raphy, using platinum black catalyst reduction and fluorometric detection. Adsorption preparative thin-layer chromatography was done on silica gel 60 F_{254} with the mobile phase petroleum ether–diethyl ether (85:15). After preparative TLC using benzene or methanol–benzene (1:2 or 1:4) as mobile phase, menaphthone (vitamin K_3) was also determined by GC (148).

A case of hemorrhage of unknown origin was observed in cattle; their liver samples were submitted to the diagnostic laboratory for assay of vitamin K by Madden and Stahr (149). After evaluating normal-phase and reversed-phase thin-layer chromatography plates with different solvents, reversed-phase TLC plates and a mobile phase of methylene chloride–methanol (7:3) were selected for the determination of vitamin K. The R_f value of vitamin K was 0.75. Levels as low as 0.2 μg were detected. Gas chromatography and densitometry can be used to quantify vitamin K in bovine liver. Mass spectroscopy can be used to confirm vitamin K present in the extracts. The method involves cleanup of tissue homogenate extracts on a Sep-Pak silica cartridge followed by separation of the vitamins by TLC on silica gel 60 F_{254} as given by Hirauchi et al. (150). Separated vitamins were extracted from the silica and determined by HPLC. The eluate was subjected to coulometric reduction. The method was used in the analysis of liver, spleen, kidney, heart, and muscle. Recoveries were 73.5–91.8%, and detection limits were in the picograms per gram or picograms per liter range.

Mazulin and Kaloshina (151) described a very simple, highly sensitive method for the quantitative determination of vitamin K in milfoil plants. A portion of extracted, filtered, coagulated, cooled, and filtered sample was used for spectrophotometric determination at 265 nm. Linear calibration was followed in the range of 2–42 μg/mL. The presence of vitamin K was confirmed by using TLC on Silufol UV254 plates (Kavalier, Czech Republic) with two mobile phases—cyclohexane–diethyl ether (4:1) and hexane–diethyl ether–acetic acid (9:1:0.1)—with phosphomolybdic acid as the detection agent. This method can be used by analytical laboratories for crude drug analysis.

Menaquinone-7 was isolated from *Pseudomonas* N.C.I.B. 10590 and identified by reversed-phase thin-layer chromatography and gas chromatography mass spectral analysis (152). Ubiquinones extracted from 24 strains of *Legionella pneumophila* and from 44 strains of other *Legionella* species were also analyzed by reversed-phase TLC on octadecylsilane-bonded reversed-phase $KC_{18}F$ TLC plates (Whatman) using acetone–water (19:1) as mobile phase. Ubiquinone profiles as determined by this method were reproducible, both qualitatively and semiquantitatively, and provided information to aid in the identification of species of *Legionella* (140). Ubiquinone was also analyzed in the rat tapeworm *Hymenolepis diminuta* and in yeast, using TLC for isolation and HPLC for determination (153,154). Menaquinone-6 and a methyl-substituted menaquinone-6 were the major isoprenoid quinones found in membrane preparations of *Campylobacter jejuni* and *Campylobacter fetus*. By reversed-phase HPLC and TLC the faster-eluting menaquinone-6 cochromatographed with a menaquinone-6 standard. The identity of menaquinone-6 was confirmed by UV spectrophotometry, mass spectrometry, and nuclear magnetic resonance (NMR). The slower-eluting methyl-substituted menaquinone-6 cochromatographed with a menaquinone-7 standard by reversed-phase TLC on C-18 RPTLC plates (Analtech, Newark, DE, USA) with a solvent system of methanol–acetone (1:1) but eluted between menaquinone-6 and menaquinone-7 standards by HPLC (155).

Menaphthone (vitamin K_3), extracted from food products (butter, margarine, yogurt, beef, pork, chicken, cheese, eggs, milk), was purified on a Sep-Pak silica cartridge and/or a Sep-Pak silica cartridge followed by TLC, then measured by HPLC on an ODS-UH column with 45 dioxane saturated with argon and containing 0.2% $NaClO_4$. The detection limit for vitamin K_3 was 50 pg/g or pg/mL in foods (156).

Sakamoto et al. (134) determined phylloquinone (K_1) and menaquinone-4 (MK-4) in plasma and liver. They used adsorption TLC on silica gel (Merck) with 85% petroleum ether–15% ethyl ether for purification. Final separation, however, was done by HPLC. This method is useful for vitamin K studies on rats, which require micro- and multisampling methods.

D. Detection of Vitamin K

All lipoquinones at levels of 0.5 μg or more are visible as dark spots on layers containing inorganic fluorescent material when illuminated with UV light, after adding Na-fluorescein or