by washing with water or buffer or a weak organic–aqueous solvent that will not elute the analyte [e.g., water (buffer)–methanol (9:1)]. The analyte is eluted with a nonpolar solvent such as methanol, acetonitrile, tetrahydrofuran (THF), hexane, or methylene chloride.

Polar extraction. A nonpolar solution containing a polar analyte is applied to an SI, CN, 2OH, or NH_2 column that was preconditioned with the nonpolar solvent in which the analyte is dissolved, such as hexane or chloroform. Viscous samples are diluted in a nonpolar solvent, and water is removed from the sample, e.g., by filtration through Whatman phase-separating paper. Nonpolar interferences are removed by washing with a nonpolar solvent or a polar–nonpolar mixture that is not strong (polar) enough to elute the analyte. The analyte is recovered by elution with a polar solvent such as methanol or isopropanol.

Anion-exchange extraction. An aqueous, low ionic strength sample (water, plasma, diluted urine) containing inorganic or organic anions is applied to an SAX, NH_2, PSA, or DEA column. Both the chosen column and the analyte must be ionic for exchange to occur. The column is conditioned with methanol followed by a buffer whose pH is 2 units above the pK_a of the analyte and <7.8 for NH_2, PSA, and DEA columns. The sample pH is adjusted as above for conditioning and applied to the column. Interferences are removed by washing with the sample buffer and with an organic solvent such as acetonitrile or methanol, if necessary. The analyte is eluted with a buffer whose pH is at least 2 units below the analyte pK_a, a buffer whose pH is 2 units above the column pK_a, or a buffer of high ionic strength (>0.1 M). The eluents can be totally aqueous or aqueous–organic mixtures; addition of an organic modifier such as methanol may improve analyte recovery.

Cation-exchange extraction. An aqueous, low ionic strength sample containing inorganic or organic cations is applied to an SCX, PRS, or CBA column preconditioned with methanol followed by a buffer whose pH is 2 units below the analyte pK_a and >6.8 for the CBA column. The sample pH is adjusted in the same manner. Interferences are eliminated by elution with the sample buffer and with an organic solvent, if necessary. The analyte is eluted with a buffer at least 2 units above the analyte pK_a, a buffer of pH <2.8 for the CBA column, or a buffer of high ionic strength (>0.1 M). Addition of an organic modifier such as methanol may improve analyte recovery.

Examples of applications of SPE prior to TLC analysis include analysis for pesticides in fruits and vegetables according to the official German multimethod S19 using SPE on silica gel and amino cartridges prior to HPTLC with gradient elution AMD (60); oxygenated cholesterol derivatives in plasma using silica gel SPE (61); quinoline and quinuclidine alkaloids in pharmaceutical preparations using cation-exchange SPE (62); rutin in glycerinic plant extracts using Envi-18 (Supelco) cartridges (63); and aflatoxins in a variety of foods using phenyl, silica, C_{18}, and Florisil-C_{18} cartridges (64). A strategy for choosing SPE cartridge elution solvents based on the PRISMA TLC mobile-phase optimization procedure was demonstrated for extraction of furocoumarin isomers and flavonoid glycosides from medicinal and aromatic plants (65).

The use of immunoaffinity columns for sample cleanup is among the newest sample preparation procedures. Immunoaffinity cleanup was used after methanol extraction for determination of aflatoxins B-1, B-2, G-1, and G-2 in various food matrices by TLC-densitometry (66).

Of the current sample preparation methods (46,48), only SPE (above) and SFE have had substantial use in combination with TLC. Automated Soxhlet extraction, microwave-assisted extraction (MAE), and accelerated solvent extraction (ASE) have good potential for preparing solid samples for TLC analysis, but published methods have not yet appeared. Stahl first interfaced SFE with TLC in 1977, and there has been increasing interest in developing new methods in recent years. Examples of SFE-TLC analyses reported include cyanizine herbicide in soil (67); flavonoids in *Scutellariae radix* (68); aloin and aloe-emodin in consumable aloe products (69); semivolatile compounds in cassia and cinnamon (70); and residues of 20 pesticides of multiple classes in soil (71). Hydroperoxides in combustion products were separated from solid matrices using SFE with on-line transfer to TLC plates (72).

6. Additional Sample Preparation Procedures

Additional procedures performed prior to TLC analysis, depending on the sample type, include drying, grinding, freeze-drying (removal of water), drying of extracts (passage through a drying