centered at the point of application, thereby facilitating direct quantification by densitometry at different wavelengths. The detection level was 0.1 ng. The quantity of sample applied and the areas and peak heights of the spots formed on the plate were directly related. The antibiotics applied were gentamycin, erythromycin, tobramycin, amikacin, ciprofloxacin, norfloxacin, ofloxacin, cefotetan, aztreonam, clavalinic acid, imipenem, vancomycin, rifampin, trimethoprim, chloramphenicol, clindamycin, nitrocefin, tetracycline, and sulfamethazole.

Multiclass qualitative detection of chloramphenicol, nitrofuran, and sulfonamide residues in animal muscle was described (117). The drugs were extracted from animal tissue with ethyl acetate and purified by silica SPE. The extract was spotted and chromatographed on the HPTLC silica gel plate. Nitrofurans were visualized first by their specific UV photochemical reaction with pyridine. Chloramphenicol was then reduced to its amino derivative, and the derivative and sulfonamides were sprayed with fluorescamine and examined under UV light. Detected residue levels were 10, 5, and 100 ppb for chloramphenicol, nitrofurans, and sulfonamides, respectively.

A simple classification method for 24 antibiotics using a TLC bioautographic procedure was described (118). The antibiotics were divided into four groups (β-lactams, aminoglycosides, macrolides, and tetracyclines) by developing silica gel plates with an ammonium chloride solution in a graded concentration range (0.5%, 1%, 2%, 3%, 5%, 10%, and 20%). For bioautographic detection *Bacillus subtilis* and *Micrococcus luteus* were used.

A TLC method was described for identification and quantification of oxytetracycline, tiamulin, lincomycin, and spectinomycin in veterinary preparations (119). Silica gel plates were developed with two mobile phases: 10% aq. citric acid–*n*-hexane–ethanol (80:1:1) and *n*-butanol–ethanol–chloroform–25% ammonia (4:5:2:5). R_f values, spot colors, and direct UV and densitometric measurements were used for identification. The results of quantitative analysis were close to the declared contents of the constituents. The recoveries ranged from 100.01% to 102.54%.

Markakis presented two papers on TLC separation and detection combined with microbiological quantification by the agar diffusion method of various antibiotics. The first paper (120) reported the determination of oxytetracycline and chlortetracycline in the presence of 11 other drugs (nitrofurans, macrolides, sulfonamides, coccidiostatics). The second paper (121) reported the determination of erythromycin and tylosin in the presence of 11 other drugs (nitrofurans, tetracyclines, sulfonamides, coccidiostatics).

A routine method was described (122) for detecting the following antimicrobial agents in feeds: avilamycin, avoparcin, Zn-bacitracin, erythromycin, flavomycin, furazolidone, lasalocid, monensin, narasin, penicillin, salinomycin, spiramycin, tetracyclines, tylosin, and virginiamycin. The method uses the agar diffusion of buffered samples, neutral extraction of polyether antibiotics followed by thin-layer chromatography, and acid extraction for other antibiotics followed by TLC. Identification after TLC was achieved by bioautography.

Vega et al. (82) described HPTLC analysis of various antibiotics in fish feed: (a) oxolinic acid on silica impregnated with 0.1 M K_2HPO_4 with chloroform–acetone (9:1), (b) erythromycin on silica with ethyl acetate–acetic acid–water (6:2:2), and (c) oxytetracycline on silca impregnated with EDTA with dichloromethane–methanol–water (59:35:6).

A TLC method was described (123) for detecting tetracycline and amino glycopeptide antibiotics on silica gel plates developed with ethanol–acetic acid–water (10:6:6) and butanol–formic acid–water (6:5:7). Detection was done by exposing the obtained spots to iodine vapor.

A TLC screening method was established for determining flumequine and doxycycline in milk; these two antibiotics are often used in veterinary treatment (124). Samples of milk spiked with the antibiotics were applied onto the concentrating zone of a silica gel plate with fluorescent indicator and predeveloped with hexane to remove lipids. The plate was then developed with the mobile phase. Several mobile phases were tested; the most effective was 0.05 M citric acid–methanol–2-propanol (1:3:1). Spots were detected under UV light at 254 nm for flumequine and at 366 nm for doxycycline. Calibration curves of the antibiotics were obtained by scanning in the reflection mode at 325 nm for flumequine standards and at 360 nm for doxycycline standards. The recoveries were close to 100%. A similar TLC method but combined with bioautography was also described by Choma et al. (125).