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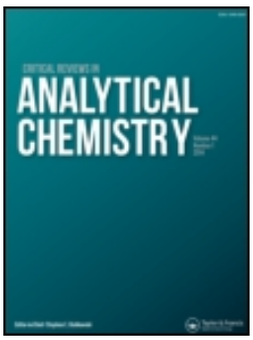

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Review of Properties and Analytical Methods for the Determination of Norfloxacin

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ABSTRACT

The first-generation quinolones have their greatest potency against Gram-negative bacteria, but newly developed molecules have exhibited increased potency against Gram-positive bacteria, and existing agents are available with additional activity against anaerobic microorganisms. Norfloxacin is a broad-spectrum antimicrobial fluoroquinolone used against Gram-positive and Gram-negative organisms (aerobic organisms). There are different analytical methods available to determine norfloxacin applied in quality control of this medicine in order to ensure its effectiveness and safety. The authors present an overview of the fourth generation of quinolones, followed by the properties, applications, and analytical methods of norfloxacin. These results show several existing analytical techniques that are flexible and broad-based methods of analysis in different matrices. This article focuses on bionalytical and pharmaceutical quality-control applications, such as thin-layer chromatography, microbiological assay, spectrophotometry, capillary electrophoresis (CE), and high-performance liquid chromatography (HPLC).

KEYWORDS

Analytical methods; fluoroquinolones; norfloxacin; pharmacokinetics; quality control

Introduction

Quinolones and fluoroquinolones are chemical groups related to nalidixic acid derivatives. The first quinolone to be introduced was nalidixic acid (1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid) in 1962; it is derived from the antimalarial drug chloroquine (Andriole, 2005; Ball, 2003; Chen and Lo, 2003; Hawkey, 2003; Izawa et al., 1980). Current agents were developed following fluoridation of the quinolone molecule, and the first to receive approval by the U.S. Food and Drug Administration (FDA) was norfloxacin (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) in 1984 and it was patented by the pharmaceutical company Kyorin Seiyaku Kabushiki Kaisha (Bolon, 2011; U.S. Food and Drug Administration, n.d.).

Norfloxacin is a broad-spectrum antimicrobial used in several countries to effectively treat infections in humans and animals. It has been used for treatment of several bacterial infections, such as *Escherichia coli*, *Citrobacter freundi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Shigella* (Vijan and Conci, 2008). In Brazil, this antimicrobial agent is commonly used as 400 mg pharmaceutical tablets and was authorized by the National Agency for Sanitary Surveillance (ANVISA) in 1983. The drug is represented by pharmaceutical companies such União Química, Sigma Pharma, Merck, Medley, and Biosintética (ANVISA, n.d.).

With the large number of products marketed by different companies in several countries, providing high-quality products remain a challenge. Quality control is an important task in the pharmaceutical industry, not only to protect the manufacturer against compensation claims, but also to guarantee the patient the use of safe and effective products. One of the important goals of quality control is to report the analytical method validation to ensure confidence in the analytical data throughout product development (International Conference on Harmonization, 2005; World Health Organization, n.d.) and ensure that the procedures of good manufacturing practices guides as well as good laboratory practice required by the U.S. FDA are applied in the pharmaceutical industry (Shabir, 2003).

This article is not intended to be a systematic review of the literature on these subjects. Rather, it provides an overview of relevant published literature and a discussion of data, highlighting progress in quinolone and fluoroquinolone development and the challenges to provide these drugs as high-quality products, with a focus on the norfloxacin.

Structural modification

The generation of quinolones can be demonstrated in two ways, the naphthyridones group and the fluoroquinolone group (Table 1). The quinolone basic molecular structure is composed of a carboxylic acid ring, pyridine ring, carbon or nitrogen, and side chain. Nalidixic acid is the original naphthyridine core, with a ketone function at C-4, which determines antibacterial activity by influencing the affinity for bacterial enzymes. This antibacterial agent has a modest activity against Gram-negative bacteria, low activity against Gram-positive bacteria, and no activity against *Pseudomonas aeruginosa*. Due to its low oral absorption and high concentration in urine, its therapeutic use has been restricted to the treatment of urinary tract infections (Bolon, 2009; Christian, 1996; Oliphant and Green, 2002; Van Oort et al., 1983). Nalidixic acid (discontinued) structure changes in position N-1 affect drug potency and

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Table 1. Quinolone classification used in human medicine.

Quinolones			
	Fluoroquinolones		
1st Generation (naphthyridines)	2nd Generation	3rd Generation	4th Generation
Nalidixic acid	Norfloxacin	Tosufloxacin	Trovafloxacin
Oxolinic acid	Pefloxacin	Temafloxacin	Moxifloxacin
Piromidic acid	Enoxacin	Sparfloxacin	Prulifloxacin
Cinonaxin	Ofloxacin	Grepafloxacin	Sitafloxacin
Miloxacin	Ciprofloxacin	Pazufloxacin	Gemifloxacin
Rosoxacin	Flumequine	Balofloxacin	Clinafloxacin
Pipemidic acid	Lomefloxacin	Levofloxacin	Besifloxacin
Droxacin	Nadifloxacin		Garenoxacin
	Rufloxacin		Gatifloxacin
	Fleroxacin		Alatrofloxacin

pharmacokinetics. Positions C-2, C-3, and C-4 determine antibacterial activity by influencing the affinity for bacterial enzymes; also positions C-3 and C-4 are involved in metal chelation and the consequent interaction with divalent and trivalent cations (Andriole, 2005).

The agents from the first generation (Figure 1) such as oxolinic acid, piromidic acid, cinoxacin (discontinued), miloxacin, rosoxacin (discontinued), pipemidic acid, and droxacin have analogue structures with nalidixic acid (discontinued) and showed no advantage in therapy over the precursor (Gadebusch and Shungu, 1991; King et al., 2000; Souza et al., 2012; Zhanel et al., 1999).

The new classification of quinolone antibiotics takes into account the expanded antimicrobial spectrum of the new fluoroquinolones (Andriole, 2005).

As was highlighted by Bolon (2011) the long period of fluoroquinolone development provides considerable insight into the effect of structural modification upon the antimicrobial activity and pharmacologic properties of these agents.

The second-generation fluoroquinolones (Figure 2) have increased Gram-negative activity as well as some Gram-positive and atypical pathogen coverage. The addition of a fluorine atom to position C-6 transforms a quinolone into a fluoroquinolone, enhancing drug penetration into the bacterial cell. Position N-1 presents low or no antimicrobial activity, possibly due to the formation the tautomers. Quinolone derivates with cyclopropryl substituent at this position show high activity against Gram-negative bacteria. The addition of a member of the group piperazine or a piperidine moiety at C-7 increases activity against *P. aeruginosa*, whereas a pyrrolidine group improves Gram-positive activity. The presence of any halogen at position C-8 can increase a drug's half-life, adsorption of the drug, and antianaerobic activity (King et al., 2000). Norfloxacin is specifically active against aminoglycoside-resistant *P. aeruginosa*, *Serratia* sp., and betalactamase-producing organisms (Naumann and Dopp, 1989). At this time, a large number of other related drugs, including ciprofloxacin, ofloxacin, flumequine, enoxacin (discontinued), lomefloxacin (discontinued), fleroxacin, nadifloxacin, pefloxacin, and rufloxacin, are used in clinical practice and others are in vigorous stages of development and clinical investigation (Appelbaum and Hunter, 2000; Bolon, 2009, 2011).

The third-generation fluoroquinolones (Figure 3) include sparfloxacin (discontinued), grepafloxacin (discontinued), levofloxacin, balofloxacin, tosufloxacin, pazufloxacin, and temafloxacin. They were subsequently developed and are all active against penicillin-resistant *Streptococcus pneumoniae* and have been proven highly effective in the treatment of lower respiratory tract infections (acute sinusitis and acute exacerbations of chronic bronchitis) (Ball, 1999). Position C-5 of the quinolone ring is important in determining in vitro potency, especially against Gram-positive bacteria (as is found on sparfloxacin). Grepafloxacin contains a methyl group at C-5 that increases the potency against Gram-positive organisms to a less extent (Bolon, 2009, 2011; Domagala, 1994).

The final group of fluoroquinolones, including trovafloxacin (discontinued), sitafloxacin, prulifloxacin, gatifloxacin (discontinued), clinafloxacin, gemifloxacin (tentative approval), moxifloxacin, besifloxacin, garenoxacin, and alatrofloxacin (discontinued), is termed fourth generation (Figure 4). This generation has potent activity against anaerobes and increased activity against pneumococci (Higgins et al., 1978). The addition an alkyl substitution of either ring type improves solubility (causing less risk of crystalluria), and activity of the fluoroquinolone also prolongs the half-life (Bolon, 2009, 2011; Domagala, 1994).

Molecule substitutions have resulted in advanced generations of fluoroquinolone that have expanded the spectra of activity, improved safety, and enhanced pharmacokinetic properties with better tissue penetration involving the gastrointestinal, genitourinary, and respiratory systems and skin and soft tissues as well, depending on dosing and the specific fluoroquinolone (Jones and Mandell, 2002).

Chemical structure

The empirical formula of norfloxacin is $C_{16}H_{18}FN_3O_3$, and physically it is a light-yellow crystalline powder with a molecular mass of 319.331 g/mol and melting point of about 220° to 221°C. The Chemical Abstracts Service (CAS) register number is 70458-96-7 (O'Neil, 2006). It is freely soluble in glacial acetic acid and very slightly soluble in ethanol, methanol, and water (*British Pharmacopoeia 2014,* 2014; *United States Pharmacopeia*, 2013). It is odorless and has a bitter taste. The partition coefficient (octanol/water) of this drug is 0.46 (O'Neil, 2006).

Norfloxacin has two receptors of protons groups, which correspond to two chemical ionization equilibria in a physiologically relevant pH range. The carboxylic group protonates up

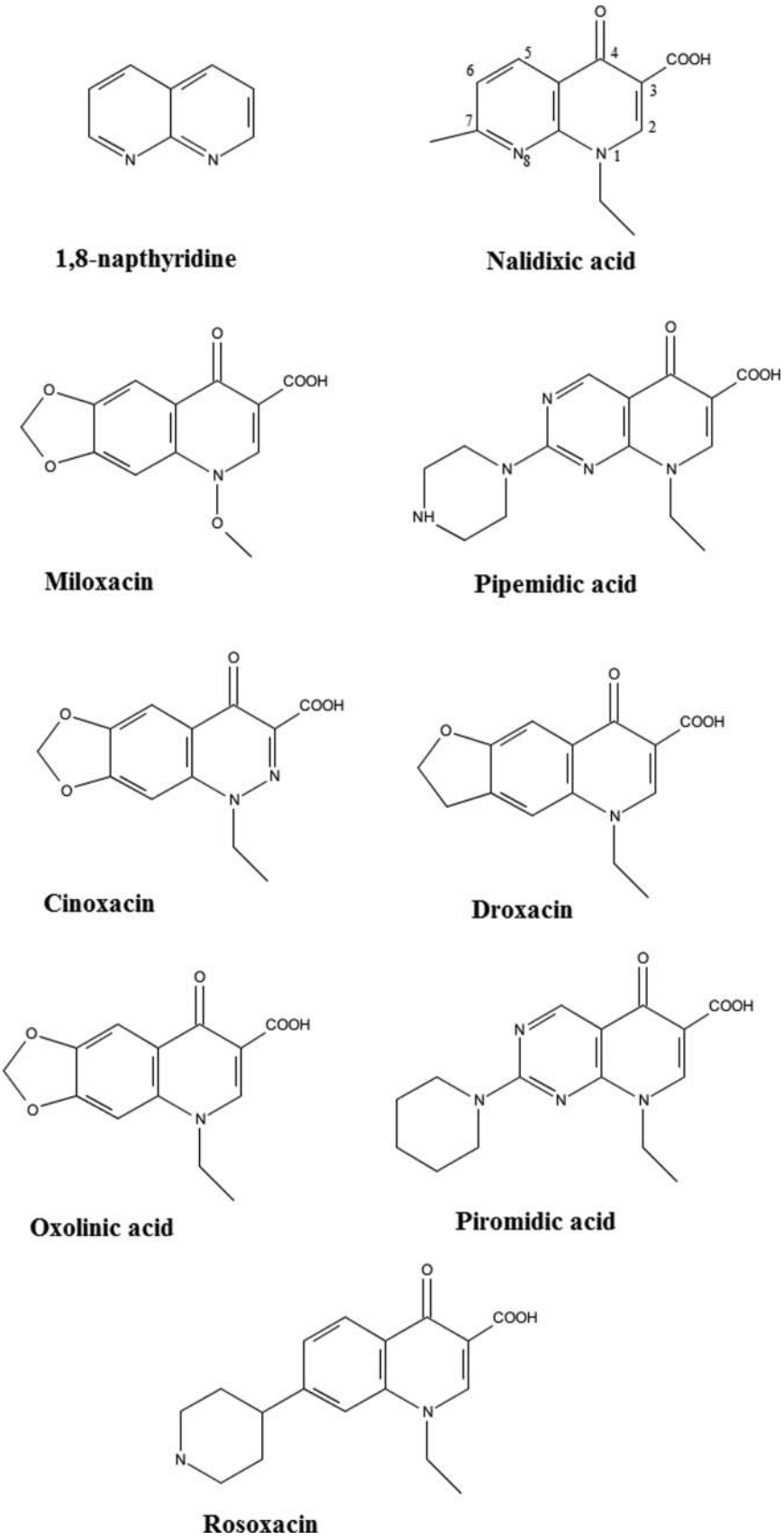

Figure 1. First-generation quinolones.

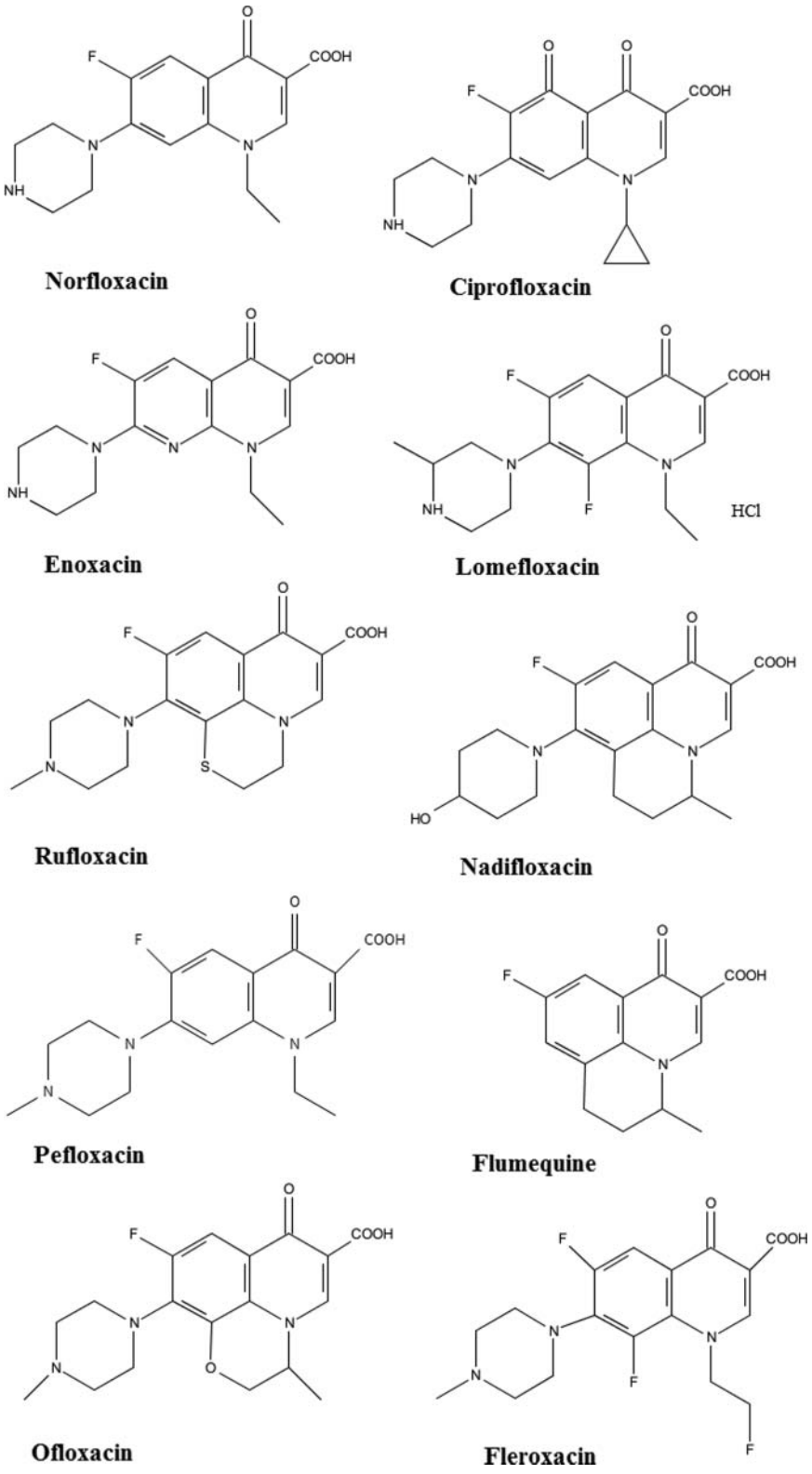

Figure 2. Second-generation quinolones.

amid slightly acid conditions, $pKa_1 = 6.34$. The group associated with the nitrogen at position four at the piperazine ring protonates in alkaline medium, $pKa_2 = 8.75$ (Mouton et al., 2005). At neutral pH, there is predominantly zwitterion (with deprotonated carboxylic group and the protonated N-4). At pH 10, more than 90% of the drug will be in ionic form; at pH to 4.5 or less it will be in cationic form. Norfloxacin exhibits greater aqueous solubility at pH below 4.5 or above 8.0 (Musa and Eriksson, 2009).

Mechanism of action

The fluoroquinolones in general rapidly inhibit deoxyribonucleic acid (DNA) synthesis by promoting cleavage of bacterial

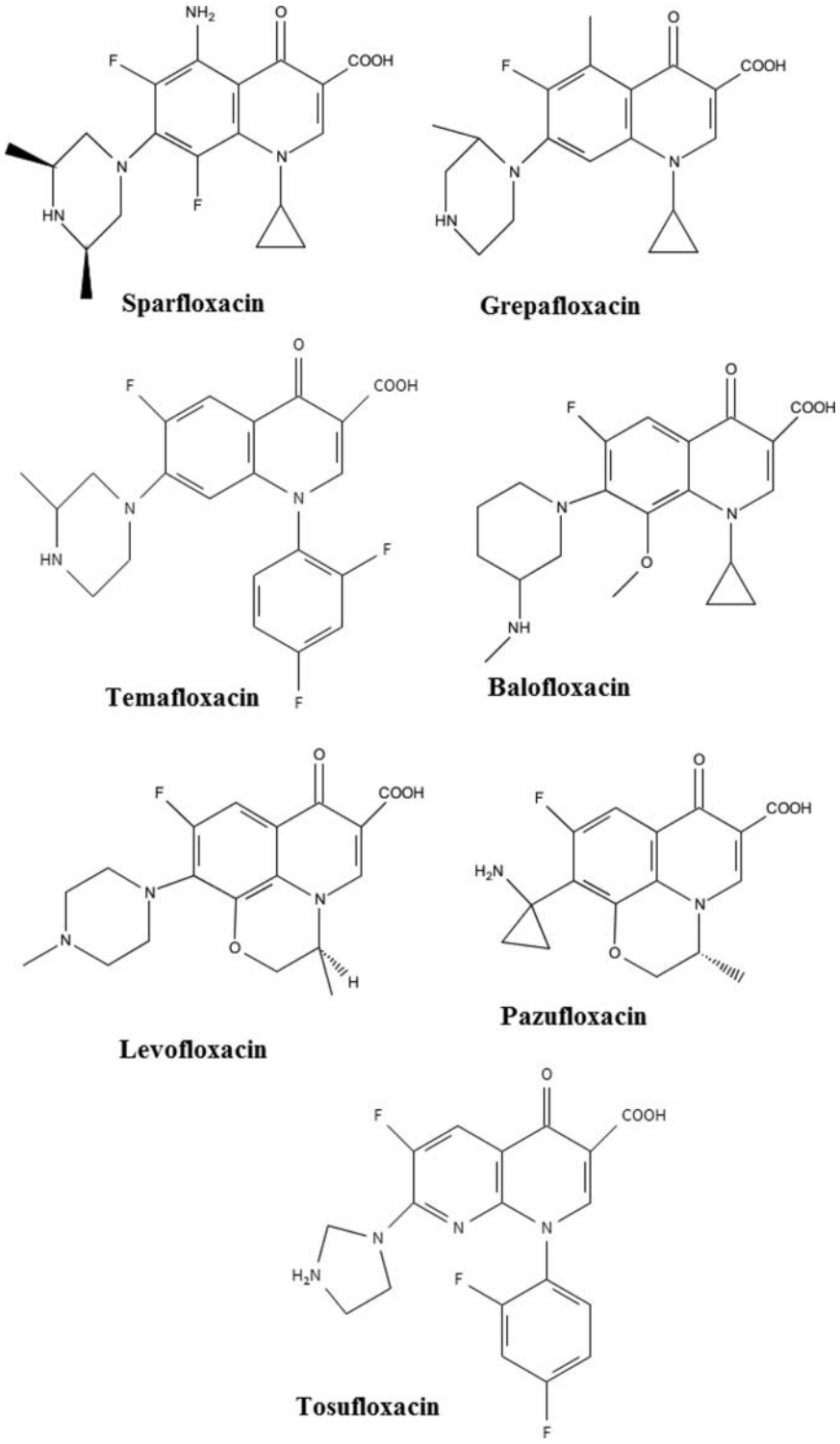

Figure 3. Third-generation quinolones.

DNA in the DNA enzyme complexes of DNA gyrase and type IV topoisomerase, resulting in rapid bacterial death (Hooper, 1999).

Norfloxacin in particular inhibits bacterial DNA gyrase (topoisomerase II), an enzyme that converts covalently closed circular DNA into negative supercoils (Sharma et al., 2008). This DNA gyrase, present in bacteria, is the only topoisomerase II known to introduce negative superhelical turns into duplex DNA (Hayashi et al., 2004). This DNA gyrase enzyme is able to accumulate the energy released from the hydrolysis of adenosine triphosphate (ATP) to drive the formation of supercoils. It is believed that the drug directly acts on DNA, producing a covalent attachment of DNA gyrase, which forms a complex that is inaccessible to the action of DNA polymerase; thus, it leads to prevention of DNA synthesis and replication, which ultimately results in rapid cell death (Goldstein, 1987; Lee and Ronald, 1987; Moellering, 1987; Schaeffer, 1987; Shen and Pernet, 1985; Shen et al., 1990).

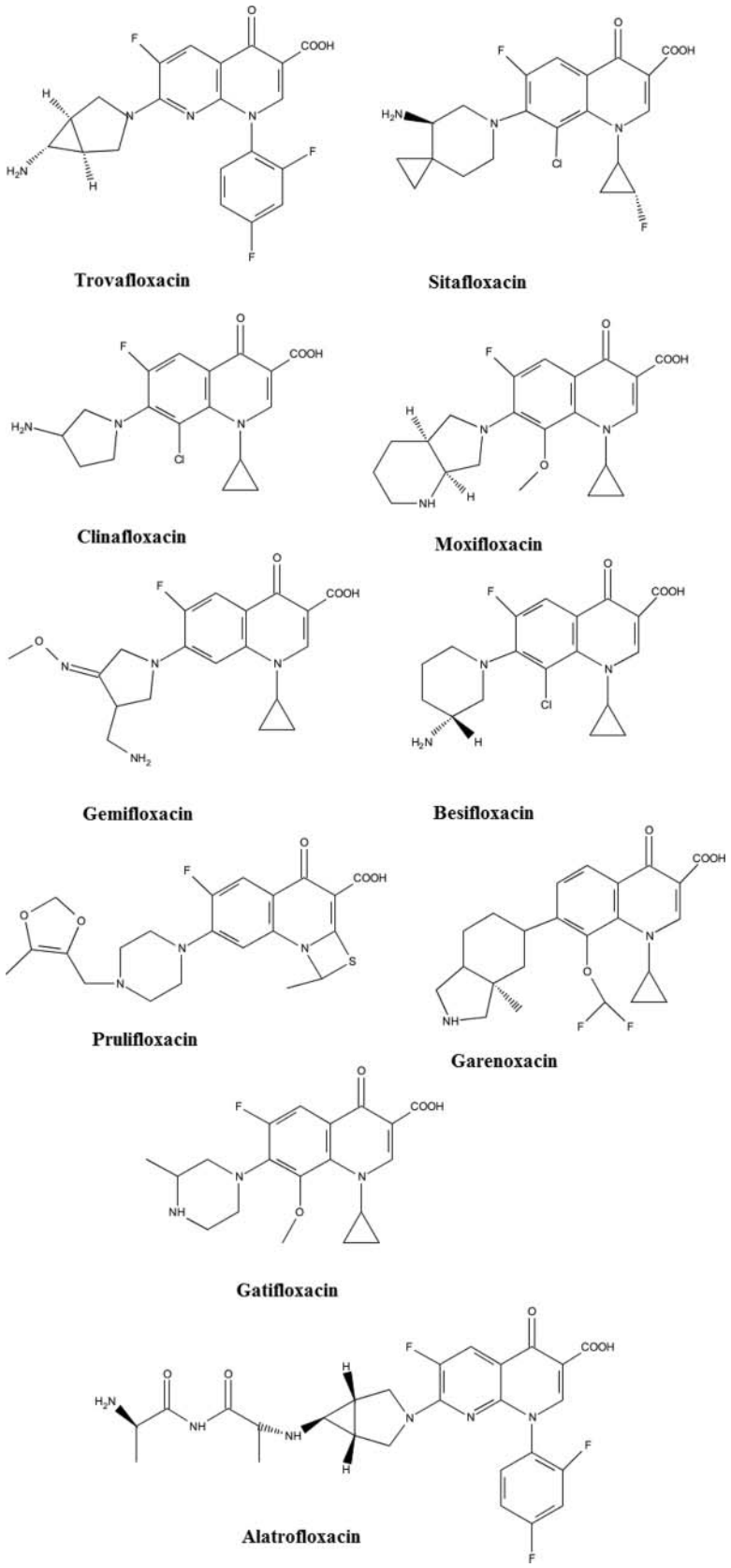

Figure 4. Fourth-generation quinolones.

Pharmacokinetics

Chen et al. (2012) reported that the bactericidal activity of fluoroquinolones is concentration-dependent. Segreti et al. (2012) have discussed the important role played by the pharmacokinetics data combined with the minimum inhibitory concentration (MIC) data in predicting antibacterial efficacy in various infection models.

The correlation between pharmacokinetics and pharmacodynamic data (PK/PD) and how it provides a better view of drug effect over time was shown by Liu et al. (2002) and highlighted by Segreti et al. (2012). In agreement with Segreti et al. (2012), since fluoroquinolones have concentration-dependent bactericidal activity, the area under the curve (AUC)/MIC and the maximum plasma concentration (C_{max})/MIC ratios are the best PK/PD indices predictive of efficacy.

As discussed by Segreti et al. (2012), for fluoroquinolones 90% of their maximum efficacy against Gram-negative bacilli can be reached at an AUC/MIC ratio of 100–125 and against Gram-positive organisms at a C_{max}/MIC90 ratio equal to or higher than 10 or an AUC(0–24)/MIC90 ratio of 30–50.

For urinary tract infections (UTIs) the peak concentrations in urine might be the key factor in the therapeutic outcome. Levofloxacin and ciprofloxacin have significant renal excretion and they are some of fluoroquinolones indicated for treatment of UTIs (Moellering, 1987).

In agreement with Chen et al. (2012), following the PK/PD parameters of fluoroquinolones, it is possible to predict clinical failure when the levofloxacin MIC of the causative pathogen is higher than 32 mg L^{-1} (when measured in urine 8–12 h after drug intake) or 64 mg L^{-1} (when measured in urine sampled 12–24 h after drug intake). A concern about the fluoroquinolones is the emergence of resistance. Chen et al. (2012) reported that previously, lower doses of fluoroquinolones (such as 250 mg of levofloxacin every 24 h) were recommended, and currently higher daily doses have been recommended (such as 750 mg of levofloxacin every 24 h).

Absorption

Absorption of the quinolone antimicrobials after oral administration is quite good and penetrates cells, extravascular compartments, and tissues extremely well.

The fluoroquinolones are rapidly and almost completely absorbed from the gastrointestinal tract. As noted by Bolon (2011) and Borcherding et al. (1996), the fluoroquinolones' favorable pharmacokinetic properties have encouraged their widespread use. Peak serum concentrations obtained after oral administration are very near those achieved with intravenous administration. Absorption of orally administered fluoroquinolones is significantly decreased when these agents are coadministered with magnesium, aluminum, iron, zinc, or calcium; these compounds make an insoluble drug cationic chelate complex in the gastrointestinal tract. The absorption of these drugs is only slightly affected by food.

Distribution

The quinolones demonstrate excellent and relatively comparable tissue penetration. Quinolones are concentrated in kidney, lung, bronchial, nasal, bone, and prostate cells, and enter into phagocytic cells and white blood cells. Peak concentration is in gall blander tissue, and pancreatic fluid concentrations are several times those in serum (Just, 1993; Robson, 1992). Distribution of the fluoroquinolones into respiratory tract tissues and fluids is of particular interest because of the activity of these agents against common respiratory pathogens (Garey and Amsden, 1999).

Metabolism and elimination

The individual fluoroquinolones differ markedly in their degree of metabolic biotransformation. The degree of metabolism explains the differences observed in the total body clearance and elimination half-life of these drugs (Stein, 1996).

The long half-lives of the fluoroquinolones allow once or twice daily dosing. The elimination of quinolones can be by two pathways: renal and nonrenal (gastrointestinal or hepatic) (Fitton, 1992). They are present in most secretions and accumulate in urine and feces. In contrast, penetration into the central nervous system is minimal, so these agents are inadequate for first-line treatment of meningitis. To avoid toxicity, dosages often need to be adjusted in patients with renal or hepatic impairment (Alghasham and Nahata, 1999).

Norfloxacin pharmacokinetics

For norfloxacin, after an oral dose of 200–400 mg, mean peak serum concentrations of 0.8 ± 0.3 and 1.5 ± 0.6 mg L^{-1} are respectively achieved within 60–90 min. The presence of food and dairy products slightly affects its absorption. Studies in animals show that the volume of distribution of norfloxacin is very large, about 50% of body weight, and its bioavailability is 50–80%. Approximately 15% of the drug in the serum is bound to plasma proteins. After a single oral dose of 200 mg, norfloxacin has not been found in human milk and has a very low central nervous system penetration due to relatively low lipophilicity. The metabolism and excretion is through biliary and kidney systems. Its elimination half-life is 3 h approximately (Chenel et al., 2004; Delon et al., 1999; Sarro and Sarro, 2001).

Clinical applications

Improvements in the spectrum of activity and tissue penetrations of fluoroquinolones have been followed by extension of its indications involving the treatment of urinary tract, gastrointestinal, respiratory tract, bone and joint, and skin and soft tissue infections, as well as sexually transmitted diseases (Jones et al., 2002).

Norfloxacin is useful for the treatment of diseases causing genitourinary infections (cystitis, pyelitis, cystopyelitis, pyelonephritis, chronic prostatitis, epididymitis, and infections associated with urological surgery and neurogenic bladder); acute gastroenteritis; bacterial infections caused by susceptible microorganisms; gonococcal cervicitis caused by strains of *Neisseria gonorrhoeae* and not producing penicilinase; infections caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Proteus mirabilis*, *Proteus vulgaris*, *Citrobacter freudii*, *Staphylococcus aureus*, *S. epidermidis*, *S. saprophyticus*, *Enterococcus faecalis*, *Enterobacter aerogenes*, and *Serratia marcescens*; superficial ocular infections involving the cornea conjunctiva; typhoid fever (Grangie et al., 1998); and infections caused by some Gram-positive organisms (*Streptococcus pneumoniae* excluded) (Gauzit and Lakdhari, 2012; Ramirez-Ronda et al., 1987; Roner et al., 2004; Wagenlehner et al., 2011).

Appropriate culture and susceptibility tests should be performed before treatment in order to isolate and identify organisms causing the infection and to determine their susceptibility to norfloxacin.

Quality control

The quinolones and fluoroquinolones are marketed all around the world and are available as generic products of several chemical entities of these antibiotical classes. These generic products accounted for more than two-thirds of their worldwide consumption in 2010 (Gauzit and Lakdhari, 2012).

As stated by Gauzit and Lakdhari (2012) the goals of a drug policy supporting the use of generic drugs are to decrease the cost of drugs for the healthcare budget of developed countries and to facilitate access to care in developing countries. According to the authors there are many published reports on problems with the quality of these products that lead to questioning the therapeutic equivalence of generic drugs.

For Cox (1987) the nonobservance of quality standards remains possible for oral antibiotics, despite a specific regulation on this issue, and there are published cases of antibiotical generic products that did not observe FDA and European Medicines Agency (EMA) quality standards, such as absence of sterility when it is required and the presence of impurities.

To stop the marketing of low-quality pharmaceutical products it is important that the regulatory agencies have specific rules available and a surveillance service that has the authority to disapprove these products as well as remove them from the market. The analytical tools available play an important role helping to identify and separate high-quality products from the others.

The development of analytical methods for the qualitative and quantitative quality control of pharmaceuticals should be based on good planning. The methods should allow for complete analysis of the product; considering aspects such as identification and determination of the active substance, the identification and determination of levels of impurity and degradation products, and verification of the stability of the active substance in the formulation is very important.

Different methods describing the determination of fluoroquinolone concentrations in pharmaceutical products, biofluids, and groundwater as well in food have previously been reported. HPLC with UV or fluorescence detection was the technique most used. Other techniques like CE, spectrophotometry, thin-layer chromatography, and microbiological assay have been used to determine fluoroquinolones. Several articles have reported the separation and simultaneous quantification of two or more fluoroquinolones.

Fluoroquinolones are amphoteric molecules obtained by the modification of the quinolone core mentioned above. Fluoroquinolones are slightly soluble in water and subject to strong UV light degradation. That molecular structure determines their solubility in water and their strong ability to form stable complexes with cations like magnesium, calcium, iron, zinc, and aluminum. Despite resistance to heat and hydrolysis, fluoroquinolones show photosensitivity. Irradiation in water leads to oxidative degradation and defluoronation of the amine side chain (Domagala, 1994). In the next sections we will present a literature review of analytical methods for identification and quantification of norfloxacin in different matrices.

Thin-layer chromatography (TLC)

In the United States Pharmacopeia (2013) the TLC method described uses water, diethylamine, toluene, chloroform, and methanol (8:14:20:40:40 v/v/v/v/v) as the mobile phase. The same methods are described in the *British Pharmacopoeia* (2014), the Brazilian pharmacopeia (*Farmacopéia Brasileira*, 2001), the Portuguese pharmacopeia (*Farmacopeia Portuguesa*, 2005), and the *European Pharmacopoeia* (2011).

Microbiological method

Froehlich and Schapoval (1990a) described the bioassay by agar diffusion (cylinders and plate) using the strain *Bacillus subtilis* (American Type Culture Collection 6633) and agar 11. Later, the Brazilian pharmacopeia also described the same 3 × 3 bioassay for norfloxacin (*Farmacopéia Brasileira*, 2001).

High-performance liquid chromatography (HPLC)

The liquid chromatographic method for the determination of norfloxacin is the choice of some pharmacopeias (*British Pharmacopoeia 2014,* 2014; *European Pharmacopoeia*, 2011; *Farmacopéia Brasileira*, 2001; *Farmacopeia Portuguesa*, 2005; *United States Pharmacopeia*, 2013). HPLC has also been applied for the determination of norfloxacin in biological samples like urine, plasma, tissues, and serum.

Forchetti et al. (1984) reported the first study to correlate the pharmacological effects of norfloxacin with its tissue concentrations. Montay and Tassel (1985) described an HPLC procedure for the quantitation of pefloxacin and its main active metabolites in human urine, norfloxacin and oxonorfloxacin, however, the sensitivity of this assay was not sufficient to determine plasma levels of the metabolite. Groeneveld and Brouwers (1986) reported an HPLC method for the analysis of norfloxacin, ciprofloxacin, and pefloxacin in serum. The quinolones were extracted using dichloromethane under neutral conditions, followed by drying under nitrogen and dissolving in mobile phase before chromatography. Morton et al. (1986) compared a standard bioassay with an HPLC method for determination norfloxacin and ciprofloxacin concentrations in body fluids.

Nilsson-Ehle (1987) improved on the HPLC reported earlier. However, the assays described for norfloxacin and ciprofloxacin involve rather elaborate sample preparation. In this method the serum and urine samples can be directly injected into the equipment. Both studies reported by Laganà et al. (1987, 1988) described an HPLC method with fluorimetric detection for the quantitative determination norfloxacin in renal and prostatic tissues and plasma. Hussain et al. (1995) reported an HPLC method using fluorescence detection; following protein precipitation with 10% trichloroacetic acid, norfloxacin and internal standard enoxacin were extracted from plasma with chloroform, dyed, and reconstituted in the mobile phase. Wallis et al. (1995) determined norfloxacin in serum with ethylnorfloxacin as the internal standard; they were

extracted with chloroform. Mascher and Kikuta (1998) described analysis of norfloxacin in human plasma and urine, deproteinized with acetonitrile. Yamada et al. (2003) determined three fluoroquinolones (levofloxacin, norfloxacin, and lomefloxacin) into the aqueous humor in human eyes. Espinosa-Mansilla et al. (2005) determined fluoroquinolones in urine and serum based in the separation of the formed irradiation photoproducts; in another work (Espinosa-Mansilla et al., 2006) the compounds were analyzed by an isocratic elution method, using a mixture of tetrahydrofuram and phosphate buffer. Bedor et al. (2007) developed a method using ultraviolet detection to analyze human plasma and applied it to a bioequivalence study between two norfloxacin formulations. Sher et al. (2010) described the determination and bioequivalence study of norfloxacin in tablet formulations by using ciprofloxacin as an internal standard. Payán et al. (2011) described three phase hollow fiber-based liquid phase microextraction combined with HPLC to analyze fluoroquinolones (Table 2).

Several reports have described analysis of norfloxacin tablets. Chen et al. (1993), described a method to study the thermal stability of the drugs by following the degradation of norfloxacin glutamate and glucuronate. Córdoba-Borrego et al. (1999) described the dissolution interference with antacids. Kassab et al. (2005) developed one method to determine ciprofloxacin and norfloxacin. Shervington et al. (2005) reported an HPLC method to separate five quinolones. Oliveira et al. (2009) prepared a new formulation of norfloxacin extended-release tablets. Patel et al. (2011) developed a method to separate norfloxacin and ornidazole in their combined dosage form. Sebaiy et al. (2011) developed a method to perform simultaneous separation of norfloxacin and tinidazole. Chierentin and Salgado (2013) reported a reversed-phase liquid chromatography (RP-LC) method to determine norfloxacin in tablets. A method to determine norfloxacin residues in pharmaceutical industry equipment was described by Simonovska et al. (1999). Determination of synthetic impurities was described by Rao and Nagaraju (2004); they first synthesized the impurities by heating 1-ethyl-6-fluoro-7-chloro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (ECA) with piperazine. During this reaction, not only the unreacted ECA but also its related analogues, 7-chloro-6-fluoro-1-methyl-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (MCA) and ethyl-7-chloro-6-fluoro-4-oxo-1,4-dihydro-3-quinolinecarboxylate (CAT), were obtained and analyzed by HPLC. Groundwater was analyzed by Mitani and Kattaoka (2006); the researchers developed a useful approach for determination of five fluoroquinolones in environmental waters, using a fully automated method consisting of in-tube solid-phase microextraction (SPME) coupled with liquid chromatography-tandem mass spectrometry (LC/MS/MS). Vázquez et al. (2012) developed an ultrasound-assisted ionic liquid dispersive liquid-liquid microextraction (US-IL-DLLME), used for extraction of eight fluoroquinolones in groundwater.

Microwave-assisted extraction (MAE) and HPLC were used to determine eight fluoroquinolones in agricultural soils by Sturini et al. (2010). A solid-phase extraction (SPE) and LC method was developed for determination of three fluoroquinolones in wastewater samples by Lee et al. (2007). Lillenberg et al. (2009) determined fluoroquinolones from sewage sludge by pressurized liquid extraction (PLE) and quantification by HPLC with electrospray ionization mass spectrometry. Shao et al. (2009) presented a multi-residue method for the analyses of 76 pharmaceutical agents including fluoroquinolones in slaughterhouse wastewater and receiving river. Yan et al. (2011) investigated pharmaceutical wastewater by UA-DLLME coupled with LC-UV. Khan et al. (2012) presented a method to determine antibiotics, antivirals, and nasal decongestants in treated sewage effluent and surface water by SPE and LC-MS/MS (Table 2).

A method to determine norfloxacin in foods was developed by Gigosos et al. (2000), using solid-phase extraction, for assaying 5 quinolones with confirmative diode-array detection in samples of bovine kidney and muscle and eggs. Pecorelli et al. (2003) described a simple multi-residue method for assaying 13 quinolones in feeds. The samples were extracted by a metaphosphoric acid/acetonitrile mixture and automatically purified. Wan et al. (2006) determined five quinolones by HPLC coupled with chemiluminescence detection. The method was successfully applied to the determination of quinolones in prawn samples. Christodoulou et al. (2007) proposed a method for the determination of 10 quinolones in chicken muscle and egg yolk. Bogialli et al. (2008) developed a sensitive procedure for determining residues of 8 widely used quinolone antimicrobials in bovine milk. The method was based on the matrix solid-phase dispersion technique with hot water as extractant followed by LC/MS/MS. Galarini et al. (2009) described an assay for 11 quinolones in feeds at sub-additive levels; the samples were extracted by a metaphosphoric acid/acetonitrile mixture. Gajda et al. (2012) developed a procedure to determine 7 fluoroquinolones and 3 quinolones in eggs. The procedure was based on dispersive solid-phase extraction technique with acetonitrile as extractant. Moema et al. (2012) determined 6 fluoroquinolones from chicken liver samples by a pretreatment method using liquid-liquid microextraction (DLLME) (Table 2).

Capillary electrophoresis (CE)

The capillary electrophoresis technique is a good choice to analyze norfloxacin in biological samples because it can be determined with several kinds of compounds, without the need to change solvents, analytical columns, and procedures. Hernández et al. (2000) developed capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) methods to separate and determine 10 quinolones in pig plasma samples. The influence of different conditions, such as the buffer and pH of the electrolyte, the surfactant and ion-pairing agents added to the electrolyte, and the organic modifier, was studied. Kowalski et al. (2003) investigated the presence of antibiotics based on the European Union–defined maximum residue limits (MRLs) for veterinary drug residues in food products; they used capillary electrophoresis with ultraviolet detector (CE-UV) to quantify residues from poultry and porcine tissues. Ferdig et al. (2004) developed a capillary electrophoresis with fluorescence detector (CE-FL) to determine residues of fluoroquinolones in food or other matrices. Deng et al. (2006) described a method by capillary electrophoresis with end-column electrochemiluminescence (ECL) detection to determine norfloxacin in human urine.

Table 2. Chromatographic systems for determination of norfloxacin in biological and pharmaceutical samples reported in the literature.

Matrices	Method	λ (nm)	Mobile phase	Column	Range	Reference
Tablets	HPLC	275	ACN: PHA (15:85 v/v)	L1	N	*United States Pharmacopeia*, 2013
Tablets	HPLC	265	ACN: PHA (5:95 v/v), pH 2.0	C_{18} (250 × 4.6 mm; 5 μm)	N	*British Pharmacopoeia 2014*, 2014
Tablets	HPLC	275	PHA 0.1%: ACN (85:15 v/v), pH 4.0	C_{18} (300 × 3.9 mm)	N	*Farmacopéia Brasileira*, 2001
Tablets	HPLC	275	ACN: PHA (15:85 v/v)	L1	N	*Farmacopeia Portuguesa*, 2005
Tablets	HPLC	275	ACN: PHA (15:85 v/v)	L1	N	*European Pharmacopoeia*, 2011
Urine, plasma and tissues	HPLC	280	ACN: FB (20:80 v/v), pH 7.0	Vydac (250 × 4.5 mm; 10 μm)	1.0–500.0 μg mL^{-1}	Forchetti et al., 1984
Tissues	HPLC	$\lambda_{(exc)} = 330$ $\lambda_{(em)} = 440$	ACN: AB (15:85 v/v), pH 4.8	Nucleosil C_{18} (100 × 5.0 mm; 3 μm)	0.06–10 μg mL^{-1}	Montay and Tassel, 1985
Serum	HPLC	278	Solution of PHA with Bu_4NOH: MetOH (70:30 v/v), pH 2.2	Chrompak Nucleosil C_{18} (250 × 4.6 mm; 5 μm)	0.3–1.5 μg mL^{-1}	Groeneveld and Brouwers, 1986
Serum and plasma	HPLC	$\lambda_{(exc)} = 278$ $\lambda_{(em)} = 456$	ACN: FB (15:85 v/v), pH 3.0	Waters Bondapak C_5 (150 × 3.9 mm; 5 μm)	0.125–20.0 μg mL^{-1}	Morton et al., 1986
Serum and urine	HPLC	$\lambda_{(exc)} = 278$ $\lambda_{(em)} = 445$	ACN: FA with Bu_4NOH (11:89 v/v), pH 3.0	Macherey-Nagel Nucleosil C_{18} (200 × 4.0 mm; 5 μm)	10.0–100.0 μg mL^{-1}	Nilsson-Ehle, 1987
Plasma and tissues	HPLC	$\lambda_{(exc)} = 300$ $\lambda_{(em)} = 420$	ACN: MetOH: FB (19:3:78 v/v/v), pH 2.5	Partisil pxs C_8 (250 × 34.6 mm; 10 μm)	5.0–1500.0 ng mL^{-1}	Laganà et al., 1987
Plasma and fluids	HPLC	$\lambda_{(exc)} = 300$ $\lambda_{(em)} = 420$	ACN: MetOH: FB (19:3:78 v/v/v), pH 2.5	Whatman Partisil pxs C_8	50.0–500 ng mL^{-1}	Laganà et al., 1988
Plasma	HPLC	$\lambda_{(exc)} = 280$ $\lambda_{(em)} = 418$	MetOH: TFA	Brockville Zorbax C_8 (80 × 4.6 mm; 5 μm)	0.025–5.0 μg mL^{-1}	Hussain et al., 1995
Serum	HPLC	279	ACN: FB (11:89 v/v), pH 2.5	Biosystems RP-18 (400 × 3.2 mm)	N	Wallis et al., 1995
Plasma and urine	HPLC	$\lambda_{(exc)} = 300$ $\lambda_{(em)} = 450$	MetOH: PA with trietilamine (30:70 v/v)	Nucleosil C_{18} (800 × 4.0 mm; 3 μm)	31.0–2507 ng mL^{-1}	Mascher and Kikuta, 1998
Vitreous	HPLC	$\lambda_{(exc)} = 290$ $\lambda_{(em)} = 470$	ACN: FA (15:85 v/v), pH 3.0	TSK-GEL ODS-80 TOSOH	0.025–1.25 μg mL^{-1}	Yamada et al., 2003
Urine and serum	HPLC	$\lambda_{(exc)} = 277$ $\lambda_{(em)} = 444$	FB: THF (96:4 v/v), pH 3.0	Waters Novapak C_{18} (150 × 3.9 mm)	6.0-14–0 ng mL^{-1}	Espinosa-Mansilla et al., 2005
Urine and serum	HPLC	$\lambda_{(exc)} = 277$ $\lambda_{(em)} = 490$	FB: THF (92:8 v/v), pH 3.0	Novapak C_{18} (150 × 3.9 mm; 4 μm)	N	Espinosa-Mansilla et al., 2006
Plasma	HPLC	280	FB: ACN (88:12 v/v), pH 3.0	Phenomenex Gemini C_{18} (150 × 4.6 mm; 5 μm)	25.0–3000 ng mL^{-1}	Bedor et al., 2007
Plasma	HPLC	280	MetOH: FB: ACN (30:30:40 v/v/v), pH 3.0	Agilent Shimpak ODS (250 × 4.6 mm; 5 μm)	30.0–200.0 ng mL^{-1}	Sher et al., 2010
Biological sample	HPLC	274	ACN: FA (14:86 v/v), pH 2.6	Star RP C_{18} (750 × 4.0 mm; 3 μm)	0.06–1000 μg L^{-1}	Payán et al., 2011
Tablets	HPLC	278	MetOH: water: MeSH (50:50:0.4 v/v/v), pH 5.5	Hypersil ODS C_{18} (100 × 4.6 mm; 5 μm)	1.0–45.0 μg mL^{-1}	Chen et al., 1993
Tablets	HPLC	278	ACN: FB (15:85 v/v), pH 3.0	LiChrosorb-RP8 (200 × 4.6 mm; 10 μm)	10–20.0 μg mL^{-1}	Córdoba-Borrego et al., 1999
Tablets	HPLC	279	Water: ACN: trietilamine (80:19.7:0,3 v/v/v), pH 3.3	LiChrospher 100 RP-18 (125 × 4.0 mm; 5 μm)	4.0–24.0 μg mL^{-1}	Kassab et al., 2005
Tablets	HPLC	275	ACN: acetate TBAA, dodecil, ammonium sulfate, and citric acid (35:65 v/v)	Phenomenex C_{18} (150 × 4.6 mm; 5 μm)	N	Shervington et al., 2005
Tablets	HPLC	272	FB:ACN (84:16 v/v), pH 3.0	Luna C_{18} (150 × 4.6 mm)	0.05–5 μg mL^{-1}	Oliveira et al., 2009
Tablets	HPLC	294	FB: ACN: MetOH (15:70:15 v/v/v), pH 2.5	Prontostl-AQ ODS (250 × 4.6 mm; 5 μm)	4.0–20.0 μg mL^{-1}	Patel et al., 2011

(*continued*)

Table 2. (*Continued*)

Matrices	Method	λ (nm)	Mobile phase	Column	Range	Reference
Tablets	HPLC	290	MetOH: FB (20:80 v/v), pH 3.0	Chromolith performance RP-18 (100 × 4.6 mm)	1.0–80.0 μg/mL	Sebaiy et al., 2011
Tablets	HPLC	277	5% acetic acid aqueous solution: MetOH (80:20 v/v)	Zorbax C18 Agilent RP-18 (150 × 4.6 mm)	10–30.0 μg mL^{-1}	Chierentin and Salgado, 2013
Residues of norfloxacin on pharmaceutical equipment	HPLC	$\lambda_{(exc)} = 277$ $\lambda_{(em)} = 446$	ACN: FB (11:89 v/v), pH 3.3	Bondapak C_{18} (250 × 4.0 mm; 10 μm)	10.0–90.0 ng mL^{-1}	Simonovska et al., 1999
Synthetic impurities	HPLC	260	FB: ACN (60:40 v/v), pH 3.0	Waters C_{18} (250× 4.6 mm; 5 μm)	N	Rao and Nagaraju, 2004
Groundwater	LC-MS	320.1 (m/z)	Ammonium solution: ACN (85:15v/v), pH 3.0	Capcel Pak C_8 Carboxen 1010 Plot	7.0–29.0 pg mL^{-1}	Mitani and Kataoka, 2006
Groundwater	HPLC	$\lambda_{(exc)} = 278$ $\lambda_{(em)} = 466$	ACN: FB (12:88 v/v)	Waters Aquasil-C_{18} (150 × 4.6 mm; 5 μm)	5.0–150.0 ng L^{-1}	Vázquez et al., 2012
Soil	HPLC	$\lambda_{(exc)} = 280$ $\lambda_{(em)} = 500$	FB: ACN	Ascentis Supelco (250 × 4.6 mm; 5 μm)	2.0–50.0 μg mL^{-1}	Sturini et al., 2010
Effluent (wastewater)	LC-MS	320 (m/z)	ACN: MetOH:FA: water (6:12:0.5:81.5 v/v/v/v)	Zorbax-sb C_8 (150 × 2.1 mm; 3.5 μm)	5.0–100.0 pg μL^{-1}	Lee et al., 2007
Effluent	LC-MS	320 (m/z)	MetOH: ammonium buffer (20:80 v/v), pH 2.8	Phenomenex Synergi (250 × 4.6 mm; 4 μm)	10.0–5000 ng mL^{-1}	Lillenberg et al., 2009
Treated effluent	LC-MS	N/I	FA 0.1%: MetOH (80:20 v/v)	Acquaty C_{18} (100 × 2.1 mm; 1.7 μm)	5.0–53.0 ng L^{-1}	Shao et al., 2009
Effluent from the pharmaceutical industry	HPLC	280	MetOH: Water: TFA (70:30:0.05 v/v)	Zorbax Eclipse XDB C_{18} (150 × 4.6 mm; 5 μm)	0.01–2.0 μg mL^{-1}	Yan et al., 2011
Effluent	LC-MS	302.1 (m/z)	FA: ACN	Hypersil Gold C_{18} (200 × 2.1 mm; 12 μm)	25.0–50.0 ng L^{-1}	Khan et al., 2012
Tissues and eggs	HPLC	280	ACN: PA (15:85 v/v)	Hypersil (250 × 4.6 mm; 5 μm)	4.0–100.0 ng mL^{-1}	Gigosos et al., 2000
Foods	HPLC	$\lambda_{(exc)} = 278$ $\lambda_{(em)} = 446$	ACN: THF: FB (50:1:49 v/v/v), pH 2.6	Phenomenex Luna C_5 (150 × 4.6 mm; 5 μm)	0.5–10.0 μg mL^{-1}	Pecorelli et al., 2003
Shrimp	HPLC	278	ACN: MetOH: AB (3:15:82 v/v/v), pH 3.65	Eclipse Zorbax C_{18} (150 × 4.6 mm; 5 μm)	0.36–2.4 ng mL^{-1}	Wan et al., 2006
Chicken muscle and egg yolk	HPLC	275	Aqueous solution TFA 0.1%: ACN: MetOH (80:10:10 v/v)	ODS-3 (250 × 4.0 mm; 5 μm)	15.0–600 μg kg^{-1}	Christodoulou et al., 2007
Milk	LC-MS	320 (m/z)	MetOH: ACN: water (35:35:30 v/v/v)	All-tech C_{18} (250 × 4.6 mm; 5 μm)	0.3–15.0 ng mL^{-1}	Bogialli et al., 2008
Animal food	HPLC	278 $\lambda_{(exc)} = 278$ $\lambda_{(em)} = 446$	ACN: PHA (20:80 v/v), pH 3.0	Phenomenex Gemini C_{18} (250 × 3.0 mm; 5 μm)	0.04–0.8 mg kg^{-1}	Galarini et al., 2009
Eggs	LC-MS	320 (m/z)	ACN: aqueus acid solution heptafluorobutyric	Phenomenex Luna C_{18} (150 × 2.0 mm; 3 μm)	0–50.0 μg kg^{-1}	Gajda et al., 2012
Chicken liver	HPLC	280	ACN: FA, pH 2.7	Waters Terra C_{18} (150 × 3.0 mm; 3.5 μm)	30.0–500.0 kg kg^{-1}	Moema et al., 2012
Plasma	CZE MEKC	260	Sodium tetraborate buffer (40 mM): MetOH 10%, pH 8.1	Fused silica (64.5 cm × 75 μm)	0.8–45.0 mg L^{-1}	Hernández et al., 2000
Bird and pig tissues	CE	280	Sodium bicarbonate buffer (30 mM), sodium tetraborate (25 mM)	Fused silica (60 cm × 75 μm)	0.01–1.0 μg mL^{-1}	Kowalski et al., 2003
Plasma	CE	$\lambda_{(exc)} = 240$ $\lambda_{(em)} = 400$	PHA (50 mM): ACN (60:40 v/v), pH 7.55	Fused silica (70 cm × 75 μm)	100–5000 μg L^{-1}	Ferdig et al., 2004
Urine	CE-CL	N	FB (15 mM), pH 8.2	Fused silica (40 cm × 75 μm)	0.05–10.0 μmol L^{-1}	Deng et al., 2006

Table 2. (*Continued*)

Matrices	Method	λ (nm)	Mobile phase	Column	Range	Reference
Rat liver	CE	$\lambda_{(exc)} = 325$ $\lambda_{(em)} = 435$	FB (50 mM), pH 4.6	Fused silica (60 cm × 75 μm)	0.01–100.0 μg mL^{-1}	Cheng et al., 2007
Urine	CE	N	FB (20 mM), pH 8.2	Fused silica (55 cm × 50 μm)	2.0–200.0 μmol L^{-1}	Liu et al., 2008
Urine	CE	254	Citrate buffer (20 mM), citric acid (4 mM), sodium sulfite (10 mM), pH 6.1	Fused silica (47.5 cm × 75 μm)	0.057–0.084 μg mL^{-1}	Yang et al., 2008
Chicken muscle	CE	N	TRIS buffer (30 mM), PHA (3 mM),pH 9.0	Fused silica (7.8 cm × 50 μm)	100–200.0 ng mL^{-1}	Qin et al., 2009
Milk	CE	220	Sodium tetaborate buffer, pH 10.0	Fused silica (50 cm × 75 μm)	0.15–4.0 μg mL^{-1}	Solangi et al., 2009
Milk	CZE	254	Tetraborate buffer (12 mM), pH 9.0	Fused silica (65 cm × 50 μm)	7.0–500.0 μg mL^{-1}	Wang et al., 2009
Water	CE-CL	325	FB (125 mM) and MetOH 36%, pH 2.8	Fused silica (70 cm × 75 μm)	0.3–1.9 ng^{-1}	Lombardo-Aqui et al., 2010
Tablets	CE	275	Sodium borate buffer (65 mM), sodium phosphate (35 mM), sodium cholate (60 mM): ACN (72:28 v/v), pH 7.3	Fused silica (67 cm × 50 μm)	25–300 μg mL^{-1}	Sun and Wu, 1999
Tablets	CE	214	FB (125 mM), pH 7.0	Fused silica (47.5 cm × 75 μm)	100–500 μg mL^{-1}	Fierens et al., 2000
Tablets	CZE	N/I	Ammonium carbonate buffer (120 mM), pH 9.12	Fused silica (34.2 cm × 50 μm)	N	McCourt et al., 2003
Tablets	CE	214	FB (50 mM), pH 8.0	Fused silica (50.2 cm × 50 μm)	N	Lin et al., 2004
Tablets	CE	301	FB (32.5 mM), pH 2.5	Fused silica (31.2 cm × 50 μm)	10–50.0 mg mL^{-1}	Alnajjar et al., 2007a
Tablets	CE	285	FB (10.0 mM), pH 2.5	Fused silica (31.2 cm × 50 μm)	1.0–50.0 mg mL^{-1}	Alnajjar et al., 2007b
Raw material	CE	214	Sodium borate buffer (50 mM), pH 7.3	Fused silica (60 cm × 50 μm)	N	Sun and Chen, 1997
Raw material	CE	280	FB (0.05 mM), pH 11.0	Fused silica (47 cm × 75 μm)	N	Barbosa et al., 1997
Raw material	CZE MEKC	280 $\lambda_{(exc)} = 325$ $\lambda_{(em)} = 420$	Sodium bicarbonate buffer (0,1 M), pH 9.2	Fused silica (57 cm × 75 μm)	0.5–10.0 mg L^{-1}	Schmitt-Kopplin et al., 1999

N = not described; HPLC = high-performance liquid chromatography; ACN = acetonitrile; MetOH = methanol; TBAA = tetrabutylammonium; FB = phosphate buffer; AB = acetate buffer; PHA = phosphoric acid; Bu_4NOH = tetrabutylammonium hydroxide; MeSH = dithylamine; TFA = trifluoroacetic acid; PA = perchloric acid; PH = potassium hydride; THF = tetrahydrofurane; CE = capillary electrophoresis; CZE = capillary zone electrophoresis; MEKC = micellar electrokinetic chromatography; CL = chemiluminescence; $\lambda_{(exc)}$ = wavelength of the excitation; $\lambda_{(em)}$ = wavelength of the emission.

Table 3. Spectrophotometric methods for determination of norfloxacin in biological and pharmaceutical samples reported in the literature.

Matrices	Method	λ (nm)	Solvent or reagent	Range	Reference
Tablets	VIS	575 485	Violet 3B Tropaeolin	5.0–40.0 μg mL^{-1}	Sastry et al., 1995
Tablets	VIS	550	Chloranilic acid	0.25–5.75 mg mL^{-1}	Amin et al., 1995
Tablets	VIS	524	Reineckato ammonium	N	Avadhanulu et al., 1999
Tablets	VIS	614	Brilliant blue G	0.4–8.0 μg mL^{-1}	Gowda and Seetharamappa et al., 2003
Tablets	VIS	547 545	Eosin and merbromin	1.0–20.0 μg mL^{-1} 0.8–16.0 μg mL^{-1}	El-Brashy et al., 2004a
Tablets	VIS	623	Cobalt thiocyanate	20.0–240.0 μg mL^{-1}	El-Brashy et al., 2005a
Tablets	VIS	453	Tetraiodide bismuth III	8.0–80.0 μg mL^{-1}	El-Brashy et al., 2005b
Tablets	VIS	290	0.1 M H_2SO_4	0.3–1.4 μg mL^{-1}	Salem, 2005
Tablets	VIS	477	NBD-Cl	2.5–15.0 μg mL^{-1}	Abdel-Hay et al., 2008
Tablets	VIS	550 520 591	Sudan II Congo red Gentian violet	0.5–4.0 μg mL^{-1} 0.5–9.0 μg mL^{-1} 0.5–6.0 μg mL^{-1}	Amin et al., 2008
Tablets	VIS	495	*p*-DAC	2.75 $\times$ 10^{-5}–3.44 $\times$ 10^{-4} mol.L^{-1}	Rufino et al., 2011
Raw material	VIS	374	Iron III	N	Lee et al., 1994
Tablets	VIS	410	Ferric chloride	0.10–0.30 mg mL^{-1}	Froehlich et al., 1990
Tablets	VIS	410	Marquis reagent	1.0–2.0 mg mL^{-1}	Froehlich and Schapoval, 1990b
Tablets	VIS	545	Eosin Palladium II	3.0–10.0 μg mL^{-1}	El-Walily et al., 1996
Tablets	VIS	567	Sudan III	0.4–12.0 μg mL^{-1}	Amin, 2000
Tablets	VIS	435	Iron III	0.2–1.4 μg mL^{-1}	Pojanagaroon et al., 2002
Tablets	FL	$\lambda_{(exc)} = 334$ $\lambda_{(em)} = 431$	Chloranilic acid	0.08–5.6 μg mL^{-1}	Du et al., 2003
Tablets	VIS	545 547	Merbromin Eosin Y	2–8 μg mL^{-1}	El-Brashy et al., 2004b
Tablets	VIS	603	Potassium permaganate	2.0–20.0 μg mL^{-1}	Rahman et al., 2004
Tablets	VIS	525	Ammonium reineckate	5.0–65.0 μg mL^{-1}	Ragab and Amin, 2004
Tablets	FL	$\lambda_{(exc)} = 277$ $\lambda_{(em)} = 453$	TCNQ	0.04–1.20 μg mL^{-1}	Du et al., 2005
Tablets	UV	322	Bismuth citrate	N	Shaikh et al., 2007
Tablets	VIS	625	*N*-vinilpiperazine	20.0–150.0 μg mL^{-1}	Darwish et al., 2009
Tablets	VIS	526	Potassium permanganate	N	Naik et al., 2009
Tablets	FL	$\lambda_{(exc)} = 278$ $\lambda_{(em)} = 355$	PABA	0.5–8.0 μg mL^{-1}	More et al., 2009
Raw material	VIS	300 297	Pipric acid 3.5-dinitrobenzoic acid	N	Refat et al., 2011
Tablets	UV	277	0.1 M HCl	1.0–2.0 mg mL^{-1}	Froehlich and Schapoval, 1990c
Tablets	UV FL	276 $\lambda_{(exc)} = 330$ $\lambda_{(em)} = 445$	0.1 M HCl	1.0–10.0 μg mL^{-1}	Córdoba-Borrego et al., 1996
Tablets	UV VIS	280 358	0.1 M HCl and NaOH Iron (II)	0.2–0.8 mg mL^{-1} 0.16–0.64 mg mL^{-1}	El-Khateeb et al., 1998
Raw material	UV FL	278 $\lambda_{(exc)} = 330$ $\lambda_{(em)} = 445$	0.1 M HCl	N	Córdoba-Díaz et al., 1998
Urine and serum	FL	$\lambda_{(exc)} = 272$ $\lambda_{(em)} = 446$	Acetate buffer pH 3.8	0.1–4.0 ng mL^{-1}	Vílchez et al., 2001
Tablets	FL	$\lambda_{(exc)} = 277$ $\lambda_{(em)} = 490$	0.1 M HCl	29.5–800 ng mL^{-1}	Ulu, 2009
Tablets	UV/VIS	277/520	0.01 M HCl/methanol	2–7 μg mL^{-1}/95–120 μg mL^{-1}	Chierentin and Salgado, 2014
Tablets	UV	277	0.1 M HCl	N	*Farmacopéia Brasileira*, 2001

VIS = visible spectrophotometry; FL = fluorescence spectrophotometry; UV = ultraviolet spectrophotometry; N = not described; NBC-Cl = 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole; TCNE = tetracyanoethylene; *p*-DAC = *p*-(dimethylamino) cinnamaldehyde; TCNQ = 7,7,8,8-tetracyanoquinodimethane; PABA = *p*-amino benzoic acid.

Cheng et al. (2007) described a CZE method to determine norfloxacin in the physiological perfusate of isolate rat liver. Norfloxacin and the internal standard triamterene were detected using laser-induced-fluorescence (LIF) detection. Liu et al. (2008) related a CE-LIF to determine norfloxacin and levofloxacin in human urine. Yang et al. (2008) developed a method by CE-ECL to quantify norfloxacin and prulifloxacin in a fortified urine sample. Qin et al. (2009) described CE-UV used to verify the influence of bovine serum albumin in determining five quinolones. Also, norfloxacin was determined in milk by Solangi et al. (2009) and Wang et al. (2009). Lombardo-Aqui et al. (2010) developed a sensitive CE-FL method to determine six fluoroquinolones of human and veterinary use in different kinds of water (Table 2).

Sun and Wu (1999) reported quantitative analyses of seven fluoroquinolones in tablet form (ciprofloxacin, enoxacin, lomefloxacin, norfloxacin, ofloxacin, pefloxacin, and sparfloxacin). Other works also determined norfloxacin tablets, like Fierens

et al. (2000), McCourt et al. (2003), Lin et al. (2004), and Alnajjar et al. (2007a, 2007b). Determination of norfloxacin in raw materials was proposed by Sun and Chen (1997), Barbosa et al. (1997), and Schmitt-Kopplin et al. (1999) (Table 2).

Spectrophotometric method

Spectrophotometric methods in the visible region for the determination of fluoroquinolones are based on the reaction of the drug with different reagents, yielding colored compounds (Marona and Schapoval, 2001). Some reagents are used for determination of norfloxacin through "complex formatting by ion pairing" (Table 3). Sastry et al. (1995) developed a method to determine some fluoroquinolone derivatives with supracene violet 3B and tropaeolin 000. Amin et al. (1995) reported the reaction to determine norfloxacin with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone, 7,7,8,8-tetracyanoquinodimethane (TCNQ), *p*-chloranil, and chloranilic acid as π-acceptors. Avadhanulu et al. (1999) determined some fluoroquinolone formed complexes with bromocresol green (BCG), bromocresol purple, bromocresol blue, bromothymol blue, and methyl orange in acidic buffer. Gowda and Seetharamappa (2003) proposed a complex with brilliant blue G in a buffer of pH 4.0. El-Brashy et al. (2004a, 2005a, 2005b) reported the follow ligands to yield ion association: BCG, *p*-chloranilic acid, tetracyanoethylene (TCNE), cobalt(II) thiocyanate at pH 2.5, and bismuth(III) tetraiodide. Salem (2005) reported a method to determine ciprofloxacin, pefloxacin, and sparfloxacin based on reaction with *p*-dimethylaminobenzaldehyde. Abdel-Hay et al. (2008) proposed the use of 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole in the presence of alkaline borate buffer. Amin et al. (2008) determined norfloxacin using Sudan II, Congo red, and gentian violet in universal buffer, and Rufino et al. (2011) proposed a reaction with *p*-dimethylaminobenzaldehyde in micellar medium.

The reactions through "forming a charge transfer complex" are described in Table 3. Lee et al. (1994) and Froehlich et al. (1990) and Froehlich and Schapoval (1990b) proposed complexation with iron(III) and Marquis reagent. El-Walily et al. (1996) described the reaction with palladium(II), and eosin. Amin (2000) reported a method with the formation of an ion pair with Sudan (II) in aqueous-acetone medium. Pojanagaroon et al. (2002) reported a reversed-flow injection colorimetric procedure based on the reaction between iron(III) and norfloxacin. A fluorescence spectroscopy method was described by Du et al. (2003) between chloranilic acid and some fluoroquinolones. El-Brashy et al. (2004b) performed a determination of pharmaceutical tablets in pure form and spiked human urine using a method based on binary complex between norfloxacin and eosin Y and merbromin. Rahman et al. (2004) described an oxidation of norfloxacin with alkaline potassium permanganate. Ragab and Amin (2004) reported a spectrometric, conductometric, and colorimetric method using reineckate dissolved in acetone. Du et al. (2005) proposed π-electron donors with TCNQ. Shaikh et al. (2007) reported a complex formation with bismuth citrate with aqueous solution of norfloxacin. Darwish et al. (2009) developed a method based on the reaction of *N*-vinylpiprazino formed from interaction of the mono-substituted piprazinyl group in norfloxacin. Naik et al. (2009) investigated the oxidation of norfloxacin by diperiodatargentate (III) in aqueous alkaline medium. More et al. (153) studied the interaction of norfloxacin and *p*-amino benzoic acid and Refat et al. (2009) reported the reaction between picric acid and 3,5-dinitrobenzoic acid acceptors.

Fluorescence and ultraviolet methods are described by Froehlich and Schapoval (1990c), Córdoba-Borrego et al. (1996), El-Khateeb et al. (1998), Córdoba-Díaz et al. (1998), Vílchez et al. (2001), Ulu (2009), Chierentin and Salgado (2004), and the Brazilian pharmacopeia (*Farmacopéia Brasileira*, 2001) (Table 3).

Conclusion

This review describes norfloxacin's properties, its antimicrobial activities, pharmacokinetic/pharmacodynamic characteristics, and therapeutic use and also presents an overview of the analytical methods for quantification of this drug. Pharmaceutical formulations have to meet regulations and provide efficacy without increasing risk to the life and treatment of the consumer. Therefore, strict quality control of this drug under study must be rigorously done. Besides the use of norfloxacin in humans, it also used in animals and added to their feed. It is important to control the use of norfloxacin for the treatment of infections in animals because it can result in resistance to bacterial treatment in humans.

Researchers are also concerned about the environment and have also quantified norfloxacin in effluent and soils, which may be a possible source of contamination by antibiotics. Finally, this literature overview is very important as norfloxacin has been used since the 1980s for various purposes, and basic and sophisticated techniques can provide crucial information about this antimicrobial.

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