acid sodium and calcium salts are often used as additives because they are easily handled and stable. Panthenol is usually used in liquid pharmaceutical preparations and in cosmetics.

A rapid, simple, and specific TLC method was developed for estimation of panthenol and pantothenic acid in pharmaceutical preparations containing other vitamins, amino acids, syrups, and enzymes (36). Panthenol and pantothenic acid were extracted with ethanol (tablets and capsules) or benzyl alcohol (liquid oral preparations) and isolated from other ingredients by TLC on silica gel 60 plates with 2-propanol–water (85:15) as solvent. β-Alanine (pantothenate) or β-alanol (panthenol) was liberated by heating for 20 min at 160°C. The liberated amines were visualized by ninhydrin reaction and estimated by spectrodensitometry at 490 nm. Recoveries for panthenol and pantothenic acid were 99.8 ± 2.25% and 100.2 ± 1.7%, respectively.

An overpressured layer chromatographic procedure with photodensitometric detection for the simultaneous determination of water-soluble vitamins in multivitamin pharmaceutical preparations was developed and evaluated (35). HPTLC on silica gel plates with 1-butanol–pyridine–water (50:35:15) as mobile phase was used. The quantification was carried out without derivatization (vitamin B_1, vitamin B_2, vitamin B_6, folic acid, nicotinamide, vitamin C) or after spraying ninhydrin reagent (calcium pantothenate) or 4-demethylaminocinnamaldehyde (vitamin B_{12}, biotin). This method was applied to the analysis of multivitamin solutions (Table 7).

VIII. BIOTIN

Biotin, hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-pentanoic acid, has been isolated from egg yolk (Fig. 8). Biotin is required by all living cells but is biosynthesized only by plant, fungi, and most microorganisms. Sources of exogenous biotin for animals are found in yeast extracts, liver, kidneys, egg yolks, milk, and cereals. Biotin is very stable and can be autoclaved without being affected. However, biotin can be easily oxidized into biotin sulfoxides and biotin sulfone in very dilute solution.

Several unidentified avidin-binding substances in human urine were analyzed and identified by TLC (37). Urine was collected before and after intravenous administration of 18.5 μmol biotin to healthy adults. As shown in Table 8, unknown substances 1, 3, and 6 were identified as biotin sulfone, bisnorbiotin methyl ketone, and tetranorbiotin-*l*-sulfoxide, respectively, by derivatization with *p*-demethylaminocinnamaldehyde after TLC separation. Recently, biotin and metabolites were accurately assayed in urine from six healthy adults (38); of that total, biotin accounted for 32 ± 12%, bisnorbiotin for 52 ± 15%, bisnorbiotin methyl ketone for 7.9 ± 5.8%, biotin-*d*,*l*-sulfoxide

Table 7 Resolution of Water-Soluble Vitamins by Overpressured Liquid Chromatography (OPLC) Versus Conventional HPTLC

	Resolution	
Vitamin	OPLC	HPTLC
Vitamin B_1	6.16	—
Vitamin B_2	1.44	0.30
Folic acid	0.37	0.37
Vitamin B_{12}	3.37	0.84
Nicotinamide	1.00	0.8
Vitamin C	1.86	1.02
Calcium pantothenate	0	0.72
Biotin	0.71	0
Vitamin B_6	—	0.64

Eluant used was 1-butanol–pyridine–water (50:35:15).
Source: Ref. 35.