gel impregnated with tricaprylmethylammonium chloride (TCMA) using methanol–water mobile phases. The retention increased with increasing TCMA concentration on the plate. The basis of the retention was not ion pairing but hydrophobic interactions due to the caprylic group of TCMA. The pH and ionic strength of the eluent had no effect on retention.

A review paper of chromatographic methods for the analysis of penicillins in food animal tissues was written by Boison (8). The review includes microbiological and immunochemical tests, gas chromatography, HPLC, and TLC.

B. Cephalosporins

Other β-lactam antibiotics are cephalosporins derived from natural cephalosporin C produced by the *Cephalosporium acremonium* fungus. They possess a cephem nucleus (7-aminocephalosporanic acid) substituted with two side chains. Chemically they are closely related to penicillins and exhibit the same mechanisms of action, i.e., they inhibit bacterial cell wall synthesis. They kill both gram-positive and gram-negative bacteria and are used mainly for treating staphylococcal and streptococcal infections in patients who cannot use penicillins. Cephalosporins are commonly divided into three generations that differ slightly in their spectrum and toxicity.

Cephalosporins can be analyzed by both normal- and reversed-phase TLC. More efficient separation is obtained on silanized gel than on bare silica gel. Mobile phases are polar and similar to those used for penicillins. Cephalosporins can be detected by bioautography and by UV detection at 254 nm. The detection limit can be diminished by applying a reagent such as ninhydrin, iodoplatinate, chloroplatinic acid, or iodine vapor.

Cephalosporin C was separated from its by-products formed during fermentation (desacetylcephalosporin C, desacetoxycephalosporin C, and penicillin N) by RP-TLC with water or aqueous phases (27).

Quintens et al. (28) described a procedure that enables identification of 30 cephalosporins on TLC silanized silica gel plates containing a fluorescence indicator. The mobile phases were composed of a buffer (15% w/v of ammonium acetate adjusted to pH 6.2 with glacial acetic acid) mixed with various organic modifiers:

A. Buffer–methanol (85:15)
B. Buffer–acetonitrile (85:15)
C. Buffer–methanol–acetonitrile (85:10:5)
D. Buffer–acetone (85:15)
E. Buffer–tetrahydrofuran (90:10)
F. Buffer–ethanol (85:15)
G. Buffer–methyl acetate (85:15)

Table 1 contains hR_f values of 30 cephalosporins developed with phases from A–G. No system separated all the cephalosporins, but 12 could be separated from all others with at least one mobile phase. The others could be identified when supplementary information was obtained from color reactions and/or an additional TLC system.

With the OPLC system described above (26), cephalosporin C, 7-aminocephalosporanic acid, 7-aminodesacetoxycephalosporanic acid, a new cephalosporin BK-218, and cefalotin can be separated. Cephalosporins were also separated using ion-pair TLC on silanized plates. Detection was done under a UV lamp and by iodine vapor (29).

Misztal et al. (30) developed new solvent systems, both normal- and reversed-phase, for the separation of seven cephalosporins: cefoxitin sodium (A), cefsulodin sodium (B), cefalotin sodium (C), cefatoxime sodium (D), ceftriaxone sodium (E) , cefalexin monohydrate (F), and cefamandole naphthate (G). The R_f values on silica gel plates for the best NP solvents are presented in Table 2. The first phase separated six analyzed drugs; the second, five. The best RP solvents, offering good separation of all analyzed substances on RP C-18, were phosphate buffer pH 2.65–tetrahydrofuran (9:1, 8:2, or 7:3); and phosphate buffer pH 2.65–methanol (8:2). All cephalosporins could be detected at 254 nm. Eight other modes of detection are presented in Table 3.

Bushan and Parshad (31) used Na_2EDTA as an impregnating agent to resolve some cephalosporins. Changes in the concentration of the impregnating reagent and in the composition of the mobile phase influenced the resolution. Three new solvent systems were successful: