system, such as oxalic acid and Na_2EDTA, do not cause any problems, because they remain on the TLC plate. To prevent diffusion of the analyte and to obtain high sensitivity from the TLC-FAB-MS, a sample condensation technique was developed (3).

Naidong et al. (69) described a procedure for identification of tetracyclines—CTC, TC, OTC, DC, DMCTC, MTC, and MINO—by TLC on silica gel previously sprayed with a 10% solution of Na_2EDTA with dichloromethane–methanol–water (59:35:6) as the mobile phase (69). The same chromatographic system was then used for assay and purity control of oxytetracycline and doxycycline (70) and tetracycline (71,72). Results were compared with those obtained previously by HPLC on a poly(styrene-divinylbenzene) phase. Chlortetracycline was assayed using the same system, whereas for demeclocycline the proportions of the mobile phase were changed to 60:35:5 (73). All the major impurities were well separated from the main components and from each other. The results were compared with those obtained on silanized silica gel with a methanol–acetonitrile–0.5 M oxalic acid (pH 2) (1:1:6) mobile phase and with HPLC on a poly(styrene-divinylbenzene) phase; good correlation was obtained. The mobile phase dichloromethane–methanol–water (59:35:6 or 60:35:5) was used for purity control by semiquantitative TLC of six tetracyclines: DC, CTC, OTC, TC, MTC, and DMCTC (74). After development, the plates were dipped in a 30% solution of liquid paraffin in hexane and inspected under UV light at 365 nm. The fluorescence was stable for long periods of time.

Minocycline was separated from impurities using the above-described TLC method. The stationary phase was silica gel impregnated with Na_2EDTA, and the mobile phase was dichloromethane–methanol–water (57:35:8) (75). 6-Deoxy-6-demethyltetracycline was selectively determined by fluorescence densitometry, and other impurities and MINO were quantified by UV densitometry. Results were compared with those obtained by HPLC on the poly(styrene-divinylbenzene) phase. A similar method was described for the assay and purity control of metacycline (76).

Using NP-TLC, TC, DC, OTC, and CTC were separated on silica gel with the aid of various mobile phases. The influence of impregnation with Na_2EDTA and activation of chromatographic plates was discussed (77). The same tetracyclines were isolated from milk and separated on silica gel plates impregnated with 5% aq. Na_2EDTA. Samples of milk spiked with tetracyclines were spotted into the middle of specially prepared trapezoidal regions created by making incisions in the concentrating zones of the plate. Development with the mobile phase chloroform–methanol–5% aq. Na_2EDTA (65:20:5) (lower phase) was preceded by predevelopment in the same direction with hexane and acetone (78).

Direct analysis of tetracycline and its impurities on the TLC plate was done by TLC/MALDI-MS (79). Using the electrospray method, the limit of detection for tetracycline from a TLC plate was found to be 1 ng.

Xie et al. (80) presented a TLC-fluorescence densitometry method for the determination of OTC, TC, and DC in honey, serum, and urine. Silica gel TLC plates impregnated with Na_2EDTA were used, and the solvent system was chloroform–methanol–acetone–1% aq. ammonia (10:22:50:18). Kang and Ebel (81) described the separation of eight tetracyclines on cyanophase HPTLC plates developed with methanol–acetonitrile–0.5 M oxalic acid (3:1:6).

F. Quinolones

Quinolone and fluoroquinolone antibiotics are a group of relatively new broad-spectrum synthetic antibiotics. Nalidixic acid, discovered in 1962 by Lescher and coworkers, was the first member of this class, though of rather minor importance. In the 1980s, synthetic fluoroquinolones were developed and became valid antibiotics with high potency and of good tolerance. Quinolone antibiotics consist of a 1-substituted 1,4-dihydro-4-oxopyridine-3-carboxylic moiety combined with an aromatic or heteroaromatic ring. Quinolones are sometimes divided into three generations. First-generation quinolones, e.g., nalidixic and oxolinic acids, have poor anti-gram-positive activity and are predominantly used to treat urinary tract infections. The second-generation quinolones, e.g., norfloxacin and ciprofloxacin, are active against both gram-positive and gram-negative bacteria, whereas those of the third generation, e.g., temafloxacin, have potency against *Staphylococcus aureus* and also against the anaerobic bacteria *Chlamydia* and *Mycoplasma*. Quinolones