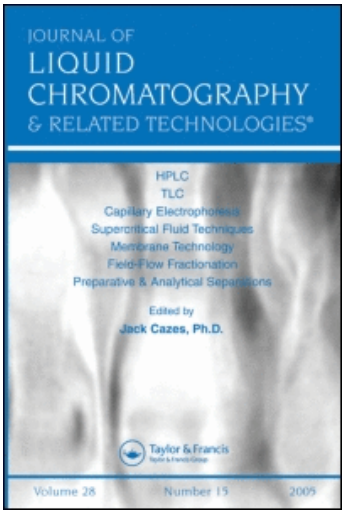

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OPTIMIZATION OF HPLC ANALYSIS OF BIOGENIC AMINES WITH REFERENCE TO MOLECULAR STRUCTURE

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ABSTRACT

The selectivity and molecular connectivity indices (χ) up to the sixth order were calculated and compared with measured reversed-phase high pressure liquid chromatographic (HPLC) retention data for biogenic amines common in foodstuffs and animal feed. The separation of seventeen dansylated amines including primary, secondary and polyamines, was investigated using an isoselective multisolvent gradient (IMGE), reversed-phase HPLC method. The mobile phase was optimized with the "PRISMA" model testing thirteen selectivity points. The compounds were divided into two groups according to their retention; the low-order valence level indices best described the retention. As the high correlations between the calculated and observed retention indicate, retention could be predicted in different selectivity points with a high degree of accuracy by the molecular connectivity indices.

INTRODUCTION

Biogenic amines are physiologically active, sometimes toxic, organic bases which are widely distributed in living material. They are usually formed by decarboxylation of amino acids, and can be found in foodstuffs and feed. Since the biogenic amine concentration rises as a result of decomposition or spoilage of foodstuffs and feed, the content of these decomposition products should be monitored.

There are many methods based on high performance liquid chromatography (HPLC) for measuring biogenic amines in different high-protein foodstuffs (1, 2, 3). In chromatography the relative differences between the affinities of the mobile and stationary phases for sample components are affected by the parameters that influence separation factor (α). The mobile phase is the chromatographic system parameter that can be most easily varied in optimizing α for HPLC analyses.

The "PRISMA" model is an efficient method to optimize systematically the mobile phase *e.g.* in HPLC (4). The "PRISMA" model is a three-dimensional model that is dependant on solvent strength (S_T) and the selectivity of the mobile phase. In the "PRISMA" model the elution can be carried out in isocratic or gradient mode. The gradient elution can be performed in order to change solvent strength (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) (5, 6).

The retention of compounds in HPLC analyses can be predicted using molecular connectivity indices, χ (7, 8, 9). The concept of molecular connectivity was introduced by Randic (10) and further developed by Kier and Hall (11). The χ terms are numerical values which are fundamental in defining and describing quantitatively the adjacency relationships in the molecular structure. When the nature of the atom is not taken into consideration the index is referred to as the connectivity level, χ. If it is, the index is described as the valence level, χ^v. Connectivity indices have

been extended to include indices of different orders, as well as subgraphs composed of paths, clusters and path/clusters, which are described by the subscripts p, c, and pc, respectively.

The behaviour of heteroatom-containing molecules is sometimes difficult to predict on the basis of molecular connectivity indices. Kier and Hall (11) have added the vertex value, δ, of the heteroatoms to the regression equation to improve the use of molecular connectivity indices for heteroatom-containing molecules. As shown by Lehtonen (2) the rentention of oxygen-containing amines could not be predicted when analysed together with non-oxygen-containing amines.

The aim of the study was to compare molecular connectivity indices with the retention of seventeen dansylated, closely related, amines common in feed and spoiled foodstuffs. The aim of the study was also to optimize the difficult HPLC separation of these compounds using the "PRISMA" model (6) with reference to molecular connectivity indices describing the molecule structure.The indices were used to study the predictability of the HPLC behaviour and optimization.

MATERIALS AND METHODS

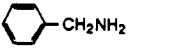
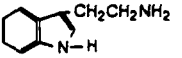
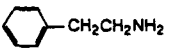
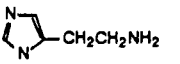
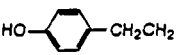
Reagents and solvents

The biogenic amines (see Table I for structures) and dansyl chloride were purchased from Fluka AG, FRG. Ammonia, ammonium acetate, sodium hydroxide and sodium bicarbonate were purchased from Merck. The water was distilled and deionised. Acetonitrile, methanol and tetrahydrofuran were of HPLC quality (Rathburn Chemicals LTD, UK).

Dansyl derivatization

The amines and fish feed were dissolved in 0.4 M perchloric acid. 5 mg of pure amines and 1 g of fish feed in 20 ml of solvent gave optimum concentrations. After centrifugation 0.2 ml of 2 M sodium hydroxide and 0.3 ml of saturated sodium bicarbonate were added to 1.0 ml of the

Table I Structure of the biogenic amines.

Compounds	Group	Structure
1.Ethanolamine	I	$HOCH_2CH_2NH_2$
2.Ethylamine	I	$CH_3CH_2NH_2$
3.Propylamine	I	$CH_3CH_2CH_2NH_2$
4.Diethylamine	I, II	$(CH_3CH_2)_2NH$
5.Butylamine	I, II	$CH_3CH_2CH_2CH_2NH_2$
6.Benzylamine	I, II	
7.Tryptamine	I	
8.Phenethylamine	I	
9.Pentylamine	I	$CH_3CH_2CH_2CH_2CH_2NH_2$
10.Histamine	I	
11.1,4-Diaminobutane	I	$NH_2CH_2CH_2CH_2CH_2NH_2$
12.1,5-Diaminopentane	I	$NH_2CH_2CH_2CH_2CH_2CH_2NH_2$
13.Hexylamine	I	$CH_3CH_2CH_2CH_2CH_2CH_2NH_2$
14.1,6-Diaminohexane	I	$NH_2CH_2CH_2CH_2CH_2CH_2CH_2NH_2$
15.Tyramine	I	
16.Spermidine	I	$NH_2(CH_2)_3NH(CH_2)_4NH_2$
17.Spermine	I	$NH2_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$

supernatant to make the solution basic. 0.5 ml of 1% dansyl chloride in acetone and 0.5 ml of acetone were added. The mixture was incubated at room temperature for 45 minutes.

After derivatization, 0.1 ml of ammonia was added to the reaction mixture to remove the nonreacted dansyl chloride as described by Hinkkanen and Rajakylä (12). After 30 minutes the volume was adjusted with acetonitrile to 5 ml. The dansyl derivatives were protected against light and stored at -20 °C.

Chromatographic apparatus

Waters 6000A liquid chromatograph pumps (Milford, MA, USA) combined with a control module (Waters 660) equipped with a PU4020 UV detector (Pye Unicam LTD, Cambridge, UK) and Hewlett Packard 3390 A integrator (Avondale, PE, USA) were used for the standard dansylated amines.

Chromatographic conditions

Columns: Novapak (Waters, Milford, MA, USA) C18 3.9 mm x 15 cm, 4 µm, in a 37 °C water bath
Mobile phase: combinations of tetrahydrofuran, acetonitrile, methanol and water (see Results and Discussion). 0.1 M ammonium acetate in water was used as a modifier.
Flow rate: 1 ml/min
Detection: UV, 254nm
Dead volume: $NaNO_2$ in water (0.2 µl) detected at 250nm.

Calculation of the molecular connectivity indices

The molecular connectivity indices for the dansylated amines were calculated using a self-made program running on BASIC, as has earlier been described (13). The following general equation proposed by Kier and Hall (11) was used for computation of an index of type t and order m

$$ {}^{m}\chi_t = \sum_{j=1}^{{}^{m}n_s} {}^{m}c_j = \sum_{j=1}^{{}^{m}n_s} \left[\prod_{i=1}^{m} \left(\delta_i \right)_j^{-1/2} \right] $$

where ${}^{m}c_j$ is the subgraph term for m:th order subgraphs and ${}^{m}n_s$, is the number of m:th order subgraphs.

Calculation of α-surfaces and correlations

The α-surfaces of the dansylated amines at selectivity points were calculated with a Macintosh SE computer using the SYSTAT (SYSTAT, Inc., Evanston, IL, USA) program. The StatView 512+™ (Brain Power, Calabas, CA, USA) procedure was used for determining the index best describing retention using linear regression analysis. The dansylated amines were divided into two groups according to their retention behaviour with reference to connectivity indices: group I (compounds 1-6, 9, 10, 13 and 15; see Table I) and group II (compounds 4-8, 11, 12, 14, 16 and 17; see Table I).

RESULTS AND DISCUSSION

Optimization of the mobile phase

Optimization of the mobile phase was carried out using the "PRISMA" model (6). The use of "PRISMA" as a geometric model makes it possible to combine 1-5 solvents systematically. Usually three of these are organic solvents, the fourth serves as a solvent strength regulator and the fifth can be a modifier. The resulting mobile phase is characterized by the solvent strength (S_T) and selectivity points (P_S).

Reversed-phase material was selected as the stationary phase for the separation of the dansylated amines. Tetrahydrofuran, (THF; S_T=4.5), acetonitrile, (ACN; S_T=3.2) and methanol, (MeOH; S_T=2.6) were selected in accordance with the Snyder classification (14) on the basis of their properties as proton acceptors, proton donors and their dipole

interactions, respectively. Water was used as the solvent strength regulator. Ammonium acetate was added to water (0.1 M) as modifier in order to decrease tailing in the separation of the dansylated amines.

Solvent strength adjustment

The solvent strength adjustment was carried out as described in Outinen *et al.* (15). The retention in isocratic runs was tested, but the separation was poor because the compounds had a broad range of polarity. According to Outinen *et al.* (15) the best separation was achieved using an IMGE gradient elution instead of an isocratic run. ACN (S_T=3.2) and THF (S_T=4.5) were diluted to the same solvent strength as MeOH (S_T=2.6) with water. Thus the solvent strength of 100% MeOH corresponds to 81.3% ACN or 57.8% THF aqueous solutions. The dansylated amines were analyzed in 57.8% THF and 81.3% ACN and 100% MeOH using the gradient run, where the solvent strength increased during the gradient run from 0.52 to 2.34, while the selectivity point (P_S) was kept constant according to IMGE (Fig. 1a).

Influence of selectivity on the separation factor (α)

After solvent strength adjustment the dansylated amines were tested at thirteen selectivity points (Fig. 1b). The selectivity points (P_S) represent the volume fractions of the three organic solvents diluted with water to the solvent strength of the weakest organic solvent in reversed phase chromatography. The edges of the "PRISMA" - P_S=100 (THF), 010 (ACN), 001 (MeOH) - which represent three pure organic solvents and water with modifier regulating the solvent strength, were tested first. Ten points inside the "PRISMA" representing the quaternary mixtures were also tested. The selectivity points chosen were one of the middle points (Ps=343), the basic selectivity points (Ps=811,181,118) and the six points along the sides of the triangle near the middle points (Ps=541, 451,154, 145, 415, 514). Nyiredy *et al.* (4) have showed that retention measurements at 12 selectivity points (P_S=118, 316, 613, 811, 631, 361, 181, 163, 136, 334, 343, 433) at a selected solvent strength level allow the calculation and prediction with high accuracy of the k' values also in

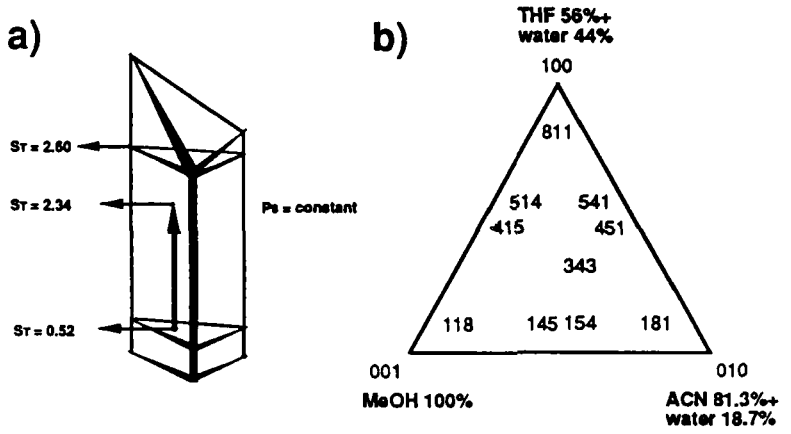


Figure 1 a) Isoselective multisolvent gradient (IMGE). The solvent strength (S_T) was increased during the chromatographic run from 0.52 to 2.34 while keeping the selectivity (P_S) constant. **b)** The thirteen selectivity points studied in the regular part of the "PRISMA".

all other selectivity points. In this study, however, the selectivity points used were appropriate to describe the three-dimensional separation factor (α)-surfaces with high accuracy.

The separation factors were calculated for all the dansylated amines at thirteen selectivity points. Changes in the separation factors in the different organic solvent combinations, as well as the changes in the mean values of the separation factors, were examined.

Figure 2 shows three-dimensional plots of the separation factor (α)-surface of the thirteen selectivity points. The three plots demonstrate different types of α-surfaces. In these plots it is clearly seen that the increase of methanol in mobile phase decreases α. The most difficult compounds to separate were four, successively eluted dansylated amines: diethylamine (4), butylamine (5), benzylamine (6) and tryptamine (7). The changes in the mean values of the separation factors of these compounds at selectivity points showed that the best separation was

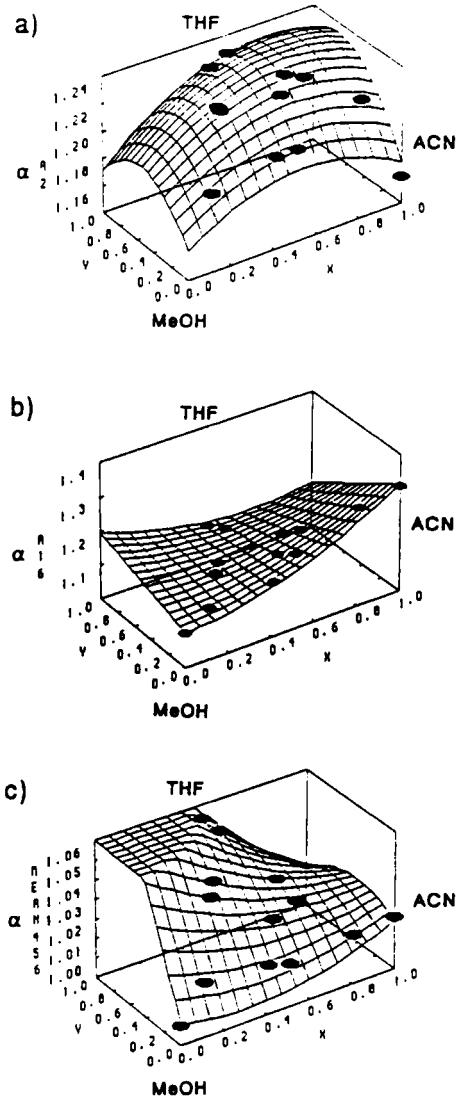


Figure 2 The values of the separation factors (α) of dansylated amines at selectivity points (see Table I for compounds, solvents as described in results and discussion): **a)** compounds 2 and 3, **b)** compounds 16 and 17, **c)** compounds 4-7 (mean value).

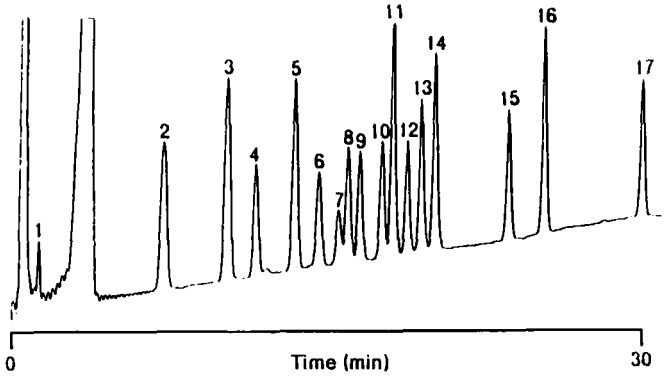


Figure 3 The chromatogram of the seventeen dansylated amines at selectivity point P_S=811. The change in the solvent strength during the gradient was from S_T= 0.52 to S_T= 2.34 in 30 minutes. Flow rate: 1 ml/min. Column: Novapak C18 3,9 mm x 15 cm, 4 µm. Detection: UV= 254 nm (see Table I for compounds).

achieved when the mobile phase contained three organic solvents at P_S = 811 (Fig. 2c). This is also evident from the chromatogram (Fig. 3).

Correlation between molecular structure and retention

The correlation between indices describing the molecular structure and retention in gradient runs was studied. The retention was correlated with the molecular connectivity path (χ_p) and valence indices for path (χ_p^v) type through to sixth order. The connectivity and valence indices for cluster (χ_c, χ_c) and path/cluster (χ_{pc}, χ_{pc}) types were equally calculated to the sixth order. The compounds were divided into two groups due to different behaviour in HPLC (see Table I for groups I and II).

The correlation of retention with the molecular connectivity indices has not much been studied for gradient runs (13, 16). Here it was investigated for thirteen solvents containing tetrahydrofuran, acetonitrile and methanol in water with S_T from 0.52 to 2.34. The observed k′ values

Table II Capacity factor, k', of the dansylated amines in three different organic solvent/water mixtures and in P_S=811 in IMGE runs.

Compound	THF	ACN	MeOH	811
1.	5.72	7.02	11.69	4.16
2.	13.49	13.39	16.05	12.86
3.	16.66	16.32	18.55	15.89
4.	17.60	20.15	20.95	17.25
5	19.47	19.10	20.95	18.75
6.	20.49	19.10	20.95	19.60
7.	20.49	19.10	20.95	20.08
8.	21,30	20.95	22.63	20.70
9.	21.76	21.88	23.05	21.27
10.	22.29	24.60	25.71	22.09
11.	22.53	22.82	24.57	21.84
12.	23.42	24.01	24.21	22.77
13.	23.70	24.60	24.99	23.55
14.	24.35	25.36	24.99	23.78
15.	26.02	29.58	27.47	26.29
16.	27.52	31.25	28.19	27.59
17.	30.63	40.84	30.89	31.45

are shown in Table II. In all thirteen solvents the index $^0\chi^v$ showed the best correlation for group I. Also $^0\chi$-indices could be used in describing the retention. For group II most of the indices showed an excellent correlation with retention; only indices of the type $^6\chi_c$ and $^6\chi_c$ showed a poor correlation. However, the index $^0\chi^v$ showed the best correlation for group II. The correlation of compounds in groups I and II with the index $^0\chi$ -values for all the selectivity points showed r values from 0.92 to 1.00. Figure 4 shows the regressions with high r-values for compounds in group I (a) and group II (b) at the selectivity point P_S=811, where the separation was excellent.

The regression equations for the edges of the "PRISMA" are given in Table III as $k' = A\chi+B$, where A is the slope of the regression curve, B is the intercept and χ the corresponding index value of the compound. The correlation coefficient of level 0.98 ($2p < 0.0001$) was achieved, when correlating the experimental k' values from gradient runs of pure solvents

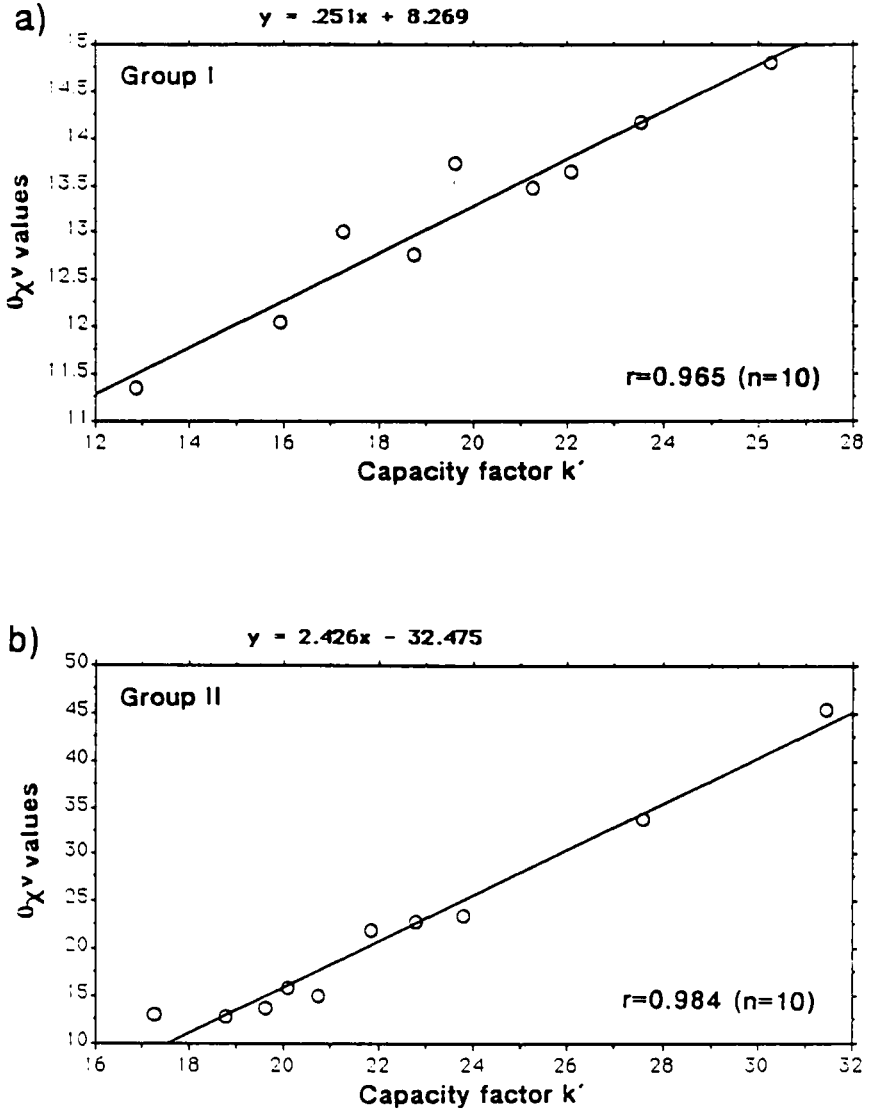


Figure 4 Plot of the measured capacity factors (k′) and molecular connectivity indices (χ) for group I (**a**) and II (**b**) for IMGE run in P_S =811 and S_T = 0.52 - 2.34 in 30 min.

Table III Regression coefficients for equations of the type $k' = A\chi + B$ in the three eluents for IMGE runs.

Solvent	THF		ACN		MeOH	
Group	I	II	I	II	I	II
A	0.27	2.65	0.21	1.51	0.22	3.06
B	7.80	-38.44	8.91	-14.76	8.36	-51.16
r	0.97	0.97	0.94	0.99	0.92	0.99
index χ	$^0\chi^v$	$^0\chi^v$	$^0\chi^v$	$^0\chi^v$	$^0\chi^v$	$^0\chi^v$

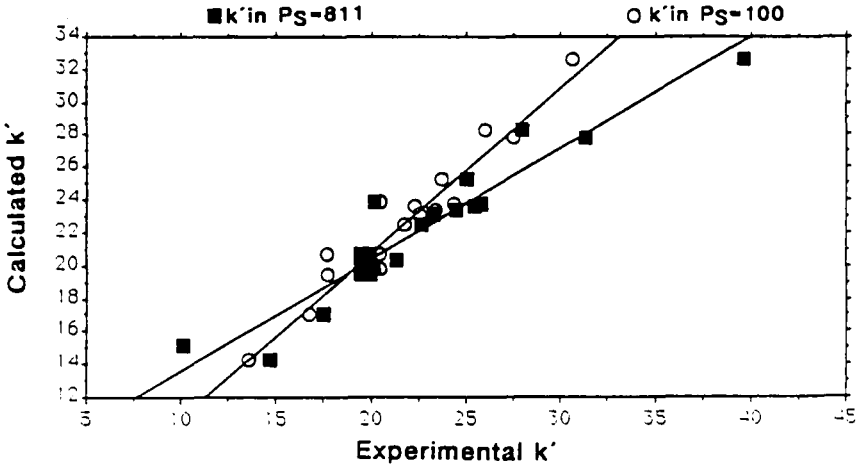


Figure 5 Plot of the observed and predicted capacity factors (k') of all the compounds for the IMGE run in P_S =811 and P_S =100; S_T = 0.52 - 2.34 in 30 min. The predicted values were calculated from equations of the type $k' = \Sigma \Pi (A\chi + B)$.

with calculated k' values obtained from the equations in Table III. In gradient runs the retention could well be predicted. This is in good agreement with results obtained by Lehtonen (13) for amines and by Härmälä *et al.* (16) for coumarins.

The retention of the dansylated amines was estimated for the IMGE runs at the selectivity point P_S= 811 using regressions obtained with pure solvents at edges of the "PRISMA" (Table III). The capacity factors were calculated from the regression curves of the type $k' = A\chi+B$ shown in Table III and it follows at selectivity points $k' = \Sigma \Pi (A\chi+B)$, where Π is the fraction of the solvent used in forming the "PRISMA", by assuming that there exists a linear correlation for retention between different selectivity points. Figure 5 demonstrates the efficiency of the used method to calculate the k' values at different selectivity points within the "PRISMA" based on the retention information at the "PRISMA" edges *i.e.* gradient runs with aqueous THF, ACN and MeOH only. The correlation (r=0.98) between the calculated and measured k' values at Ps=811 is highly significant ($2p < 0.0001$) in regression analysis. The slope of the corresponding regression curve for retention data at P_S=100 differs significantly ($2p < 0.001$) from that at P_S=811. It shows that the calculated as well as the measured retention can clearly be distinquised from those at different P_S.

CONCLUSIONS

An excellent separation between the seventeen dansylated amines was achieved using the "PRISMA" model in the optimization process of the mobile phase. The retention of dansylated amines under different HPLC conditions correlated well with the indices describing molecular structure. The retention could be well predicted for the different selectivity points in the "PRISMA" using the molecular connectivity indices.

REFERENCES

1. Antila, P., Antila, V., Mattila, J., Hakkarainen, H., Milchwissenschaft, 39, (2), 81 (1984).
2. Lehtonen, P., Z. Lebensm. Unters. Forsch., 183, 177 (1986).
3. Mietz, J.L., Karmas, E., J.Food Sci., 42, 155 (1977).
4. Nyiredy, Sz., Dallenbach-Toelke, K. and Sticher, O., J. Liq. Chromatogr. 12, 95 (1989).
5. Kirkland, J.J., Glajch, J.L., J. Chromatogr., 255, 27 (1983).
6. Nyiredy, Sz., Sticher, O., HRC & CC, 10, 208 (1987).
7. Lehtonen, P., Chromatographia, 19, 316 (1984).
8. Wells, M.J.M., Clark, C.R. and Patterson, R.M., Anal. Chem., 58, 1625 (1986).
9. J. Burda, M. Kuras, J. Kriz and L. Vodicka, Z., Anal. Chem., 321, 549 (1985).
10. Randic, M., J. Am. Chem. Soc., 97, 6609 (1975).
11. Kier, L.B. and Hall, L.H. , Molecular Connectivity in Chemistry and Drug Research, Academic Press, Inc., London 1976.
12. Hinkkanen, R., Rajakylä, E., Proceedings of 4th Nordic Symposium on Analytical agricultural Chemistry, 1st Symposium of the section of analytical agricultural chemistry in NJF, Espoo, Finland, 1987.
13. Lehtonen, P., Behaviour of Amine Derivatives in Reversed-phase Liquid Chromatography with reference to Molecular Structure, Academic Dissertation, University of Helsinki, Helsinki 1987.
14. Snyder, L.R., J. Chromatogr. Sci., 16, 223 (1978).
15. Outinen, K., Vuorela, H., Hinkkanen, R., Hiltunen, R., Acta Pharm. Fenn. (in press).
16. Härmälä, P., Vuorela, H., Lehtonen, P., Hiltunen, R., J. Chromatogr., 507, 367 (1990).