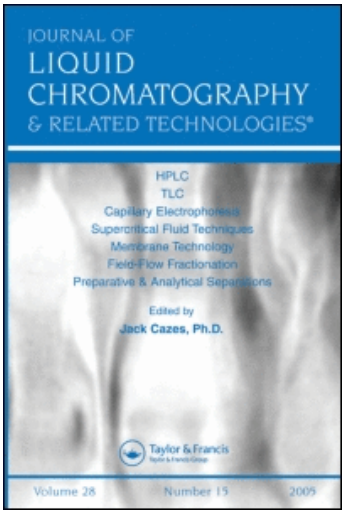

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Some Solvent Systems for the Resolution of Dansyl Amino Acids by Silicagel Thin Layer Chromatography

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SOME SOLVENT SYSTEMS FOR THE RESOLUTION OF DANSYL AMINO ACIDS BY SILICAGEL THIN LAYER CHROMATOGRAPHY

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ABSTRACT

The dansyl amino acids have been divided into two groups depending on their chromatographic behaviour on silicagel thin layers in ten new solvent systems, five for each group. The systems reported herein provide rapid and effective resolution (35-45 min) for 20 dansyl amino acids in all. The systems also provide separations of Dns-Thr/Dns-Ser, Dns-Asp/Dns-Glu and Dns-Arg/Dns-ϵ lys which were earlier reported[13] to be unresolved even by two-dimensional thin layer chromatography.

INTRODUCTION

The resolution and identification of PTH-DNP-, and dansyl amino acids is required for the N-terminal sequence determination of proteins. We have earlier reported several successful thin layer chromatographic methods for the resolution and identification of PTH-amino acids[1,2] and DNP-amino acids[3]. Quantitative determination of amino acids as their dansyl derivatives in plants, insects, and human serum, urine and sweat[4-7] has been carried out by various analytical methods, however, TLC has been considered simple, inexpensive and widely used. Literature reveals several solvent systems for two-dimensional thin-layer chromatography of dansyl amino acids[8-12]. However, even after two-dimensional chromatography many Dns-amino acid pairs such as Dns-Asp/Dns-Glu, Dns-Thr/Dns-Ser and Dns-His/Dns-Arg/Dns- α -Lys/Dns- ε -Lys[13] remained unresolved and required an additional run in a third solvent[8]. Long analysis time is characteristic for the two-dimensional mode and quantitative evaluation is also problematic. Therefore attempts were made to develop effective solvent systems for one dimensional thin layer chromatographic resolution of dansyl amino acids,

and the results are reported in the present communication.

EXPERIMENTAL

The Dns-amino acids were obtained from Sigma Chemical Company. Solvents and reagents were from SISCO Research Laboratory Bombay, and B.D.H.(England) AR grade. The TLC plates of 20 x 20 cm x 0.5 mm were prepared by spreading a slurry of silicagel G in distilled water. The plates were dried at a constant temperature of 60 ± 2°C for 12 h. Standard solutions of Dns-amino acids (10^{-4}M) were prepared in methanol. The individual compounds and their mixtures were applied at the 500 ng level using 25 µl Hamilton syringe. The chromatograms were developed at 30 ± 3°C with different solvent systems as given in Table 1 and 2. The migration distance was 10 cm in all the cases. Dns-amino acids were located under a long-wavelength UV lamp as fluorescent spots.

RESULTS AND DISCUSSION

The hR_f values for dansyl derivatives of twenty amino acids in ten solvent systems S_1-S_5 and A_1-A_5 have been given in Tables 1 and 2, for

TABLE - 1

hR_f values of 10 Dansyl amino acids on silicagel thin layers.

S.No.	Dansyl amino acid	Solvent systems				
		S_1	S_2	S_3	S_4	S_5
1.	Dansyl-L-Alanine	62	61	60	50	27
2.	Dansyl-L-Isoleucine	80	92	85	85	49
3.	Dansyl-L-Leucine	83	85	80	89	45
4.	Dansyl-L-Methionine	65	64	62	55	31
5.	Dansyl-L-Proline	60	84	72	30	39
6.	N-O Didansyl-L-Tyrosine	55	73	40	60	18
7.	N-α-dansyl-L-Tryptophan	51	53	46	40	21
8.	Dansyl-L-Phenylalanine	77	76	74	52	40
9.	Dansyl-L-Valine	72	88	65	48	35
10.	Dansyl-L-Norvaline	75	81	68	45	37

S_1 : n-heptane-BuOH-HOAc (20:8:3).

S_2 : Dichloromethane-MeOH-propionic Acid (30:1:0.5).

S_3 : Chloroform-HOAc-Ethylacetate(24:5:4).

S_4 : Chloroform-MeOH-Ethylacetate (23:8:2).

S_5 : Chloroform-Propionic acid-Ethylacetate (23:6:4).

R_f values are average of five determinations.

two sets of ten derivatives respectively. It was observed that the Table 1 contained dansyl derivatives of amino acids having aliphatic or aromatic side chain except methionine, while Table 2 contained derivatives of amino acids having acidic

TABLE-2

hR_f values of 10 Dansyl amino acids on silicagel thin layers

S.No.	Dansyl amino acid	Solvent systems				
		A_1	A_2	A_3	A_4	A_5
1.	N-α-dansyl-L-Asparagin	56	75	53	30	35
2.	Dansyl-L-Asparatic acid	66	72	60	64	30
3.	α-Dansyl-L-Arginine	7	12	3	2	3
4.	N-N-didansyl-L-Cystine	84	83	68	45	18
5.	Dansyl-L-Cysteic acid	82	80	25	15	11
6.	Dansyl-L-glutamic acid	80	90	84	74	55
7.	Dansyl-L-glutamine	62	77	63	41	40
8.	N-ϵ-dansyl-L-Lysine	16	20	10	6	8
9.	N-dansyl-L-Serine	72	85	72	58	32
10.	Dansyl-L-Threonine	76	88	76	68	45

A_1 : Dichloromethane-MeOH-Propionic acid (28:3:2)

A_2 : Ethylacetate-MeOH-Propionic acid (22:10:3)

A_3 : Chloroform-MeOH-HOAc (28:4:2)

A_4 : Chloroform-Acetone-HOAc (20:8:4)

A_5 : Chloroform-Acetone-Propionic acid (24:10:5)

R_f values are average of five determinations.

or basic side chain except Serine and Threonine. There was a difference of more than three units in the hR_f values of any two derivatives in each set which indicated an effective resolution. However, the experiment with actual mixtures of ten derivatives of each set resulted into resolution of five to six components in the solvents of Table 1, and seven to eight components in the solvents of Table 2. It was interesting to note that the dansyl amino acids given in Table 2 did not move from the base line in the solvent systems S_1-S_5, (Table 1), on the other hand the dansyl amino acids given in Table 1 moved to solvent front in the systems A_1-A_5 (Table 2). Therefore, it can be suggested that using appropriate solvents from the two tables twenty dansyl amino acids can be resolved and the hR_f can be used to identify them, without resorting to two-dimensional mode where in-system calibration with the standards is not possible. Besides, the solvent systems provided resolution for certain difficult combinations[13], e.g. Dns-Thr/Dns-Ser, Dns-Asp/Dns-Glu, Dns-Arg/Dns-lys, and Dns-Aspn/Dns-Glun were resolved in all the solvent systems except in A_2.

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