Table 10 R_f Values for Dansyl Amino Acids in Various Solvent Systems on Polyamide Sheets

Dansyl amino acid	R_f in solvent system									
	A	B	C	D	E	F	G	H	I	J
1. Ala	0.53	0.48	0.49	0.69	0.69	0.57	0.81	0.68	0.43	0.79
2. Arg	0.05	0.03	0.03	0.91	0.39	0.09	0.76	0.22	0.01	0.06
3. Asp	0.08	0.07	0.10	0.69	0.88	0.10	0.88	0.37	0.12	0.19
4. Cys	0.03	0.03	0.04	0.19	0.43	0.22	0.78	0.09	0.03	0.06
5. Glu	0.15	0.10	0.15	0.66	0.88	0.02	0.88	0.34	0.05	0.30
6. Gly	0.32	0.21	0.32	0.69	0.63	0.48	0.80	0.48	0.28	0.69
7. His	0.07	0.05	0.13	0.96	0.76	0.32	0.84	0.36	0.06	0.18
8. Ile	0.77	0.54	0.65	0.40	0.57	0.71	0.78	0.76	0.60	0.84
9. Leu	0.70	0.49	0.59	0.34	0.57	0.71	0.78	0.75	0.54	0.80
10. Lys (mono)	0.35	0.21	0.38	0.22	0.09	0.63	0.72	0.58	0.09	0.79
11. Lys (di)	0.53	0.37	0.48	0.78	0.69	0.35	0.82	0.40	0.39	0.76
12. Met	0.52	0.36	0.51	0.43	0.59	0.68	0.80	0.62	0.55	0.81
13. Phe	0.57	0.38	0.53	0.31	0.43	0.68	0.77	0.62	0.51	0.81
14. Pro	0.85	0.66	0.71	0.55	0.74	0.46	0.84	0.75	0.69	0.90
15. Ser	0.12	0.07	0.16	0.81	0.71	0.49	0.82	0.42	0.10	0.44
16. Thr	0.15	0.10	0.26	0.81	0.74	0.57	0.82	0.56	0.16	0.56
17. Tyr	0.63	0.47	0.61	0.00	0.00	0.84	0.73	0.65	0.58	0.91
18. Val	0.72	0.56	0.61	0.47	0.67	0.71	0.81	0.80	0.61	0.88
19. Dns-OH	0.00	0.01	0.00	0.51	0.54	0.16	0.74	0.00	0.04	0.04
20. Dns-NH_2	0.51	0.38	0.47	0.71	0.17	0.96	0.49	0.60	0.40	0.91

Solvent systems: A, benzene–acetic acid (9:1); B, toluene–acetic acid (9:1); C, toluene–ethanol–acetic acid (17:1:2); D, water–formic acid (200:3); E, water–ethanol–ammonium hydroxide (17:2:1); F, ethyl acetate–ethanol–ammonium hydroxide (20:5:1); G, water–ethanol–ammonium hydroxide (14:15:1); H, *n*-heptane–*n*-butanol–acetic acid (3:3:1); I, chlorobenzene–acetic acid (9:1); J, ethyl acetate–methanol–acetic acid (20:1:1). All of the proportions are based on volume.

In all cases, dansyl amino acids, because they are fluorescent, have been detected under a UV lamp (254 nm).

C. Dimethylamino Azobenzeneisothiocyanate Derivatives of Amino Acids

Dimethylamino azobenzene isothiocyanate (DABITC) reacts with the NH_2-terminal end of an amino acid in basic medium to give a DABTH amino acid via a DABTC derivative, in a manner similar to the Edman method, where PTH amino acid is obtained by the reaction of PITC. The use of DABITC reagent during amino acid sequencing of proteins (140) has distinct advantages over the use of dansyl chloride; for example, the color difference between DABITC, DABTC derivatives, and DABTH amino acid greatly facilitates direct visualization and identification with TLC. DABTH amino acids are colored compounds with absorption maxima at 520 nm in acid media (ε = 47,000). Thus, using the visible region, the quantification and identification of these derivatives become more convenient and sensitive (10 pmol by polyamide TLC).

1. Preparation of Standard α-Mono-DABTH Amino Acids*

Amino acids (0.5 mg) are dissolved in 100 μL of triethylamine–acetic acid buffer (50 mL water + 50 mL acetone + 0.5 mL triethylamine + 5 mL of 0.2 M acetic acid, pH 10.65) and treated with DABITC solution (50 μL, 4 nmol/μL in acetone). The mixture is heated at 54°C for 1 h,

*Method of preparation from Refs. 141 and 142.