

philic or water-soluble vitamins. A satisfactory separation for α -, β -, γ -, and δ -tocopherol and vitamin A acetate was obtained on silica gel G using cyclohexane-*n*-hexane-isopropyl ether-ammonium hydroxide (40:40:20:2) as mobile phase (159). Perisic-Janjic et al. (160) described a method for the quantitative analysis of lipophilic vitamins by thin-layer chromatography on starch, cellulose, and talc impregnated with paraffin oil. Vitamins A acetate, A palmitate, K₁, DL- α -tocopherol, DL- α -tocopherol acetate, D₂, and D₃ were separated with acetone-concentrated acetic acid (3:2) while vitamins K₃, K₄, and K₅ migrated with the front (Table 11). Differences between the R_f values were satisfactory (except for vitamins D₂ and D₃) and allowed for accurate identification.

On silica gel plates using diphacinone, pindone, valone, warfarin, and bromadiolone, vitamins K₁ and D₃ were separated with three mobile phases. No phase used alone could separate all seven compounds. However, vitamins K₁ and D₃ were separated with a mixture of dichloromethane-methanol-acetic acid (45:4:1) (R_f 0.75 and 0.59, respectively) and with a mixture of chloroform-methanol (97:3) (R_f 0.92 and 0.68, respectively) (82).

Thielemann (161,162) separated vitamins A, D₂, and E from vitamins B₁, B₂, B₆, and C; nicotinamide; and panthenol, which occur in the multivitamins Summavit® (Jenapharm) and Turigeran® (Jenapharm). Lipophilic vitamins were separated on silica gel with benzene-petroleum ether-acetic acid (35:65:1). R_f values for vitamins A, D₂, and E were 0.71, 0.18, and 0.07, respectively. This method can be used in pharmaceutical investigations. Bączyk et al. (163) described a method for the determination of vitamins D₂ and K₁ in the presence of rutin added as stabilizer and assessed the rate of breakdown of these vitamins when exposed to ultraviolet light. TLC was done on silica gel H. The best developing agent was chloroform. The R_f values were 0.60 for vitamin K₁, 0.34 for vitamin D₂, and 0 for rutin. Quantitative determination of vitamins D₂ and K₁ was done by spectrophotometric analysis. Riboflavin, ascorbic acid, and nicotinamide in pharmaceutical preparations were separated by TLC on silica gel H F₂₅₄ with chloroform-95% ethanol-acetic acid-water (54:27:9:4) as mobile phase by Ni et al. (164). Vitamin A, vitamin D, and α -tocopherol were extracted from the sample with light petroleum, and the extract was subjected to TLC on silica gel H F₂₅₄ with light petroleum-ethyl ether (4:1) as mobile phase. Detection was by scanning at 440 nm (700 nm reference wavelength) for riboflavin and at 250, 260, and 300 nm (400 nm reference wavelength) for ascorbic acid, nicotinamide, and vitamin A, respectively. Recovery was 99.9%, and the corresponding coefficient of variation was 1.9% for vitamin A.

A method was described for the simultaneous determination of retinol and α -tocopherol in plasma by Chavan and Khatri (165). The sample was mixed with methanol, then extracted with heptane containing α -tocopheryl acetate (internal standard). After vortex mixing, a portion of the extract was applied to a silica gel F₂₅₄ HPTLC plate, which was developed with chloroform-cyclohexane (11:9) and evaluated densitometrically with a Camag TLC Scanner II or by diffuse reflectance absorbance at 290 nm. Calibration graphs were rectilinear for 0.2-1.4 and 3-21 μ g/mL of retinol and α -tocopherol, respectively. The corresponding detection limits were 0.16 and 1.2 μ g/mL.

Avocado (*Persea americana*) is a fruit of unusually high oil content and is relatively rich in chlorophyll. TLC detected the presence of avocado seed oil in various avocado oils. Avocado oils, extracts, and mixtures were subjected to cold ethanol precipitation. The precipitate was discarded and the ethanol was evaporated. Samples (10 mg) were applied as a single spot on a TLC plate and eluted with petroleum ether (60-80°C)-ethyl ether (1:1). Standards of β -sitosterol, α -tocopherol, squalene, β -carotene, and β -amyryn were used to characterize unsaponifiable components and separated by TLC. The plates were sprayed with 50% H₂SO₄, and the color was developed at 115°C for 10 min (166).

Most of the prenillipids, such as chlorophylls, carotenoids, and prenilyquinones, as well as tocopherols and vitamin K₁, which occur in plant lipid extracts, can be separated by TLC using silica gel plates or special mixtures of silica gel with other adsorbents (142,167). But the compounds with one double bond per isoprene and others with a partially or fully unsaturated isoprenoid chain can be separated efficiently by argentation TLC. The separations of prenilyquinones and prenols using adsorption TLC and argentation TLC are demonstrated in Fig. 8. The R_f values