Table 17 hR_f Values of 15 Amino Acids on Silica Gel Impregnated with Zn, Cd, and Hg Salts

	A	B	C	D	E	F	G	H	I	J
Thr	25	55	42	41T	35	36	42	33	50	40
Ser	12	38	39	28T	32	29	31T	15	40	31T
Gly	10	35	29	23T	28	25	28	16	35	27T
Lys	03	13	07	05	51	08	05	04	10	05
Ala	30	48	40	31	38	36	38	20	45	35
Tyr	60	60	52	50	48	45	51	62	55	56
Ile	55	67	56	52	50	48	54	50	60	53
Leu	50	65	55	55	52	50	56	47	65	55
Cys	00	00	00	00	00	00	00	00	00	00
Met	45	62	48	48	48	42	48	39	54	45
Glu	18T	43	38	36T	34	27	38T	18	36	34T
Trp	57	60	53	51	51	44	54	45	60	47
Phe	54	67	57	55	55	46	57	58	68	52
Val	50	63	45	50	52	42	56	47	57	45
Arg	07	19	13	13	09	11	11	10	15	08

Solvent, butyl acetate–methanol–acetic acid–pyridine (20:20:5:5). Developing time, 30 min. Detection limit, 10^{-4} M. Solvent front, 10 cm. A, plain silica gel; B, C, D, 0.5%, 0.2%, 0.1% Zn^{2+}-impregnated, respectively; E, F, G, 0.5%, 0.2%, 0.1% Cd^{2+}-impregnated, respectively; H, I, J, 0.5%, 0.2%, 0.1% Hg^{2+}, respectively.
Source: Ref. 25.

V. HPTLC/OPTLC

Improvements in all practical aspects of the TLC process culminated in a performance breakthrough, resulting in an increase in separation efficiency, sample detectability limits, and reduced analysis time; the specific advance in instrumentation was termed HPTLC. Chapters 5 and 7 in this volume describe basic and theoretical aspects of the application of instrumentation in TLC and OPLC. That HPTLC could be used with advantage for the separation of PTH amino acids was recognized by Bucher (150m) and Yang (150n). But they could not achieve separation of all 20 common PTH amino acids. Schuette and Poole (150o) used continuous multiple development on silica gel and were able to separate 18 samples and standards simultaneously using five development steps with four changes in mobile phase and scanning densitometry; typical results are given in Table 24. Butler et al. (150p) separated PTH-Leu/Ile/Pro by HPTLC using multiwavelength detection. Fater (150q) carried out separation and quantification of PTH amino acids by OPLC using chloroform–ethanol–acetic acid (90:10:2) for the resolution of polar PTH derivatives and CH_2Cl_2–ethyl acetate (90:10) for the resolution of nonpolar PTH amino acids. The method was claimed to be superior to HPTLC methods (150m,150n,150p) in having relatively increased migration distance resulting in the resolution of complex mixtures containing a large number of derivatives. OPTLC (149) and HPTLC on RP-8, RP-18, and homemade ammonium tungstophosphate layers (150) have also been used for the analysis of DNP amino acids.

Norfolk et al. separated 18 amino acids on cellulose, silica gel, and chemically bonded C_{18} modern HPTLC plates, all of which except the cellulose contained a preadsorbent zone (72a); quantification was carried out by scanning standard and sample zones at 610 nm. hR_f values of amino acid standards on reversed-phase and normal-phase layers in various solvents are given in Tables 25 and 26, respectively. Amino acids were identified in water conditioned by four strains of snails using cellulose HPTLC plates developed with 1-propanol–water (2:3), and detection with ninhydrin spray reagent and valine content was quantified by densitometric scanning at 610 nm (72b).