**Table 6** Detection Modes of the Quinolone Drugs on HPTLC Plates

| | | Limit of detection[a] ($\mu$g) | | |
|---|---|---|---|---|
| No. | Detection reagent | SPA | FLE | CIN |
| 1 | UV irradiation ($\lambda$ = 254 nm) on Si 60 $F_{254}$ | 0.10 | 0.10 | 0.01 |
| 2 | Acidified phosphomolybdenate reagent | 0.10 | 0.10 | — |
| 3 | Folin–Ciocalteu reagent | 0.50 | 0.50 | — |
| 4 | Alkaline solution of $KMnO_4$ | 0.50 | 0.50 | — |
| 5 | Iodine reagent | 0.50 | 0.20 | 0.10 |
| 6 | Dragendorff reagent (after Amelink) | 0.05 | — | — |
| 7 | Forrest reagent | 0.05 | 0.05 | 0.05 |
| 8 | 15% $FeCl_3$ in 5% HCl solution | 0.10 | 0.10 | 0.05 |

[a]SPA, sparfloxacin; FLE, fleroxacin; CIN, cinoxacin.
*Source*: Ref. 87.

were used (91): (a) silica gel developed with 0.19 M $(NH_4)_2HPO_4$–methanol (1:1); (b) silanized silica gel developed with 0.357 M $NH_4NO_3$–methanol (7:3); and (c) silanized silica gel developed with 0.1 M hexane sulfonic acid–methanol (1:1). After development in a saturated tank, plates were dried at 100°C for 30 min, sprayed with ninhydrin, and heated at 100°C for 2 min. The bleomycin components appeared as purple spots on a white background. The first TLC system was combined with bioautographic detection using *Bacillus subtilis* or *Mycobacterium smegmatis*.

Vancomycin, discovered in 1956, is a major antibiotic for the treatment of life-threatening gram-positive bacterial infections. Four TLC systems were developed for the separation of vancomycin, related antibiotics, and degradation products (92):

1. Reversed-phase plates developed with 5% $NH_4HCO_3$–dioxane (3:7)
2. Silica gel developed with 1% $NH_4HCO_3$–methanol (9:1)
3. Silica gel developed with 1% $(NH_4)_2CO_3$–2-propanol (9:11)
4. Carboxymethylcellulose developed with 0.9% $(NH_4)_2HPO_4$, pH 8.3

The plates were dried and sprayed with a freshly prepared aqueous solution of *p*-nitrobenzenediazonium tetrafluoroborate. Alternatively, bioautography was performed with *Bacillus subtilis*. To enhance the contrast between the zones of inhibition and the background, the bioautogram was sprayed with *p*-iodonitrotetrazolium.

Gramicidin in fermentation samples and bulk products was quantified on HPTLC silica gel plates developed with the solvent acetic acid–butyl acetate–1-butanol–methanol–water (20:40:7.5:2.5:12) (93). The plates were dried for 15 min at 115°C, sprayed with 4-dimethylaminobenzaldehyde, heated for 3 min at 90°C, and scanned at 570 nm.

A variety of RP-TLC systems were investigated to find optimal conditions for analysis of 19 peptide-type antibiotics: cycloserine, hadacidin, azaserine, viomycin, echinomycin, polymyxin $B_1$, colistin S, actinomycin $C_2$, bacitracin A, phleomycin, bleomycin S, thiostrepton, saramycetin, gramicidin A, cinnamycin, duramycin, neocarzinostatin, restrictocin, and largomycin F-II (94). For that purpose, 27 mobile phases were used, representing three organic modifiers, three buffers, and three pH values. With only slight modifications of mobile phases, those systems were used for RP-HPLC.

Virginiamycin fermentation broth contains seven antibiotics and a number of pigments as contaminants. The HPTLC method using silica gel plates prewashed with methanol–chloroform (1:1) and developed with chloroform–methanol (92:8) was established for their separation (95). The developed plates were dried and quantified at 235 nm. Because of the lack of commercially available standards, they were isolated from fermentation broth and purified. Their purity (98%) was confirmed by UV spectrophotometry and HPLC. The chromatograms obtained for standards and components of fermentation broth are presented in Fig. 5. Separation and quantification of