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X-ray powder diffraction patterns for certain fluoroquinolone antibiotic drugs

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X-ray powder diffraction (XRD) data for six pure fluoroquinolone antibiotic drugs, ciprofloxacin, norfloxacin, enrofloxacin, ofloxacin, pefloxacin and sparfloxacin, have been obtained using a powder diffractometer. The drugs were scanned from a Bragg angle (2θ) of 10° to 70°. The obtained data were tabulated in terms of the lattice spacing (Å) and relative line intensities (I/I_I). This new information may be useful for the identification of these drugs.

Keywords: fluoroquinolones, antibiotic drugs, identification, X-ray diffraction, powder

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Fluoroquinolones are synthetic antibiotics, chemically related to nalidixic acid [1-ethyl--1,4-dihydro-7-methyl-1,8-naphthyridin-4-one-3-carboxylic acid], with which they share the same mode of action. These drugs form a group of antimicrobial agents with different chemical structures and spectra of activity. The general molecular structure of fluoroquinolone antibacterial agents consists of a 1-substituted-1,4-dihydro-4-oxopyridine--3-carboxylic moiety combined with an aromatic or heteroaromatic ring (Fig. 1). Mitscher *et al.* (1) reviewed the structure-activity relationship of new fluoroquinolones.

Powder X-ray diffraction (XRD) is widely used in the pharmaceutical industry for the identification of polymorphs (2). The XRD technique has the advantage over the other methods of being non-destructive and requiring only minimal sample preparation. This technique is unique since it combines absolute specificity with a high degree of accuracy. X-ray powder diffractometry is a powerful technique for identification of crystalline solid phases by their unique diffraction patterns (3) and characterization of pharmaceutical solids for both scientific and regulatory purposes. It is also routinely used for phase identification and quantitative phase analysis. The X-ray diffraction method has

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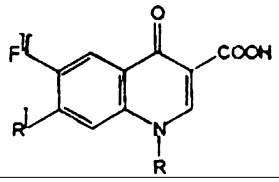

Fluoroquinolones	R	R^1
Ciprofloxacin {1-cyclopropyl-6-fluoro -1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carbocylic acid)}	1-cyclopropyl	1-piperazinyl
Norfloxain {1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid}	1-ethyl	1-piperazinyl
Pefloxacin {1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)-3-quinoline carboxylic acid}	1-ethyl	4-methyl-1-piperazinyl
Enrofloxacin {1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-{4-ethyl-1-piperazinyl)-3-quinoline carboxylic acid}	1-cyclopropyl	4-ethyl-1-piperazinyl
Sparfloxacin {5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-7-(cis-3,5-dimethyl-1-piperazinyl)-3-quinoline carboxylic acid}	5-amino-1-cyclopropyl	3,5-dimethyl-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

Fig. 1. General molecular structure of fluoroquinolones

become one of the most useful tools for qualitative characterization of materials in the pharmaceutical industry.

There have been a number of applications of XRD analysis to organic materials. Among the many compounds that have been characterized by powder XRD methods are solid aromatic hydrocarbons (4, 5). Recently, X-ray powder diffraction has been also found useful in elucidating the structures and in identification of natural products (6), analgesics (7), amines (8, 9) and antibiotics (10, 11). Also, it has become one of the most powerful tools in the forensic analysis (12, 13).

The six fluoroquinolone antibiotic drugs, ciprofloxacin, norfloxacin, enrofloxacin, ofloxacin, pefloxacin and sparfloxacin, are covered by the Martindale Extra Pharmacopoeia (14). Ciprofloxacin, norfloxacin, ofloxacin and sparfloxacin and their formulations are included in USP XXIV (15). Ciprofloxacin and norfloxacin and their formulations are included in BP (16) and IP (17) as well. Powder XRD method has not been suggested in the USP, BP and IP for identification of fluoroquinolone antibiotic drugs, but infrared absorption, thin layer chromatography, high performance liquid chromatography and polarographic methods are suggested.

A review of the literature reveals that no considerable attention has been paid to the powder XRD in identification and determination of fluoroquinolone antibiotics. However, some literature reports deal with crystal structures of ciprofloxacin, norfloxacin and pefloxacin (18-20). The highlight of this work was to provide X-ray powder diffraction data for the six above mentioned fluoroquinolone antibiotic drugs.

EXPERIMENTAL

Samples

Pure samples of ciprofloxacin ($C_{17}H_{18}FN_3O_3$), norfloxacin ($C_{16}H_{18}FN_3O_3$), ofloxacin ($C_{18}H_{20}FN_3O_4$), enrofloxacin ($C_{19}H_{22}FN_3O_3$), pefloxacin ($C_{17}H_{20}FN_3O_3$) and sparfloxacin ($C_{19}H_{22}F_2N_4O_3$) were provided as gift samples by Dr. Reddy's Research Foundation, Hyderabad, India. The drugs were of 99.8% to 98.0% purity.

Apparatus

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. Each sample was scanned from 10° to 70° (2θ) and in step sizes of 0.020, count time of 2.00 seconds, 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

A powdered specimen is usually packed and prepared in a specimen holder made of aluminum or glass. In setting up the specimen and apparatus, coplanarity 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 (21). 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.

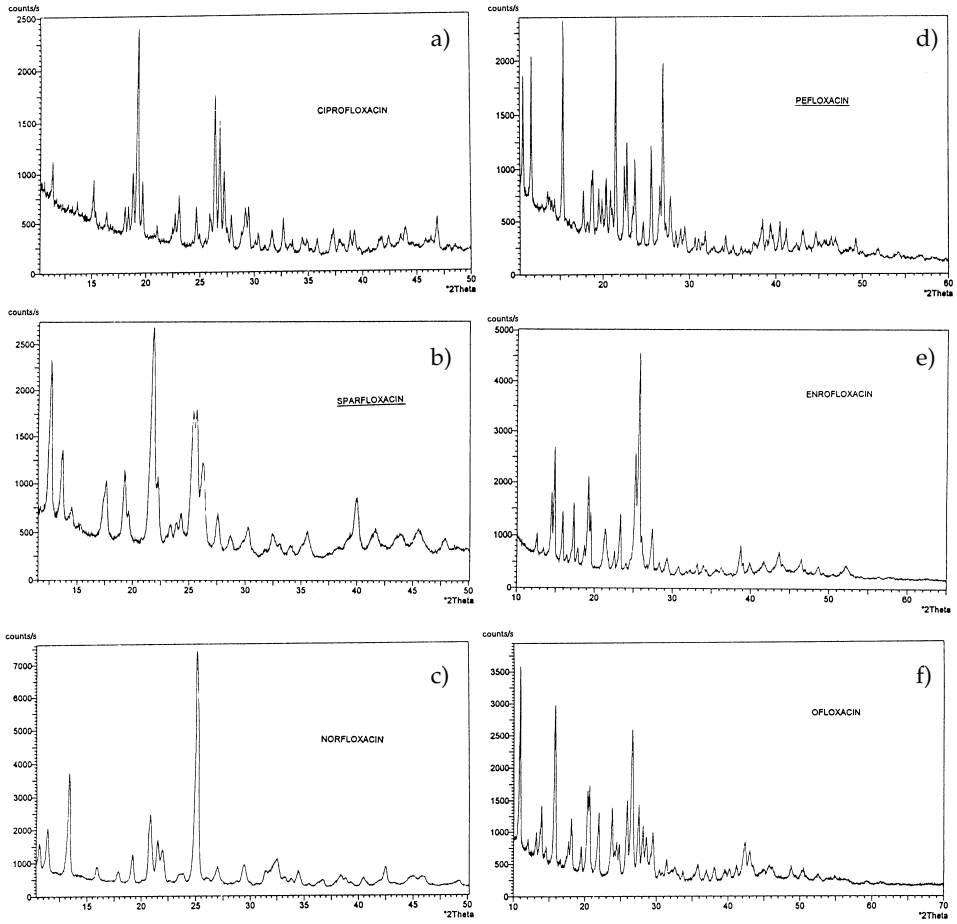

Fig. 2. X-ray diffraction patterns for a) ciprofloxacin, b) norfloxacin, c) pefloxacin, d) enrofloxacin, e) sparfloxacin and f) ofloxacin.

RESULTS AND DISCUSSION

Table I gives the data obtained for the six fluoroquinolone antibiotic drugs 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. The powder

Table I. X-ray diffraction data in terms of lattice spacing and relative intensities for certain fluoroquinolone antibiotic drugs

Ciprofloxacin		Norfloxacin		Enrofloxacin		Ofloxacin		Pefloxacin		Sparfloxacin	
Å	(I/I_I)	Å	(I/I_I)	Å	(I/I_I)	Å	(I/I_I)	Å	(I/I_I)	Å	(I/I_I)
7.80	18.73	8.32	19.14	7.00	8.87	8.09	100.00	8.23	77.32	8.70	29.59
6.47	7.08	8.25	17.91	6.56	2.15	7.35	21.38	7.81	30.02	7.00	84.35
5.82	21.24	7.75	25.55	6.10	29.62	6.71	24.79	7.55	81.43	6.45	46.41
5.40	8.42	6.60	44.52	5.94	49.54	6.34	36.48	6.85	24.27	6.08	21.26
4.89	13.67	6.07	4.83	5.56	21.36	6.07	16.85	6.51	30.08	5.77	15.39
4.81	13.41	5.58	8.19	5.37	2.28	5.58	72.71	6.39	26.94	5.02	33.18
4.70	31.80	5.54	8.16	5.10	25.12	5.34	11.94	6.28	26.31	4.59	36.67
4.60	100.00	4.94	6.93	4.97	6.37	4.99	20.49	6.16	25.42	4.51	19.65
4.49	28.19	4.61	13.60	4.95	6.64	4.89	31.48	5.74	97.00	4.08	100.00
4.21	6.46	4.42	2.92	4.73	8.95	4.56	17.24	5.49	18.44	3.99	33.26
3.95	7.37	4.29	23.65	4.61	41.87	4.35	44.00	5.33	16.53	3.98	30.21
3.90	14.55	4.25	31.10	4.54	21.43	4.29	44.10	4.98	29.87	3.80	14.90
3.84	22.71	4.13	19.50	4.18	12.97	4.04	33.94	4.86	16.63	3.72	16.01
3.60	20.10	4.03	15.36	4.15	18.69	3.84	6.58	4.74	33.82	3.66	19.70
3.56	6.31	3.93	2.58	4.13	16.85	3.73	34.26	4.70	39.20	3.51	62.18
3.49	3.52	3.79	4.91	4.06	4.50	3.63	19.31	4.53	30.62	3.46	59.77
3.43	16.79	3.72	5.62	3.93	8.67	3.58	18.03	4.45	26.37	3.39	40.85
3.36	75.05	3.53	100.00	3.80	23.94	3.43	37.83	4.34	33.38	3.23	20.02
3.31	62.70	3.43	3.62	3.69	3.35	3.34	69.45	4.24	30.07	3.11	10.67
3.26	38.75	3.30	8.59	3.61	5.12	3.24	36.65	4.20	21.27	2.95	13.91
3.23	8.55	3.10	2.29	3.52	54.44	3.17	26.36	4.12	100.00	2.81	4.69
3.19	16.68	3.02	8.92	3.45	100.00	3.12	21.82	3.94	39.85	2.76	11.31
3.09	7.50	2.84	6.79	3.42	16.60	3.02	24.61	3.89	49.79	2.70	7.54
3.05	19.69			3.33	3.29	2.94	6.74	3.76	22.17	2.63	6.69
3.02	19.80			3.25	20.10	2.89	5.84	3.74	40.21	2.52	12.15
2.97	4.75			3.14	5.39	2.84	12.20	3.60	16.01	2.35	5.71
2.94	7.21			3.04	6.89	2.74	8.60	3.47	46.46	2.30	8.59
2.88	1.45			2.89	3.82	2.66	6.45	3.34	32.16	2.25	24.99
2.82	10.00			2.81	1.39	2.50	9.10	3.30	79.88	2.17	13.19
2.73	16.04			2.76	2.17	2.43	7.49	3.26	15.98	2.08	9.67

Table I. continued

Ciprofloxacin		Norfloxacin		Enrofloxacin		Ofloxacin		Pefloxacin		Sparfloxacin	
Å	(I/I_I)	Å	(I/I_I)	Å	(I/I_I)	Å	(I/I_I)	Å	(I/I_I)	Å	(I/I_I)
2.67	6.19			2.69	5.22	2.36	7.86	3.20	27.13	2.07	11.56
2.60	7.51			2.63	4.28	2.28	6.85	3.13	13.07	1.99	12.46
2.57	5.50			2.60	2.48	2.24	7.31	3.07	13.38	1.90	9.58
2.50	6.85			2.52	3.20	2.19	8.64	3.03	14.04	1.86	5.90
2.41	12.85			2.48	4.02	2.13	20.04	2.91	10.06	1.83	5.48
2.38	7.47			2.40	1.04	2.10	15.55	2.88	9.75	1.76	4.35
2.35	4.59			2.32	12.83	2.08	8.91	2.86	6.84	1.73	3.72
2.32	11.04			2.30	6.48	2.01	6.99	2.81	12.83	1.69	2.04
2.29	11.35			2.26	6.11	1.98	8.52	2.72	6.54	1.66	3.03
2.27	2.74			2.16	6.93	1.96	7.98	2.65	6.84	1.64	2.85
2.23	2.69			2.07	11.33	1.86	9.41	2.62	10.34	1.49	0.32
2.17	6.57			1.95	7.99	1.80	7.24	2.55	7.14	1.45	0.70
2.16	9.18			1.93	3.57	1.74	4.97	2.49	6.40		
2,13	9.32			1.90	2.36	1.71	2.99	2.46	4.91		
2.08	10.22			1.86	5.62	1.67	3.79	2.43	4.99		
2.05	13.72			1.84	2.22	1.63	2.46	2.40	8.03		
1.98	7.67			1.80	1.71	1.56	1.65	2.34	17.94		
1.96	9.80			1.75	5.91	1.51	1.06	2.31	9.26		
1.94	18.54			1.69	0.96	1.46	0.49	2.29	15.93		
1.90	4.41			1.63	0.88	1.38	0.32	2.27	11.54		
1.88	5.26			1.59	0.85			2.22	15.98		
1.81	3.60			1.58	0.91			2.19	13.46		
1.78	2.67			1.54	0.37			2.13	7.40		
1.74	2.04			1.48	0.52			2.09	10.50		
1.71	3.16							2.06	6.71		
1.67	1.59							2.03	12.79		
1.65	0.83							2.01	8.24		
1.63	1.92							1.99	8.73		
1.61	1.68							1.95	11.22		
1.59	1.80							1.94	9.77		
1.49	1.27							1.87	5.69		
1.35	1.75							1.85	9.54		
								1.82	3.87		
								1.76	5.10		
								1.69	3.76		
								1.68	2.22		
								1.59	1.82		
								1.53	1.51		
								1.49	2.19		
								1.43	1.31		
								1.41	1.02		

X-ray diffraction patterns for these drugs are given in Fig. 2. All the high intensity lines (relative intensity) observed in the powder pattern of the fluoroquinolone drugs were observed in the pure form (Table I). The diffraction patterns of low absorption drugs measured in para-focusing Bragg-Brentano geometry exhibit severe line broadening. Identification of a structure from its powdered diffraction pattern is based upon the position of lines and their relative intensities. Each powder pattern is characterized by the interplanar d spacing and the relative intensities (I/I_I) of the three strongest lines in the pattern under the Hanawalt system.

Nevertheless, complete diffraction patterns are still characteristic as can be seen from Table I. Examination of the X-ray data shows that a compound can be easily distinguished from other members. Despite the fact that the six fluoroquinolones have almost the same molecular structure, their powder diffraction patterns are suprising different (Fig. 2). In general, there are only three or four of the most intense lines that are important for the characterization of the compound. Since the goal of the project 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. However, the relative intensities of the lines are also probable.

XRD has the potential ability to identify not only the active ingredient, but also the crystalline excipients in a formulation. The fluoroquinolone antibiotic drugs 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 fluoroquinolone antibiotic drugs. Our current findings provide preliminary data which make the basis for validation of the new method.

CONCLUSIONS

The powder XRD patterns for all fluoroquinolone antibiotics studied were sufficiently unique to make their identification possible.

The current preliminary studies make the basis for validation of powder XRD as a new monitoring official method for identification of fluoroquinolone antibiotic drugs.

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S A Ž E T A K

Difrakcija rentgenskim zračenjem nekih fluorokinolskih antibiotika

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Difraktometrom za praškaste tvari provedena je difrakcija rentgenskim zračenjem šest fluorokinolonskih antibiotika (ciprofloksacin, norfloksacin, enrofloksacin, ofloksacin, pefloksacin i sparfloksacin). Ispitivani uzorci su pretraživani pod Braggovim kutom (2θ)

od 10° do 70°. Određeni su razmaci između kristalnih rešetki (Å) i relativni linijski intenziteti (I/I_I). Dobiveni podaci mogli bi biti korisni za indentifikaciju fluorokinolonskih antibiotika.

Ključne riječi: fluorokinolonski antibiotici, identifikacija, difrakcija rentgenskim zračenjem, prašak

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