

Review

Analytical profile of the fluoroquinolone antibacterials. I. Ofloxacin

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Accepted 30 January, 2008

In recent years, there has been rapid progress in quinolone research and development resulting in the production of many clinically important fluoroquinolones which have been subjected to diverse analytical or assay methods. This is the first review article in this series of the fluoroquinolone antibacterial agents with focus on the analytical profile of ofloxacin. Ofloxacin and indeed all the other fluoroquinolones are synthetic antibacterial agents structurally related to nalidixic acid. This article examines the synthesis, physico-chemical properties and analytical methods that have been used for the determination of ofloxacin in pharmaceutical dosages forms and biological fluids. The current relevant analytical trends and prospective analytical methods for ofloxacin have been presented and discussed.

Key words: Ofloxacin, fluoroquinolones, synthesis, physicochemical properties, pharmacokinetics, analytical methods, antibacterial.

INTRODUCTION

The fluoroquinolones are important antimicrobial agents that have demonstrated activity against a wide range of Gram-positive and Gram-negative organisms and have proved useful against micro-organisms that are resistant to other antibacterial agents. Some examples include ofloxacin, ciprofloxacin, perfloxacin, levofloxacin and norfloxacin with newer ones entering the scene almost every five years. Ofloxacin which is the focus of this review is a second generation fluoroquinolone with a 6-fluoro substituent and a 7-piperazinyl substituent on the quinolone ring structure (Sato et al., 1982). It is a well known fact now that all the clinically useful quinolones bear a fluorine group at the C-6 position and such quinolones which are described as fluoroquinolones are produced by laboratory synthesis. They have excellent pharmacokinetic profile and attain appreciable concentrations well above their MICs in biological tissues (Wise

and Lockley, 1988). The fluoroquinolones have been analyzed by various methods which have been described in different literatures. This review has become needful in view of the rapid progress in quinolone research and development, its assay in both bulk and pharmaceutical dosage forms as well as their determination in biological fluids.

DESCRIPTION

The chemical name of Ofloxacin is (±)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (Figure 1) while the chemical formula is C₁₈H₂₀FN₃O₄ (C - 59.83%; H - 5.58%; F - 5.26%; N - 11.63%; O - 17.71%). The molecular weight is 361.4 g (Merck Index, 1997).

Molecular structure

Levofloxacin is the S(-) form of ofloxacin and it is an active antimicrobial agent. It is substantially more active

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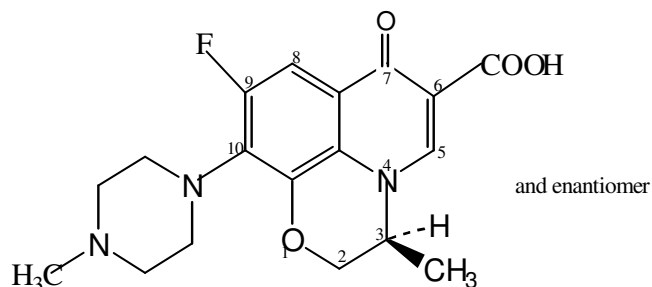


Figure 1. Structure of ofloxacin.

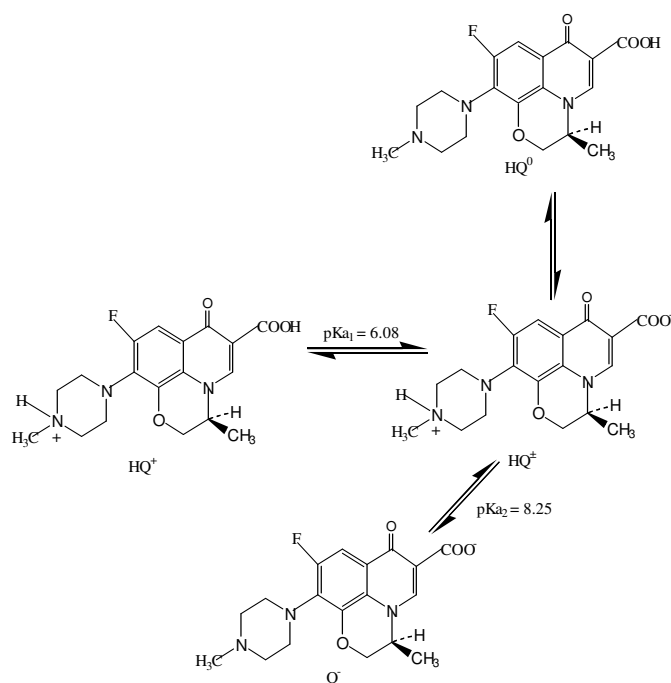


Figure 2. Macroscopic ionisation of ofloxacin (adapted from Goyne et al., 2005). HQ^0 = Ofloxacin; HQ^+ = cationic ofloxacin; HQ^\pm = zwitterionic ofloxacin; Q^- = anionic ofloxacin.

than ofloxacin (8 to 125 times depending on the bacterial species) (Martin, 1998).

Physical properties

a. Crystal properties

Appearance

Ofloxacin occurs as a pale yellow or bright yellow crystalline powder, and with a bitter taste (Macias et al. 2002). It occurs as colourless needles from ethanol. Ofloxacin should be stored at 4°C in the dark to minimize photolytically induced degradation (Park et al., 2000).

Melting point

Ofloxacin melts at 270 to 273°C (Ross and Riley, 1990). Levofloxacin which is the S-(-) form, is prepared as a hemihydrate and melts at 225 to 227°C and has an optical rotation of;

$$[\alpha]_D^{23} = -76.9^\circ$$

(Where the concentration is 0.385 M in 0.5 N sodium hydroxide) (Clarke, 2006).

Solubility

Ofloxacin is freely soluble in acetic acid, slightly soluble in water, methanol, ethanol or acetone. The octanol/water partition coefficient for ofloxacin was reported to be -0.48 by (Hiral et al., 1986; Ross et al., 1992). The pH-solubility profile shows that the dissociation and isoelectric constants for ofloxacin include $pK_{a1} = 6.05$, $pK_{a2} = 8.22$ (value for the more basic nitrogen of the piperazine group) and $pI = 7.14$ (this is the isoelectric point which is obtained by calculating the average of pK_{a1} and pK_{a2}). This means that ofloxacin has two ionizable functional groups, the 6-carboxylic group and the N_4 of the piperazine substituent. Since a carboxylic acid is normally a stronger acid than the ammonium group, the first ionization constant (pK_{a1}) corresponds to the dissociation of a proton from the carboxyl group while pK_{a2} corresponds to the dissociation of proton from the N_4 in the piperaziny group. At most physiologically relevant pH values, significant dissociation of both the 6-carboxylic acid and the basic 10-(1-piperazino) groups occur leading to significant fractions of zwitterionic species (Figure 2) (Ross and Riley, 1992; Goyne et al., 2005). The dominant Bjerrum species are the zwitterions (HQ^\pm) and the neutral form (HQ^0). The fraction of the zwitterions to neutral species at physiological pH (i.e., $(HQ^\pm)/(HQ^0) = 146$). The comparatively high pK_a values are attributed to the acid weakening effect of hydrogen bonding of the 6-carboxyl group to the adjacent carbonyl group (Ross and Riley, 1990; Ross and Riley, 1994). Ofloxacin is primarily cationic below pK_{a1} (N_4 in the piperaziny group), anionic above pK_{a2} (6-carboxyl group), and zwitterionic i.e., net neutral between pK_{a1} and pK_{a2} .

b. Spectral properties

Mass spectrum

Clarke reported that the mass spectrum of ofloxacin (Figure 3) shows principal ions at m/z 71, 375, 70, 246, 305 and 290 (Clarke, 2006).

Infrared spectrum

The infrared spectrum of ofloxacin in a KBr pellet for

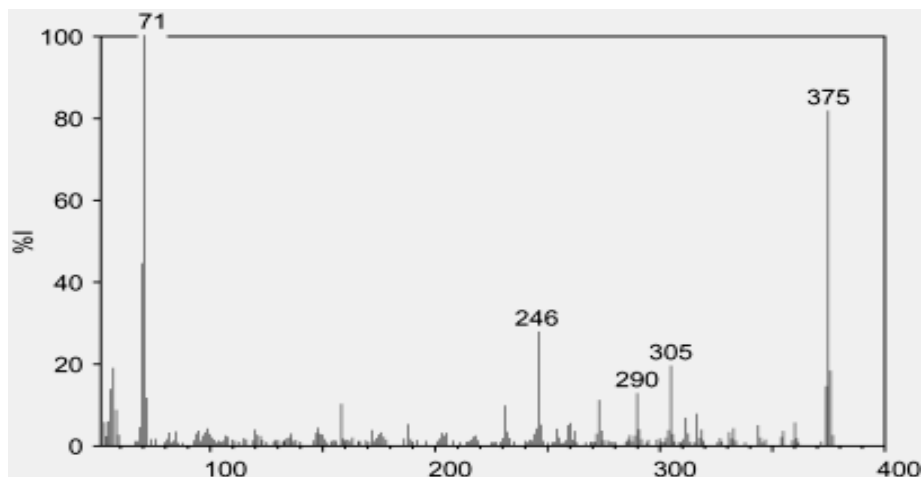


Figure 3. Mass spectrum of ofloxacin (adapted from Clarke's Analysis of Drugs and Poisons).

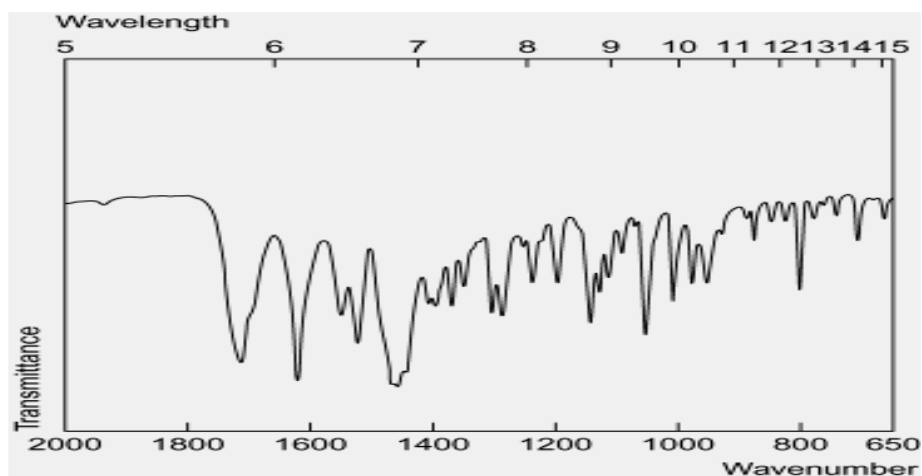


Figure 4. Infrared spectrum of ofloxacin (adapted from Clarke's analysis of drugs and Poisons).

wavenumber range of 2000 – 650 cm^{-1} is presented in Figure 4. The principal peaks are at wavenumber 1459, 1621, 1715, 1086 cm^{-1} . Generally, the carboxylic acid OH band will be in the region of 3300 – 2500 cm^{-1} . This is usually a weak band indicating that hydrogen bonding with the carbonyl group is present. The peak at 1715 cm^{-1} shows the carbonyl stretching (C=O) of carboxylic acid (Sanchez et al., 1994). The 1621 cm^{-1} peak may be due to the carbonyl stretch of the carbonyl at position 4 or the C=C stretch of C-2 and C-3. Other peaks are the 1400 cm^{-1} which is vibration associated with the protonation of N_4 in the piperazinyl group, 1530 cm^{-1} which correspond to the C=O aromatic stretching and 1055 cm^{-1} which correspond to the C-O-C stretching of the ether group (Mattioda et al., 2003).

Goyne et al. (2005) reported Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy for aqueous ofloxacin (9.0 mM) prepared in 0.06 M NaCl and 0.02 M CaCl_2 from pH 5 to 10. The spectra

were obtained by calculating the average of 400 scans at 2 cm^{-1} resolution on a Nicolet 560 spectrometer (Goyne et al., 2005).

UV spectrum

Figure 5 shows the UV spectra of Ofloxacin in aqueous acid (225, 226, 256 and 326 nm) and aqueous base (288 and 332 nm). The UV spectrum of levofloxacin in ethanol has bands at 226 and 300 nm (Clarke, 2006).

Park et al. (2000) also reported the UV spectra of ofloxacin in aqueous solution and the spectra were recorded by Unikon (model 943) spectrophotometer. They showed that the absorption spectra of 5×10^{-5} M ofloxacin in aqueous solution have two peaks (a strong peak at 287 nm and a weak peak at 332 nm). They reported that the observed strong peak corresponds to the chromophore involving N-1 position to the carboxylic

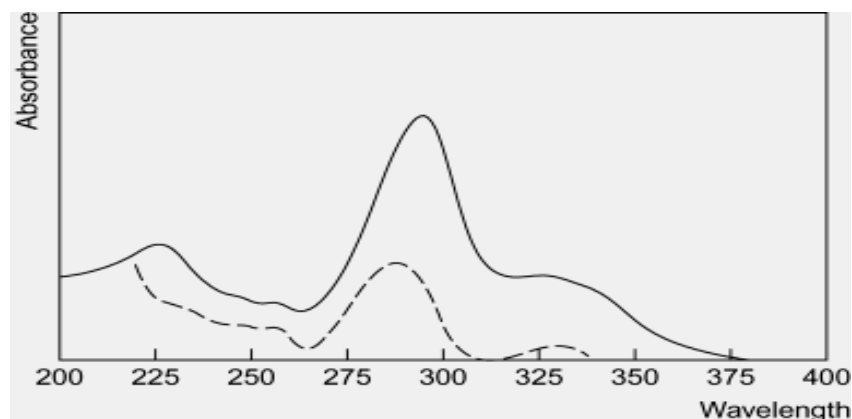


Figure 5. UV spectrum of ofloxacin (adapted from Clarke's analysis of drugs and Poisons).

group while the weak absorption peak corresponds to the chromophore involving the nitrogen of the piperazinyl group to the carbonyl group. This spectrum is affected when ofloxacin form complexes with metallic cations, especially divalent cations, leading to a red shift of the strong absorption peak to 285 nm and then a blue shift of the weak absorption peak to 330 nm (Park et al., 2000).

Optical rotation

The angle of optical rotation is -0.10° to $+0.10^{\circ}$ (Mitscher et al., 1987). Because ofloxacin has an asymmetric carbon atom, it is obtained and supplied commercially as a racemate. The racemate mixture has been resolved and the enantiomers independently synthesized and evaluated for antibacterial activity (Mitscher et al., 1987).

Synthesis of ofloxacin

The synthesis of ofloxacin as reported by Hayakawa et al. (1984) and Tanaka et al. (1984). The synthetic steps are as shown in Figure 6. First is the alkaline hydrolysis of the starting material (2, 3, 4-trifluoronitrobenzene) using potassium hydroxide in the presence of dimethylsulphoxide (DMSO) to yield 2-hydroxy-3, 4-difluoronitrobenzene. The DMSO ensures selective hydroxylation of the halogen atom adjacent to the nitro group. The second step is the alkylation of the 2-hydroxy-3, 4-difluoronitrobenzene with chloroacetone ($\text{ClCH}_2\text{COCH}_3$) in the presence of acetone and in alkaline medium to give 2-hydroxyacetone-3, 4-difluoronitrobenzene. This product (2-hydroxyacetone-3,4-difluoronitrobenzene) undergoes reductive cyclization with Raney nickel (Raney nickel is produced by mixing 14 ml of 50% aqueous Raney nickel catalyst slurred in 100 ml of absolute alcohol) in the presence of hydrogen resulting in an addition-elimination sequence to yield 7,8-dihydro-2,3-dihydro-3-methyl-4H-1,4-benzoxazine. This is

followed by cycloacylation to give 7,8-dihydro-2,3-dihydro-3-methyl-4-methylenemalonate-1,4-benzoxazine and then condensation by heating at 145°C in polyphosphoric acid to yield an ethyl ester (9,10-difluoro-3-methyl-7-oxo-2,3-dihydro-7H-pyrido-[1,2,3-de]-1,4-benzoxazine carboxylic acid ethyl ester). Removal of the carboxylic ethyl group yields 9, 10-difluoro-3-methyl-7-oxo-2, 3-dihydro-7H-pyrido-[1, 2, 3-de]- 1, 4-benzoxazine carboxylic acid and finally the condensation with N-methylpiperazine to give ofloxacin (Mitscher, 2005).

Drug impurities

The substances (as in Figures 7 to 10) are present as impurities which are normally introduced in the process of the manufacture of ofloxacin (B.P., 2003).

PHARMACOKINETIC PROFILE

Ofloxacin is characterized by a good pharmacokinetic profile. Following oral administration, there is rapid and extensive oral absorption from the gastrointestinal tract achieving peak serum concentration within 1 – 3 h and levels in excess of 100 $\mu\text{g}/\text{ml}$ in the urine and bladder (Tanaka et al., 1984; Hooper and Wofson, 1985; Lode et al., 1987; Hemanth and Gurumurthy, 2004). Food does not affect the absorption of ofloxacin (Verho et al., 1986; Yuk et al., 1991). The maximum serum concentration after 400 mg oral dose was reported to vary from 3.5 to 5.3 mg/l after a time of 1.1 to 1.4 h (Dudley et al., 1991). It has good tissue and fluid penetration. The concentrations are higher than the corresponding serum concentrations for lung tissue and many other organs. The elimination half-life is between 5 and 7 h and it is almost completely eliminated in the unchanged form by the kidneys and about 80% is excreted in 24 h. The elimination half-life is considerably increased in patients with impaired renal function where the kidneys excrete

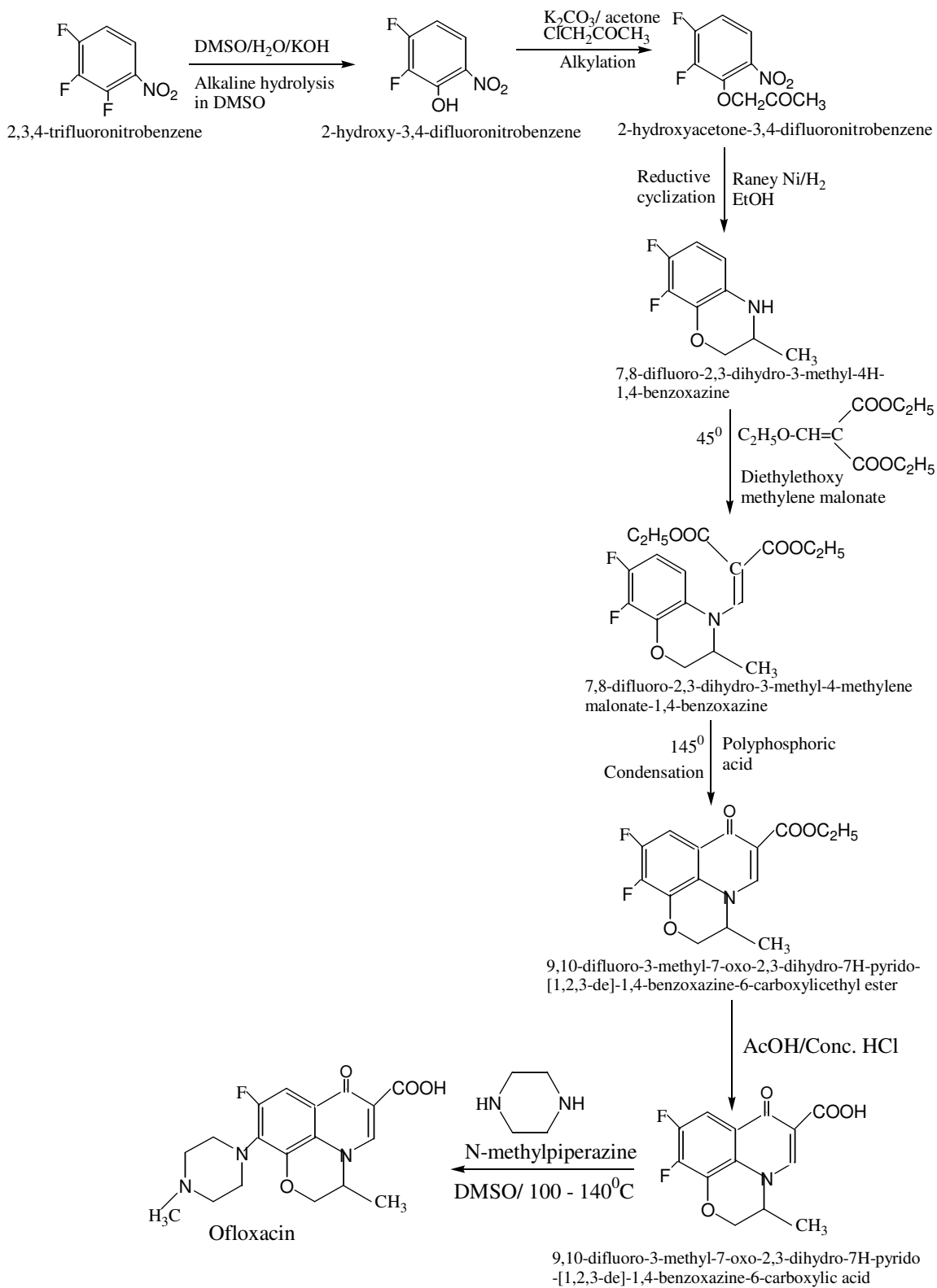


Figure 6. Synthesis of ofloxacin.

only about 20% of the drug in 24 h. This shows that the clearance of ofloxacin is dependent on the glomerular

filtration rate; therefore, caution must be exercised when administering high doses of ofloxacin in patients with re-

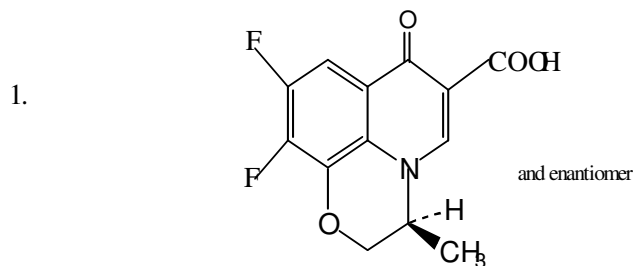


Figure 7. (RS)-9,10-difluoro-3-methyl-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid.

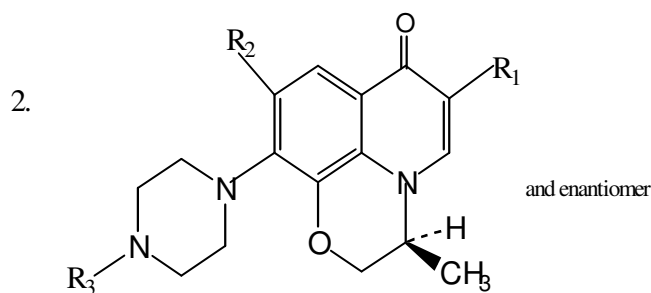


Figure 8. A- Where $R_1=H$, $R_2=F$ and $R_3=CH_3$. (RS)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoxazin-7-one. **B-** Where $R_1=COOH$, $R_2=H$ and $R_3=CH_3$. (RS)-3-methyl-10-(4-methylpiperazin-1-yl)-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid. **C-** Where $R_1=COOH$, $R_2=F$ and $R_3=H$. (RS)-9-Fluoro-3-methyl-7-oxo-10-(piperazin-1-yl)-2,3-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid.

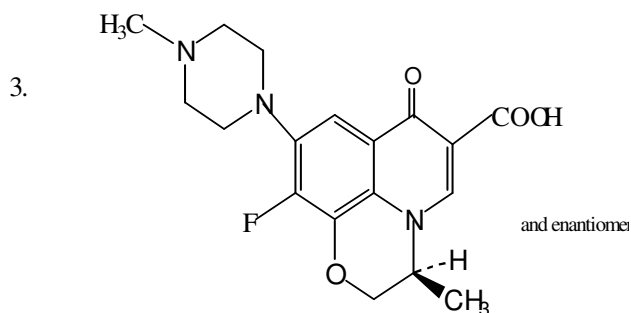


Figure 9. (RS)-10-fluoro-3-methyl-9-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid.

nal dysfunction (Monk and Campoli-Richards, 1987; Vree et al., 1986). Although renal insufficiency did not significantly modify the peak plasma level, the apparent volume of distribution, the fractional clearance, or the non-renal clearance of ofloxacin, the time to peak level was delayed in patients with creatinine clearance of less than 30 ml/min (Fillastre et al., 1987). Farinotti et al. (1988) has also reported the pharmacokinetics of ofloxacin after sin-

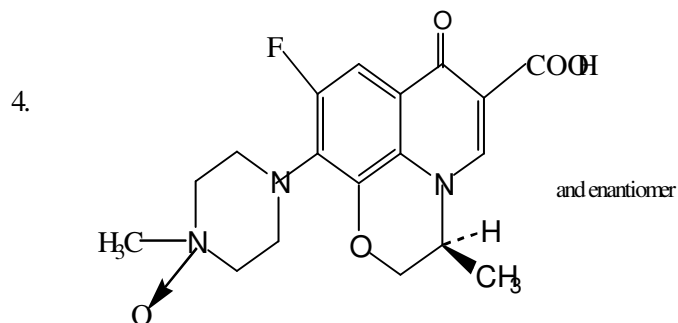


Figure 10. 4-[(RS)-6-carboxy-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoxazine-10-yl]-1-methylpiperazine-1-oxide.

gle and multiple intravenous infusions in healthy volunteers. They reported that the ofloxacin kinetics after a single 200 mg dose were best described by a two-phase curve with a total body clearance of 241 ± 43.3 ml/min a volume of distribution of 112 ± 23.1 l and an elimination half-life of 5.4 ± 0.8 h. This pharmacokinetic profile was not significantly modified by repeated administration of ofloxacin (Farinotti et al., 1988). It has also been reported that gender affects the pharmacokinetics of ofloxacin such that the volume of distribution (unadjusted for weight) is about 23% smaller in women than men. However the difference is not evident when expressed in relation to total body weight.

Generally, there is a problem resulting from complexation and chelation interactions of fluoroquinolones with a number of other therapeutic agents including antacids, metoclopramide, N-butylscopolamine bromide, xanthines and metallic cations (Maesen et al., 1987; Janknegt, 1990; Flor et al., 1990; Lomaestro and Bailie, 1991; Akerele and Okhamafe, 1991; Navarro et al., 1994; Lehto and Kivistö, 1994;). These interactions lead to reduced absorption of the drug, and hence reduced bioavailability and antibacterial activity. Furthermore, it has been observed that ofloxacin behaves differently from ciprofloxacin and some other fluoroquinolones. Infact, the effect of polyvalent cations on the oral absorption of ofloxacin has been reported to be lower than that observed with other fluoroquinolones (Lode et al., 1988; Navarro et al., 1994; Eboka and Okeri, 2005). The 4-oxo-3-carboxylic acid moiety that is required for antibacterial activity of the fluoroquinolones have been found to be the site of complexation and chelation interactions with the various metallic cations, and since all the fluoroquinolones possess this functional moiety it is anticipated that all fluoroquinolones will interact with the cations alike although the extent of interaction will vary with the quinolone type (Schentag et al., 1998; Polk, 1989; Hemanth and Gurumurthy, 2004).

Ofloxacin is metabolized to a very small extent compared to other quinolones. In man, approximately 4 – 5% of the drug is recovered as either desmethyl ofloxacin

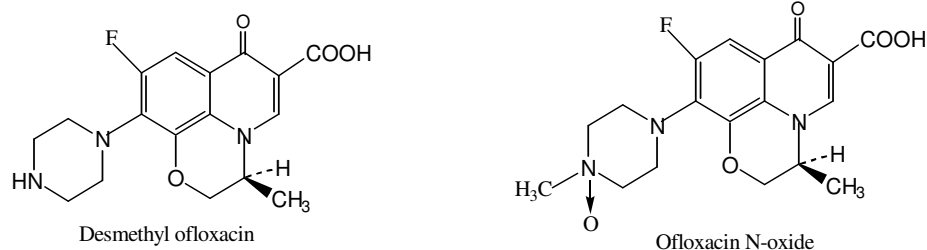


Figure 11. Metabolic products of ofloxacin.

which is still active against bacteria or ofloxacin N-oxide which is inactive. Ofloxacin has a pyridobenzoxazine ring that appears to decrease the extent of parent compound metabolism. Between 65 and 80% of an administered oral dose of ofloxacin is excreted unchanged via the kidneys within 48 h of dosing. Studies indicate that less than 5% of an administered dose is recovered in the urine as the desmethyl or N-oxide metabolites (Figure 11). 4 to 8% of an ofloxacin dose is excreted in the faeces. This indicates a small degree of biliary excretion of ofloxacin. (Flor, 1989; Outman and Nightingale, 1989).

CLINICAL USES OF OFLOXACIN

The uses include treatment of urinary tract, prostate, skin, urinary and respiratory tract infections. It is also used to treat certain sexually transmitted diseases. Ofloxacin is used as an antibacterial agent in the treatment of infections caused by a wide range of both Gram-positive and Gram-negative bacteria as well as Chlamydia infections (Monk and Campoli-Richards, 1987; Djurdjevic and Jelkic-Stankov, 1999; Mizuki et al., 1996; Detailed uses of ofloxacin can be found in Medscape as accessed on 02/22/2007).

Elemental analysis

The chemical formula of ofloxacin base is represented by $C_{18}H_{20}FN_3O_4$. The Merck Index has given the percentage composition of the constituent elements as follows: Carbon = 59.83%; Hydrogen = 5.58%; Fluorine = 5.26%; Nitrogen = 11.63% and Oxygen = 17.71% (Merck Index, 1997).

Non-aqueous titration

The official method of assay of ofloxacin as reported in the British Pharmacopoeia (B.P.) is the non-aqueous titration method. This involves the titration of 0.3 g of ofloxacin in 100 ml of anhydrous acetic acid with 0.1 M perchloric acid with the endpoint determined potentiometrically. Each ml of 0.1 M perchloric acid is equivalent

to 36.14 mg (0.03614 g) of ofloxacin (B.P., 2003).

Spectrophotometric method

Add 3 ml of 5% w/v ammonium vanadate and 2 ml of concentrated sulphuric acid to different aliquots of standard ofloxacin stock solution containing 0.2 – 1.0 mg of the drug in a 10 ml volumetric flask. Mix well and boil gently for about 20 min in a water bath. Cool and make to 10 ml mark with distilled water. The ammonium vanadate oxidizes the ofloxacin drug in sulphuric acid resulting in the development of a greenish blue colour measured at 766 nm against reagent blank (omitting the addition of drug). This colouration is attributed to the vanadium (IV) produced by reduction of vanadium (V) by ofloxacin. For the ofloxacin tablets, transfer an accurately weighed amount equivalent to 10 mg of ofloxacin from composite of 20 powdered tablets into a 100 ml volumetric flask. Dilute to mark with distilled water and sonicate for 20 min. Filter off to obtain solutions of $100 \mu\text{g ml}^{-1}$. Make further dilutions to obtain sample solutions and then follow the general procedure above (Hesham, 2005).

Soledad et al. (2005) reported a flow injection method for the determination of ofloxacin in pharmaceuticals and urine based on the formation of a yellow complex between Fe (III) in sulphuric acid and ofloxacin. The absorbance was measured at 420 nm and the calibration plot was over a range of 1.8 – 289 mg/l (Soledad et al., 2005). Hopkala and Kowalczyk used derivative UV spectrophotometry for the determination of ofloxacin, ciprofloxacin and norfloxacin in pharmaceutical tablets with a calibration range of 2.5 – 15.0 $\mu\text{g/ml}$ for ofloxacin (Hopkala and Kowalczyk, 2000).

Gracia et al. reported a flow-injection spectrophotometric method for the determination of ofloxacin in pharmaceuticals and urine. The method is based on the formation of a yellow complex between ofloxacin and Fe (III) in sulphuric acid medium (Gracia et al., 2005).

Colorimetric method

Eboka et al. (1997) developed a colorimetric method for the determination of ofloxacin, norfloxacin and ciproflo-

xacin using iron (III) nitrate nonahydrate to form complexes with the named fluoroquinolones. The solutions of the complexes obeyed Beer's law at 370 nm (the wavelength of maximum absorption) and the $A_{1cm}^{1\%}$ for ofloxacin was 207. The iron (III) nitrate nonahydrate (2.0×10^{-3} M) solution was prepared in 0.05 M sulphuric acid which was itself prepared with deionized water. Stock solution containing 500 mg ml^{-1} was also prepared. Varying volumes of ofloxacin (0 – 2.5 ml) were measured into different 20 ml volumetric flask and varying volumes of the 0.05 M sulphuric acid was added to each volumetric flask to bring the total volume to 2.5 ml. 8 ml of buffer solution was then added to each flask before 2.5 ml of the iron (III) nitrate solution was added. The resulting solution was shaken and made up to volume with deionized water. The absorbance of the solutions were measured against the reagent blank (omitting the addition of drug) and a Beer's calibration plot was prepared. The above procedure was repeated for the ofloxacin tablets after reducing twenty tablets to powder and accurately weighing an amount equivalent to 500 mg of ofloxacin. The corresponding amount of the drug was determined by interpolation from the Beer's plot of the standard solution as prepared above (Eboka et al., 1997).

Spectrofluorimetry

Heshan (2005) reported the use of Shimadzu recording spectrophotometer (model RF-540) connected to a printing recorder for the determination of ofloxacin and some other fluoroquinolones. In preparing the standard, $3 \text{ }\mu\text{g/ml}$ of stock solution of ofloxacin was prepared in ethanol and 1 ml of this solution was transferred into a 100 ml measuring flask and made up to volume with 0.1 N sulphuric acid. Different portions of the previous solution within the concentration range of 3 – 140 μg were diluted to 100 ml with 0.1 N H_2SO_4 . The fluorescence was recorded at 450 nm when excitation was at 290 nm. A blank of 0.1 N H_2SO_4 was measured and the calibration graph was rectilinear from 0.3 – 1.4 $\mu\text{g/ml}$. For the tablets, accurately weighed amount (10 μg) of the finely powdered tablets (20) was transferred into 100 ml volumetric flask and diluted to the mark with the 0.1 N H_2SO_4 . The suspension was filtered; appropriate dilutions of the filtrate were made and the fluorescence was also recorded (Hesham, 2005). Another determination of ofloxacin by fluorescence spectrophotometry was within a concentration range of 0.5 – 25 ppm (Tu and Liu, 2000).

Atomic absorption spectroscopy

A Shimadzu atomic absorption spectrophotometer (model AA.640-13) and 0.01 M cobalt sulphate was used for the precipitation of the ion associates formed from the reac-

tion with ofloxacin. The precipitates were washed with redistilled deionized water until metal free. The method depends on direct determination of the ions in the precipitate or indirect determination of the ions in the filtrate by Atomic Absorption Spectroscopy at 240.7 nm, slit width 0.2 nm, relative noise 1.0, detection limit 0.01 $\mu\text{g/ml}$, lamp current 10 mA and integration time of 3 s. The blank was prepared by omitting ofloxacin (Hesham, 2005). The optimum conditions for precipitation to obtain a rectilinear calibration plot were in the range of 3 – 30 $\mu\text{g/ml}$ and the molar ratios of the formed chelates were determined by Job's method of continuous variation (Job, 1928).

Just recently, Salem et al. (2007) also used atomic absorption spectrometric method to determine ofloxacin and certain other fluoroquinolones in pharmaceutical dosage forms and in biological fluids. The method was based on the precipitation of the ions associates formed when the fluoroquinolones reacted with silver nitrate, copper acetate and ferric chloride (Salem et al., 2007).

High performance liquid chromatography (HPLC)

Various researchers have used different high performance liquid chromatographic (HPLC) and reverse performance liquid chromatographic (RPLC) methods for the assay of the fluoroquinolones in dosage forms, serum, urine and other biological fluids (Groeneveld and Brouwers, 1986; Nangia et al., 1990; Auten et al., 1991; Samanidou et al., 2003).

Groeneveld and Brouwers (1986) extracted ofloxacin with dichloromethane under neutral conditions, followed by drying under nitrogen and dissolving in the mobile phase before chromatographic analysis. The stationary phase consisted of a stainless steel column with Nucleosil C18 (5 microns) and a mobile phase of 0.04 M phosphoric acid, tetrabutylammonium as ion-pairing reagent and methanol (pH 2.2). UV absorbance was used for detection (Groeneveld and Brouwers, 1986). Samanidou et al. (2003) used a Kromasil 100 C (8) 250 mm x 4 mm, 5 μm analytical column with an eluting system consisting of a mixture of acetonitrile: methanol: citric acid 0.4 mol/l in the ratio 7: 15: 78% v/v. Hydrochlorothiazide (2 ng/ μl) was used as internal standard and UV absorbance was used for detection at 275 nm and the limit of detection for ofloxacin was 0.01 ng. The rectilinear relationship was observed up to 12 ng/ μl for ofloxacin (Samanidou et al., 2003).

Pistos et al. (2005) described a reverse-phase and ion-interaction high performance liquid chromatographic method for the determination of zwitterionic fluoroquinolones like ofloxacin. They used an octadecylsilane stationary phase and acetonitrile as organic modifier while sodium hexanesulphonate and tetrabutylammonium hydroxide were used as sources of counter ions in ion-interaction chromatography (Pistos et al., 2005).

Griggs and Wise reported a simple isocratic high pres-

sure liquid chromatographic method for the assay of quinolones in serum. The reverse phase column was used with an acidic mobile phase containing heptane sulphonic acid and the eluate was monitored by UV or fluorescence detection (Griggs and Wise, 1989).

By using optically active mobile phase, high performance liquid chromatography has been used to determine the optical purity of ofloxacin. The chiral mobile phase contained either D- or L-phenylalanine-copper (II) complex and detection was by means of UV or fluorescence detector (Arai and Kuroda, 2006).

Chemiluminescence

This method is based on the measurement of luminescence emission resulting from the direct transformation of chemical energy into light energy. Some researchers have exploited this principle in the analysis of many substances including ofloxacin. Francis and Adcock used chemiluminescence's method for the determination of ofloxacin based on its direct oxidation with tris (2, 2'-bipyridyl) ruthenium (III) or permanganate. The CL emission was then enhanced from the oxidation of sulfite or the reaction between sodium nitrite and hydrogen peroxide (Francis and Adcock, 2005). Sun et al. (2006) then used flow-injection chemiluminescence for the determination of ofloxacin and levofloxacin in pharmaceutical preparations and biological fluids based on the chemiluminescence reaction of ofloxacin with Ce (IV)- sodium thiosulphite- sulphuric acid system (Sun et al., 2006). Also, Murillo et al. (2007) used a flow-injection chemiluminescence (CL) method to resolve mixtures of fluoroquinolones containing ofloxacin based on their reaction with tris (2,2'-bipyridyl) ruthenium (II) and cerium (IV) in sulphuric acid medium (Murillo et al., 2007).

Microbiological assay

The British Pharmacopoeia (1988) and British Pharmacopoeia (2003) specifies a microbiological method and this was modified by Akerele and Okhamafe (1991) using Petri dishes filled with 30 ml molten nutrient agar to a depth of approximately 4 mm. The nutrient agar was previously inoculated with 0.2 ml of an overnight culture of the indicator stains (*Escherichia coli* rec. A Str[®]lac.). The plates were allowed to dry for 30 min at room temperature before four circular wells of 10 mm diameter each were bored in the medium with a sterile borer. 0.2 ml of the ofloxacin sample was then placed in the wells. The zones of inhibitions were determined and the concentration of ofloxacin was calculated (Akerele and Okhamafe, 1991). Ev Lda and Schapoval (2002) used a strain of *Micrococcus luteus* ATCC 9341 as the test organism with concentration of ofloxacin ranging from 12 – 27 µg/ml (Ev Lda and Schapoval, 2002), while Mandal et al. (2003) used *Salmonella enterica* Serovar Typhi iso-

lates and the MIC for ofloxacin-sensitive isolates was 0.0125 – 0.075 µg/ml with inhibitory zone diameter of ≥ 24 mm. They also reported that some of the *Salmonella enterica* Serovar Typhi isolates showed resistance and this is vital information in the use of ofloxacin for the treatment of typhoid fever.

The various methods of susceptibility of ofloxacin in strains of *Mycobacterium tuberculosis* has also been reported (Sulochana et al., 1999).

Other methods

A capillary electrophoresis method has been developed for the analysis of ofloxacin in urine with an on-column amperometric method for its detection (Zhang et al., 2001) and another method based on the measurement of phosphorescence has been developed for the analysis of ofloxacin in tablets (Kita et al., 2003).

FUTURE PROSPECTS

Although voltammetric method has been applied for the analysis of lomefloxacin, (the levorotatory form of ofloxacin), it was not applied to ofloxacin itself and this can be a future prospect for the analysis of ofloxacin. (El Ries et al., 2005). An example of a voltammetric method of analysis is polarography which was also used for the analysis of norfloxacin and some other second generation quinolones but was not applied to ofloxacin (Corti et al., 1994).

Other prospective methods that can be exploited are pre-column and post-column derivatization high performance liquid chromatographic method. Derivatization in which polar functional groups are treated with various suitable reagents can lead to improved selectivity and/or selectivity of the analysis and also improve chromatographic performance.

Simple titrimetric methods such as acid-base methods, conductimetric methods need to be developed for the determination of ofloxacin and indeed other fluoroquinolones where sophisticated instruments are not available especially in developing countries of the world.

Also it is possible that ofloxacin will form coloured complexes with various reagents which can be exploited for its colorimetric and spectrophotometric determination. Also derivative (first, second or higher derivative spectrum) can be developed for the analysis of ofloxacin.

CONCLUSION

This review is targeted at outlining the various analytical methods and other related aspects of ofloxacin. It is however pertinent to state that newer analytical methods are being developed with respect to advancing technology and this may necessitate a future review.

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