

Analysis of macrolide antibiotics

Isadore Kanfer, Michael F. Skinner and Roderick B. Walker

Abstract

The following macrolide antibiotics have been covered in this review: erythromycin and its related substances, azithromycin, clarithromycin, dirithromycin, roxithromycin, flurithromycin, josamycin, rokitamycin, kitasamycin, mycinamycin, mirosamycin, oleandomycin, rosaramicin, spiramycin and tylosin. The application of various thin-layer chromatography, paper chromatography, gas chromatography, high-performance liquid chromatography and capillary zone electrophoresis procedures for their analysis are described. These techniques have been applied to the separation and quantitative analysis of the macrolides in fermentation media, purity assessment of raw materials, assay of pharmaceutical dosage forms and the measurement of clinically useful macrolide antibiotics in biological samples such as blood, plasma, serum, urine and tissues. Data relating to the chromatographic behaviour of some macrolide antibiotics as well as the various detection methods used, such as bioautography, UV spectrophotometry, fluorometry, electrochemical detection, chemiluminescence and mass spectrometry techniques are also included.

1. Introduction

The most commonly used macrolide antibiotics consist of a macrocyclic lactone ring containing 14, 15 or 16 atoms with sugars linked via glycosidic bonds [1]. The clinically useful macrolide antibiotics can be conveniently classified into three groups based on the number of atoms in the lactone nucleus. Erythromycins A, B, C, D, E and F, oleandomycin, roxithromycin, dirithromycin, clarithromycin and flurithromycin are 14-membered macrolides whereas azithromycin is a 15-membered compound. 16-Membered macrolides include josamycin, rosaramicin, rokitamycin, kitasamycin, mirosamicin, spiramycin and tylosin, the latter two compounds being used almost exclusively in veterinary medicine [2].

Except for rosaramicin and mirosamycin, which are isolated from *Micromonospora* species, and the semisynthetic derivatives of erythromycin A (roxithromycin, dirithromycin, clarithromycin, flurithromycin and azithromycin), macrolides are produced from various *Streptomyces* organisms. Consequently, the macrolide antibiotics obtained from macrolide-producing organisms commonly consist of mixtures of homologous components.

All these macrolide antibiotics display similar antibacterial properties and are active against Gram-positive and some Gram-negative bacteria and are particularly useful in the treatment of *Mycoplasmas*, *Haemophilus influenzae*, *Chlamydia* species and *Rickettsia*. In particular, macrolide antibiotics constitute an important alternative for patients exhibiting penicillin sensitivity and allergy.

2.1. Erythromycin

Erythromycin is a macrolide antibiotic that is produced by the actinomycete species, *Streptomyces erythreus*. The chemical structures of erythromycin A (EA), which is the major component of erythromycin base, and its related substances are depicted in [Fig. 1](#).

Erythromycin is a polyhydroxylactone that contains two sugars. The aglycone portion of the molecule, erythranolide, is a 14-membered lactone ring. An amino sugar, desosamine, is attached through a β -glycosidic linkage to the C-5 position of the lactone ring. The tertiary amine of desosamine confers a basic character to erythromycin (pK_a 8.8). Through this group, a number of acid salts of the antibiotic have been prepared. A second sugar, cladinose, which is unique to erythromycin, is attached via a β -glycosidic linkage to the C-3 position of the lactone ring.

2.1.1. Related substances and degradation products of erythromycin

The fermentation process that produces commercial grade erythromycin is not entirely selective. It results in the production of small quantities of erythromycin B (EB), C(EC), D(ED), E(EF) and F(EF), in addition to EA, which is the major component. EB, EC and EF are the most important impurities found in commercial samples of erythromycin. In addition to the related substances, the metabolite, demethylerythromycin(dMeE), and acidic and basic degradation products are also present in small quantities in commercial samples of erythromycin. These include erythromycin enol ether (EEE), anhydroerythromycin (AE), erythrolosamine (ESM), pseudoerythromycin A hemiketal (psEAHK), pseudoerythromycin A enol ether (psEAEE) and dehydroerythromycin (DE) (Fig. 2). Other related substances such as erythromycin A *N*-oxide (EANO), erythromycin oxime (EOXM) and erythromycylamine also exist and are structurally very similar, differing by only hydrogen, hydroxyl and/or methoxy groups [3, 4].

2.1.2. Salts and ester derivatives of erythromycin

Several salts and esters of erythromycin (Fig. 3) have been prepared for use in pharmaceutical dosage forms and include, erythromycin stearate (ES), erythromycin ethylsuccinate (EESC), propionyl erythromycin (PE), erythromycin estolate (EES), erythromycin lactobionate (EL), erythromycin glucoheptonate (EG), erythromycin ethyl carbonate (EEC) and erythromycin acistrate.

2.2. Thin-layer chromatography and paper chromatography

Relevant thin-layer (TLC) and paper chromatographic (PC) details extracted from the references cited in this review are summarised in Table 1.

Initial attempts to analyze erythromycin involved the use of TLC to separate EA and EB, psEAHK and AE [5]. Separation was effected on silica-gel TLC plates using various mixtures of organic solvents and the relevant compounds visualized by spraying with 50% aqueous sulfuric acid and charring. A visualization spray consisting of cerium sulfate (1%) and molybdic acid (2.5%) in sulfuric acid (10%) has also been used [6]. Semiquantitative analysis of erythromycin in methanol solution by TLC [7] and PC of an alcoholic solution of ES using bioautography [8] has also been described to visualize the separated components of a mixture containing EA, EB, EC, AE and ESM [6].

Bioautography in combination with paper chromatography has been described for the separation of PE in blood [9] whilst TLC on silica gel followed by densitometry has been applied to separate erythromycin base and EES in capsules [10]. A similar TLC system was also used to separate a mixture containing erythromycin, PE, EES, ES, EL, EG, EESC and anhydroerythromycin A (AEA) in methanol [11]. The separation of erythromycin from ES tablets and EES suspensions by TLC on silica gel was reported in

1976 [12] and a semiquantitative method for the separation of erythromycin, EOXM, erythromycylamine and their acyl derivatives was described in 1977 [13]. Esterbook and Hersey [14] described the TLC separation of erythromycin base and EES using bioautography and Bossuyt et al. [15] separated erythromycin and oleandomycin in milk. Kibwage et al. [16] separated EA, EB, EC and ED, AEA, EEE and des-*N*-methylerythromycin A (dMeEA) using TLC on Kieselgel GF₂₅₄ and sprayed with a mixture of anisaldehyde–sulfuric acid–ethanol (1:1:9) and heated. R_F values for midecamycin, josamycin, tylosin, troleandomycin, oleandomycin, leucomycin, EEC, ES, EES, spiramycins I, II and III, rosamycin and megalomycin were also provided. A similar TLC method for the semiquantitative analysis of EA and several metabolites in rat urine and faeces has also been described [17]. Cachet et al. [18] described the separation of EF, EANO and pseudoerythromycins formed from ring contraction of the macrocyclic lactone (C₁₃–C₁₁ translactonization). The latter compounds are biologically inactive and not usually present in commercial bulk erythromycins. However, some pharmaceutical formulations such as solutions for veterinary use and creams may contain pseudoerythromycins. Separation was effected on TLC plates coated with Kieselgel 60 F₂₅₄ and sprayed with 4-methoxybenzaldehyde-sulfuric acid–ethanol (1:1:9) followed by heating. A TLC method for the separation of erythromycin, tylosin, oleandomycin and spiramycin in livestock products has also been reported [19]. The plates were sprayed with xanthydrol and heated at 110°C for 5 min. Semiquantitative analysis was carried out by densitometry at 525 nm and bioautography.

2.3. Gas chromatography

Gas–liquid chromatography has been used for the quantitative analysis and separation of erythromycin in mixtures containing EA, EB, EC, AEA, ESM and PE using flame-ionization detection (FID) [20]. Similarly, EA and EB were separated and quantitated in the presence of EC, AEA and ESM in erythromycin tablets [21] whereas gas chromatography coupled to mass spectrometry (GC–MS) has been used to determine

erythromycin in beef and pork by single-ion monitoring (SIM) at m/z of 200 [22]. A procedure for the qualitative identification of erythromycin in EESC capsules using pyrolysis–gas chromatography (Py-GC) has also been reported [23]. Relevant chromatographic conditions are listed in [Table 2](#).

2.4. High-performance liquid chromatography

High-performance liquid chromatography (HPLC) is the most extensively used chromatographic method for the analysis of erythromycin and related substances.

Quantitative analysis has been successfully performed in a variety of matrices including raw material, pharmaceutical dosage forms, biological fluids and various tissues.

Chromatographic conditions and details relating to the analysis of erythromycin in raw materials and dosage forms are listed in [Table 3](#) and details relating to its analysis in biological samples are shown in [Table 4](#).

An early report describing the application of liquid chromatography for the analysis of EA, EB and leucomycins was published in 1973 [24].

Erythromycin has a low molar absorptivity as it lacks a suitable chromophore. Thus, specific, selective and sensitive UV detection of this compound is difficult. To overcome this problem low UV wavelengths, where substantial UV absorption occurs, have been used. This generally necessitates the use of extensive precolumn extraction procedures in order to eliminate potentially interfering components, particularly when using complex matrices such as biological fluids and tissues. Generally, a wavelength of 215 nm has proved to be the most useful wavelength to monitor erythromycin and related compounds and has been extensively used in most applications.

Several HPLC methods were published in 1978 [25, 26, 27, 28], three of which by the same authors [25, 26, 27] utilized reversed-phase columns with similar mobile phases consisting of an organic modifier and ammonium acetate buffer (pH 6.2, 7.0 and 7.8, respectively) using a UV detection wavelength of 215 nm. An elevated separation temperature (70°C) allowed shortened analysis time and increased resolution due to a drastic decrease in the height equivalent of theoretical plates (HETP) of some of the compounds of interest. EA, EB, EC, AE, EEE and dihydroerythromycin were analyzed in solution [25], erythromycin and EESC determined in bulk powder and oral-liquid formulations and separated from EA, EB, EC and AE [26]. Erythromycin ethyl carbonate was also chromatographed and resolution obtained between erythromycin base and the ethyl carbonate derivative. Similar chromatographic systems were used to monitor EA, EB, EC and various epimers and degradation products in fermentation broths [27, 29] and were also applied to determine erythromycin in dissolution studies on ES tablets [30]. EESC in bulk powder and tablets has also been determined by a similar HPLC procedure [31]. The mobile phase previously used [26, 32] was modified by incorporating TBA (tetrabutylammonium) which allowed a lower temperature (35°C) and pH (6.5) to be used, thereby conserving the lifetime of the column.

A stability-indicating method for the separation of EA, EB, EC and AE from erythromycin enteric film-coated tablets has also been described [32]. PE, EES and EEC were also chromatographed and the effects of temperature on the HETP of two different C₁₈ columns were described.

Preparative HPLC coupled with differential refractometry was used to isolate various erythromycins and related substances from fermentation residues [33]. These included EA, EB, EC, ED, 8,9-anhydroerythromycin A-6,9-hemiketal, which is identical to EAEE, EA and EC 6,9; 9,12-spiroketal (anhydroerythromycins) and *N*-demethylerythromycin A-6,9; 9,12-spiroketal. The compounds were subsequently analyzed on an analytical reversed-phase column at 40°C using UV detection at 215 nm. A similar procedure was described for the isolation of EE from commercial erythromycin raw material [34]. The same chromatographic system was also used to study erythromycin degradation. The

mobile phase was adjusted to pH 4 as opposed to pH 6.5 previously used and EA, AE, psEAEE and EAEE were successfully separated [35].

Geria et al. [36] described a method for the assay of capsules containing erythromycin base and also for ES capsules. This method utilized a detection wavelength of 288 nm but EB could not be separated from erythronolide B.

During the development of a stability-indicating assay of EES in tablets and suspensions, Stubbs and Kanfer [37] observed the instability of EES in methanol. Substitution by acetonitrile proved beneficial and this phenomenon was later confirmed from the results of a study of the stability of erythromycin and esters in methanol and acetonitrile using a similar HPLC method [38].

HPLC with column-switching was used to separate EA, EB, EC, EE, EF, psEAEE and EAEE [39]. The reversed-phase columns and mobile phase were similar to the system previously used for the separation of analogous compounds [40].

Attempts to separate EA, psdMeEA, erythromycin E propionate (EEP), erythromycin F propionate (EFP), AEA, ECP, EAEE, EAP, anhydroerythromycin propionate (AEAP) and erythromycin B propionate (EBP) in commercial samples using a reversed-phase C₁₈ column have been described [41]. However, psdMeA could not be separated from EFP and EEP was not separated from EAP.

Gradient elution has recently been described for the separation of *N*-dMeEA, EC, EE, EA, AE and EB where a wavelength of 205 nm was used to monitor the chromatography [42]. A similar chromatographic system was also used to determine erythromycin and related impurities in pharmaceutical formulations by the same authors [43].

Optimization of the HPLC of erythromycin and related substances was described by Cachet et al. [40]. These authors investigated the influence of pH on k' for EAEE, EB, AEA, EA, ED, dMeEA and EC. EAEE was significantly influenced by an increase in pH from 6 to 7.5, EB to a lesser extent, while the others were influenced only slightly. The addition of quaternary ammonium compounds such as tetramethylammonium (TMA) and

tetrabutylammonium (TBA) salts into the mobile phase improved peak shape and reduced retention times.

The effect of ageing on the performance of reversed-phase columns (C_2 and C_8) has been described [44]. Ageing was effected by rinsing columns with an acidic mobile phase containing methanol as the organic modifier, followed by closing each column end and heating in an oven at 120°C. Bulk stationary phase was also aged by heating in a sealed glass tube with the same mobile phase. Although ageing of most C_8 columns resulted in improved column performance, some C_8 phases showed a loss of separation which was attributed to the conditioning time of 15 h being excessive in those instances. Generally, peak symmetry and selectivity improved whereas, surprisingly, retention times of most components increased. Shorter chain (C_2) reversed-phase columns needed only 1.5 h of heating. Ageing resulted in loss in part, of the bonded-phase and the shorter the carbon chain, the larger the loss. Ageing thus appears to increase the activity of silanol groups and a certain level of silanol activity seems beneficial to the chromatography of erythromycins implying that mixed separation mechanisms are involved. Cachet et al. [45] described an improved separation for erythromycin and related compounds using reversed-phase C_{18} columns which had been aged by heating at 120°C as previously described using C_2 and C_8 stationary phases [44]. EC, dMeEA, EA, EB, AEA and EAEE were separated and the effects of ageing on EA and the longest retained compound EAEE, were studied. The gain in plate number was dramatic for EAEE, increasing from 800 on unaged columns to 12 760 plates per metre on aged RSil C_{18} columns. In general, C_{18} columns were found to be more stable during conditioning than the shorter-chain materials. Since improvement of chromatography was seen during the initial short conditioning period, and based upon the stability of C_{18} phases, it was concluded that a large loss of bonded phase did not occur. Hence degradation of the stationary phase appeared not to be the reason for the improved chromatography as thought to be the case from the ageing of shorter carbon chain bonded phases. The improvement was attributed to removal of metal impurities from the phase by conditioning with an acidic mobile phase. The loss of bonded phase with consequent increase in silanol activity which occurred more rapidly on shorter chain reversed-phase materials was thus considered a

secondary consequence of the conditioning process. Ageing of the C₁₈ columns did not reduce retention times of the erythromycin derivatives as was seen when short chain reversed-phase columns were so conditioned [44].

Eleven reversed-phase silanol-deactivated columns were used to study the HPLC of erythromycin. TBA was added to the mobile phase. The inclusion of TBA should not affect peak shape when the silanol groups have been deactivated since TBA is used to block interactions with residual silanols. The addition of TBA resulted in a decrease in retention times and the symmetry factor for EA and EAEE always decreased when TBA was included in the mobile phase. HETP for EA always increased when TBA was present and it was seen that TBA improved the quality of separation. The results showed that residual silanol activity still existed with silanol-deactivated stationary phases when used with a mobile phase of neutral pH [46].

An HPLC method for the separation of erythromycin and degradation products and also metabolites of erythromycin using poly(styrene–divinylbenzene) co-polymers (PS–DVB) as the stationary phase was described by Kibwage et al. [47]. EA, EB, EC, AE, erythromycin A enol ether (EAEE), dMeEA and metabolites found in rats dosed with erythromycin (including anhydro-*N*-demethyl-erythromycin and *N*-demethylerythromycin A enol ether) could be separated on PS–DVB columns. These nonionic stationary phases are stable over a wide pH range of 1–14 compared to the previously used derivatized silica reversed-phase packing materials which are unstable above pH 7. It was found that, in contrast to bonded silica reversed-phase columns, the retention of erythromycins increased with increasing temperature. Column temperature also affected selectivity and increasing the methanol content of the mobile phase resulted in a general decline in capacity factors (k') without compromising selectivity. Increasing the pH caused an increase in retention, similar to the pH effect observed on bonded silica reversed-phase columns. However, bonded silica reversed-phase columns were seen to offer better baseline separation and peak shapes. PS–DVB columns were used to separate EE and EA [48] since this separation had previously proved to be extremely difficult on reversed-phase silica columns. Three

different pore sizes of PS–DVB (100Å, 300Å and 1000Å) were investigated and EA, EF, dMeEA, EC, EE, AEA and EB were chromatographed at elevated temperature (70°C). The selectivity of the PS–DVB material was found to depend upon the pore size of the particles. EE and EA could be separated only on wide-pore material (8 µm, 300 Å and 1000 Å). On the narrow-pore materials, the retention times were longer and peak symmetry was poor. The larger pore size (1000 Å) showed better overall selectivity. At pH<8, capacity factors decreased rapidly with a subsequent loss of selectivity.

Further work utilizing PS–DVB columns has also been reported [49]. The method was derived from a previously reported study [48] and a subsequent collaborative study [50] where the stability of erythromycin in neutral and alkaline solutions was investigated. Compounds studied included psEAEE, psEAHK and a hydrolysis product of erythromycin A where the lactone ring had been opened. Using a buffer pH of 6.5 instead of the previously used pH 9.0, better separation between EA and psEAHK was achieved. The column temperature was also reduced from 70°C to 35°C.

Tsuji [28] described the analysis of erythromycin and EESC in serum using a C₁₈ reversed-phase column, on-line derivatization and extraction, followed by fluorometric detection using 360 nm as the excitation wavelength and emission above 440 nm. A column temperature of 70°C was used to separate erythromycin, ESM, AE and EESC.

The application of electrochemical detection for EA and AE in plasma and urine using a novel coulometric detector was reported in 1983 [51]. This involved the use of dual electrodes operated in the oxidative screen mode. The first (upstream) electrode was used to screen potentially interfering compounds at a lower potential whilst the second (downstream or analytical) electrode effected quantitation at a higher potential which resulted in a high degree of selectivity and improved sensitivity allowing a detection limit of 5–10 ng/ml. Coulometric detection using the same dual electrode system was also used by Duthu [52] who assayed erythromycin in serum using a diphenyl 5-µm column. Sodium perchlorate was included in the mobile phase which was continuously recycled and then replaced when the electrode response decreased by about 50%. Inclusion of sodium perchlorate in the mobile phase served to stabilize the electrode sensitivity and

resulted in more efficient oxidation of erythromycin, thereby enhancing detection sensitivity and allowing a limit of quantitation of 50 ng/ml following a 1 ng on-column load. In view of the relatively low UV absorbance of erythromycin, HPLC with UV detection for the analysis of the drug in biological fluids has proved extremely difficult. However, an HPLC method using a low UV wavelength of 200 nm was successfully applied for the assay of erythromycin in serum and urine [53]. The success of this method was largely due to the use of solid-phase extraction (SPE) on C₁₈ extraction cartridges coupled with a micro-phase separation step. These authors had previously established that erythromycin was strongly retained on C₁₈ reversed-phase material when a buffer-free mobile phase was used. Hence, erythromycin could be selectively separated and retained on the SPE cartridge while interfering components were washed off with water and acetonitrile and the drug eluted with an acetonitrile–buffer mixture. In view of the use of a nonselective UV wavelength of 200 nm, further sample concentration and clean-up was necessary. This was achieved by evaporating the extraction column eluate and selectively concentrating the erythromycin and internal standard (oleandomycin) by phase separation into acetonitrile which was then injected onto the column. In addition, use of the nonselective wavelength of 200 nm was facilitated by the incorporation of an in-line solvent degasser. This imparted good baseline stability at this low wavelength and also circumvented possible detection interference by dissolved gases. Furthermore, the introduction of in-line degassing has proved extremely beneficial for use with electrochemical detection.

A stability-indicating assay for erythromycin in serum and urine using amperometric detection has been reported [54]. This system is capable of separating erythromycin from its degradation product, AE. HPLC with amperometric detection has also been described for the separation of erythromycin base and EES in plasma and erythromycin and EESC in urine [55] as well as for erythromycin in plasma and whole blood [56] whilst the determination of erythromycin base and the propionyl ester in serum and urine following administration of EES tablets using coulometric detection, was reported by Stubbs and Kanfer [57]. C₁₈ reversed-columns were used for these analyses, the former utilizing liquid–liquid extraction with diethyl ether whereas the latter involved SPE. A further

HPLC method for erythromycin in plasma using amperometric detection involved separation on a PS–DVB column [58] whilst an octyl reversed-phase column was used with coulometric detection to determine erythromycin in plasma or serum using roxithromycin as internal standard and liquid–liquid extraction with a mixture of diethyl ether–isopentane (3:2) [59]. Erythromycin acistrate (2'-acetyl erythromycin) has been determined in tonsil tissue using a C₁₈ reversed-phase column with coulometric detection. However, peak shapes were poor and baseline separation of the relevant components was not achieved [60]. The same compounds were also determined in plasma using similar methodology [61].

Phase-system switching continuous-flow fast atom bombardment–liquid chromatography–mass spectrometry (LC–FAB-MS) has been applied to measure erythromycin 2'-ethylsuccinate in plasma. This involved a heart cutting procedure after separation and enrichment on a trapping column followed by desorption after washing [62].

Erythromycin A has also been determined in salmon tissue using HPLC [63]. The tissue was extracted by SPE on aminopropylsilica cartridges and chromatographed on a sterically shielded, small bore (2.1 mm I.D.) reversed-phase C₈ column using an amperometric detector. AEA, EA, EC, EAEE and ECEE were resolved, however, AEC was not separated from EA. Good temperature control was shown to minimize detector noise and drift and very good separation and peak shape were observed using this system. The same authors attempted separation using two 150-mm PS–DVB columns connected in series. However, both sensitivity and separation selectivity were extremely poor on that system.

Khan et al. [64] determined EA and metabolites in biological samples with postcolumn ion-pair extraction. Detection involved derivatization with on-line fluorescence monitoring following separation on a wide-pore (1000 Å) PS–DVB column at 70°C. Derivatization was effected with sodium 9,10-dimethoxyanthracene-2-sulfonate (DAS).

However, dMeEA and AdMeEA were not well-resolved and both peaks showed distinct shoulders. Similarly, AEA was not resolved from EA.

An HPLC method for the determination of erythromycin in gastric juice has recently been reported [65]. This method was also applied to analyse plasma and the separation was performed at 65°C using coulometric detection. The use of an elevated temperature and alkaline pH (8.0) was shown to improve peak shape using a base-deactivated C₁₈ reversed-phase column compared to poor peak shape when lower temperatures at pH values 5 or 6 were used.

More recently, a novel HPLC method using a microbore C₁₈ column (1 mm I.D.) and chemiluminescence detection for the determination of erythromycin in urine and plasma has been described [66]. The chemiluminescence reagent, tris(2,2'-bipyridyl)ruthenium(III) ([Ru(bpy)₃]⁺) was incorporated into the mobile phase. However, relatively broad peaks were obtained following injection of samples which were simply filtered by ultrafiltration (blood) or diluted and filtered (urine) with no further extraction. These authors mentioned that very small sample sizes were used but the actual sample size, however, was not stated.

An HPLC method using amperometric detection for the determination of erythromycin in rat plasma and liver has also recently been reported [67]. This method is purportedly suitable for investigations of pharmacokinetics in small animals since very small samples (200 µl) may be used. However, the erythromycin peak showed tailing on the C₁₈ column and the internal standard, oleandomycin, was seen to have a distinct shoulder. The extraction procedure involved alkalization of samples with 1 M sodium hydroxide followed by liquid extraction with *tert.*-butylmethyl ether, which resulted in 100% recovery of erythromycin compared to much lower recoveries when lower molar concentrations of alkali were used.

It is therefore clearly obvious that HPLC has proved to be the most extensively used and valuable analytical procedure for erythromycin analysis and has been applied to determine this antibiotic in a wide range of matrices. The advent of capillary

electrophoresis (CE) and its application for the separation and quantitative analysis of drugs and mixtures thereof will undoubtedly make a significant impact as yet another important analytical procedure. Although CE has already been successfully applied to analyze many drugs, only a few publications to-date have been reported using this technique for the separation and quantitation of erythromycin. The method of Flurer [68] described methods to study various mixtures of macrolide antibiotics. Selected macrolides were analyzed by CE in two separation schemes. Both systems separated oleandomycin from its triacetate derivative, troleandomycin, and erythromycin from some of its derivatives. Laloo and Kanfer have recently described the development of a CE method for the separation of erythromycin, josamycin and oleandomycin [69] and a subsequent paper by the same authors describes a CE method for the quantitative determination of erythromycin and related substances. [70].

3. Other macrolide antibiotics

3.1. Azithromycin

Azithromycin is a novel semisynthetic macrolide or azalide similar to erythromycin but composed of a 15-membered lactone ring. As in erythromycin, cladinose and desosamine sugar residues are attached at positions 3 and 5 (Fig. 4). Azithromycin exhibits a more extensive spectrum of activity, greater acid stability and more favourable pharmacokinetic parameters than erythromycin [2].

HPLC with ultraviolet detection has been used for the analysis of azithromycin in bulk samples, for the separation of related compounds produced during synthesis and for acid degradation studies [71, 72]. Methods for the HPLC analysis of azithromycin in biological samples have also been described using various methods of detection in order to overcome the limitations of poor UV absorbance. Shepard et al. [73] used both coulometric and amperometric methods for the detection of azithromycin in human and animal tissues and serum. Optimum chromatography was obtained using alkylphenyl or polymer coated alumina (γ RP-1) materials with the pH of mobile phases adjusted to neutral or alkaline pH values respectively, to obtain optimum peak shape, resolution and detector sensitivity. The coulometric method has since been used by a number of investigators to characterise the pharmacokinetics of azithromycin in humans [74, 75, 76, 77]. A sensitive HPLC assay using a C₁₈ stationary phase with detection by atmospheric

pressure chemical ionisation mass spectrometry (APCI-MS) has since been developed, specifically for the analysis of azithromycin in children, which requires only a minute sample volume of 50 μ l [78]. Chromatographic details are listed in [Table 5](#).

3.2. Clarithromycin

Clarithromycin is a semisynthetic macrolide derived from erythromycin A and consists of a 14-membered lactone ring as well as cladinose and desosamine residues at positions 3 and 5 of the ring, respectively ([Fig. 4](#)). Like erythromycin, it has no conjugated double bond in the lactone ring, hence significant UV absorbance is only obtained at wavelengths below 210 nm. Whilst UV detection of clarithromycin may be suitable for most in vitro samples, electrochemical detection has proved to be most effective when quantitation of low concentrations of the drug in biological samples is required. Specific HPLC conditions used for the analysis of clarithromycin are listed in [Table 6](#).

Morgan et al. [79], using reversed-phase chromatography at 50°C with UV detection were able to separate clarithromycin and 8 related compounds produced during the synthetic process. They found that separation was largely dependent on the organic–aqueous ratio of the mobile phase and, in contrast to erythromycin, almost unaffected by temperature and pH, although an elevated column temperature was used to maintain peak symmetry and resolution. Gorski et al. [80] subsequently developed a UV method for the determination of nonpolar compounds produced during clarithromycin synthesis, while Morgan et al. [81] separated several acid and base degradation products in a stability-indicating assay for stability studies and the analysis of dosage forms. Erah et al. [82] also used UV detection in a stability-indicating assay developed to monitor the acid degradation of clarithromycin at gastric pH values. The parent and the major degradation product were separated on reversed-phase material with an ion-pairing agent incorporated in the mobile phase. Another in vitro, but nonspecific assay, using electrochemical detection for the determination of clarithromycin as a contaminant on surfaces and used as part of a cleaning validation procedure, was published by Rotsch et al. [83]. Early methods for the determination of clarithromycin in biological fluids used both ultraviolet and electrochemical detection [84, 85, 86], but latterly electrochemical

detection has been preferred [87, 88, 89]. The method of Chu et al. [87] described an assay in which clarithromycin and several metabolites were separated on C₈ columns following liquid–liquid extraction and quantitatively determined at concentrations as low as 30 ng/ml in serum using electrochemical detection. This method was successfully used to determine pharmacokinetic parameters of clarithromycin following oral administration [90, 91]. Subsequently, Hedenmo and Eriksson [89] described an electrochemical assay for roxithromycin and clarithromycin which used solid-phase CN material for in-line sample clean-up followed by chromatography on C₁₈ material. They found that an increase in temperature increased retention times but improved peak shape. The analytical column was therefore maintained at 55°C. The mobile phase was buffered at pH 7.0 to optimise detector sensitivity. However, since only a small amount of serum was used (ca. 100 µl), the sensitivity of this method was compromised to some extent.

3.3. Dirithromycin

Dirithromycin is a 9-*N*-11-*O*-oxazine derivative of erythromycin (Fig. 4) that exhibits antibacterial activity against a variety of gram-positive and gram-negative bacteria [92]. Whitaker and Lindstrom [93] developed a sensitive, selective and rapid assay for dirithromycin, its metabolite erythromycylamine (Fig. 4) and the experimental macrolide, LY281389, in dog plasma using a liquid–liquid extraction procedure, followed by reversed-phase HPLC and coulometric detection in the oxidative screen mode. Separation was achieved using diphenyl columns maintained at 40°C. The concentration of buffer in the mobile phase was reported to affect peak symmetry with low concentrations showing prolonged retention times, due to amine–silanol interactions. HPLC conditions used for dirithromycin analyses are shown in Table 7.

3.4. Roxithromycin

Roxithromycin (Fig. 4) is a semisynthetic macrolide antibiotic derived from erythromycin [1] and contains a 14-membered lactone ring. In 1987, Croteau et al. [55] described an HPLC method using amperometric detection for the analysis of erythromycin using roxithromycin as an internal standard. Grgurinovich and Matthews [59] developed an HPLC method using coulometric detection in the oxidative screen mode for the analysis of roxithromycin in plasma or serum, with erythromycin as the internal standard. Despite specificity for the metabolites not being established, the method was used to elucidate the pharmacokinetics of roxithromycin and erythromycin after single and chronic dosing [94]. A simple, sensitive micro-method for the determination of roxithromycin in plasma and urine using electrochemical detection has been reported [95]. The effects of higher pH (7.0–11.0) were investigated and despite an

increased electrochemical response, retention times increased. HPLC conditions for the analysis of roxithromycin are listed in [Table 7](#).

3.5. Flurithromycin

Flurithromycin ([Fig. 4](#)) is a novel macrolide antibiotic used as the ethylsuccinate salt [[2](#)]. Colombo et al. [[96](#)] reported both TLC systems and an HPLC system for the chromatographic identification, quantitation and subsequent structural identification of flurithromycin ethylsuccinate. Separation by TLC was effected and the spots visualised by exposure to iodine vapour or by spraying with an anisaldehyde–sulfuric acid–glacial acetic acid–methanol mixture and warming ([Table 1](#)). With each solvent system and detection technique, a single discrete flurithromycin spot was observed.

The same authors also described an HPLC method using a C₁₈ reversed-phase column with detection at 214 nm. The method was used to monitor the equilibrium between flurithromycin and the hemiketal derivative ([Table 7](#)).

3.6. Josamycin

Josamycin (leucomycin A₃) is a member of the leucomycin group of macrolides which contain a 16-membered lactone ring ([Fig. 5](#)). Josamycin also contains a mycaminose residue attached at position 5 of the ring with a mycarose residue attached at position 4 of the mycaminose moiety [[1](#)]. It is produced by *Streptomyces narbonensis* var. *josamyceticus* [[2](#)] and is used mainly for the treatment of gram-positive infections in man and also in veterinary medicine. Other leucomycins include rokitamycin (leucomycin A₅ ester derivative) and kitasamycin which is a mixture of various leucomycins.

As for other macrolides, early analytical data were obtained using microbiological assays. However, the metabolites of josamycin are microbiologically active which renders this type of assay nonselective for the parent compound. Several HPLC methods have been developed to determine josamycin in biological samples, but the selectivity of the majority of these assays has not been specifically demonstrated. Methods published to-date have predominantly utilised reversed-phase HPLC with buffered mobile phases to obtain separation. Detection has involved either fluorescence, or UV at about 230 nm which is facilitated by a conjugated double bond within the lactone ring. HPLC conditions and related details are listed in Table 8.

Fourtillan et al. [97] published an early HPLC assay for josamycin using a modified system originally developed by Tsuji [28] for the determination of erythromycin and erythromycin ethylsuccinate in serum. Although sensitive, the method involved rather complex on-line derivatisation with Tinopal[®] for subsequent fluorometric detection. Several simple assays utilising UV detection were later published [98, 99, 100, 101, 102] with the latter two exhibiting greater sensitivity than previously published methods. Both methods used solid-phase extraction techniques. Räder et al. [101] used a direct injection technique with valve switching for sample clean-up and applied the assay to the determination of josamycin in plasma and blood cells. Skinner and Kanfer [102] used solid-phase extraction for the analysis of josamycin in serum and urine in pharmacokinetic studies. More recent assays have, however, favoured fluorescence detection. Tod et al. [103] derivatised josamycin and rokitamycin (a semisynthetic macrolide similar to the leucomycins) with dansylhydrazine to determine both compounds at a limit of quantitation of 50 ng/ml in plasma. Leroy et al. [104] derivatised josamycin with cyclohexa-1,3-dione to determine the compound in porcine tissue and achieved similar sensitivity.

HPLC analysis has also been applied to josamycin raw materials. Skinner et al. [105] separated several acid and alkali degradation products on a C₁₈ stationary phase and

monitored the decomposition of josamycin in acid and alkali, while Paesen et al. [106] separated a series of 10 leucomycins in josamycin raw material using a PS–DVB column.

3.7. Mycinamycin

The mycinamycins (Fig. 6) are a group of macrolide antibiotics produced by *Micromonospora griseotubida* [1]. Preparative and semipreparative HPLC methods coupled with analytical HPLC (Table 7) and TLC (Table 1) for the facile separation and monitoring of the mycinamycins have been described by Mierzwa et al. [107]. These techniques coupled with spectroscopic investigations resulted in the discovery of two new macrolide antibiotics, compound II and compound V (Fig. 6).

Mirosamycin (mycinamycin II) is the principle component of the mycinamycins. Horie et al. [108] developed an HPLC method using SPE for sample clean up to determine mirosamycin levels in animal tissues. Separation at ambient temperature was performed on a C₁₈ column and the analyte detected at 230 nm. The use of an end-capped column

reduced amine–silanol interactions and thus peak tailing. In addition, the low pH reduced retention times and improved peak shape (Table 7).

3.8. Oleandomycin

Oleandomycin (Fig. 7) is a broad spectrum macrolide antibiotic isolated from *Streptomyces antibioticus* with a similar range of activity to that of erythromycin [1]. Lees et al. [109] described the use of a paper chromatographic analysis in conjunction with bioautography for the separation of the acetylated oleandomycins in multicomponent antibiotic mixtures (Table 1). Four useful solvent systems of increasing polarity were identified as suitable for the separation of oleandomycin base and its acetylated derivatives, including triacetyl-oleandomycin (Fig. 7). Iglóy et al. [8] investigated the effects of varying mixtures of polar and nonpolar solvents in different proportions. In addition, they investigated the effects of pH of the paper and of the solvent system on the chromatographic separation of magnamycin, ES, oleandomycin phosphate, picromycin and methymycin. When using buffered paper, separation was considerably affected by solvent pair and paper pH but the system produced elongated spots causing difficulty in evaluation. However, the use of buffered solvents completely eliminated the tailing of spots (Table 1).

A precise, selective and sensitive HPLC method [110] for the analysis of oleandomycin in serum and urine using erythromycin as internal standard was published in 1986 (Table 7). The success of this method, which involved the use of a nonselective UV wavelength of 200 nm, was attributed to the efficiency of the solid-phase extraction procedure which incorporated a micro-phase separation step. In view of the similar chromatographic behaviour of oleandomycin and erythromycin, oleandomycin has proved to be extremely valuable as an internal standard in many HPLC methods used for the determination of erythromycin and other macrolide antibiotics [37, 38, 53, 54, 56, 57, 58, 61, 65, 67, 102].

A qualitative chromatographic procedure, using Py-GC (Table 2) has also been reported for oleandomycin [23].

3.9. Rosaramicin

Rosaramicin contains a 16-membered lactone ring and a single sugar residue, desosamine, glycosidically linked at position 5 of the macrocyclic ring (Fig. 8). It is produced by *Micromonospora rosaria* and *Micromonospora capillata* and has activity against both gram-positive and gram-negative bacteria [1]. Lin et al. [111] developed a precise, accurate and specific reversed-phase HPLC method for the determination of rosaramicin in serum or plasma with desepoxyrosaramicin as the internal standard (Table 7). Separation was achieved at ambient temperature on a reversed-phase column. Following a liquid-liquid extraction procedure, UV detection at 254 nm was used to monitor the serum/plasma samples of 12 human subjects who received a 500-mg dose of rosaramicin.

4. Macrolide antibiotics for veterinary use

4.1. Spiramycin

Spiramycin (Fig. 9) consists predominantly of three closely related substances [112] together with a number of other minor compounds. Spiramycin I (SI) is the major component and contributes about 63% of the mixture while Spiramycins II (SII) and III (SIII) contribute about 24 and 13%, respectively. The members of this series are composed of 16-membered lactone rings with two or three sugar substituents, mycaminose, forosamine and mycarose, together with various other substituent groups. Mycaminose and forosamine are present in all spiramycin-related substances and are attached to the lactone ring at positions 5 and 9 via β -glycosidic linkages, respectively. SI, SII and SIII all contain mycarose attached at position 4 of the mycaminose moiety, also via a glycosidic linkage but in the remaining minor related substances, mycarose may or may not be present.

The spiramycins are produced as a product of fermentation by *Streptomyces ambofaciens* and used predominantly as a veterinary medicine in much the same way as tylosin [2].

As for other macrolides obtained directly from a fermentation process, the chromatographic analysis of spiramycins has largely been concerned with separation of the major spiramycins to enable the analysis of these compounds with a sensitivity and selectivity not afforded by traditional microbiological assays. Selective HPLC methods have been developed to assay raw material, as well as for the determination of spiramycin in biological samples and in tissues. Methods published to-date have utilised both normal and reversed-phase chromatography with UV detection at about 230 nm, which is

facilitated by a conjugated double bond within the lactone ring. HPLC conditions and related details are listed in [Table 9](#).

An early chromatographic procedure for the analysis of spiramycin was reported by Frères and Valdebouze [113] in a publication which described the TLC of a number of anti-infective agents used in veterinary practice ([Table 1](#)). Omura et al. [24] described an HPLC assay for the separation of SI and SIII which could also be used for some other macrolide antibiotics ([Table 3](#)). Later, Mourot et al. [114] developed a selective HPLC assay capable of resolving all three major components of spiramycin. They used a reversed-phase C_8 , 10- μm stationary phase with an acidic mobile phase which eluted SI, SII and SIII in that order with increasing retention corresponding to an increasingly lipophilic substituent group at position 3 of the lactone ring. Separation of these three compounds was also accomplished by Bens et al. [115] on both reversed-phase C_{18} material (at 50°C) and on silica gel (at 25°C) with the aid of an ion-pairing agent (diethylamine) in both instances. The method was subsequently used to characterise the composition of a number of different batches of raw materials. Bens et al. [116] further investigated the effects of isohydric solvent systems and temperature on the retention of

the spiramycins on normal-phase material and easily obtained rapid separation of SI, SII and SIII with baseline resolution. Optimal separation was effected with an organic mobile phase containing a small percentage of water together with diethylamine, at temperatures of 45–65°C. As expected, elution was in reverse order from that previously obtained by Mourot et al. [114] with reversed-phase chromatography. An unusual chromatographic method for the qualitative determination of spiramycin has been described by Danielson et al. [23]. They demonstrated the use of Py-GC to identify spiramycins in raw material. This method was also used for the identification of oleandomycin, troleandomycin and tylosin (Table 2). Recently, a PS–DVB stationary phase has been used to identify and separate nine spiramycin and spiramycin related compounds (Fig. 9) present in raw materials [117, 118]. As with similarly specific methods published for erythromycin [48], tylosin [119] and josamycin [106] by the same group of researchers, this degree of selectivity was obtained with a mobile phase of pH 9.0 at an elevated temperature of up to 60°C.

Dow et al. published an HPLC method for the determination of spiramycin in biological samples in 1985 [120]. Following in-line sample enrichment and clean-up of human serum, chromatography was performed on reversed-phase C₈, 5-µm material at ambient temperature with the mobile phase acidified with perchloric acid. The method was specific for SI and SII (internal standard), however, the retention of SIII and possible degradation products derived from the hydrolysis of spiramycin in plasma, as observed by Inoue et al. [121], were not determined. The reported limit of quantitation (LOQ) of 50 ng/ml facilitates the utilisation of the assay for pharmacokinetic studies in man. This method was subsequently slightly modified and used to determine concentration vs. time profiles of SI in bovine plasma [122], and further modified to simultaneously determine SI and the demycarosil residue of spiramycin, neospiramycin, in bovine plasma and milk [123]. Separation of spiramycin and neospiramycin was achieved on a C₁₈ column at ambient temperature with the mobile phase acidified with sulfuric acid. Furthermore, a sensitivity substantially higher than obtained by the unmodified assay was achieved.

In addition to the aforementioned assays for biological samples, methods for the determination of spiramycin residue in chicken and bovine tissue have been published. Nagata and Saeki [124] used a C₈ stationary phase with an ion-pairing agent, and UV detection, for the determination of residual spiramycin in chicken tissues. Sanders and Delphine [125] used a C₁₈ stationary phase for chromatographic separation followed by particle beam mass spectrometry for the confirmatory analysis of spiramycin in bovine muscle.

4.2. Tylosin

The tylosin series of macrolides is composed of several related substances of which tylosin A (TA) is the major component. Other minor constituents include tylosin B or desmycosin (TB), tylosin C or macrocin (TC), tylosin D or relomycin (TD), lactenocin (TL), 5-*O*-mycaminosyltylonolide (OMT) and desmycosinyl tylosin (DMT). They consist of a 16-membered lactone ring with up to three sugar substituents, mycaminose, mycinose and mycarose, together with various other substituent groups (Fig. 10). Mycaminose is present in all the related substances and is attached to the lactone ring at position 5 via a β -glycosidic linkage. TA, TC and TD all contain mycinose, attached at position 14 of the lactone ring, and mycarose which is attached at position 4 of the mycaminose moiety, also via glycosidic linkages. The remaining related substances contain either one or neither of these two sugars.

The tylosins are produced during the fermentation process by *Streptomyces fradiae*, *S. rimosus* and *S. hydroscopicus* with *S. fradiae* used for commercial production [126]. It is a relatively new antibiotic solely for veterinary use as a feed additive to maintain health and promote growth in chickens, pigs and cattle, and also for the treatment of various infections in livestock [2].

The analysis of tylosin in biological samples has predominantly been directed towards detecting residual levels of tylosin in food products of animal origin eg. meat, eggs and milk. This was initially accomplished using microbiological methods. However, besides their nonselectivity, these methods are barely sensitive enough to detect the maximum levels permitted by regulatory authorities, which is usually in the 0.2 ppm range. HPLC methods have therefore been developed which provide additional sensitivity as well as allowing for the selective determination of TA and its' related compounds. The majority of methods have utilised reversed-phase chromatography with UV detection between

254–290 nm (Table 10). Tylosin has also been determined in animal derived foodstuffs using TLC [19], (Table 1).

An early HPLC assay for tylosin was developed by Bhuwapathanapun and Gray [127] to follow the fermentation process. They used reversed-phase chromatography with an inorganic, aqueous mobile phase devoid of buffer salts or other modifiers. Thus, only limited selectivity was obtained which required the use of an additional normal-phase chromatographic system, also of limited selectivity, in order to obtain separation of TA, TB, TC and TD. An HPLC assay for the determination of tylosin in animal tissues and foodstuffs was published by Kennedy [128] who used a reversed-phase C₁₈ end-capped stationary phase with a mobile phase containing monoethanolamine as an ion-pairing agent. Separation of the four main tylosin-related compounds was achieved at a pH of 11.7, which is too high for long-term use with silica-based stationary phases. Kennedy later separated nine tylosin-related components on both C₈ and C₁₈ reversed-phase

materials although baseline resolution was not obtained for the majority of them [129]. In this instance, separation was accomplished at pH 3.5 using an elevated column temperature (55°C) with sodium 1-pentanesulfonate as an ion-pairing agent. Investigations into the effect of elevated temperatures demonstrated that retention increased with an increase in temperature. This behaviour was attributed to the influence of temperature on the formation of ion-pairs between the analyte and the ion-pairing agent. Furthermore, the elution behaviour of the tylosins could be predicted to some extent by considering the nature of the functional groups attached to the lactone ring. Subsequent methods have all used acidic mobile phases which are more suitable for reversed-phase chromatography. Yeh et al. [130] adapted Kennedy's method to separate TA, TC and TD in order to monitor the biosynthesis of tylosin. They achieved particularly good separation with baseline resolution of these three compounds using a C₈ stationary-phase maintained at 55°C with 1-pentanesulfonic acid in an acidic mobile phase. Moates [131, 132] also used an acidic mobile phase with an end-capped column but with buffer salts as the modifier rather than an ion-pairing agent. However, these assays were not specific, but suitable to monitor residual tylosin and related compounds in foodstuffs. The good peak symmetry and adequate separation from endogenous compounds was attributed to the presence of buffer salts in the mobile phase. Comparison of this method with a microbiological assay showed the HPLC assay to be significantly more sensitive with a limit of detection of 0.1 ppm [133].

A substantially more sensitive assay for the determination of tylosin in bovine tissue has subsequently been published [134]. Although separation of the individual tylosins was not achieved, the presence of erythromycin, oleandomycin and tilmicosin did not interfere with the HPLC analysis. Determination of tylosin in tissues has traditionally been performed after liquid-liquid extraction of tissue homogenate, which in most cases has proved time consuming and labourious. These authors utilised faster SPE techniques with silica gel cartridges to effect extraction and managed to attain a detection limit of 15 ng/g (0.015 ppm) on a C₁₈ column. A simple SPE procedure was also utilised by Chan et al. [135] who used C₁₈ extraction columns for sample clean-up prior to analysis and attained a detection limit of 0.02 ppm.

Fish and Carr [136] described a method which resolved TA, TB, TC, TD and DMT and allowed accurate quantitative analysis. The assay was developed to replace cumbersome, nonspecific, paper chromatographic methods for the characterisation of tylosin in raw materials and veterinary dosage forms. Separation was performed on a variety of C₈ and C₁₈ columns. With the use of sodium perchlorate as an ion-pairing agent, a mobile phase of pH 2.5 could be used. This overcame the disadvantage inherent with the use of a mobile phase of high pH which was previously found to be necessary in order to obtain separation of all these compounds [128]. This low pH optimised the protonation of the tylosin compounds and facilitated the subsequent formation of the ion-pair complex. Furthermore, separation was obtained at ambient temperature. A previously unknown compound, later identified as an aldol condensation product of tylosin (TAD-tylosin A aldol), which probably formed by base catalysis in the sample solution at pH 9.0, was also detected and eluted adjacent to TA. Optimal separation of this compound from TA whilst maintaining resolution between the remaining compounds was obtained on C₁₈ material.

Quantitative analysis of the tylosins in raw materials and dosage forms was further improved by Roets et al. [137]. They claimed that their method exhibited better selectivity and reproducibility on different stationary phases than did previously published methods, and with the added advantage of using alternative ion-pairing agents in preference to the potentially corrosive, previously used, pentanesulfonic acid. Good separation and baseline resolution was achieved for TA, TB, TC, TD and DMT as well as the aldol TAD using C₈ and C₁₈ columns at the slightly elevated temperature of 30°C. An 8-µm C₈ stationary-phase was ultimately chosen for the analysis of raw materials from various sources. TBA was used in preference to TMA as the noncorrosive ion-pairing agent, in a mobile phase acidified using phosphoric acid. Paesen et al. [119] developed an assay using a polymeric stationary phase with an alkaline mobile phase (pH 9.0) and succeeded in separating TA from potential impurities and related substances, including acidic and alkaline degradation products. This method was subsequently used to determine the pH stability profile of tylosin in acid and alkali solutions [138]. It was also used as an aid in the purification of the TA aldol decomposition product (TAD), first

detected by Fish and Carr almost 10 years earlier [136], where separation of two TAD epimers as well as a photochemical reaction product, isotylosin A, was effected [139]. Recently, LC-MS (Table 10) has been used as an alternative method for the determination of residual levels of tylosin in bovine muscle [140]. This method utilises SIM at a number of m/z ratios to determine the concentration of tylosin and is capable of detecting residual tissue concentrations as low as 10 ng/g (0.01 ppm).

5. Conclusion

In spite of the overwhelming success of HPLC as a valuable tool for the quantitative analysis of macrolide antibiotics, microbiological and nonchromatographic methods are still extensively used. In particular, some official compendia, such as the United States Pharmacopeia, retain microbiological assays for erythromycin and other macrolide antibiotics in some monographs whereas HPLC methods to quantitatively determine some of the newer macrolides, such as flurithromycin and dirithromycin, are conspicuously absent from the published literature.

Further efforts to use modern chromatographic techniques, including CE, for the quantitative analysis of macrolide antibiotics will undoubtedly continue. The main goals to be addressed in the future include improved selectivity, sensitivity, analytical simplicity and efficiency.

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