

in the late 1950s, whereas florphenicol is more recent. Chloramphenicol is now banned in the United States and in the European Union because it is believed to cause blood toxicity. Chloramphenicol, florphenicol, and thiamphenicol show strong UV absorption and can be determined by TLC.

A method of determining chloramphenicol in serum has been published (110). An ethyl acetate extract of serum and the chloramphenicol standard were spotted on an HPTLC silica gel plate and developed with heptane–chloroform–methanol (6:12:3). The spots were scanned at 280 nm.

Eyedrops containing chloramphenicol were spotted, after dilution with chloroform, directly on the silica gel sheet together with chloramphenicol standards dissolved in chloroform. The plate was developed with diethyl ether (111).

According to the European Pharmacopoeia, chloramphenicol dissolved in acetone is determined on silica gel plates prewashed with methanol–methylene chloride (8:2) and developed with water–methanol–chloroform (1:10:90) phase (112). Plates are dried in air and examined at 254 nm. Sterilization of drugs prior to their application can cause formation of by-products other than those arising from conventional degradation mechanisms. Chloramphenicol samples sterilized by γ -irradiation were screened for impurities by thin-layer chromatography according to the European Pharmacopoeia (113). A significant impurity spot was detected that was found not to be a γ -irradiation by-product of chloramphenicol. What the impurity turned out to be was a cyclic ketale, a product of the condensation of chloramphenicol and acetone catalyzed by traces of acid formed during the irradiation process. Therefore, to avoid artifacts, instead of acetone, methanol should be used as the solvent for TLC investigation of the purity of chloramphenicol.

3. Rifamycins

Rifamycins (ansamycins) are structurally similar macrocyclic antibiotics produced by *Streptomyces mediterranei*. All of them possess the characteristic “ansa” structure consisting of aromatic rings spanned by an aliphatic bridge. They are active against gram-positive streptococci and mycobacteria. They are mainly used in treating tuberculosis.

Grassini-Strazza et al. (114) separated four rifamycins (rifamycins S and SV, rifampicin, and 3-formyl-rifamycin) using RP (diphenyl and C-18) plates. A variety of mobile-phase systems were applied, from neat organic solvents (hexane, cyclohexane, chloroform, tetrahydrofuran, acetone, and C₁–C₄ alcohols) to binary nonaqueous solvents (different proportions of hexane–chloroform and hexane–ethanol) and binary aqueous–organic solvents (e.g., methanol–water or acetonitrile–water). Rifamycins are colored compounds and do not require special detection.

4. Nitrofurans

Nitrofuran drugs are synthetic broad-spectrum chemotherapeutic agents that are derivatives of nitrofuran. Their application in the treatment of humans is limited. Nitrofurazone is used externally, furazolidone is used to treat infections of the intestine, and nitrofurantoin for urinary tract infections. Nitrofurans are used as growth promoters and to prevent and treat diseases in poultry and swine.

Abjean (115) described the TLC screening of nitrofurazone, furaltadone, furazolidone, and nitrofurantoin in meat. Extracts were cleaned up on a Sep-Pak silica column, and the standards were spotted on silica gel plates with a concentrating zone. The plate was developed with dioxane–chloroform (1:1), and after drying it was sprayed with pyridine and immediately illuminated with UV light at 366 nm. After a few seconds, nitrofurazone appeared as a yellow spot and three other nitrofurans as yellow-green spots. The positive detection of nitrofuran drug was possible when it was spiked at the 5 ppb level.

J. Miscellaneous Classes of Antibiotics

Scanning densitometry was described for direct quantification of many classes of antibiotics on a hydrocarbon-impregnated silica gel HPTLC plate without solvent elution (116). Standards and samples were dissolved in water and spotted on the plate. Each sample remained as a single spot