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# A REVIEW ON QUALITATIVE DETERMINATION OF DIFFERENT MEMBERS OF FLUOROQUINOLONE ANTI-BACTERIALS BY HPLC METHODS

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## ABSTRACT

Occurrence of new drug resistant strains of bacterial infections leads to the invention newer antibiotics. Fluoroquinolones are one of the most promising and vigorously pursued areas of contemporary anti-infective depicting broad spectrum and potent active class of drugs. Fluoroquinolones are the drugs in order to overcome the drawbacks of natural antibiotics. This review first time covers the applications of novel HPLC methods particularly to the determination of 3rd and 4th generation of Fluoroquinolones antibacterials either in dosage form, blood serum or in the biological fluids. In this review Tables 2-11 show the reported methods for nalidixic acid, Ciprofloxacin, Levofloxacin, Ofloxacin, Norfloxacin, Balofloxacin, Prulifloxacin, Moxifloxacin, Sparfloxacin, and Gatifloxacin respectively. Table 12 is concerned with the reported methods for the combination of different members of quinolones antibacterials and this review covers the HPLC methods for the analysis of various fluoroquinolone antibiotics.

**Key words:** Fluroquinolones, Dosage forms, Biological fluids Estimation, Antibiotics.

## 1. INTRODUCTION

The discovery of antibiotics like Penicillins, Cephalosporins, amino glycosides tetracyclines and anti bacterials like Sulfonamides is a breakthrough in the treatment of infectious diseases. In order to overcome the drawbacks of natural antibiotics and synthetic antibacterial agents, a new class of antibiotics called semi-synthetic anti bacterial agents was introduced in the treatment of infectious diseases.

Starting with nalidixic acid, quinolone derivatives were introduced as anti-bacterials in 1962. Quinolone ring structure is shown in the following figure 1. Later some structural modifications were made to increase activity, safety and efficacy.

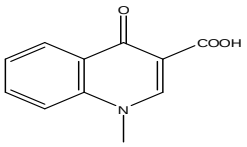


**Figure.1.Quinolone ring structure**

## 2. CHEMISTRY

Prototype quinolone Anti-bacterials, such as nalidixic acid (naphthpyridin nucleus) have narrow spectrum of activity. Addition of piperazinyl ring at 7th position broadened the activity. The molecular weight of fluoroquinolines is between 300-500 Daltons. Most of them occur in Zwitter ionic form. Pharmacophore required for significant anti-bacterial activity is 4-pyridone-3-carboxylic acid with a ring at 5th or 6th position. The brand names of different sorts of fluoroquinolones are shown in the Table 1 and the schematic representation of classification of fluoroquinolones is shown in the following scheme.

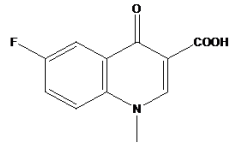


**Figure.2.Quinolone ring with fluorine atom that broadens the activity**

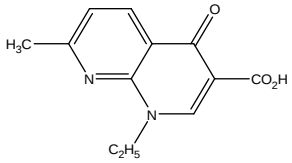


**Figure.3.Nalidixic acid drug Structure**

**Table.1.Different brand names of fluoroquinolones**

| Fluoroquinolone | Marketed brand names |
|---|---|
| Norfloxacin | Norbactin, Norflox, Norwin, Norvit, Alflox, Normax, Quinobid, Norilet. |
| Gatifloxacin | Tequin, Segat, Algat, Eligat, Gatilox, Gatispan, Q-Gat, Zigat, Zyquin, Ecogat. |
| Lomefloxacin | Loma day, Lomflox-400, Floxaday, Relom. |
| Ciprofloxacin | Cifran, Adcip, Alcip, Anocip, Baycip,Cadiwin, Cipfun,Cipgen, Ciplo, Ciplox. |
| Ofloxacin | Tarivid, Aflox, Aloflox, Armbid, Avicin, Bact-O, Bactorax, Bestoflox. |
| Pefloxacin | Pelox, Peflobid, Perti, Piflasyn. |
| Sparfloxacin | Sparflox, Sparact, Sparcin, Spardac, Spartin, Sparzid, Spicy, Spike, Sap. |
| Levofloxacin | Tavanic, Alefox, Day-5, Elflox, Enatome, Leon, Leviza, Levloc, Levobact. |
| Moxifloxacin | Avelox, Mahaflox, Morris, Moxicip, Staxom, Unomox. |
| Gemifloxacin | Factive, EG1, G-cin, Gametop, Gamiflox, Gembax, Gamibit, Gemibid, Gex. |
| Prulifloxacin | Alpruli, Percin, Prudila, Pruflox, Prulif, Prulin, Punox, Prulisoz, Pruibact. |

**Nalidixic acid:** It is the first of the synthetic quinolone antibiotics. In the technical sense, it is a naphthyridone, not a quinolone: its ring structure is a 1, 8 - naphthyridine nucleus that contains two nitrogen atoms, unlike quinoline, which has a single nitrogen atom. The structure of nalidixic acid is represented in the Figure 3 and the Application of HPLC to the determination of Nalidixic acid is represented in Table 2.

**Table.2.Application of HPLC to the determination of Nalidixic acid**

| Material | Column | Mobile phase | Detection |
|---|---|---|---|
| Formulations | Machery- Nagel $RP_8$, 5 µm | $H_2O$/ acetonitrile/triethylamine ( 680 : 320: 1 ) | UV, 310 nm |
| Pharmaceuticals | Alltech Anion R, 10 µm | 0.001 M $TBCl_3$ and 0.005M heptanesulphonate in acetic acid pH 6.8 /methanol ( 11: 9) | Fluor. 318, 545 nm |
| Serum brain, CSF | Wakosil 5 $C_{18,}$ 5 µm | $CH_3OH$ / acetonitrile/ 0.015 M $KH_2$ $PO_4$ containing sodium lauryl sulphate ( 3: 2 : 5 ) | UV, 255 nm |
| Formulations | Hypersil, $C_{18}$ | water/ acetonitrile/triethylamine ( 600: 400 : 1 ) | UV, 254 nm |
| Cultured fish | Nucleosil, $C_{18}$ | THF/ acetonitrile/ $H_3PO_4$ / $H_2O$ (29:1:0 :06:69:94 ) | UV, 260 nm |
| Fish | Nucleosil 3,$C_{18,}$ 3µm | Acetonitrile / methanol / 0.01 M oxalic acid of pH 3 ( 3: 1 : 6 ) | UV, 290 nm |

**Ciprofloxacin:** Ciprofloxacin is a synthetic anti bacterial agent shows broad spectrum of activity comes under the group of fluoroquinolones. The substance was developed in 1981 by the company Bayer corporation. Ciprofloxacin was determined in pharmaceuticals through its reaction with ferric chloride at 430 nm. The structure of Ciprofloxacin is shown in Figure 4 and the Application of HPLC to the determination of Ciprofloxacin is represented in Table 3.

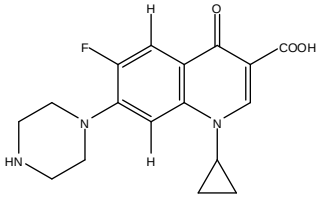


**Figure 4: Ciprofloxacin drug structure**

**Table.3.Application of HPLC to the determination of Ciprofloxacin**

| Material | Column | Mobile phase | Detection |
|---|---|---|---|
| Pharmaceuticals | Invertsil ODS 2 | THF/ acetonitrile/ hexane sulphometa (2 : 1 : 17) | UV, 254 nm |
| Human aqueous humor | Novapak $C_{18}$ cartridge | Methanol/ acetonitrile/ 0.4M citric acid (3 : 1 : 10) | Fluor. 278 nm, (450nm) |
| Body fluids | 5 µm PLRP – S | 0.02 M TCA / acetonitrile / methanol (37: 11: 2 ) | Fluor. 277 nm, (418nm) |
| Formulations | 10 µm $C_{18}$ | Methanol / $H_2O$/acetic acid (840 : 159 : 1) | UV, 254 nm |
| Biological materials | Inertsil OSD -2 | 50% Aqueous methanol of pH 2.5 | Fluor. 320, 545 nm |
| Plasma | CLC – shim pack ODS , 5 µm | Methanol : 0.2 M ammonium acetate (8: 17 ) | UV, 280 nm |
| Plasma and chinchilla middle ear effusion | Hypersil $C_{18,}$ 5 µm | $NaH_2PO_4$ + triethylamine + SDS of pH 3/ acetonitrile (3: 2 ) | Flour. 278 nm ; 456 nm |

**Table.3.Application of HPLC to the determination of Ciprofloxacin continution**

| Material | Column | Mobile phase | Detection |
|---|---|---|---|
| Serum | Bonadapak $C_{18}$ | Methanol / acetonitrile / phosphate buffer pH 2.8 (5: 4: 11) | Flour. 338 nm; 455nm |
| Urine, serum , saliva | Sphere – 5- OD-5A | 0.1M $KH_2PO_4$ / acetonitrile (1: 1) | UV , 280 nm |
| Serum | KYWG-$C_{18,}$ 10 µm | $KH_2PO_4$/ methanol/ acetonitrile (56: 33: 11). | Flour .280 , 455 nm |
| Serum | Nucleosil $C_{18}$, 3 µm | $KH_2PO_4$ / $H_2O$/ tetrabutylammonium bromide/ acetonitrile (12 : 6: 1: 1 ) | UV, 277 nm |
| Brain and CSF | Wakosil 5 $C_{18}$ | Sodium dodecylsulphonate in methanol / 0.02 M $KH_2PO_4$ (3: 2, adjusted to pH 2.5) | Flour .277, 445 nm |
| Plasma micro samples | MB $C_{18}$ Radial pak 10 µm | ( $NH_4)_2$ $HPO_4$ (0.1 M) / acetonitrile / methanol/ (80/ 13/7) | Flour .277, 453 nm |

**Levofloxacin:** Levofloxacin (trade names are Levaquin (US), Tavanic (EU), and others) is a broad spectrum antibiotic of the fluoroquinolone drug class, and the levo isomer of its predecessor Ofloxacin. Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections, including Gram negative (*Escherichia coli, Haemophilus influenza, Klebsiella pneumoniae, Legionella pneumophila, Moraxella catarrhalis, Proteus mirabilis, and Pseudomonas aeruginosa*), Gram positive (*Streptococcus pneumoniae, Staphylococcus epidermidis, Enterococcus faecalis, and Streptococcus pyogenes*), and atypical bacterial pathogens (*Chlamydophila pneumonia and Mycoplasma pneumonia*). Compared to earlier antibiotics of the fluoroquinolone class such as Ciprofloxacin, Levofloxacin exhibits greater activity toward Gram-positive bacteria but lesser activity toward Gram-negative bacteria, especially Pseudomonas aeruginosa. Levofloxacin and later generation fluoroquinolones are collectively referred to as "respiratory quinolones" to distinguish them from earlier fluoroquinolones which exhibited modest activity toward the important respiratory pathogen Streptococcus pneumonia.

Levofloxacin and other fluoroquinolones are valued for their broad spectrum of activity, excellent tissue penetration, and for their availability in both oral and intravenous formulations. Levofloxacin is used alone or in combination with other antibacterial drugs to treat certain bacterial infections including pneumonia, urinary tract infections, and abdominal infections. The structure of Levofloxacin is shown in the Figure 5 and the Application of HPLC to the determination of Levofloxacin is represented in Table 4.

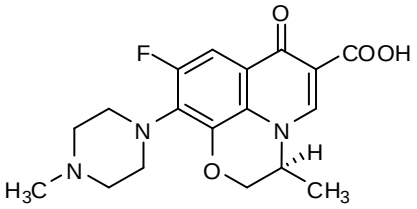


**Figure 5: Levofloxacin drug structure.**

**Table.4.Application of HPLC to the determination of levofloxacin**

| Material | Column | Mobile phase | Detection |
|---|---|---|---|
| Human serum | Nucleosil $C_{18}$ | Water : acetonitril ( 6: 5) | UV, 293 nm |
| Pharmaceutical dosage forms | ACE $C_{18}$ column | Mixture of ) 5 % (V/V) triethylamine in sodium dihydrogen ortho phosphate | UV, 294 nm |
| Rat plasma saliva | Phenomenex Luna $C_{18}$ | Acetonitrile : water ( 80 : 20 V/V ) | UV, 296 nm |

**Ofloxacin:** Ofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class considered to be a second-generation fluoroquinolone. The original brand, Floxin, has been discontinued by the manufacturer in the United States on 18 June 2009, though generic equivalents continue to be available. Ofloxacin was first patented in 1982 (European Patent Daiichi) and received approval from the U.S. Food and Drug Administration (FDA) on December 28, 1990. Ofloxacin is sold under a wide variety of brand names as well as generic drug equivalents, for oral and intravenous administration. Ofloxacin is also available for topical use, as eye drops and ear drops (marketed as Ocuflox and Floxin Otic respectively in the United States and marketed as Optiflox, eylox respectively in Jordan and Saudi Arabia.

Ofloxacin is a racemic mixture, which consists of 50% Levofloxacin (the biologically active component) and 50% of its "mirror image" or enantiomer dextrofloxacin. When Levofloxacin disks were not available in early clinical trials, a 5-pg Ofloxacin disk was substituted. The U.S. Food and Drug Administration (FDA) medical reviewers considered the two drugs to be one and the same and hence interchangeable. The structure of Ofloxacin is represented in the Figure 6 and the Application of HPLC to the determination of Ofloxacin is represented in Table 5.

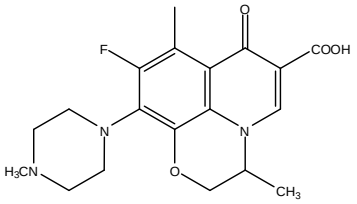


**Figure 6: Ofloxacin drug structure**

**Table.5.Application of HPLC to the determination of Ofloxacin**

| Material | Column | Mobile phase | Detection |
|---|---|---|---|
| Aqueous humor | Novapak $C_{18,}$ 4 µm | 0.4 M citric acid / methanol/ acetonitrile (10 : 3 : 1) | Flour. 290, 500 nm |
| Human saliva | Develosil ($C_{18}$), 5 µm | 0.06 M sodium acetate of PH 2.5 / acetonitrile (21 : 4) | UV, 300 nm |
| Human plasma | Separon SGX $C_{18}$ | 5.5 % THF in 0.06 M $KH_2PO_4$ of pH 2.6 | Flour. 282, 450 nm |
| Human scalp hair | ODS 120 -T, 5 µm | $KH_2PO_4$ pH 2.6 / acetonitrile (41 : 9) | Flour. 290, 460 nm |
| Plasma and lung tissue | Ultrabase RP 8, 5 µm | Citrate buffer of pH 4.8 / acetonitrile (17 : 3) | Flour. 280, 500 nm |
| Hair | TSK gel ODS – 120 T , 5 µm | Phosphate buffer of pH 2.6 / acetonitrile (41 : 9) | Flour. 290, 460 nm |
| Dosage form | Anion exchange vydac , 10 µm | 0.05 M phosphate buffer pH 7/ acetonitrile (1: 4) | UV, 297 nm. |
| Human serum | Develosil ODS - 5 µm | 0.5 % sodium acetate pH 2.5 / acetonitrile (87 : 13) | UV, 300 nm |
| Serum | Develosil CN, 5 µm | 0.04 M $NaH_2PO_4$ / 0.04 M $H_3PO_4$/methanol (2: 5 : 3) | UV, 300 nm |
| Body fluids | Nucleosil 5, $C_{18}$ | Acetonitrile / 0.005M tetrabutylammonium phosphate pH 2 | Flour. 295, 418 nm |
| Body fluids | Nucleosil 5, $C_{18}$ | Acetonitrile / 0.05 M $H_3PO_4$ containing triethylamine, pH 2.8 (9 :41) | Flour. 310, 487 nm |

**Norfloxacin:** Norfloxacin is a synthetic chemotherapeutic antibacterial agent occasionally used to treat common as well as complicated urinary tract infections. It is sold under various brand names with the most common being Noroxin. In form of ophthalmic solutions it is known as Chibroxin. Norfloxacin is a first generation synthetic fluoroquinolone (quinolone) developed by Kyorin Seiyaku K.K. (Kyorin). The structure of Norfloxacin is represented in the Figure 7 and the Application of HPLC to the determination of Norfloxacin is represented in Table 6.

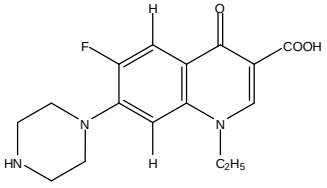


**Fig 7: Norfloxacin drug structure.**

**Table.6.Applications of HPLC to the determination of Norfloxacin**

| Materials | Column | Mobile phase | Detection |
|---|---|---|---|
| Serum | $C_{18,}$ 5 µm | 10mM Triethylaminephosphate in 55% acetonitrile of pH 4.8 | UV, 226 nm |
| Dosage form | $C_{18}$ , 5 µm | 0.2 % $H_3PO_4$ , pH 2 / acetonitrile ( 87 : 13 ) | UV, 275 nm |
| Serum | Spheri- 3, $C_{18}$ , 3 µm | 11% acetonitrile in 0.01 M $NaH_2PO_4$ , pH 2.5 containing 0.001 M triethylamine | UV, 279 nm |
| Blood | Zorbax $C_{18,}$ 5 µm | Methanol / TFA , 0.01 % ( 1: 3 ) | Flour. 280, 418 nm |
| Tablets | Micropak– $NH_2$-10, 10 µm | Acetonitrile /tetrabutylammonium hydroxide / o-phosphoric acid/ water ( 10: 1.5 : 0: 167 : 100 ) | UV, 278 nm |

**Balofloxacin:** The new fluoroquinolone Balofloxacin is a broad spectrum fluorinated quinolone anti- biotic prescribed for sinusitis chronic bronchitis UTI and exhibits excellent antibacterial activity against gram positive bacteria such as multiple drug resistant staphylococci and pneumococci it is sold under brand name of Q - roxin. The structure of Balofloxacin is represented in the Figure 8 and the Application of HPLC to the determination of Balofloxacin is represented in Table: 7.

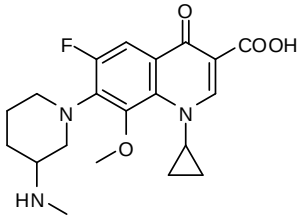


**Figure.8.Balofloxacin drug structure**

**Table.7.Application of HPLC to the determination of Balofloxacin**

| Material | Column | Mobile phase | Detection |
|---|---|---|---|
| Formulation | Welchrom $C_{18}$ column 5 µm | Acetonitrile ( 70 : 30 v/v ) | UV, 293 nm |
| Formulation | RP $C_{18}$ F 254 | Methanol : water : triethylamine (6: 4: 0.5 V/V/V) | UV, 266 nm |
| Formulation | Welchrome $C_{18}$ | Phosphate buffer : acetonitrile (70: 30 % V/V) | UV, 293 nm |

**Prulifloxacin:** Prulifloxacin is a prodrug and it is metabolized in the body to convert the active compound ulifloxacin. Prulifloxacin appeared as effective as Ciprofloxacin, co-amoxiclav in the treatment of bronchitis exacerbations or lower urinary tract infections. It was tolerated as well as Ciprofloxacin. Prulifloxacin has a long half-life and may therefore be taken only Once a day. Prulifloxacin has been approved for use in Japan. In the United States, it is undergoing phase III clinical trials for the treatment of traveler's diarrhea. It has been proven that Prulifloxacin is more effective than Ciprofloxacin in the treatment of adults with complicated urinary tract infections.

PRF is not official in any pharmacopoeia. Literature survey revealed that few Chromatographic methods have been reported, which include LC-MS, HPLC, with fluorescence detection, capillary zone electrophoresis and capillary electrophoresis chemiluminescence methods for the determination of the active metabolite of Prulifloxacin in human plasma and other biological fluids. It has been also reported that there is a sensitive determination of Prulifloxacin by its fluorescence enhancement on terbium (III)-sodium dodecyl benzene sulfonate system. The structure of Prulifloxacin is represented in the Figure 9 and the Application of HPLC to the determination of Prulifloxacin is represented in Table 8.

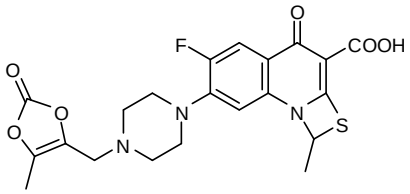


**Figure.9.Prulifloxacin drug structure**

**Table.8. Application of HPLC to the determination of Prulifloxacin**

| Material | Column | Mobile phase | Detection |
|---|---|---|---|
| Formulation | Phenomenex $C_{18}$ column | Acetonitrile (60 : 40) | UV, 275 nm |
| Tablet dosage form | $C_{18}$ column | $KH_2PO_4$ buffer : acetonitrile pH 7.3 with triethylamine (10 : 90 V/V) | UV, 273 nm |
| Tablet dosage form | $C_{18}$ column | Acetonitrile : water : triethylamine (40 : 60 : 0.3 % V/V/V ) pH 3.3 | UV, 273 nm |

**Moxifloxacin:** Moxifloxacin is a fourth-generation synthetic fluoroquinolone antibacterial agent developed by Bayer AG (initially called BAY 12-8039). It is marketed worldwide (as the hydrochloride) under the brand names Avelox, Avalox, and Avelon for oral treatment. In most countries, the drug is also available in parenteral form for intravenous infusion. Moxifloxacin is also sold in an ophthalmic solution under the brand names Vigamox, Moxeza for the treatment of conjunctivitis (pink eye). A united state patent application was submitted on 30 June 1989, for Avelox (Moxifloxacin hydrochloride). In 1999 Avelox was approved by the United States Food and Drug Administration (FDA) for use in the United States.

In the United States, Moxifloxacin is licensed for the treatment of acute bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis community acquired pneumonia, complicated and uncomplicated skin and skin structure infections, and complicated intra-abdominal infections. In the European Union, it is licensed for acute bacterial exacerbations of chronic bronchitis, non-severe community-acquired pneumonia, and acute bacterial sinusitis. Based on its investigation into reports of rare but severe cases of liver toxicity and skin reactions, the European medical agencies recommended in 2008 that the use of the oral (but not the IV) form of Moxifloxacin be restricted to infections in which other antibacterial agents cannot be used or have failed. In the US, the marketing approval does not contain these restrictions, though the label contains prominent warnings against skin reactions.

Avelox (Moxifloxacin) was launched in the United States in 1999 and is currently marketed in more than 80 countries worldwide. In the United States, Avelox is marketed by Bayer's partner Merck. In 2011 the FDA added two boxed warnings for this drug in reference to spontaneous tendon ruptures and the fact that Moxifloxacin may cause worsening of myasthenia gravis symptoms, including muscle weakness and life-threatening breathing problems. The structure of Moxifloxacin is represented in the Figure 10 and the Application of HPLC to the determination of Moxifloxacin is represented in Table 9.

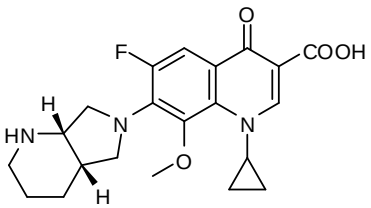


**Figure.10.Moxifloxacin drug structure**

**Table.9.Application of HPLC to the determination of Moxifloxacin**

| Material | Column | Mobile phase | Detection |
|---|---|---|---|
| Formulation | Welchrom $C_{18}$ Column | 10mM Phosphate Buffer(pH-3.1): Acetonitrile (70 : 30 v/v) | UV, 293 nm |
| Formulation | Phenomenox $C_{18}$ column | Ammonium formate : acetonitrile ( 70:30 v/v) | UV, 295nm |
| Tablet dosage form | Welchrom $C_{18}$ column | Buffer 2.5 with triehylamine and orthophosphoric acid : methanol ( 55 : 45 v/v ) | UV, 293 nm |
| Formulation | Hypersil BDS $C_{18}$ column | Acetonitrile : Buffer : pH -4 ( 60 : 40 v/v ) | UV, 294 nm |

**Sparfloxacin:** The new fluoroquinolone Sparfloxacin (SPF) is (5-Amino-1- cyclopropyl-7-(*cis*-3,5-dimethyl-1-piperazinyl)-6,8-difluoro-1,4 dihydro-4-oxo-3-quinolinecarboxylic acid is a broad spectrum fluorinated a quinolone antibiotic used in the treatment of bacterial infections and commonly prescribed for infective opthalmitis and sinusitis, acute exacerbation of chronic bronchitis, community-acquired pneumonia, eye infections, urinary tract infection. SPF is a new difluorinated quinolone with similar activity for gram-negative and gram-positive bacteria and a spectrum of activity that embraces anaerobes, Chlamydiatrachomatis,

Mycoplasma and mycobacteria. The quinolones and SPF compounds are bactericidal in nature. The molecular target of quinolones is considered to be DNA gyrase, since quinolones inhibit gyrase activities and gyrases isolated from quinolone-resistant strains are resistant to quinolones. Escherichia coli gyrase consists of subunits A and B which are the products of the gyrA and gyrB genes, respectively since the unexpected finding by Shen and Pernet that [3H] Norfloxacin binds to DNA but not to purified gyrase it has been proposed that SPF exerts its antibacterial activity by inhibiting DNA gyrase which is a bacterial topoisomerase. DNA gyrase is an essential enzyme which controls DNA topology and assists in DNA replication, repair, and deactivation. The structure of Sparfloxacin is represented in the Figure 11 and the Application of HPLC to the determination of Sparfloxacin is represented in Table 10.

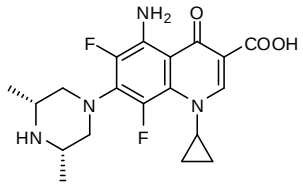


**Figure 11: Sparfloxacin drug structure.**

**Table.10. Application of HPLC to the determination of Sparfloxacin**

| Material | Column | Mobile phase | Detection |
|---|---|---|---|
| Formulation | Welchrom $C_{18}$ 5 µm | Acetonitrile (70 : 30 v/v ) | UV, 291 nm |
| Human plasma | Novapak $C_{18,}$ 4 µm | 5% acetic acid / acetonitrile /methanol ( 14 : 3: 3) | UV, 364 nm |
| Serum and urine | Nucleosil 100SA, 5 µm | Acetonitrile / 0.1 M $H_3PO_4$ ( 3 :1 ) | Flour 295, 525 nm |
| Tablet | Purospher star $C_{18}$ | Methanol : water : acetonitrile (54: 41 : 5 v/v/v pH 2.7 ) | UV, 232 nm |
| Formulation | Welchrom $C_{18}$ | Phosphate buffer ( pH 2.8 ): acetonitrile (70 : 30 v/v) | UV, 291nm |
| Tablet dosage form | $C_8$ | Methanol & 0.02 M phosphate buffer pH 3.0 ( 60 : 40 v/v ) | UV, 270 nm |
| Formulation | Chromolith Rp- $C_{18}$ | Methanol : 0.025M $KH_2PO_4$ pH 3 ( 20 : 80 v/v ) | UV, 290 nm |

**Gatifloxacin:** Gatifloxacin sold under the brand names Gatiflo, Tequin and Zymar, is an antibiotic of the fourth-generation fluoroquinolone family, that like other members of that family, inhibits the bacterial enzymes DNA gyrase and topoisomerase IV. Bristol-Myers Squibb introduced Gatifloxacin in 1999 under the proprietary name Tequin for the treatment of respiratory tract infections, having licensed the medication from Kyorin Pharmaceutical Company of Japan. Allergan produces it in eye-drop formulation under the names Zymar and Zymaxid. Gatifloxacin is also available as tablets and in various aqueous solutions for intravenous therapy in many countries. The structure of Gatifloxacin is represented in the Figure 12 and the Application of HPLC to the determination of Gatifloxacin is represented in Table 11.

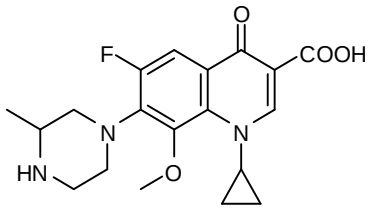


**Figure.12.Gatifloxacin drug structure**

**Table.11.Application of HPLC to the determination of Gatifloxacin**

| Material | Column | Mobile phase | Detection |
|---|---|---|---|
| Tablet dosage form | Hypersil $C_{18}$ | Acetonitrile: methanol : water ( 50 : 37.5 : 12.5 v/v/v ) | UV, 280 nm |
| Bulk & dosage form | $C_{18}$ | Acetonitrile : 0.025 M potassium dihydrogen phosphate buffer ( 50 : 50 v/v ) | 300 nm |

HPLC is most frequently applied technique for the determination of fluoroquinolones in biological fluids, blood serum, fish feed and tablet formulations. Table 12 represents the HPLC methods for the simultaneous estimation of different drugs of quinolone antibacterials.

**Table.12. HPLC methods for the simultaneous determination of different drugs of quinolone anti-bacterials**

| Drugs | Material | Column | Mobile phase | Detection |
|---|---|---|---|---|
| Nor., dif., cipro., sara | Fish feed | Alltech $C_{18}$, 10 µm | Sodium citrate –citric acid, pH2.4/ acetonitrile (13:7) | UV,280 nm |
| Several quinolons | Fish and animal tissues | Nucleosil $C_{18,}$ 5 µm | 0.02 M acetonitrile / THF (1:1) | Flour, 336 and 375 nm |
| OA, NA, fl., POA, beno., dano., ofl. | Fish and meat | Wakosil II s $C_{18}$ , HG | Phosphate buffer, pH 2.5 /acetonitrile (65 : 35v/v) | UV, 280 nm and Flour, 325 / 365 nm |
| Cipro, enox, flero., nor, ofl, PMA | Raw material | Lichrosphere 100 $C_{18}$ 5µm | Tetrabutylammonium bromide in $H_3PO_4$, pH 3.89 / acetonitrile (93 : 7) | Potentiometry |
| Fluoroquinolones | Human plasma | $C_{18}$ | Acetonitrile / phosphate buffer pH 2 | UV, 257 nm |
| Fluroquinolones | Clinical specimens | Lichrosphere 100 C $_{18,}$ 10µm | Acetonitrile / 0.4% M citric acid (1: 5) | Flour , 275 , 340 nm |
| Quinolonic and cinolonic acid derivatives | Urine | Nova pack $C_{18}$ | Acetonitrile / 0.4 % acetic acid (7 : 18) | UV, 265 nm |
| -Eno.+ ofl .+ nor., cipro+pef. + enro | Dosage forms | Shimpak CLC- ODS | Tetrabutylammonium hydroxide/ acetonitrile ( 9: 1) | UV, 280 nm |
| -Beno. + enro.+ dano.+ ofl | Chicken tissues | Wakosil II 5 $C_{18}$ | Phosphate buffer pH 2.4 acetonitrile ( 4: 1) | Flour, 245, 445 nm |
| Gati, levo, lome, Peflo | Tablets and injections | Lichrosper 100 RP-$C_{18}$ | Water : acetonitrile ( 80:20 v/v) | 279-295 nm |
| Pipemidic aci marbo eno, ofl, nor, cipro, dano, lome, enro, sara, diflo, oxolonic acid, nalidixic acid, flumequine and piromidic acid. | Urine and pharmaceutical samples | RP $C_{18}$ | Methanol – ACN- 10mM citrate buffer at pH 3.5., 4.5 | UV,280 nm |
| Levo, cipro, gati, moxi, trova, cino | Human plasma | Phenomenex ODS $C_{18}$ | 35% (v/v) Aqueous acetonitrile: tetrabutyl-ammonium acetate, sodium dodecyl sulphate and citric acid ( pH 3.4) | 235, 254, 275, 300 nm |

**Abbreviations:** nor:norfloxacin; NA:nalidixic acid; ofl: Ofloxacin; cino:cinoxacin; dif:difloxacin; fl:flumequine; pef: pefloxacin; fluor:fluorometrically; Cipro:Ciprofloxacin; PMA:Pipemidic acid; OA:Oxolonic acid; Sara:sarafloxacin; beno: Benofloxacin; dano:Danofloxacin; POA:Piromidic acid; eno:Enoxacin; enro:Enrofloxacin; trova:travafloxacin; levo:Levofloxacin; gati:Gatifloxacin; moxi: Moxifloxacin; lome: lomefloxacin

## CONCLUSION

This paper describes the individual and simultaneous estimation of different important class of Quinolone Antibacterial drugs. A number of HPLC methods have been reported for analyzing fluoroquinolones in biological fluids and pharmaceutical formulations for the determination of fluoroquinolones individually or in combination. Presentation of various methods for the determination of fluoroquinolones in formulation and in body fluids is useful for the researchers who are interested to study this class of antibacterials further. The information compiled

in this article reduces valuable time and money spent on the analytical method development in the analysis of fluoroquinolines from the first step onwards. A researcher can use this information and go a point forward.

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