involved TLC of a plant extract on aluminum-backed silica gel HPTLC plates developed with chloroform–ethanol (4:1). After separation, the track to be measured was removed from the plate and glycerol and dimethyl sulfoxide (DMSO) cosolvent was applied prior to attaching the track to a commercial TLC/MS probe. Mass spectrometry and MS-MS spectra of individual components were obtained by liquid secondary ion mass spectrometry (LSIMS) (30 keV cesium ions) using a tandem mass spectrometer. In the second, insect egg extract was analyzed with similar methodology except that samples were introduced manually after scraping the appropriate layer material and adding glycerol and cosolvent. Mass spectra obtained for polypodine B and 2-deoxyecdysone are illustrated in the Wilson and Morden paper.

VI. QUANTITATIVE ANALYSIS

Steroids have been quantified by TLC using scraping and elution of separated analyte zones in sample and standard chromatograms, usually followed by visible or UV spectrometry or fluorimetry. An unspotted layer area adjacent to the zones is analyzed simultaneously and identically as a blank for background correction.

More common today is in situ quantification by optical densitometry with a slit-scanning densitometer (see Chaps. 5 and 10). Compounds that absorb UV light (e.g., corticosteroids) are scanned directly with the UV mode of the densitometer on a nonfluorescent layer at the wavelength of maximum absorption, or more commonly as quenched zones on an F-layer containing a fluorescent indicator at 254 nm. Compounds that are detected as colored zones with a postchromatographic derivatization reagent are scanned in the visible mode at the wavelength of maximum absorption in the 370–700 nm range. Compounds that become fluorescent by pre- or postchromatographic reaction with a fluorogenic reagent or by thermal activation are scanned with the fluorescence mode of the densitometer using a mercury or deuterium UV source lamp for excitation and visible range emission measurement. Reflectance scanning is most often performed. If they are to be meaningful, the results of quantitative analyses should be validated in terms of factors such as accuracy, precision, detection limit, range of applicability, linearity, specificity (resolution), and robustness.

The remainder of this section contains references to papers that have been published on the quantitative determination of steroids in various samples by TLC-densitometry.

Steroid sapogenins (diosgenin, hecogenin, manogenin), steroid alkaloids (solasodine, tomatidine), and total steroids have been assayed by visible absorbance-reflectance scanning after detection of zones with anisaldehyde–sulfuric acid reagent. The method was found to be faster, simpler, and cheaper than HPLC or GC (3).

Corticosteroids and their esters (e.g., betamethasone dipropionate, dexamethasone, hydrocortisone, testosterone) in pharmaceutical preparations of creams and ointments were identified and quantified by silica gel TLC for the purpose of quality control. Mobile phases were chloroform–ethyl acetate (2:1) for esters and chloroform–ethyl acetate (1:1) for free corticosteroids, and detection was with tetrazolium blue chromogenic reagent. Densitometry was performed at 240 nm before spraying with the reagent or at 520 nm after spraying (59).

Three papers (60–62) reported method development and results for the determination of the ecdysteroids 20-hydroxyecdysone and polypodine B from *Serratula tinctoria* and *Serratula wolffii* (Asteraceae) using silica gel layers, successive development with dichloromethane–ethanol (4:1) and chloroform–methanol–benzene (25:5:3), and reflectance densitometry at 254 nm. Plant material was extracted with methanol, and extracts were cleaned up on a polyamide column prior to TLC. Seasonal dependence of the steroid levels in leaves was studied.

Cholic acid and deoxycholic acid were determined in commercial bile salts on silica gel with isooctane–ethyl butyrate–acetic acid–water (18:5:3:1) as mobile phase. Detection was by spraying with 30% sulfuric acid in ethanol and heating at 110°C, and quantification was by scanning at 385 nm (63). Bile acids were quantified in artificial Niuhuang by TLC on silica gel with chloroform–diethyl ether–acetic acid (2:2:1), detection with 10% PMA acid in ethanol, and visible absorbance densitometry at 540 nm (64).