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Research Article

FTIR AND XRD INVESTIGATIONS OF SOME FLUOROQUINOLONES

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ABSTRACT

The vibrational spectroscopy, such as FTIR has been used to measure the vibrational modes of fluoroquinolones, provides information about structural differences of its individual members. From the interpreted spectral data, Norfloxacin, Ofloxacin and Ciprofloxacin have been distinguished by the presence of different substituents in their parent nucleus. X-ray powder diffraction (XRD) data of those three pure fluoroquinolones have been obtained using a powder diffractometer. The drugs were scanned from a Bragg's angle (2θ) of 10˚to 70˚. The powder XRD patterns of all fluoroquinolones studied were sufficiently unique to make their identification possible. Both FTIR and XRD studies provide the most direct and definitive identification of fluoroquinolones and offer a means for qualitative analysis of pure drugs. The current preliminary studies make the basis for validation of both FTIR and powder XRD as a good monitoring official method for identification of fluoroquinolones.

Keywords: Fluoroquinolones, Norflox, Oflox, Cipro, FTIR, XRD

INTRODUCTION

Fluoroquinolones are today's blockbusters of the antibacterial market due to their therapeutic efficacy having tolerable side-effects and thus challenging the predominance of well- established β-lactam antibiotics which are becoming more prone to the resistant pathogenic bacteria. The fluoroquinolone market is heavily dominated by norfloxacin, ofloxacin, ciprofloxacin, etc. The benzopyridone nucleus (quinolone) proved to be more responsive to chemical manipulation in order to enhance antibacterial potency, and subsequent discovery of fluorine atom and piperazinyl ring on the quinolone ring revolutionized the chemistry and clinical importance of fluoroquinolones[1,2].

Norfloxacin (Norflox) has an ethyl group at the N-1 position. Norflox, having a C-7-piperazinyl group in addition to a C-6 fluorine substituent, has antibacterial potency far superior to that of the earlier classical quinolones against Gram-positive and Gram-negative bacteria, including many strains of *Ps. aeruginosa*. In case of Ofloxacin (Oflox), the substitution of methyl group at the C-4 position of the piperazinyl moiety enhances the Gram-positive antibacterial activity of the parent compound with a slight decrease in Gram-negative activity, especially against *Ps. aeruginosa*. Ciprofloxacin (Cipro), with an N-1-cyclopropyl substituent, is by far the most potent of the marketed quinolones *in vitro* against members of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa* (Fig. 1). The fluoro group at position C-6 seems to improve both the DNA gyrase complex binding {2- to17-fold), and cell penetration (1- to 70-fold) of the corresponding derivatives with no substitution at the C-6 position. Because of such an enhancement of antibacterial potency, nearly all of the recently synthesized quinolones carry a C-6 fluorine substituent[3,4].

In Fourier Transform Infrared (FTIR) spectroscopy, the resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint, no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis. The information that can be provided by FTIR is identification of unknown materials along with quality, purity and consistency of the sample. FTIR is preferred over dispersive or filter methods of infrared spectral analysis, as it is a non-destructive technique[5-7].

The X-ray powder diffractometry (XRPD) technique has also advantages over the other methods, being a non-destructive and requiring only minimal sample preparation. This technique is unique since it combines absolute specificity with a high degree of accuracy. XRPD is a powerful technique for identification of crystalline solid phases by their unique diffraction patterns[8] and characterization of pharmaceutical solids for both scientific and regulatory purposes. The X-ray diffraction method has become one of the most useful tools for qualitative characterization of materials in the pharmaceutical industry.

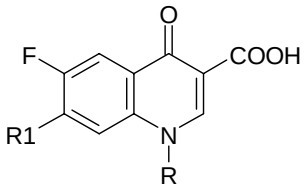

Fig. 1: Molecular structures of Norfloxacin, Ofloxacin and Ciprofloxacin

Fluoroquinolones	R	R1
Norfloxacin {1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid}	1-ethyl	1-piperazinyl
Ciprofloxacin {1-cyclopropyl-6-fluoro -1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid}	1-cyclopropyl	1-piperazinyl
Ofloxacin {9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid}	1,4-benzoxazine	4-methyl-1-piperazinyl

Norflox, Oflox and Cipro, and their formulations are included in USP XXIV[9,10]. Cipro and Norflox, and their formulations are included in BP[11] and IP[12] as well. Powder XRD method has not been suggested in the USP, BP and IP for identification of fluoroquinolones, but infrared absorption, thin layer chromatography, high performance liquid chromatography and polarographic methods are suggested. A review of the literature reveals that very little attention has been paid to the powder XRD in identification and determination of fluoroquinolone antibacterials.

It has already been mentioned that both gyrase inhibition and cell penetration are enhanced by the presence of a C-6 fluorine atom and the cell permeability is predominantly controlled by the nature of the C-7 substituent. So, the presence of groups which occupy the N-1, C-6, C-7, and C-8 positions are responsible for yielding potent antibacterial activity. Taking into consideration of above positive aspects of FTIR analysis and XRPD study, we have used these analytical tools to know the purity of some fluoroquinolones.

MATERIALS AND METHODS

Materials

Pure samples of norfloxacin ($C_{16}H_{18}FN_3O_3$), ofloxacin ($C_{18}H_{20}FN_3O_4$) and ciprofloxacin ($C_{17}H_{18}FN_3O_3$) were provided as gift samples by Dr. Reddy's Research Foundation,

Hyderabad, India. The drugs were of 99.8% to 98.0% purity.

Methods

Apparatus

FTIR spectroscopic analysis

FTIR Spectroscopy is an important analytical technique which detects various characteristic functional groups in molecules of any matter. On interaction of an infrared light with the matter, chemical bonds would stretch, contract and bend, and as a result each chemical functional group tends to absorb infrared radiation in a specific wavelength range regardless of the structure of the rest of the molecule. Based on this principle, functional groups present in composite materials are identified. It is performed in a FTIR Spectrophotometer interfaced with infrared (IR) microscope operated in reflectance mode. The microscope is equipped with a video camera, a liquid Nitrogen-cooled Mercury Cadmium Telluride (MCT) detector and a computer controlled translation stage, programmable in the x and y directions. The FTIR imaging in the present investigation was carried out using a Perkin Elmer Spectrum RX. Here KBr pellet method was used for sample preparation for FTIR study[13]. The spectra were collected in the 400 cm^{-1} to 4000 cm^{-1} region with 8 cm^{-1} resolution, 60 scans and beam spot size of 10 µm-100 µm.

X-ray diffraction study

XRD measurements were obtained using the Philips X'Pert on powder diffraction system (Philips Analytical, The Netherlands) equipped with a vertical goniometer in the Bragg-Brentano focusing geometry. The X-ray generator was operated at 40 kV and 50 mA, using the CuKα line at 1.54056 Å as the radiation source. 10 mg of each sample was scanned from 10˚ to 70˚ (2θ) and in step sizes of 0.020; count time of 2.00 s, using an automatic divergence slit assembly and a proportional detector. The samples were scanned at 25°C. Relative intensities were read from the strip charts and corrected to fixed slit values.

Procedure

FTIR spectroscopic analysis

A KBr pellet was prepared by grinding the solid sample with solid potassium bromide (KBr) and applying great pressure to the dry mixture. KBr is chosen because it is transparent to infrared radiation. If the pellet is prepared properly, one can actually see through it, as through a pane of glass. 2 mg of each drug sample were taken with dry IR-grade KBr at about 2% sample to KBr ratio in an agate mortar. The grinding was carried out until it was uniformly distributed throughout the KBr. Some amount of the mixture was transferred to the pellet making die and by applying a small pressure to the die before pulling the vacuum. Then full pressure of 10,000 pounds to 16,000 pounds was applied to the die for 2 min. First vacuum was released then pressure. KBr pellet of the drug sample was prepared. Then a vacuum was pulled for 1 to 2 min. The die set was disassembled by removing the base by twisting it off and releasing the 'O' ring seal. The pellet was discharged with the clear cylindrical pellet extractor located above the end of the bore and the plunger located beneath the assembly. Along with this a blank KBr pellet was prepared. Normally background was first scanned by using blank potassium bromide pellet. Then the sample was scanned. The spectra were collected in the 400 cm^{-1} to 4000 cm^{-1} region with 8 cm^{-1} resolution, 60 scans and beam spot size of 10 µm-100 µm[14,15].

XRD study

A powdered specimen is usually packed and prepared in a specimen holder made of glass. In setting up the specimen and apparatus, co-planarity of the specimen surface with the specimen holder surface and the setting of the specimen holder at the position of symmetric reflection geometry have to be assured. The powders were passed through a 100 mesh sieve and were placed into the sample holder by the side drift technique. The holder consisted of a central cavity. In order to prepare a sample for analysis, a glass slide was clipped up to the top face of the sample holder so as to form a wall. Each powder was filled into the holder, gently tapped and used for the XRD analysis[16].

RESULTS

FTIR spectroscopic analysis

The infrared spectra are recorded on Fourier Transform Spectrometer in the mid –infrared region (MIR) within the range (400-4500 cm^{-1})[17]. Due to the complex interaction of atoms within the molecule, IR absorption of the functional groups may vary over a wide range. However, it has been found that many functional groups give characteristic IR absorption at specific narrow frequency range. Multiple functional groups may absorb at one particular frequency range but a functional group often gives rise to several characteristic absorptions. Thus, the spectral interpretations should not be confined to one or two bands only; actually, the whole spectrum should be examined.

While the FTIR band at 4000-1300 cm^{-1} represented functional group region, the appearance of strong absorption bands in the region of 4000 to 2500 cm^{-1} was due to stretching vibrations between hydrogen and some other atoms with a mass of 19 or less. The O-H and N-H stretching frequencies were in the 3700 to 2500 cm^{-1} region with various intensities. Hydrogen bonding has a significant influence on the peak shape and intensities, generally causing peak broadening and shifts in absorption to lower frequencies. The C-H stretching vibration occurred in the region of 3300 to 2800 cm^{-1} [13,18].

In FTIR spectra of Norfloxacin, one prominent characteristic peak was found between 3550 and 3500 cm^{-1}, which was assigned to stretching vibration of OH group and intermolecular hydrogen bonding by single bridge. A band at 3500 to 3300 cm^{-1} suggested the NH stretching vibration of the imino-moiety of piperazinyl groups. The peak at 2750-2700 cm^{-1} indicated the presence ethyl group. The peak at 2500 cm^{-1} was due to the υOH group of the carboxylic acid. The band at 1700 cm^{-1} represented the carbonyl C=O stretching i.e., $\upsilon_{C=O}$. The peak at 1650 to 1600 cm^{-1} was assigned to υN-H bending vibration of quinolones. The bands at the 1500 to 1450 cm^{-1} represented υ_{O-C-O} of acids and at 1300 to 1250 cm^{-1} suggested bending vibration of O-H group, which indicated the presence of carboxylic acid. In addition, a strong absorption band between 1050 and 1000 cm^{-1} was assigned to C-F group. The band in the region 950-900 cm^{-1} suggested the δNH bending vibration of amines. The peak at 800 cm^{-1} was due to the meta distribution of the aromatic protons (Fig. 2 and Table 1[a])[13,19,20].

In FTIR spectra of Oflox, one prominent characteristic peak was found between 3050 and 3000 cm^{-1}, which was assigned to stretching vibration of OH group and intramolecular hydrogen bonding (Fig. 3). This band also suggested the NH stretching vibration of the imino-moiety of piperazinyl groups which was less prominent due to intense OH stretching vibration. The peak at 2700 cm^{-1} was assigned to υCH_3 of methyl group. The band at 1750-1700 cm^{-1} represented the acidic carbonyl C=O stretching i.e., $\upsilon_{C=O}$[21]. The peak at 1650 to 1600 cm^{-1} was assigned to υN-H bending vibration of quinolones. The 1550 to 1500 cm^{-1} represented the υCH_2 of the aromatic ring. The band at 1450-1400 cm^{-1} was assigned to the stretching vibration of CH_2 confirming the presence of methylene group in benzoxazine ring. The peak at 1400-1350 cm^{-1} represented the bending vibration of hydroxyl group. The band at the 1250 to

1200 cm^{-1} suggested the stretching vibration of oxo group. In addition, a strong absorption peak between 1050 and 1000 cm^{-1} was assigned to C-F group. The band at 900-800 cm^{-1} represented the out of plane bending vibration of double bonded enes or =CH groups (Table 1[b])[13,18,22,23].

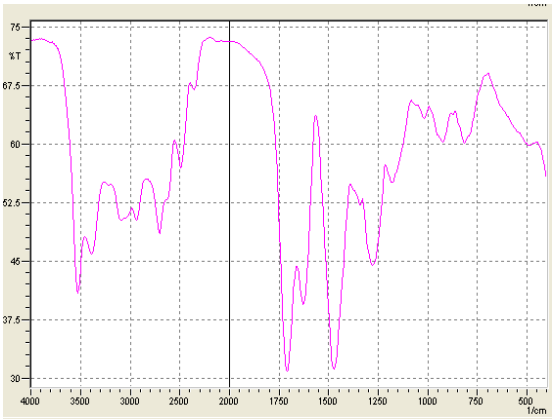

Fig. 2: FTIR Spectra of Norfloxacin

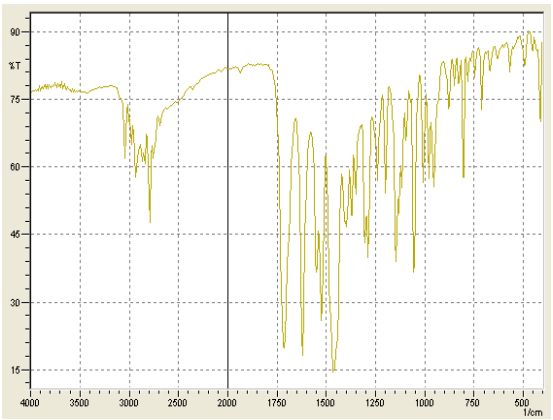

Fig. 3: FTIR Spectra of Ofloxacin

Table 1: Comparative FTIR peaks analysis of fluoroquinolones

(a) Prominent FTIR Peaks of Norfloxacin		
Peaks(cm-1)	**Groups**	**Peak assignment**
3550-3500	Hydroxyl group	Intermolecular H -bonding by single bridge
3500-3300	Imino-moity of Piperazinyl groups	NH stretching vibration
3000-2950	Aromatic,cyclic enes	υ=CH & Ar-H
2750-2700	Ethyl group	υCH_3 of C_2H_5
2500	Acid group	υOH group
1700	Carbonyl of acids	υC=O stretching vibration
1650-1600	Quinolones	υN-H bending vibration
1500-1450	O-C-O group of acid	υ_s stretching vibration of O-C-O group
1300-1250	Hydroxyl group	δO-H bending vibration
1050-1000	C-F groups	υC-F
950-900	Amines	δNH bending vibration
800	Aromatic m – distribution	δAr-H
(b) Prominent FTIR peaks of Ofloxacin		
Peaks(cm-1)	**Groups**	**Peak assignment**
3050-3000	Hydroxyl group	O-H stretching vibration, intremolecular H-bonded
3000-2950	Aromatic,cyclic enes	υ=CH & Ar-H
2750	Alkyl groups	υCH_3
1750-1700	C=O group of acids	υC=O stretching vibration
1650-1600	Quinolines	δN-H bending vibration
1550-1500	Alkyl groups	υCH_3 and υCH_2
1450-1400	Methylene group in Benzoxazine	stretching vibration of CH_2
1400-1350	Hydroxyl group	δO-H bending vibration
1250-1200	Oxo group	C-O-C stretching vibration
1050-1000	Fluorine group	C-F stretching
950-800	Aromatics & enes	=C-H out of plane bending vibration
(c) Prominent FTIR peaks of Ciprofloxacin		
Peaks(cm-1)	**Groups**	**Peak assignment**
3500-3450	Hydroxyl group	O-H stretching vibration, intermolecular H-bonded
3000-2950	Aromatic,cyclic enes	υ=CH & Ar-H
2900	Cyclopropyl group	C-H stretching vibration
1750-1700	CO group of acid	C=O stretching vibration
1650-1600	Quinolines	δN-H bending vibration
1450-1400	Carbonyl group	υC-O
1300-1250	Hydroxyl group	δO-H bending vibration
1050-1000	Fluorine group	C-F stretching

In FTIR spectra of Cipro, one prominent characteristic peak was found between 3500-3450 cm^{-1}, which was assinged to stretching vibration of OH group and intermolecular hydrogen bonding (Fig. 4). The possible peak of imino moiety of the piperazinyl group was less prominent due to intense OH stretching vibration. Another band at 3000-2950 cm^{-1} represented the alkene and aromatic C-H stretching, mainly $\upsilon_{=C-H}$. The peak at 2900 cm^{-1} was assigned to C-H stretching vibration of cyclopropyl group. The 1950 to 1450 cm^{-1} region exhibited FTIR absorption from a wide variety of double-bonded functional groups. The band at 1750 to 1700 cm^{-1} represented the

carbonyl C=O stretching i.e., $\upsilon_{C=O}$. The peak at 1650 to 1600 cm $^{-1}$ was assigned to quinolones. The band at the 1450 to 1400 cm^{-1} represented υ_{C-O} and at 1300 to 1250 cm^{-1} suggested bending vibration of O-H group which proved the presence of carboxylic acid. A strong absorption peak between 1050 and 1000 cm^{-1} was assigned to C-F group (Table 1[c])[13,24,25]. FTIR spectra of Norflox, Oflox and Cipro have been compared in Fig. 5.

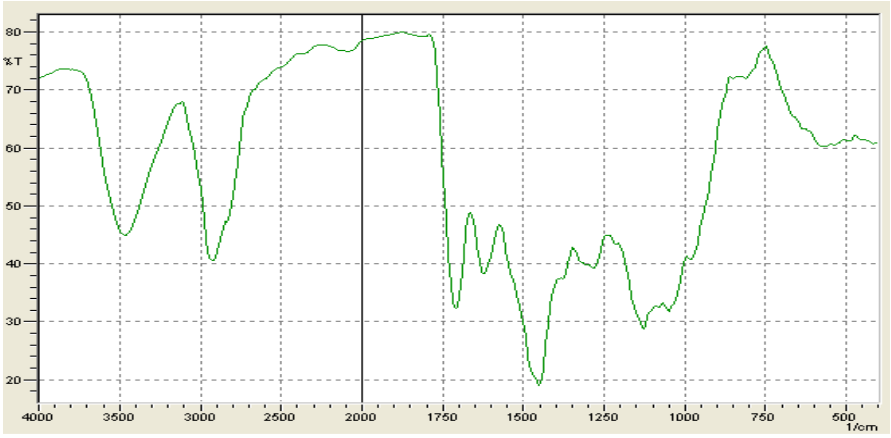

Fig. 4: FTIR spectra of Ciprofloxacin

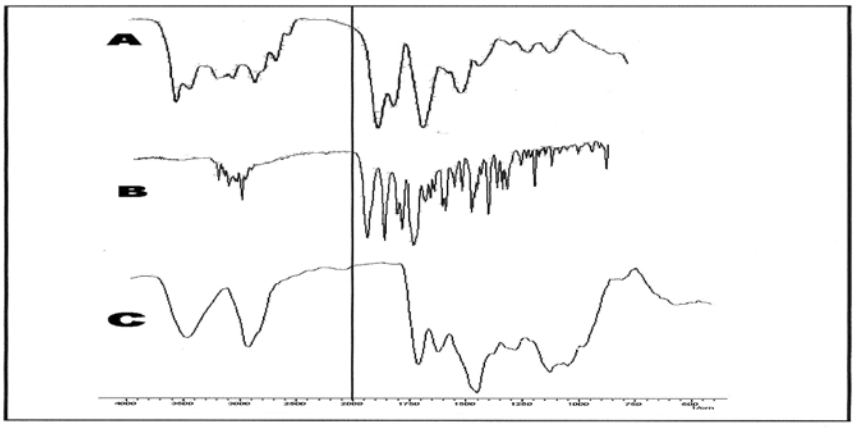

Fig. 5: FTIR spectra of pure Norfloxacin (A),Ofloxacin (B), Ciprofloxacin(C)

X- ray diffraction study

The powder X-ray diffraction patterns of Norfloxacin, Ofloxacin and Ciprofloxacin are given in Fig. 6.

Table 2 and 3 give the data obtained for the three fluoroquinolones in terms of the lattice spacing and the relative line intensities. Most of the characteristic lines in the diffraction patterns were generally prominent and sharp, so measurement of the angles and hence of d-values was accurate. Proper sample preparation helps attain accurate peak positions for qualitative analysis. If the sample surface is irregular or if it is displaced from the focusing circle, peak locations and intensities will vary.

All the high intensity peaks (relative intensity) observed in the XRD pattern of the pure fluoroquinolones were compared (Table 2 and 3). The pure fluoroquinolones were found to show similar XRD patterns. Identification of a structure from its powdered diffraction pattern is based upon the position of peaks and their relative intensities. Each XRD pattern is characterized by the interplanar d-spacing (Å) and the relative intensities (I/I_0) of the three strongest peaks in the pattern under the Hanawalt system (Table 2).

Table 2: Three strongest peaks in the XRD pattern of some fluoroquinolones under the Hanawalt system

Name of Drugs	2θ	d-spacing	I/I_0	H
	13.2243	6.68947	39.70	1348
Norfloxacin	20.7229	4.28272	33.52	1139
	24.9791	3.56180	100.00	3397
	8.14385	8.14385	100.00	2383
Ofloxacin	15.7701	5.61186	99.43	2375
	26.5541	3.35101	83.15	1993
	19.2242	4.61309	54.85	900
Ciprofloxacin	26.3925	3.37417	100	1642
	29.1625	3.05967	28.47	467

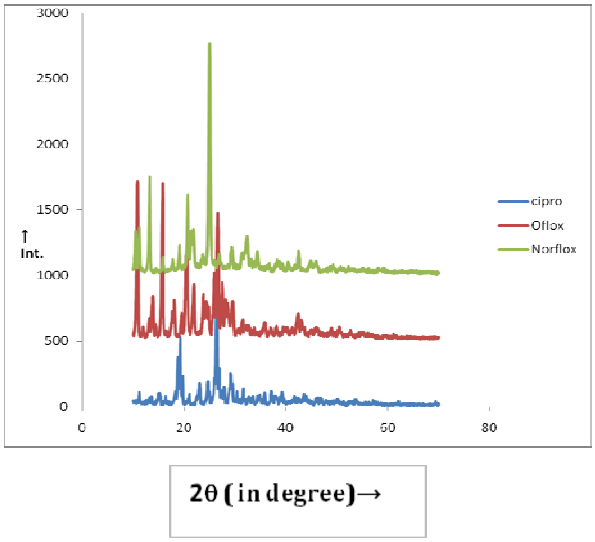

Fig. 6: Comparative X-ray diffraction patterns of Pure Norfloxacin, Ofloxacin and Ciprofloxacin

Table 3: X-ray diffraction data of some fluoroquinolones in terms of lattice spacing and relative intensities

Sl.No	Norfloxacin		Ofloxacin		Ciprofloxacin	
	d-spacing	I/I_0	d-spacing	I/I_0	d-spacing	I/I_0
01	8.39137	15.61	8.14385	100.00	7.85768	9.10
02	7.85364	19.22	7.38179	5.96	5.49944	5.37
03	6.68947	39.70	6.72177	12.87	5.84815	10.98
04	6.07254	0.96	6.39190	27.32	5.41838	5.76
05	5.58651	5.23	6.09752	8.28	4.71198	38.92
06	4.98324	5.42	5.61186	99.43	4.61309	54.85
07	4.64229	11.36	5.35897	4.49	4.50865	22.06
08	4.28272	33.52	5.03979	14.48	4.23066	7.98
09	4.13894	17.81	4.80979	23.09	3.95584	5.97
10	4.07080	18.53	4.55891	15.04	3.85834	18.01
11	3.75223	6.86	4.33077	56.42	3.59959	19.61
12	3.56180	100.00	1.05769	34.24	3.37417	100.00
13	3.31130	8.33	3.73706	28.13	3.31286	27.18
14	3.11694	3.33	3.64542	24.05	3.25759	13.26
15	3.04064	10.94	3.57991	18.17	3.19967	15.74
16	2.84944	8.41	3.44295	43.00	3.05967	28.47
17	2.76768	16.68	3.35101	83.15	2.94445	11.48
18	2.70768	6.19	1.24494	36.49	2.82854	13.70
19	2.60958	8.72	3.17246	24.65	2.73887	6.81
20	2.53713	2.00	3.12187	22.56	2.67646	7.16
21	2.45129	3.55	3.02711	24.41	2.60664	5.69
22	2.35204	5.31	2.91144	5.32	2.57495	8.94
23	2.29726	3.10	2.84559	11.68	2.50843	9.22
24	2.22986	4.37	2.79051	7.51	2.41797	11.24
25	2.16093	2.72	2.74245	8.47	2.37372	7.84
26	2.13003	8.92	2.65870	6.95	2.29794	10.40
27	2.01871	4.85	2.50697	10.14	2.26902	4.49
28	1.97484	4.24	2.42810	6.91	2.22554	2.69
29	1.87436	2.04	2.36127	8.05	2.16814	7.79
30	1.85171	2.89	2.27920	7.13	2.13214	5.80
31	1.80162	1.95	2.24461	7.28	2.08098	7.46
32	1.77813	1.70	2.19062	7.53	2.06287	7.29
33	1.74976	1.79	2.13523	16.91	1.97752	5.70
34	1.63676	1.25	2.10167	12.23	1.93964	5.03
35	1.58599	1.22	2.01552	5.44	1.89912	3.51
36	1.44383	0.54	1.98135	7.18	1.82118	4.07
37			1.86434	5.68	1.78018	1.53
38			1.84422	3.43	1.75580	1.76
39			1.80903	6.57	1.73713	2.86
40			1.73921	4.31	1.70660	3.80
41			1.69624	2.56	1.65710	2.03
42			1.67052	4.05	1.61064	2.77
43			1.62188	2.40	1.49844	1.03
44			1.55661	1.66	1.41232	0.52
45			1.51005	1.04		
46			1.44979	1.00		

DISCUSSION

In case of FTIR spectra of fluoroquinolones, it has been found that there was prominent peak for $\upsilon_{C\text{-}O}$ / $\delta_{O\text{-}H}$ (Fig. 5). Here, we may expect the –CO-, -CHO and -COOR groups give peak for stretching vibration of C=O group in the same range. They can be distinguished by the presence of fermi resonance band for –CHO, $\upsilon_{C\text{-}O\text{-}C}$ bands for esters, and absence of these two for ketones. So, the presence of –COOH group in fluoroquinolones is confirmed (Table 1).

Another probability of interaction is hydrogen bonding i.e., intermolecular hydrogen bonding due to prominent FTIR peaks between 3550 and 3500 cm^{-1}, 3500 and 3450 cm^{-1} and 3050 and 3000 cm^{-1}. The hydrogen bonded -OH stretching vibration occurs over a wide range,

3550-2600 cm^{-1}. The band at 3500-3300 cm^{-1} indicates the presence of piperazinyl group. The peak at 1650-1600 cm^{-1} corresponds to the quinolone moiety of the fluoroquinolones. The bending vibration of O-H group gives medium to strong band in the region around

1450-1250 cm^{-1}, which confirms the presence of COOH group. Here, the FTIR peak at

950-800 cm^{-1} gives the probability of out of plane bending of –ene bond and m-substitution of $\delta_{Ar\text{-}H}$ hydrogen atom. The peak at 1050-1000 cm^{-1} indicates the presence of C-F group which takes a major role in its antimicrobial activity (Table 1)[13,17,18].

The different members of fluoroquinolones like Norfloxacin, Ofloxacin and Ciprofloxacin can be distinguished by the presence of different substituents of the fluoroquinolone moiety. In case of Norfloxacin the presence of ethyl group is confirmed by the appearance of a sharp peak at

2750-2700 cm^{-1}. The band at 2700 cm^{-1} corresponds to methyl group in Ofloxacin. The band at 1450-1400 cm^{-1} corresponds to the methylene group in benzoxazine ring which distinguishes Ofloxacin from other two drugs[13, 22, 23].

Examination of the X-ray data shows that a compound can be easily distinguished from other members. Despite the fact that the three fluoroquinolones have almost the same molecular structure, their powder diffraction patterns are suprisingly different (Fig. 6 and Table 3). It is considered that there are only three or four of the most intense lines which are important for the characterization of those compounds according to Hanawalt system. Since the goal of the study is phase identification, our predominant interest is the position of X-ray lines (d-spacing). Therefore, our discussion is restricted to the d-spacing of the X-ray line.

XRD has the potential ability to identify not only the active ingredient, but also the crystalline excipients in a formulation. The fluoroquinolones under investigation should not only be distinguished one from the other but from the most components of the pharmaceutical preparations. The aim of this paper is to produce the basis for the powder XRD as a promising new official method for identification of fluoroquinolones. Our current findings provide preliminary data which make the basis for validation of the new method.

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