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QUANTIFICATION OF FLUOROQUINOLONES FROM BULK, PHARMACEUTICAL FORMULATIONS AND BIOLOGICAL MATRICES USING CHROMATOGRAPHIC TECHNIQUES

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ABSTRACT: Fluoroquinolones are the most commonly prescribed synthetic antibacterial agents having improved pharmacokinetic properties and broad spectrum of activity. Due to their broad clinical activity they are the most promising drug for antibacterial chemotherapy. The current study focused on the application of similar chromatographic system for the analysis of a number of fluoroquinolones from bulk, pharmaceutical formulations and biological matrices. We have also summarized how a single chromatographic system can be used for the quantification of a number of fluoroquinoloes from their respective marketed formulation. A detailed systemic survey of the sample preparation techniques, the chromatographic conditions and detection of the respective fluoroquinolones has been presented in this study. In the course of this discussion the advantages and applicability of the high performance liquid chromatographic techniques are also examined.

1. INTRODUCTION:

1.1: Quinolones as antibacterial agents:

A number of chemically synthesized antibacterial agents are available for the treatment of local, systemic and/or urinary tract infections. Chemically these can be classified into sulfonamides, certain nitro-heterocyclics – imidazole derivatives like metronidazole, ornidazole etc. Quinolones also belongs to this class of these synthetic antibacterials.

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Initially these molecules were found to show anti-infective properties against gram negative urinary pathogens. Further structure activity study led to the development of more potent and biologically active molecules having a broad spectrum of activity. Almost all of these quinolones have a fluorine atom at the C_6 position of the quinolone, napthyride and benzene ring system and so is the name "fluoroquinolones".

These agents are chemically related to nalidixic acid [1] and share a similar mode of action. The activity is known to result from the inhibition of bacterial DNA sysnthesis. These molecules in general are bacterial DNA-gyrase (topoisomerase II) and topoisomerase IV enzyme inhibitors responsible for bacterial DNA synthesis (**Fig.1**), leading to cell death [2]. The highly polar quinolones are believed to enter the bacterial cells through the

highly charged porin channels present in the bacterial membrane [2, 3]. Thus alteration in these poring channels due to mutation or energy dependant efflux in many bacterial species may result in the development of resistance against quinolones [3].

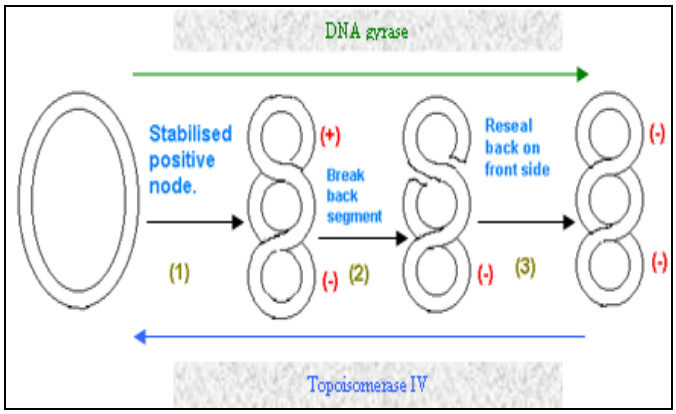

FIG.1: STAGES OF BACTERIAL DNA SYNTHESIS

These antibacterial agents have a broad spectrum of activity against both gram-positive and gram-negative bacteria as well as anaerobes [3] that affects both human and animals. Chemically the general structure of these molecules is 1-substituted-1,4-dihydro-4-oxopyridine-3-carboxylic combined with aromatic and heteroaromatic ring [3, 4] (**Fig. 2**). A detailed structure activity study presented the importance of 1, 4-dihydro-4-oxo-3-pyridinecarboxylic acid moiety in their antibacterial activity. Association of aromatic ring with heterocyclic ring is essential for its activity. Isosteric replacements of nitrogen for carbon atoms at positions 2 (cinnolines), 5 (1,5- napthyridines), 6 (1,6-naphthyridines), and 8 (1,8-naphthyridines) are consistent with retention of antibacterial activity. Substitutions at the 5, 6, 7 and 8 positions of the annulated ring improve activity.

For example, piperazinyl and 3-aminopyrrolidinyl substitutions at position 7 have been shown to convey enhanced activity on members of the quinolone class against *P. aeruginosa*. Fluorine atom substitution at position 6 is also associated with significantly enhanced antibacterial activity (Fluoroquinolones). The alkyl substitution at the 1-position is essential for activity, and a progressively improved potency is observed with lower alkyl (methyl, ethyl, cyclopropyl) substituent with increase in the number of carbon atoms. Aryl substitution at the 1-position is also consistent with antibacterial activity, with a 2, 4-difluorophenyl group providing optimal potency. Ring condensations at the 1, 8-, 5, 6-, 6,7-, and 7,8-positions also lead to active compounds [3-6] (**Fig. 2**). As discussed earlier, the earlier quinolones starting with nalidixic acid are found to show gram negative activity. The newer members having 6-fluoro, 7-piperzinyl substitution improves its spectrum of activity for the gram negative pathogens and efficacy towards gram positive cocci. It is however found to show poor activity towards most anaerobic bacteria and shows efficacy against organisms that have developed resistance against other antibacterial antibiotics like penicillin, methicillin and their derivatives [5, 6].

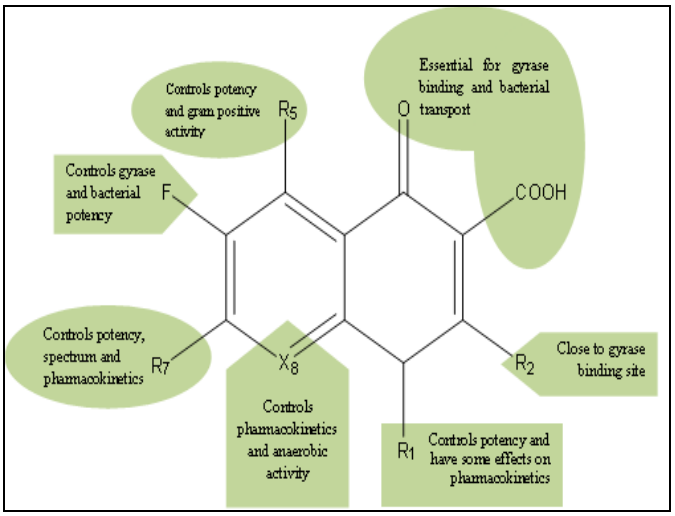

FIG. 2: THE GENERAL STRUCTURE OF FLUOROQUINOLONE WITH STRUCTURE ACTIVITY RELATIONSHIP

Attempts have been made to subdivide fluoroquinolones depending upon the period of their introduction, spectrum of activity, potency and pharmacology. However till date no classifications have been accepted although a classification into first, second, third generation is occasionally used in order to distinguish them on the basis of their introduction and spectrum of activity. Higher efficacy of these quinolone derivatives promoted extensive study on their structure activity relationships, microbiology and associated synthetic chemistry [5-7]. The structures of the available fluoroquinolones are presented in **Fig.3**.

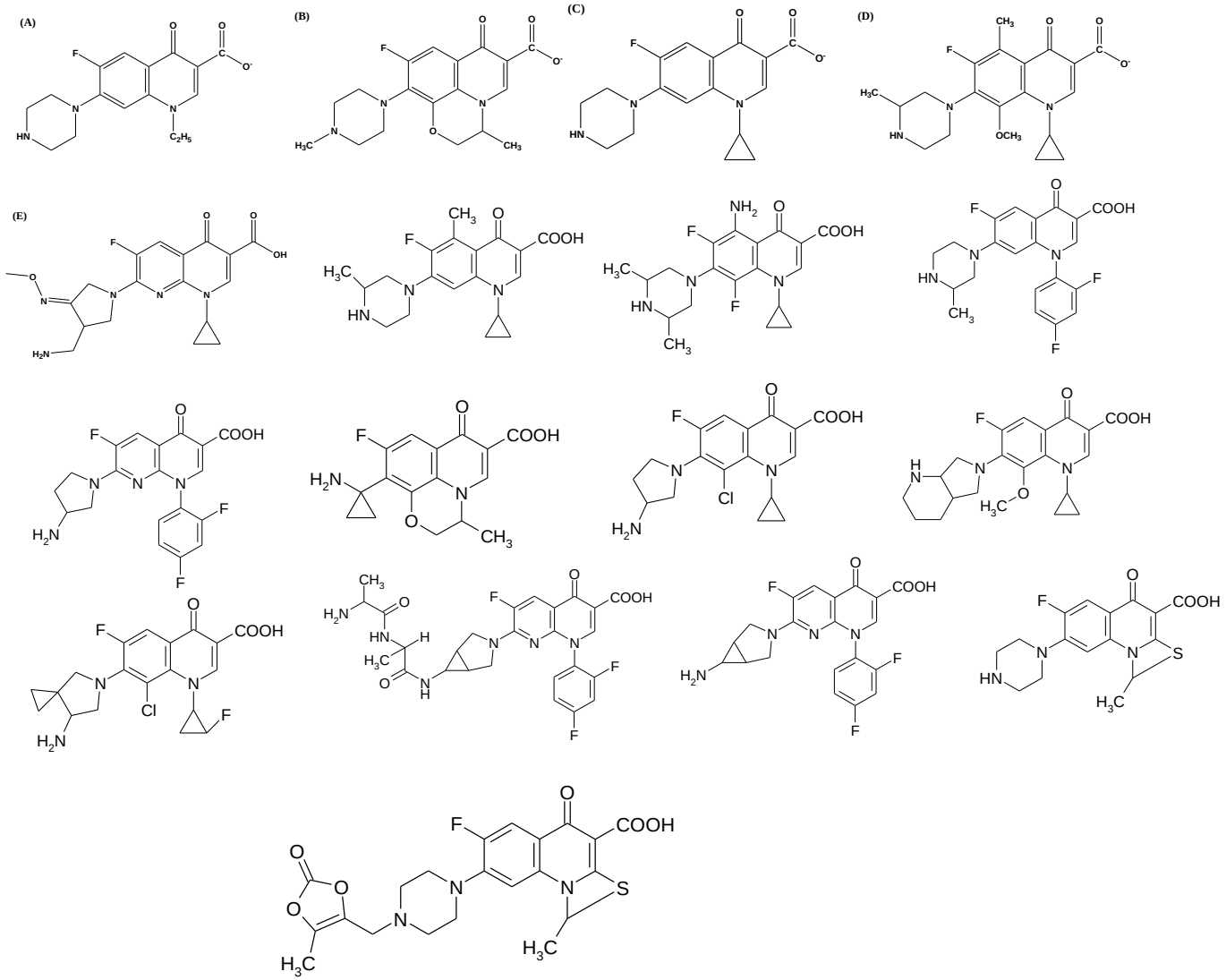

FIG.3: STRUCTURE OF FLUOROQUINOLONES UNDER STUDY; (A) NORFLOXACIN (2ND GENERATION), (B) OFLOXACIN / LEVOFLOXACIN (2ND GENERATION), (C) CIPROFLOXACIN (2ND GENERATION), (D) GATIFLOXACIN (3RD GENERATION), (E) GEMIFLOXACIN (4TH GENERATION), (F) GREPAFLOXACIN, (G) SPARFLOXACIN, (H) TEMAFLOXACIN, (I) TOSUFLOXACIN, (J) PAZUFLOXACIN, (K) CLINAFLOXACIN, (L) MOXIFLOXACIN, (M) SITAFLOXACIN, (N) ALATROFLOXACIN, (O) TROVAFLOXACIN, (P) ULIFLOXACIN, (Q) PRULIFLOXACIN.

1.2. Available analytical techniques for the quantification of quinolones from formulations, biological fluids and waste water:

Numerous techniques have been developed for the analysis of fluoroquinolones in biological fluids and pharmaceutical preparations [8-13]. Initially microbiological methods of analysis had been developed but the method was found to be slow and suffers from poor precision and specificity. Other reported physical and chemical techniques suffered from a variety of disadvantages. The clinical and molecular studies of the pharmacological profile presented a better correlation between the observed effects of fluoroquinolones and its plasma concentration. This encouraged the development and validation of a number of liquid chromatographic methods for the regular quantification of this antimicrobial agent from bulk, formulations and different types of tissues and body fluids.

Also chromatography in the last few years has emerged as an important tool for therapeutic drug monitoring and also supports clinical and non clinical drug development and quality control of pharmaceutical formulations. A number of chromatographic techniques have been developed in last two decades for the quantification of newer fluoroquinolones [14-22] from a variety of sample matrices. The introduction of chromatographic

techniques has revolutionized the process of detection, separation and quantification of the parent components and the respective metabolites with higher selectivity, sensitivity, precession and accuracy form a variety of sample matrices including biological and environmental matrices [22-35]. Moreover these chromatographic techniques can be derived from available techniques with slight modification or can be developed on the basis of knowledge gathered through experience and physical properties of the analyte under consideration.

The aim of this review is to focus on chromatographic techniques available in literature with special focus on the application of a similar chromatographic system validated for the quantification of all available fluoroquinolones from bulk and marketed formulations. The objective is to provide an appropriate basis for the development of new chromatographic techniques for newer fluoroquinolones or molecules under development or structurally related to fluoroquinones, and also to present universal technique for the analysis of these compounds using a single chromatographic system.

2. Methodology:

2.1. Sample preparation:

The sample preparation, pretreatment or sample cleanup constitutes a major issue for the successful quantification of any analyte from any type of matrix and constitutes the most important and time consuming part of any analysis. This also have impact on the quality of the data obtained from analysis. Most of the chromatographic analysis involves the quantification of analytes, their metabolites and different degradation products from biological matrices in presence of different forms of interfering substances like, salts, acids, bases, proteins, cells, lipids, and enzymes. At the same time for the quantification of the analyte from marketed formulations and in bulk a proper sample preparation procedure is often required when the excepients in formulations (suspensions, lotions, creams) interferes with the analyte [36]. In such cases sample pretreatment is often necessary [27-36]. Thus possible chromatographic interferences and clogging or detoriation of the column can be avoided. The technique for sample preparation depends on the chemical properties of the analyte under consideration, the matrix and the specific requirement for the investigation. Most commonly found techniques include extraction of the fluoroquinolone from the matrix - protein precipitation, liquid-liquid extraction, solid phase extraction, ultrafiltration, dilution and subsequent chromatographic analysis **(Table 1)**.

In bioanalytical application, protein precipitation followed by solvent evaporation and dilution are most commonly used for the simplicity of the process. In this case the plasma protein is denatured by the addition of organic solvent, acidification or by heating the sample. During the process of denaturation, protein looses their secondary and tertiary structures and the analyte bound with these proteins becomes freely soluble in the medium. The sample solution is centrifuged to precipitate out the protein in forms of pellets and the clear supernatant is available for chromatographic analysis. Common precipitating agents used for the quantification of fluoroquinolones are acetonitrile, methanol and perchloric acid **(Table 1)**.

Among these solvents acetonitrile is found to be most effective in precipitating out the protein however a greater peak height and area is obtained when methanol is used, explaining its higher extraction power for fluoroquinolones in comparison with acetonitrile [53]. So, methanol is often used for higher sensitivity [53]. On the other hand the use of acids in protein precipitation often results in the co-precipitation of the analyte and reduces the sensitivity of the process [54]. Apart from these literature reports presents the application of Liquid Liquid Extraction (LLE) and Solid Phase Extraction (SPE) as important tool for protein separation and sample preparation [40, 41, 52].

Sample preparation is not that complex when we are analyzing the active pharmaceutical ingredients from formulation, however there are instances where the excepients interferes with the analyte peaks. In such cases a proper sample preparation procedure becomes necessary. Recent studies on a number of fluoroquinolones estimated from a wide range of formulations reported a common and simple technique of sample preparation [45-50]. A pre-weighed powder sample is dissolved in 0.1(M)

hydrochloric acid solution and finally diluted using the mobile phase. This finally diluted sample is injected into the chromatographic system. The observed peaks are found to be spectrally homogeneous [45-50].

TABLE 1: SAMPLE PREPARATION PROCEDURE APPLIED FOR THE ANALYSIS OF NEWER FLUOROQUINOLONES

Fluoroquinolones	Matrix	Sample preparation	Sample Volume (µL)	Solvent used	API Recovery %	Ref.
Moxifloxacin	Saliva	Deproteinisation	-	Perchloric acid	101	37
Levofloxacin	Human Plasma	Deproteinisation	100	Acetonitrile	99.48-104.81	38
Levofloxacin	Rat plasma and prostrate tissue	Microdialysis	30.0	Ringer's solution pH =7.2	-	39
Gemifloxacin	Rat serum	Liquid Liquid Extraction	200	Chloroform/ isoamyl alcohol (9:1 v/v) 3ml	79.32-88.21	40
Ulifloxacin	Rat and rabbit plasma	Liquid Liquid Extraction	250	Chloroform/ isoamyl alcohol (85:15, v/v) ≈ 2.5ml	96.90-99.01	41
Pazofloxacin Ciprofloxacin Levofloxacin	Human Serum	Deprotination	200	6.0% (w/v) perchloric acid 0.1ml + methanol 0.1ml	97 (paz) 88 (Cip) 90 (Lev)	42
Sparfloxacin	Rat plasma	Deprotination	100	Acetonitrile 0.2ml	> 90.0	43
Ciprofloxacin Ofloxacin Moxifloxacin	Human plasma	Protein precipitation and evaporation/ reconstitution	400	Acetonitrile 1.0ml	95(OFL), 86.4 (Cip), 94.2 (Mox)	44
Norfloxacin	Drug formulations	-	-	0.1(M) HCl solution/ Buffer of pH 3.0	95 to 100%	45
Ciprofloxacin						46
Gatifloxacin						47
Gemifloxacin						48
Ofloxacin						49
Sparfloxacin						50
Ciprofloxacin	Human Plasma	Protein precipitation	-	Acetonitrile	About 100%	51
Gatifloxacin	Human Plasma	Liquid liquid extraction	500	Ethyl acetate 3 ml	56.39-57.75	52

2.2. Chromatographic separation:

High performance liquid chromatography is an important tool for the analysis of different types of organic entities including drugs and their metabolites from different complex matrices. Last decades have seen considerable attempts for the development of the different steps of chromatography and currently it has proven itself as a well established technique for separation and quantification processes. A number of newer methodologies have been developed for the quantification of fluoroquinolones using reverse phase chromatography **(Table 2)**. Each of these techniques is specific for the quantification of single fluoroquinolone. A cumulative data from six studies also presented the use of a single chromatographic technique for the analysis of six fluoroquinolones available from marketed formulations [45-50]. However, some difficulties are also identified in the quantification of these molecules using reverse phase chromatography. As discussed earlier, these molecules are weak heterocyclic amino acids having an active protonation site (amine group) and a deprotonation site (carboxylic group) [55].

As a result in solution a fluoroquinolone can exist in cationic, neutral, zwitterionic and anionic forms. A difference in the hydrophobicity of the same molecule during analysis results a differential migration through the column, a differential retention in the column affecting the peak bandwidth. This together with column particle size, polarity modifiers in mobile phase, gradient elution and sample nature may affect the resolution, peak shape and sensitivity [56]. The neutral and zwitterionic forms are expected to get retained by the reverse phase columns. However in aqueous

solution the fluoroquinolones are expected to remain in their ionic forms as a result the use of buffer or ion suppressor in the mobile phase or paired ion chromatographic reagents and adjustment of pH of the mobile phase is common in reverse phase chromatographic analysis of these molecules [55]. An optimized mobile phase and column selection involves consideration of several issues like the percentage of organic modifier, the pH, the type and the concentration of buffers or ion pair reagents. Acetonitrile and methnol are commonly used organic modifiers in reverse phase chromatography and their concentration in the mobile phase is a critical parameter to be considered.

TABLE 2: DESCRIPTION OF THE LIQUID CHROMATOGRAPHIC TECHNIQUES USED FOR THE SEPARATION OF THE FLUOROQUINOLONES

Fluoro-quinolone Under consideration	Chromato-graphic technique	Detection (m/z; nm)	Sample matrix	Column used	Elution type	Mobile Phase used	Flow rate (ml/min)	Run time (min)	Calibra-ion range (µg/ml; µg/g)	LOQ (µg/ml; µg/g)	Year	Ref.
Norfloxacin	HPLC	278.0	Tablets and bulk formulations	Xterra C18 (5µm) 150mm x 4.6mm	Isocratic	Phosphate Buffer pH 3.5	0.5	5.0	0.01 to 0.50	0.23	2014	45
Ciprofloxacin		275.0								0.20		46
Gatifloxacin		298.0								0.27		47
Gemifloxacin		272.0								0.19		48
Ofloxacin		295.0								0.26		49
Sparfloxacin		298.0							4.0 to 32	0.29		50
Besifloxacin, Gatifloxacin, Moxifloxacin	LC-MS/MS	m/z 394.2→376.2 (Bes), m/z 376.2→358.2 (Gat), m/z 402.2→384.2 (Mox)	Human aqueous humour	X Bridge C18 (3.5µm)	Isocratic	0.2% Formic acid / acetonitrile (80:20 v/v)	-	-	0.001 - 1.000	0.001	2011	57
Gemifloxacin mesylate	HPLC	287nm	Bulk drug	Itersil ODS3V C18 (5µm)	Gradient 27ºC	0.1% Trifluoroacetic acid (pH 2.5, liquid ammonia)/ methanol (55:45 →20:80 v/v)	1.0	15	0.1 – 200	1.0	2011	58
Levofloxacin	UPLC	288	Pharmaceutical formulations	Waters Acquity HSS T-3 C18 (1.8µm)	Gradient 50ºC	0.1% trifluoroacetic acid/ acetonitrile (92:08)	0.45	5.0	20-45	0.05	2010	59
levofloxacin	HPLC	295	Pharmaceutical formulations	Zorbax Eclipse XBD-C8(5µm)	Isocratic	Acetonitrile : Aqueous phosphoric acid (15:85)	0.5	10	0.005-0.600	0.600	2008	60
Prulifloxacin, Ulifloxacin	HPLC	278	Human plasma	HILIC stationary phase	Isocratic	ammonium acetate buffer (5 mM, pH 5.8)/acetonitrile (both with 1% Et_3N, v/v)	-	-	1-25	1.0	2015	61
Levofloxacin, pazufloxacin, gatifloxacin, moxifloxacin and trovafloxacin	HPLC	Fluorescence spectroscopy excitation/emission wavelengths of 260/455nm	Human plasma	LiChroCART(®) Purospher Star C18 column (55mm×4mm, 3µm)	Gradient	0.1% aqueous formic acid adjusted to pH 3.0 with triethylamine, acetonitrile and methanol	1.0	-	0.005-5µg/mL for GAT, 0.02-5µg/mL for LEV, PAZ and MOX and 0.04-5µg/mL for TRO	0.005µg/mL for GAT, 0.02µg/mL for LEV, PAZ and MOX and 0.04µg/mL for TRO	2013	62
Gatifloxacin	HPLC	293nm	Bulk and pharmaceutical preparations	UPELCO® 516 C-18-DB, 50306-U, HPLC column (250 mm × 4.6 mm, 5 µm)	Isocratic	Disodium hydrogen phosphate buffer:acetonitrile (75:25, v/v) and with orthophosphoric acid pH was adjusted to	1.0	5.0	4.0–40 µg./mL	-	2015	63

Gemifloxacin	HPLC	Fluorescence spectroscopy excitation/emission wavelengths of 272/395nm	Human breast milk	C18 column (150 × 4.6 mm, 5 µm I.D.)	Isocratic	3.3 methanol:50 mM ortho-phosphoric acid solution (40:60)	1.0	-	0.1–2.5 µg/mL	-	2015	64
Moxifloxacin	HPLC	254	Pharmaceutical formulations	C8 column (250 × 4.6 mm, 5 µm)	Isocratic	phosphate buffer (20 mM) containing 0.1% (v/v) triethylamine, at pH 2.8 (adjusted with dilute phosphoric acid) and methanol (38.5:61.5 v/v)	1.5		50–350 µg m L^{-1}	-	2014	65
Levofloxacin	HPLC	UV detection	Human Plasma	Symmetry C_{18} column	Isocratic	acetonitrile and 0.01 Мpotassium dihydrogen aqueous solution (pH 3.4; 14:86 v/v)	-	4.5	0.1–12 µg/mL	-	2015	66
Levofloxacin	HPLC	287nm Extinction/ emission wavelengths = 300/500nm	Tablets	C18 column	Isocratic	acetonitrile-methanol-phosphate buffer 0.1M15:25:60 (v/v/v)	1.0	-	-	-	2015	67
Ciprofloxacin	HPLC	278 nm	Pharmaceutical formulations	X Bridge C18 (4.6×150 mm and 3.5 µm.)	Isocratic	0.025 M phosphoric acid (adjusted to pH 3.0±0.1 with triethanolamine) and acetonitrile (60:40)	1.0	-		0.35	2014	68
Ciprofloxacin	HPLC	277nm	Human plasma	ACE® 5 C18 column (250 mm × 4.6 mm, 5 µm)	Isocratic	phosphate buffer (pH 2.7) and acetonitrile (77:23, v/v)	-	-	0.05–8 µg/m	-	2015	69

Literature reports present the use of surface response techniques for optimizing the concentration of organic part in the mobile phase [70, 71]. The pH of the mobile phase is also an important parameter to be considered and the average pH of analysis was observed [72, 73] between 2.5 to 3.5. The use of paired ion chromatography reagents are also reported for fluoroquinolones. Due to their ionizable properties in aqueous media, these reagents are often used to increase their retention [71]. Liang et. al. used SDS as ion pair reagent to separate fluoroquinolones in a mixture at pH 3.0 and found a concentration 10.0 mM to be optimum [74]. Band broadening and peak tailing is common with reverse phase chromatography for fluoroquinolones. This may be due to interaction of

the amine functional group with the uncapped acidic silanol groups present in the bonded stationary phase of the column and may be the principle cause for peak tailing. Use of mobile phase having low pH protonates the amine functional group and the silanolic hydroxyl group and thereby reduces silanophilic interaction [75]. The same objective is achieved by using amine modifiers in the mobile phase [72-75]. The use of paired ion chromatographic reagents or competing base in the mobile phase is a common practice in most of the techniques reported.

In developing separate analytical technique for newer fluoroquinolones an optimized mobile phase consisting of phosphate buffer of pH =3.0 and acetonitrile in the ratio 60:40 and a flow rate of 1.0ml per minute is used. This mobile phase combination is found suitable for the analysis of fluoroquinolones irrespective of the matrix under consideration for pharmaceutical formulations and

is found to provide better performance in regular analysis. These methods are found to be rugged, accurate and precise and each has been validated as per ICH guidelines [76]. The results and validation data are presented in **Table 2**.

The choice of column for chromatographic analysis is also an important parameter for consideration as all separation takes place in this medium. Literature study presents C18 column to be the most acceptable one for the reverse phase chromatographic analysis of the fluoroquinolones. A C18 column (125 mm × 4.6 mm; 5µm; 100 Å, Xterra, Waters, MA, USA) is used for the analysis of the newer fluoroquinolones **(Table 2)**.

2.2.1. Similar chromatographic systems used for the analysis of Fluoroquinolones:

Among the available chromatographic techniques the high performance liquid chromatographic (HPLC) techniques have high sensitivity, specificity and rapidity became the most acceptable tool for estimation of fluoroquinolones for formulations and biological fluids. Most of these techniques involve either an isocratic or gradient elution technique and a C18 column for the separation purpose. Among the different reported techniques the choice of mobile phase varied with the fluoroquinolone under consideration, however the pH of the system remained between 2.5 to 3.5 **(Table 2)**. All of these molecules are polar and most of these analysis reports the application of reverse phase for analysis using a C18 column. The mobile phase is usually a combination of a buffer or a solution of paired ion chromatographic reagent along with a polar modifier like methanol or acetonitrile.

2.3. Detection:

In chromatographic analysis the detection of the components in the eluent after separation is an important parameter to be considered. Three types of chromatographic systems are commonly cited in literature – UV-Vis, Fluorescence and Mass Detectors **(Table 2)**. Among these the commonly used detectors includes the UV-Vis and the fluorescence detectors. As most of these molecules have chromophoric groups in their structure the analysis is mainly carried out on the basis of molar UV absorbance and fluorescence efficacies [77]. Recently, the MS/MS-detectors are in use. Practically speaking the choice of detector is dependent upon the sensitivity (expressed in terms of detection and quantitation limits) and selectivity required for the purpose of analysis.

In case of UV detection the choice of absorbance maxima is selected upon the absorbance characteristics of the analyte and this corresponds to the wavelength showing maximum absorbance to enhance sensitivity. However, certain endogenous substances in the matrix may interfere with the analytes at this characteristic wavelength of the molecule. In such cases some alternative wavelength may be chosen. For example, Kamberi et al [78] chose 290 nm as lambda max for sparfloxacin while analyzing the same from plasma and 364 nm for analysis from urine not because of the degree of sensitivity but to reduce the interference of endogenous substances. UV detectors are also compatible with gradient elution as most of the components present in the mobile phase like buffer, water, methanol, acetonitrile and tetrahydrofuran are mostly transparent in the wavelength range under consideration for the analysis of fluoroquinolones. However, in combination they are not always transparent and in case of gradient elution certain fluctuations in the baseline may be observed which is usually eliminated by selecting a single wavelength specific for the compound and analyzing with respect to reference value [79]. With a objective to develop a single chromatographic system for the analysis of fluoroquinolones, Bera et. al. has used an isocratic elution technique and specific wavelengths for each component under analysis [45-50].

CONCLUSION: Reverse phase high performance liquid chromatography in particular is the most commonly used technique for the quantification of fluoroquinolones from variable types of matrices like biological matrices and pharmaceutical formulations. The selectivity, sensitivity, accuracy and reproducibility of these techniques make them a suitable tool of the quantification of these molecules. Several such techniques have been discussed in the course of this review. The column commonly used for the separation of fluoroquinolones is mostly C18 column with a very

few exceptions **(Table 2)**. The mobile phase offering optimal resolution, peak separation and analysis time is acidic with pH just below the respective pK values. Ammine derivatives and peak reagents are commonly used to control peak broadening and tailing common with fluoroquinolones. The detectors associated with these types of chromatographic separations includes mainly UV-Vis detectors, however use of fluorescence and mass detectors are also reported.

The UV-Vis detector is cheap, commonly available in analytical laboratories and has got maximum applications. The fluorescence detectors are costly in comparison to UV-detectors, but sensitive and selective and are suitable for the analysis of fluoroquinolones. The mass detectors are expensive and sophisticated and are used for very specific applications only. Sample preparation is also a very important issue for consideration in order to achieve a clean chromatographic separation in presence of complex matrix. Specifically for the quantification of the analyte from the serum samples procedures like protein precipitation, liquid liquid extraction and solid phase extraction techniques are commonly used. Apart from these, other techniques used includes ultrafiltration and dilution, common for the quantification of the fluoroquinolones from complex formulations and bulk preparations. Certain automated techniques are also available as good alternatives which are reliable and less time consuming.

Keeping in mind the pH of the mobile phase, better resolution and the stationary phase commonly used for most separation, it may be concluded that for the quantification of fluoroquinolones one should start with a mobile phase pH of 2.5 to 3.5 using a buffer to impart ion-suppression and if required a paired ion chromatography reagent like sulphonate salts of long chain carboxylic acids. A C18 column is most suitable stationary phase for separation. Studies have also shown the use of similar chromatographic condition is suitable for the separation of ciprofloxacin, gatifloxacin, sparfloxacin, gemifloxacin, norfloxacin, ofloxacin and can be predicted to be an optimal chromatographic separation technique for all these components. It can therefore be expected that the chromatographic techniques discussed through this review can be helpful in the development of further analytical techniques for newer fluoroquinolones and compounds having structural similarity with the molecules under study.

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