## D. Macrolides

Macrolides are bacteriostatic antibiotics composed of a macrocyclic lactone ring containing 14, 15, or 16 atoms with one or more deoxy sugars attached to it via glycosidic bonds. The main representative of the class, erythromycin, was discovered in 1952 as a metabolic product of *Streptomyces erythreus*. Except for rosaramicin and mirosamycin, isolated from *Micromonospora*, macrolides are obtained from *Streptomyces*. The others are semisynthetic derivatives of erythromycin A. Macrolide antibiotics are active against gram-positive and some gram-negative bacteria and some fungi. They are used in the treatment of pneumonia caused by fungi, streptococcus, and syphilis infections, especially when the patient is allergic to penicillin. Erythromycin is highly active against many new, dangerous bacteria such as *Campylobacter* or *Legionella*. Spiramycin and tylosin are used almost exclusively in veterinary medicine. Separation of macrolides is performed on silica gel, kieselguhr, cellulose, and reversed-phase layers. Silica gel and polar mobile phases are frequently used, usually with the addition of methanol, ethanol, ammonia, or sodium or ammonium acetate. Because macrolides lack chromophore groups, bioautography or postchromatographic derivatization is often used.

The early papers on macrolide antibiotics, mainly erythromycin, were published in the 1960s and considered paper chromatography or TLC on silica gel and kieselguhr with methanolic mobile phases and detection with charring and bioautography. These papers are summarized, together with several newer ones, in a review by Kanfer et al. (7).

Various commercially available macrolides were separated on silica gel using ethyl acetate–ethanol (or 2-propanol)–15% ammonium acetate (9:4:8), pH 9.6. The components of erythromycin as well as various macrolides were separated on silanized silica gel with methanol–water–ammonium acetate (5:2:1), pH 7.0. Eight spraying reagents were described, e.g., sulfuric acid, anisaldehyde, or Dragendorff reagent (49).

Kibwage et al. (50) developed a TLC method for the separation of erythromycins using silica gel and diisopropyl ether–methanol–25% ammonia (75:35:2). The results obtained with several other phases were also discussed. Detection was achieved by spraying with anisaldehyde–sulfuric acid–ethanol (1:1:9) and heating. A similar TLC method was described for semiquantitative analysis of erythromycin A and its metabolites in rat urine and feces (51). Similar phases were also used for the separation of erythromycins, pseudoerythromycins, and erythromycin A enol ether and *N*-oxide, degradation products possibly present in preparations of erythromycin (52). Spots were sprayed with 4-methoxybenzaldehyde–sulfuric acid–ethanol (1:1:9) and heated.

Dichloromethane–methanol–25% ammonia (90:9:1.5) was used for quantitative analysis of bulk erythromycin (53). Detection by postchromatographic color reaction was achieved by spraying with 0.15% xanthydrol in hydrochloric acid–acetic acid (90:7.5) and heating. Spots were quantified by scanning at 525 nm, using troleandomycin as an internal standard.

Separation of erythromycin, tylosin, oleandomycin, and spiramycin in livestock products has been reported (54). The TLC system was silica gel and *n*-butanol–water–acetic acid (6:2:2). The plates were sprayed with xanthydrol or anisaldehyde–sulfuric acid–ethanol (1:1:9) and heated at 110°C. Semiquantitative analysis was carried out by densitometry at 525 nm and bioautography using *Bacillus subtilis*.

Flurithromycin, a novel macrolide antibiotic, was analyzed by TLC and HPLC by Colombo et al. (55). Silica gel plates were developed with methylene chloride–methanol–ammonium hydroxide or methylene chloride–methanol. The spots were visualized by exposure to iodine vapor or by spraying with an anisaldehyde–sulfuric acid–glacial acetic acid–methanol mixture and heating.

The TLC method of monitoring mycinamycins in raw materials was coupled with preparative and semipreparative HPTLC methods (56). TLC was done on silica gel using chloroform–methanol–ammonium hydroxide as a mobile phase and with bioautography as a detection method.

Vega et al. (57) established a method for determining erythromycin and tylosin in chicken meat. The antibiotics were extracted with a mixture of acetonitrile and aqueous solution of potassium chloride (9:1), defatted with hexane, and reextracted with chloroform–ethyl acetate (2:1). The extract was spotted on silica gel HPTLC plates and developed with ethyl acetate–