A decade later a scientific team lead by Howard Florey, Ernst Chain, and Norman Heatley isolated and purified the ingredient responsible, penicillin, and established its effectiveness against many serious bacterial infections. In 1941 the first doses of an injectable form of the drug were available for therapeutic use, and by the end of World War II industrial production had begun. The basic structure of penicillins is a thiazolidine ring linked to a β-lactam ring to form 6-aminopenicillanic acid, the so-called penicillin nucleus. This acid, obtained from *Penicillium chrysogenum* cultures, is a precursor of semisynthetic penicillins (ampicillin, amoxicillin, oxacillin, cloxacillin, dicloxacillin, and methicillin) produced by attaching different side chains to the "nucleus." Benzylpenicillin (penicillin G) and phenoxymethylpenicillin (penicillin V) are naturally occurring penicillins.

The most widely used stationary phase for analysis of penicillins is silica gel, but reversed-phase (RP) or cellulose plates are also used. It is advantageous to add acetic acid to the mobile phase and/or to spot acetic acid before sample injection in order to avoid the decomposition of β-lactams on silica gel. RP phases usually contain pH 5-6 buffer and organic solvents.

Penicillin V in fermentations (14) was controlled by chromatography on silica gel HPTLC plates with toluene–ethyl acetate–acetic acid (40:40:20) as the mobile phase. After scanning at 268 nm, R_f values for 4-hydroxypenicillin V, penicillin V, and phenoxyacetic acid were calculated as 0.34, 0.50, and 0.60, respectively.

Hendrickx et al. (15) described identification of 18 penicillins on silica gel and on silanized silica gel, using 35 mobile phases. They concluded that TLC on silica gel was not a valuable general method, because RP systems gave better results. No system could separate all 18 penicillins, but it was easy to find systems appropriate for groups of related products.

Two-dimensional TLC on cyano phases with dichloromethane–hexane–acetic acid (9:10:1) in the first dimension and acetonitrile–methanol–water (40:37:32) in the second was used for the separation of a *Penicillium* fungal extract (16). Detection was carried out under UV light at 254 and 366 nm.

Dhanesar (17) described the use of scanning densitometry for direct quantification of six penicillins on a hydrocarbon-impregnated silica gel HPTLC plate without solvent elution. Penicillin standards and samples were dissolved in water and spotted onto the plate. The sample remained as a single spot centered at the point of application, thereby facilitating direct quantification by densitometry at different wavelengths. The detection level was 0.1 ng.

For the quantitative analysis of ampicillin in urine (18), the following method was used. Ampicillin was isolated from urine by extraction with chloroform containing benzalkonium chloride. Extracts were evaporated and resolved in chloroform. The obtained samples and standards were then developed with dioxane–water–1-butanol–formic acid (75:15:15:1.25) on HPTLC SiF_{254} plates. Scanning was performed at 480 nm after spraying the plate with ninhydrin and heating it at 110°C for 5 min. The limit of detection was 0.05 mg/mL.

Saesmaa (19) described a number of TLC systems and spray reagents that are capable of separating the cation and anion parts of benzathine and embonic acid salts of ampicillin, amoxicillin, cefalexin, and talampicillin embonate. These TLC methods were used for assessment of the purity of the synthesized embonate and benzathine salts of β-lactam antibiotics.

The residues of penicillins in food can be dangerous for a consumer because of possible allergic reactions. Sensitive persons may suffer an anaphylactic reaction after consumption of a single bite of meat contaminated with penicillin, which usually occurs when the producer does not follow prescribed withdrawal periods for the animal (20). The traces of penicillins in meat were extracted with methanol and analyzed by TLC bioautography (TLC-B) on silica gel or cellulose using polar mobile phases and detection with *Bacillus subtilis* (21–23). Different solvents and sorbents were tested for their suitability for TLC-B of several antibiotics—among others, penicillin, ampicillin, and amoxicillin (24). The best results for these antibiotics were obtained on silica gel and alumina tested with *Escherichia coli*. The solvents usually used for TLC were tested, and only three of them gave inhibition zones. They were tetrahydrofuran, 5% sulfuric acid, and 5% hydrogen chloride. Recently, a new bioautographic test kit was produced by Merck and tested by Eymann and Hauck (25) for procaine penicillin G, monensin, and chlorotetracyline, with good results.

The retention behavior of some penicillin and cephalosporin derivatives was studied by Kovács-Hadady and Szilágyi (26) by means of overpressured layer chromatography (OPLC) on silica