a Osborne 386SX personal computer (USA) and Epson LX-850 integrator (Japan). The UV detector operated

at 254 nm. A Nova-pak C18, 4 μm, 150 × 3.9 mm I.D. (Waters Millipore, Part No. 863344) reversed-phase column was used.

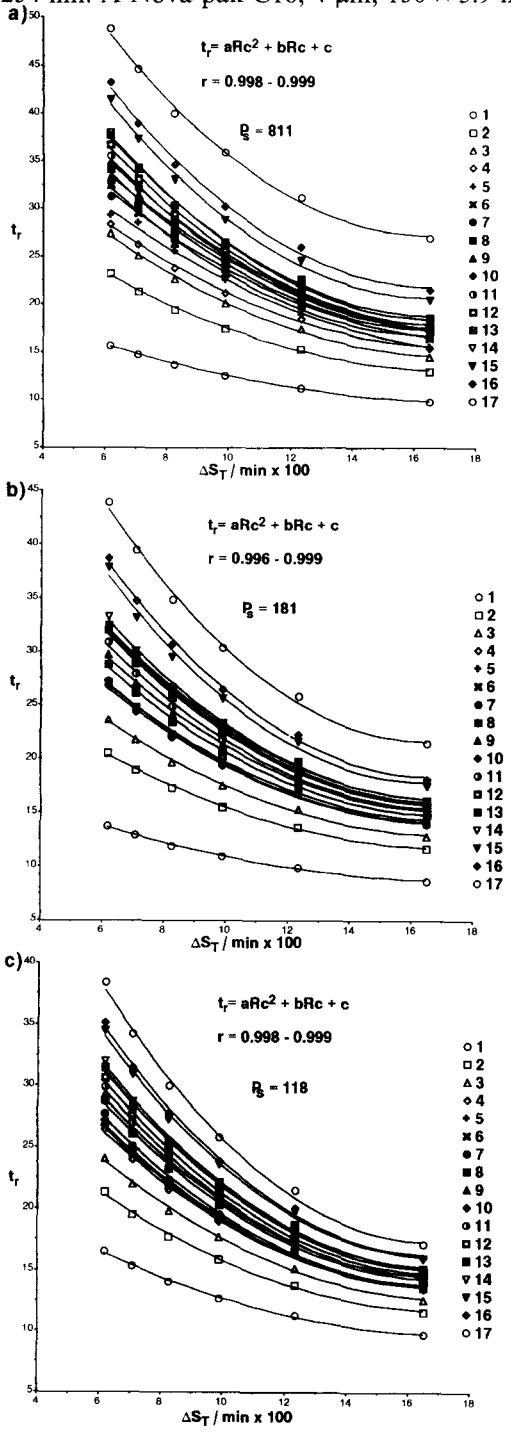


Fig. 2. The dependences between the t_r values of 17 dansyl amides and rate of change of S_T at (a) $P_s = 811$, (b) $P_s = 181$ and (c) $P_s = 118$ (see Table 1 for compounds).

The amines were dissolved in 0.4 M perchloric acid; 5.0 mg of pure amines in 20.0 ml of solvent gave optimum concentrations. After centrifugation, 0.4 ml of 2 M sodium hydroxide and 0.6 ml of saturated sodium bicarbonate were added to 3.0 ml of the supernatant to make the solution basic. Then, 1 ml of 1% dansyl chloride in acetone and 1 ml of acetone were added. The mixture was incubated at room temperature for 45 min. After derivatization, 0.2 ml of ammonia was added to the reaction mixture to remove the nonreacted dansyl chloride. After 30 min the volume was adjusted with acetonitrile to 10.0 ml. The dansyl derivatives were protected against light and stored at −20°C [24].

Combinations of tetrahydrofuran (THF), acetonitrile (MeCN), methanol (MeOH) and water were used as the mobile phase (see Results and Discussion). Six linear gradients (15, 20, 25, 30, 35 and 40 min) with a flow-rate of 1 ml min^{-1} were used. The system was re-equilibrated for 10 min after each run. The gradients were performed at the 13 selectivity points (see Results and discussion). The reliability of measurment the retention times was tested for 17 amines by the reproducibility of the intra-assay (C.V.% = 0.04–0.09, $n = 6$ for 17 amines in each P_s).

The statistics were performed on Macintosh versions of StatView II and Systat 5.1

4. Results and discussion

4.1. Construction of the mobile phase

Reversed-phase material was selected as the stationary phase for the separation of the dansyl amides. Tetrahydrofuran, acetonitrile and methanol were selected in accordance with the Snyder classification of solvents. Water was used as the solvent strength regulator.

The solvent strength of acetonitrile ($S_{MeCN} = 3.2$) and tetrahydrofuran ($S_{THF} = 4.5$) was first adjusted by dilution with water to the same solvent strength as methanol ($S_{MeOH} = 2.6$) (Fig. 1a). Thus, the solvent strength of 100% MeOH corre-

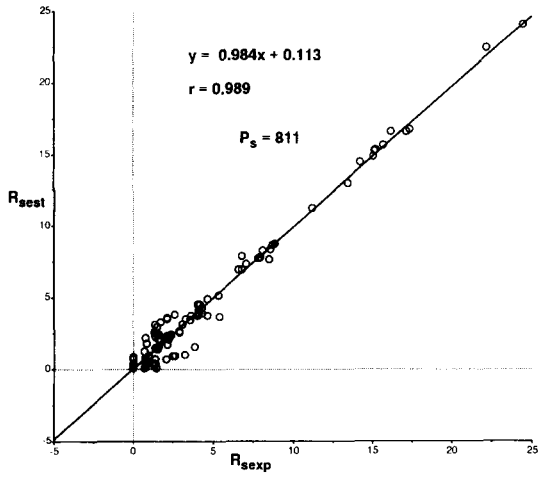


Fig. 3. The dependences between the experimental (R_{sexp}) and estimated (R_{sest}) resolutions at $P_s = 811$ (see Table 1 for compounds).

sponds to 81.3% MeCN or 57.8% THF aqueous solutions. The increase in the solvent strength from 0.13 to 2.6 during the gradient run corresponds to a change in the organic solvent from 5 to 100%. The dansyl amides were tested using six linear gradients: 15, 20, 25, 30, 35 and 40 min gradients. The gradients were performed at 13 selectivity points ($P_s = 811$, 181, 118, 631, 361, 163, 136, 316, 613, 433, 343 and 334, and the middle selectivity point 333) (Fig. 1b). For example, the selectivity point 811 at $S_T = 2.6$ represents solvent fractions of 46.2% THF, 8.1% MeCN, 10.0% MeOH and 35.6% H_2O. Tables 2 and 3 show the examples of the used retention data at the $P_s = 631$ in every six gradients and in 30 min gradient at 13 selectivity points.

4.2. The dependence between the S_T and retention data

The retention behaviour of the 17 dansyl amides was studied using the six different gradients at 13 solvent combinations. The retention times (t_r values) of dansyl amides were plotted against the rate of change of solvent strength (R_c), ranging from 0.062 to 0.165 ΔS_T min^{-1}. The S_T value changed from 0.13 to 2.6 in 15, 20, 25, 30, 35 and 40 min. The rate of change of S_T described the steepness of the gradient, i.e. the change of concentration of organic modifier per unit time. The linear, quadratic and cubic regression functions for the measured retention data were studied.

There were clear quadratic dependences between the t_r values of 17 amine derivatives and gradient elutions, as can be seen from the basic selectivity points 811, 181 and 118 in Fig. 2. The results closely followed the quadratic regression functions at all the studied selectivity points:

$$t_r = aR_c^2 + bR_c + c \quad (1)$$

where a, b and c are coefficients and R_c is the rate of change of solvent strength. The correlation coefficients, r, varied 0.996–0.999 ($n = 6$ for each of the 17 amines at the 13 P_s). The spreading of t_r values between the dansyl amines at these three selectivity points was greatest in the 40 min gradients (0.062 ΔS_T min^{-1}) and largest at the $P_s = 811$ ($15.72 < t_r < 48.87$) (Fig. 2).

The availability of these functions in calculating resolution (R_s) were investigated at four selectivity points; 811, 181, 118 and the middle point 333. The retention times (t_r values) were first estimated using the quadratic regression functions Eq. (1). The resolutions for the dansyl amides were calculated using the estimated and experimental t_r values:

$$R_s = \frac{1}{2}N^{1/2}(t_{ri} - t_{rj}/t_{ri} + t_{rj}) \quad (2)$$

where N is the average number of theoretical plates for the two peaks and t_{ri} and t_{rj} are the retention times of the adjacent peaks [25]. High dependences were achieved for each of the 16 peak pairs between the experimental (R_{sexp}) and estimated (R_{sest}) R_s values expressed as the linear regression functions ($r = 0.989$–0.999 using six gradient times at each P_s) (Fig. 3).

4.3. The three dimensional resolution (R_s) maps

The second aim of this study was to show descriptively how the different gradients and solvent combinations affect the separation of 17 dansyl amides. The quality of a separation was measured on the basis of the resolution (R_s) Eq. (2). A total of 78 different chromatographic con-

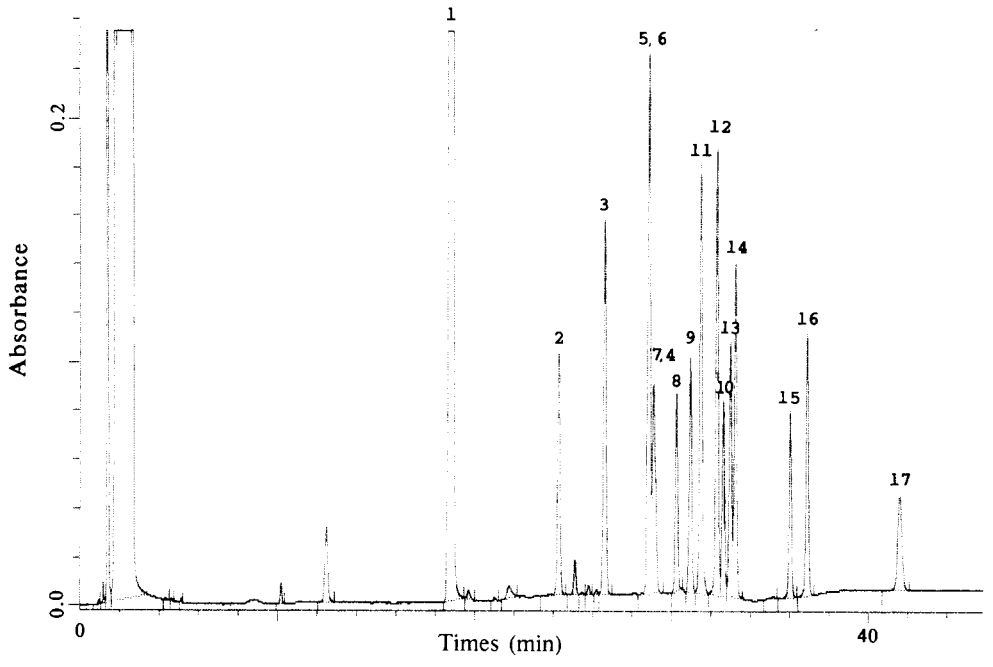


Fig. 4. The chromatogram of 17 dansyl amides at $P_s = 181$. S_T changed from 0.13 to 2.6 in 35 min. Flow rate: 1 ml min^{-1}. Column: Nova-pak C18, 4 μm, 150 × 3.9 mm I.D. Detection: UV 254 nm. (see Table 1 for compounds).

ditions for the separation of 17 dansyl amides were investigated.

The resolutions changed considerably with different gradients and selectivity points. The compounds eluting at the beginning and end of the chromatographic run (compounds 1–3 and 17) were always easily separated. The middle-eluting dansyl amides (compounds 4–6 and 10, 13 and 14) were difficult to separate, often being only partially separated or coeluted. The retention order of compounds 4, 10, 13 and 14 also changed depending on the gradients and P_s. The shortest gradients, 15 and 20 min, were found to be too short to achieve a good separation. The eluents containing mainly MeCN or MeOH ($P_s = 181$ and 118) gave the poorest separation for the dansyl amides (Fig. 4).

The data obtained with the 78 different chromatographic conditions (1248 R_s values) proved to be difficult to treat as a whole. Therefore an approach was developed to get an overview about separation in various P_s. An R_s value of 1.2 was used as the benchmark for a goor average separation because it resulted in a less than 1% overlap for the peaks studied. The separation of the dansyl amides was examined using three-dimensional resolution maps. R_s values below 1.2 were omitted and values above 1.2 were chosen for each P_s and gradients. The number of resolution values exceeding $R_s \geq 1.2$ ($R_{s(n)}$) was counted and three dimensional resolution maps were plotted using these values. The changes in resolution between the different S_T and P_s values could be more descriptively presented using these $R_{s(n)}$ values because the whole dataset could subsequently be expressed with six maps (Fig. 5.). The selectivity points are coordinated by the x and y axes and the number of resolution values exceeding $R_s \geq 1.2$ ($R_{s(n)}$) by the z axis. The changes in resolution between the P_s could be clearly seen especially from the shape of the maps of the longer gradients (Fig. 5d–f). The best separations for the 17 dansyl amides were achieved at the THF corner of the prism; this was the case for all gradients. The number of resolution values exceeding $R_s \geq 1.2$ decreased on moving towards the MeCN and MeOH corners.

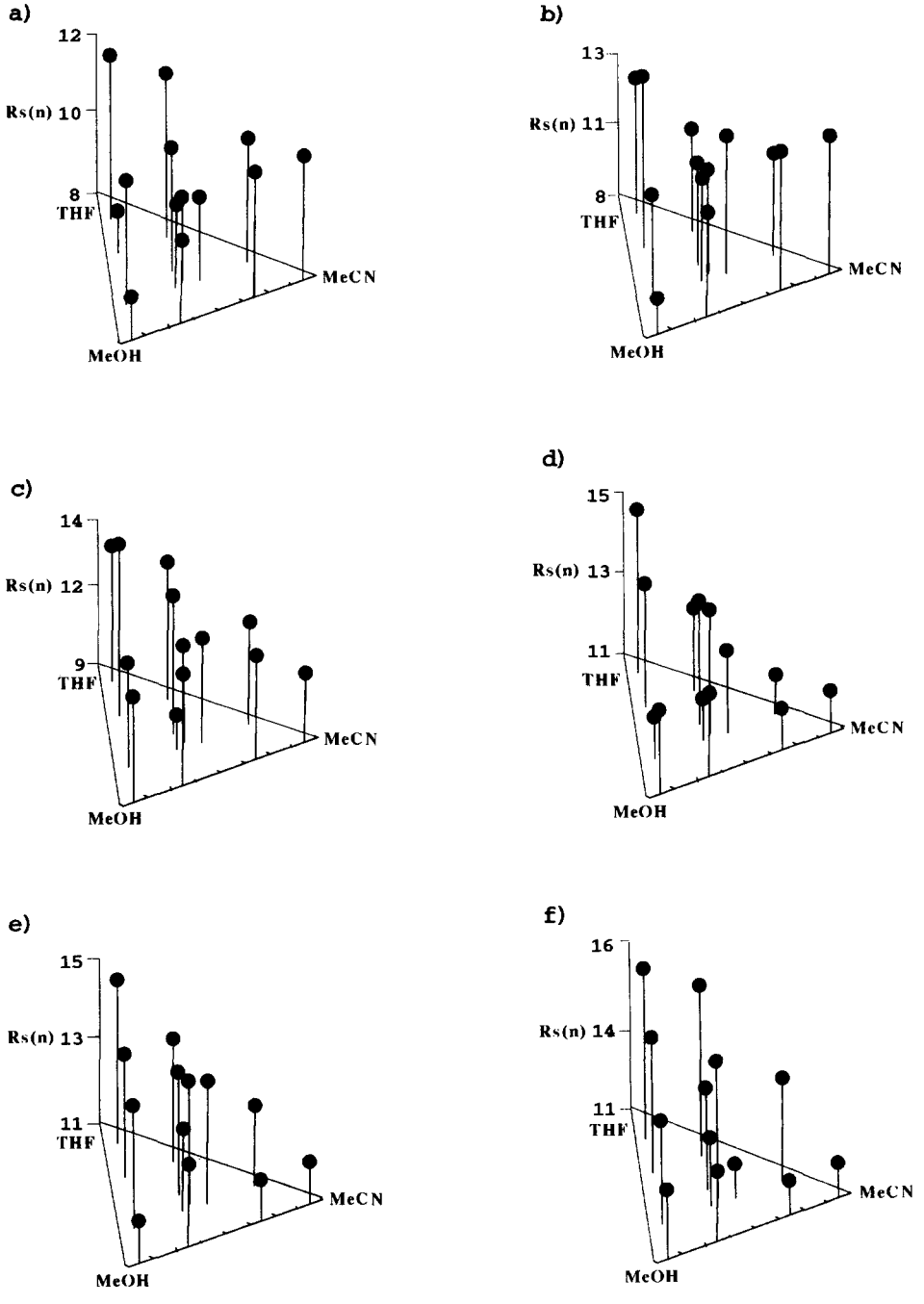


Fig. 5. The three dimensional resolution (R_s) maps of (a) 15 min, (b) 20 min, (c) 25 min, (d) 30 min, (e) 35 min and (f) 40 min gradients at the 13 selectivity points.

The changes in resolution were also depicted using a two-dimensional line chart (Fig. 6.). The best eluents mainly contained THF; $P_s = 811$, 631, 613 and 433 (Fig. 7). These eluents maintained good separation with both short and longer gradients. The run time of the first three gradients was

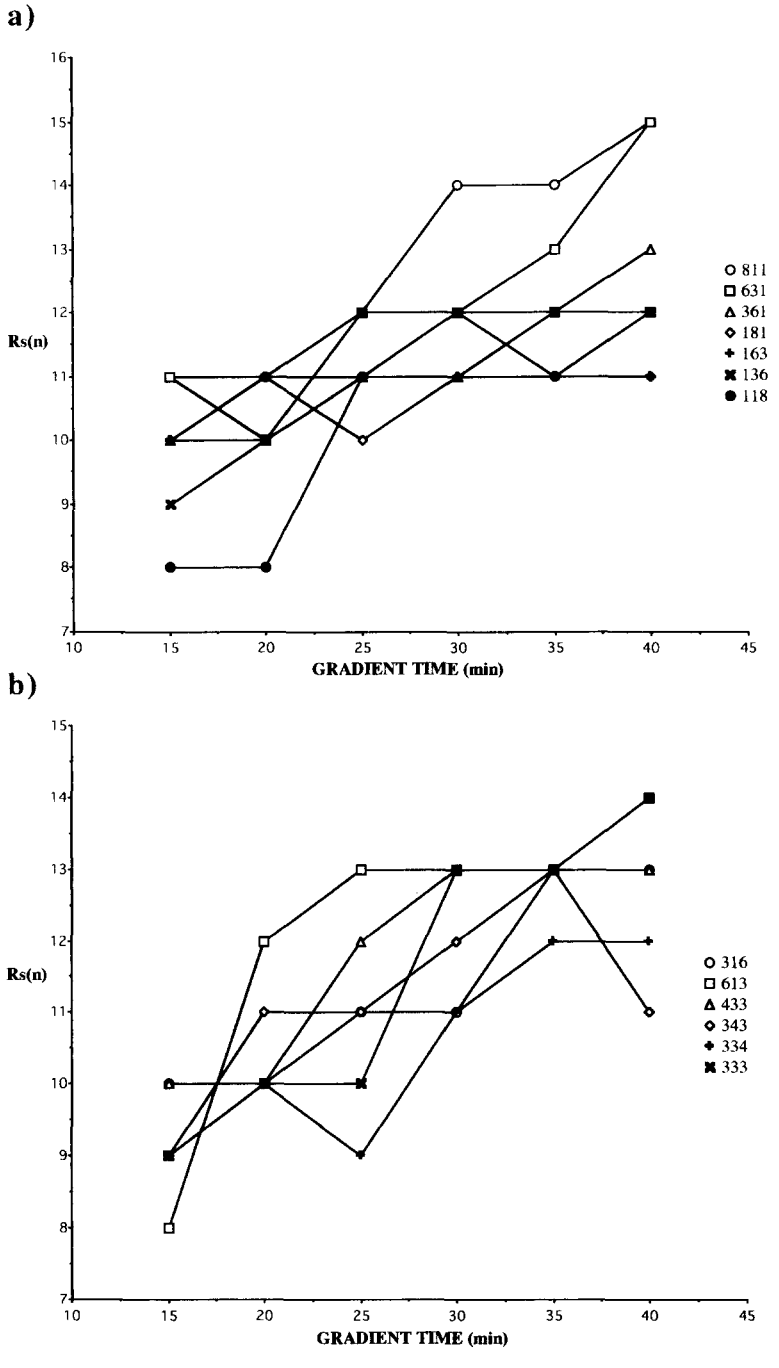


Fig. 6. The two dimensional line charts describing the changes of resolution at the different gradients and selectivity points (a) at $P_s = 811$, 631, 361, 181, 163, 136 and 118; (b) at $P_s = 316$, 613, 433, 343, 334 and 333.

much too short to achieve a good separation. When the gradient time was increased, the resolution increased. However, the differences between the 35 and 40 min gradients were quite small, and thus both can be used as the acceptable gradient. Further optimization can be performed based on

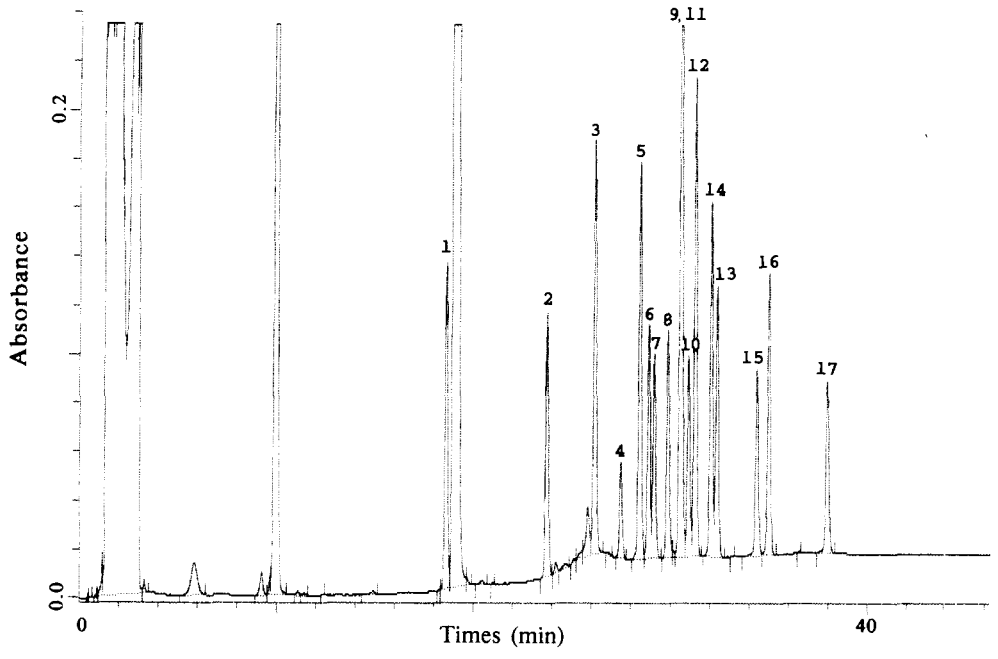


Fig. 7. The chromatogram of 17 dansyl amides at $P_s = 631$. (see Fig. 4 for the other conditions and Table 1 for the compounds)

these results. For example at $P_s = 631$ using a 35 min gradient, the separation can be improved by changing the initial and final solvent strength. Depending on the aim of the analysis, the critical pairs can be examined in detail by changing the level of the R_s values. The separation quality of the eluent can then be easily defined with this method without difficult and time-consuming calculations.

5. Conclusions

High dependences were found between the different gradient times and retention data of 17 dansyl derivatized biogenic amines expressed as the quadratic regression functions. These functions can be used to estimate the resolution and, furthermore, to predict the resolution in other gradient elutions. An easy method to describe the influence of solvent strength and eluent mixtures on resolution by $R_{s(n)}$ values was developed and this can be constructed by the three-dimensional resolution maps. The study showed that the best eluents retained their separation power regardless of the gradient steepness. However, the gradient steepness expressed by the rate of change of solvent strength influenced significantly on resolution.

Acknowledgements

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