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R. Bhushan^a; G. P. Reddy^a ^a Department of Chemistry, University of Roorkee Roorkee, India

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

R. BHUSHAN AND G. P. REDDY

Department of Chemistry University of Roorkee Roorkee - 247 667 India

ABSTRACT

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

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INTRODUCTION

The resolution and identification of PTHand dansyl amino acids is required for the DNP-, sequence determination of proteins. N-terminal We have earlier reported several successful thin layer chromatographic methods for the resolution and identification of PTH-amino acids[1,2] DNPand 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 outby 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 twodimensional chromatography many Dns-amino acid pairs such Dns-Asp/Dns-Glu, Dns-Thr/Dns-Ser as Dns-His/Dns-Arg/Dns- ≪ -Lys/Dns- € -Lys[13] and remained unresolved and required additional an 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

	-					
			ystem	ns		
S.N	. Dansyl amino acid	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.	$N- \prec -dansyl-L-Tryptophar$	n 51	53	46	40	21
8.	Dansyl-L-Phenylalanine	77	76	74	52	40
9.	Dansyl-L-Valine	72	88	6 5	48	35
10.	Dansyl-L-Norvaline	75	81	68	45	37

TABLE - 1

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

S₁ : n-heptane-BuOH-HOAc (20:8:3).

- S₂ : Dichloromethane-MeOH-propionic Acid (30:1:0.5).
- S₂ : Chloroform-HOAc-Ethylacetate(24:5:4).
- S₄ : Chloroform-MeOH-Ethylacetate (23:8:2).
- S₅ : Chloroform-Propionic acid-Ethylacetate (23:6:4).

R_f values are average of five determinations.

sets of ten derivatives respectively. two It was observed that Table 1 the 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

Dansyl amino acid			Solvent systems					
S.No. Dansyl amino acid			^A 3	A ₄	A ₅			
N-∝-dansyl-L-Asparagin	56	75	53	30	35			
Dansyl-L-Asparatic acid	66	72	60	64	30			
🛩 -Dansyl-L-Arginine	7	12	3	2	3			
N-N-didansyl-L-Cystine	84	83	68	45	18			
Dansyl-L-Cysteic acid	82	80	25	15	11			
Dansyl-L-glutamic acid	80	90	84	74	55			
Dansyl-L-glutamine	62	77	63	41	40			
N- ϵ -dansyl-L-Lysine	16	20	10	6	8			
N-dansyl-L-Serine	72	85	72	58	32			
Dansyl-L-Threonine	76	88	76	68	45			
	N-∝-dansyl-L-Asparagin Dansyl-L-Asparatic acid ∝-Dansyl-L-Arginine N-N-didansyl-L-Cystine Dansyl-L-Cysteic acid Dansyl-L-glutamic acid Dansyl-L-glutamine N- € -dansyl-L-Lysine N-dansyl-L-Serine Dansyl-L-Threonine	N-∝-dansyl-L-Asparagin 56 Dansyl-L-Asparatic acid 66 ∝-Dansyl-L-Arginine 7 N-N-didansyl-L-Cystine 84 Dansyl-L-Cysteic acid 82 Dansyl-L-glutamic acid 80 Dansyl-L-glutamine 62 N-€-dansyl-L-Lysine 16 N-dansyl-L-Serine 72 Dansyl-L-Threonine 76	N- \checkmark -dansyl-L-Asparagin5675Dansyl-L-Asparatic acid6672 \checkmark -Dansyl-L-Arginine712N-N-didansyl-L-Cystine8483Dansyl-L-Cysteic acid8280Dansyl-L-glutamic acid8090Dansyl-L-glutamine6277N- ε -dansyl-L-Lysine1620N-dansyl-L-Serine7285Dansyl-L-Threonine7688	N- \checkmark -dansyl-L-Asparagin567553Dansyl-L-Asparatic acid667260 \checkmark -Dansyl-L-Arginine7123N-N-didansyl-L-Cystine848368Dansyl-L-Cysteic acid828025Dansyl-L-glutamic acid809084Dansyl-L-glutamine627763N- \pounds -dansyl-L-Lysine162010N-dansyl-L-Serine728572Dansyl-L-Threonine768876	N- \checkmark -dansyl-L-Asparagin56755330Dansyl-L-Asparatic acid66726064 \checkmark -Dansyl-L-Arginine71232N-N-didansyl-L-Cystine84836845Dansyl-L-Cysteic acid82802515Dansyl-L-glutamic acid80908474Dansyl-L-glutamine62776341N- \pounds -dansyl-L-Lysine1620106N-dansyl-L-Serine72857258Dansyl-L-Threonine76887668			

A ₁	:	Dichloromethane-MeOH-Propionic	acid
т		(28:3:2)	

- A₂ : Ethylacetate-MeOH-Propionic acid (22:10:3)
- A₂ : Chloroform-MeOH-HOAc (28:4:2)
- A_{A} : Chloroform-Acetone-HOAc (20:8:4)
- A₅ : 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 without resorting to two-dimensional mode them, in-system calibration with the standards where not possible. Besides, the solvent systems is 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 A₂.

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