INFRARED SPECTRA AND THE STRUCTURE OF DRUGS OF THE FLUOROQUINOLONE GROUP

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Drugs of the fluoroquinolone group are antimicrobial agents with a broad spectrum of activity. While extensive literature is available about the pharmacological properties and clinical use of these drugs, information concerning the physicochemical properties (in particular, spectral characteristics) of these compounds is insufficient [1 – 7].

IR absorption spectroscopy is widely used in pharmaceutical analysis for the identification of drugs. The use of standard spectra instead of reference samples allows this identification procedure to be considerably simplified and the cost of analysis to be significantly reduced.

This study was aimed at the accumulation and interpretation of the IR absorption spectra of the parent substances of drugs belonging to the fluoroquinolone group. These data are necessary for successful use of IR spectroscopy as a means of monitoring the quality of such drugs.

MATERIALS AND METHODS

Objects of investigation. The series of parent substances studied represented nine drugs belonging to the group of fluoroquinolones:

(1) Norfloxacin: reference sample (KRKA, Slovenia);

(2) Pefloxacin methanesulfonate (mesylate) dihydrate: working standard (Dr. Reddy's Laboratories Ltd., India);

(3) Ciprofloxacin hydrochloride monohydrate: parent substance (Ranbaxy Laboratories Ltd., India);

(4) Ciprofloxacin: reference sample, 99.8% (Bayer AG, Germany);

(5) Moxifloxacin hydrochloride: reference sample, 96.1% (Bayer AG, Germany);

(6) Ofloxacin: working standard, 99.5% (Aventis Pharma Ltd., France);

(7) Levofloxacin hemihydrate: working standard (Aventis Pharma Ltd., France);

(8) Lomefloxacin hydrochloride: parent substance (Searle, France);

(9) Sparfloxacin: working standard (Dr. Reddy's Laboratories Ltd., India);

It should be noted that, in the discussion of results, the term "fluoroquinolone base" refers to parent substances in the state other than salt. Of course, this concept is rather conditional, since all fluoroquinolones are ampholytes.

Sample preparation. The samples for spectroscopic measurements were prepared so as to meet all requirements of the State Pharmacopoeia (RSP XI, Vol. 1, p. 37). In accordance with this, a sample containing 15 mg of each parent substance or reference compound was triturated in an agate mortar with 1 – 2 drops of Vaseline oil (special grade for IR spectroscopy). The obtained suspension was placed between two KBr plates and this sample was used to measure the IR spectrum.

Measurement conditions. The IR spectra were measured using a computer-controlled single-beam interference IR spectrophotometer with the inverse Fourier transform, Infralum FT-02 (Lumex company, Russia). The spectra were measured in a 4000 – 400 cm^{-1} range of wavenumbers at a resolution of 1 cm^{-1}. The spectra were accumulated in a cyclic mode (20 scans; standard apodization regime).

The background spectrum (air) was recorded immediately before measuring each spectrum of a drug sample. The instrument operation and data processing were controlled by a Spectralum routine for Windows (Lumex company, Russia) and an ACD/SpecViewer (Freeware Version) program package (Advanced Chemistry Development, Canada). The obtained spectra were interpreted using published data [8 – 11] and an IR-Wizard program package (Institute of Chemistry, University of Potsdam) available online (http://www.chem.uni-potsdam.de/tools).

RESULTS AND DFISCUSSION

The structural formulas of compounds studied are presented in Table 1. Figure 1 shows the typical IR spectrum of ofloxacin. The assignment of absorption bands in the IR spectra of fluoroquinolones is given in Table 2.

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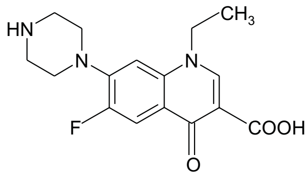
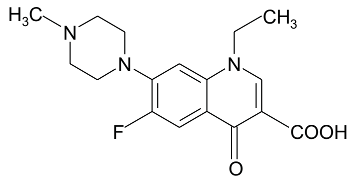
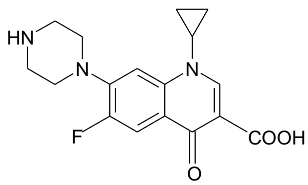
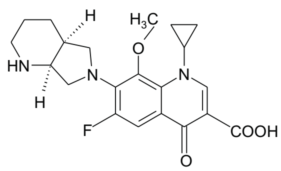
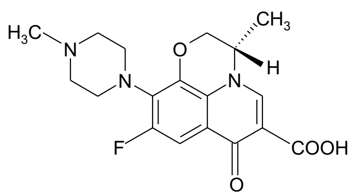
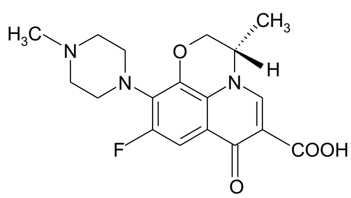
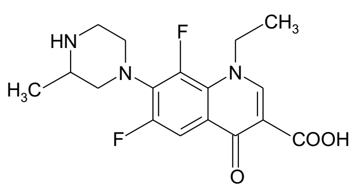
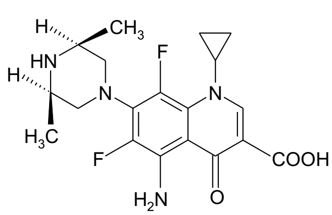
TABLE 1. Structural Formulas of Fluoroquinolones

Fluoroquinolone (INN*)	Structural formula	Chemical name and remarks
Norfloxacin		4-Oxo-7-(1-piperazinyl)-6-fluoro-1-ethyl-1,4-dihydroquinoline-3-carboxylic acid; parent substance: norfloxacin
Pefloxacin		7-(4-Methyl-1-piperazinyl)-4-oxo-6-fluoro-1-ethyl-1,4-dihydroquinolin-3-carboxylic acid; parent substance: pefloxacin methanesulfonate (mesylate) dihydrate
Ciprofloxacin		4-Oxo-7-(1-piperazinyl)-6-fluoro-1-cyclopropyl-1,4-dihydroquinoline-3-carboxylic acid; parent substance: ciprofloxacin hydrochloride monohydrate
Moxifloxacin		7-[(*S*,*S*)-2, 8-Diazabicyclo[4.3.0]non-8-yl]-8-methoxy-4-oxo-6-fluoro-1-cyclopropyl-1,4-dihydroquinoline-3-carboxylic acid; parent substance: moxifloxacin hydrochloride
Ofloxacin		(±)-3-Methyl-10-(4-methyl-1-piperazinyl)-7-oxo-9-fluoro-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid; parent substance: ofloxacin
Levofloxacin		(–)-3-Methyl-10-(4-methyl-1-piperazinyl)-7-oxo-9-fluoro-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid; parent substance: levofloxacin hemihydrate
Lomefloxacin		6,8-Difluoro-7-(3-methyl-1-piperazinyl)-4-oxo-1-ethyl-1,4-dihydroquinoline-3-carboxylic acid; parent substance: lomefloxacin hydrochloride
Sparfloxacin		5-Amino-7-(3,5-dimethyl-1-piperazinyl)-6,8-difluoro-4-oxo-1-cyclopropyl-1,4-dihydroquinoline-3-carboxylic acid; parent substance: sparfloxacin

* International nonpatented name.

TABLE 2. Assignment of Absorption Bands in the IR Spectra of Fluoroquinolones (cm^{-1})

Compound*	$\nu_{C=O}$ in COOH	$\nu_{C=O}$	$\nu_{as}(COO^-)/\nu_s(COO^-)$	$\nu_{as}(S=O)/\nu_s(S=O)$ in SO_3^-
Norfloxacin	1731	1628	—	—
Pefloxacin mesylate	1711	1629	—	1175/1055
Ciprofloxacin · HCl	1709	1624	—	—
Ciprofloxacin	—	1618	1591/1377	—
Moxifloxacin · HCl	1709	1625	—	—
Ofloxacin	1714	1623	—	
Levofloxacin	1726	1621	—	—
Lomefloxacin · HCl	1725	1625	—	—
Sparfloxacin	1717	1644	—	—

Compound*	$\nu_{C\text{-}H}$ in Ar nucleus and/or in $R_1R_2 = CHR_3$	$\nu_{C\text{-}H}$ in $N–CH_3$ and/or in R-O-Ar	$\delta_{C\text{-}H}$ in Ar nucleus	$\delta_{C\text{-}H}$ in $R_1R_2 = CHR_3$
Norfloxacin	3048	—	887	806
Pefloxacin mesylate	3048/3026	2729b [overlap of $\nu_{(NH^+)}$ and $\nu_{C–H}$ in $N–CH_3$]	890	806
Ciprofloxacin · HCl	3100 – 3011	—	890	805
Ciprofloxacin	3146 – 3014	—	868	—
Moxifloxacin · HCl	3068	$\nu_{C–H}$ in R–O–Ar masked by $\nu(NH_2^+)$	876	804
Ofloxacin	3041	2797 – 2687	879	804
Levofloxacin	3082	2804 – 2691	874	802
Lomefloxacin · HCl	3055	—	885	808
Sparfloxacin	3031	—	—	809

Compound*	$\nu_{N–H}$	$\nu_{as(N–H)}/\nu_{s(N–H)}$ in NH_2	$\nu(NH_2^+)$ or $\nu(NH^+)$	$\nu_{C=C}$ in Ar nucleus
Norfloxacin	3600 – 3200	—	—	1618/1582/1552/1523 (and $\delta_{N–H}$)
Pefloxacin mesylate	—	—	2729b [overlap of $\nu_{(NH^+)}$ and $\nu_{C–H}$ in $N–CH_3$]	1552/1518/1489
Ciprofloxacin · HCl	—	—	2765 – 2461	1611/1553/1515/1495 (and $\delta(NH_2^+)$)
Ciprofloxacin	3600 – 3200	—	—	1544/1500/1485 (and $\delta_{N–H}$)
Moxifloxacin · HCl	—	—	2800 – 2427 (overlap with $\nu_{C–H}$ in R–O–Ar)	1590/1580/1519 (and $\delta(NH_2^+)$)
Ofloxacin	—	—	—	1550/1523
Levofloxacin	—	—	—	1541/1519/1495
Lomefloxacin · HCl	—	—	2796 – 2460	1614/1559/1545/1526/1494 (and $\delta(NH_2^+)$)
Sparfloxacin	not manifested	3464/3340	—	1587/1559/1534/1516/1495 (and $\delta_{N–H}$)

* HCl = hydrochloride.

In the IR spectra of all samples, the bands at 2955, 2924, and 1855 cm^{-1} correspond to the $\nu_{C–H}$ stretching vibrations, and the bands at 1462, 1378, and 722 cm^{-1} correspond to the $\delta_{C–H}$ bending vibrations in Vaseline oil, while the bands at 2361, 2346, 2334, and 666 cm^{-1} belong to the $\nu_{C–O}$ stretching vibrations of carbon dioxide molecules present in the atmosphere.

In the IR spectra of fluoroquinolones, a very strong band in the region of 1731 – 1709 cm^{-1} corresponds to the stretching vibrations of carboxy carbonyls ($\nu_{C=O}$ in COOH). The decrease in the $\nu_{C=O}$ wavenumber in COOH can be related to the formation of dimers and/or to the participation of carboxy groups in complex conjugated systems. However, analysis based on IR data only cannot unambiguously establish whether the fluoroquinolone molecules form dimers.

Another very strong absorption band, which is observed in the region of 1644 – 1618 cm^{-1}, corresponds to the stretching vibrations of carbonyls ($\nu_{C=O}$) in position 4. The strong shift of this band toward lower frequencies is explained by the participation of the oxo group in a complex conjugated system and by the influence of an intramolecular hydrogen bond formed between carboxy and carbonyl groups.

Medium or weak bands observed in the IR spectra of fluoroquinolones at 809 – 802 cm^{-1} correspond to the $\delta_{C–H}$ bending vibrations characteristic of alkenes with the general formula R_1R_2=CHR_3 [10].

In the IR spectrum of ofloxacin, the bands characteristic of $\nu_{C=O}$ in COOH and $\delta_{C–H}$ in alkenes are missing, which is explained by ionization of the carboxy groups with the for-

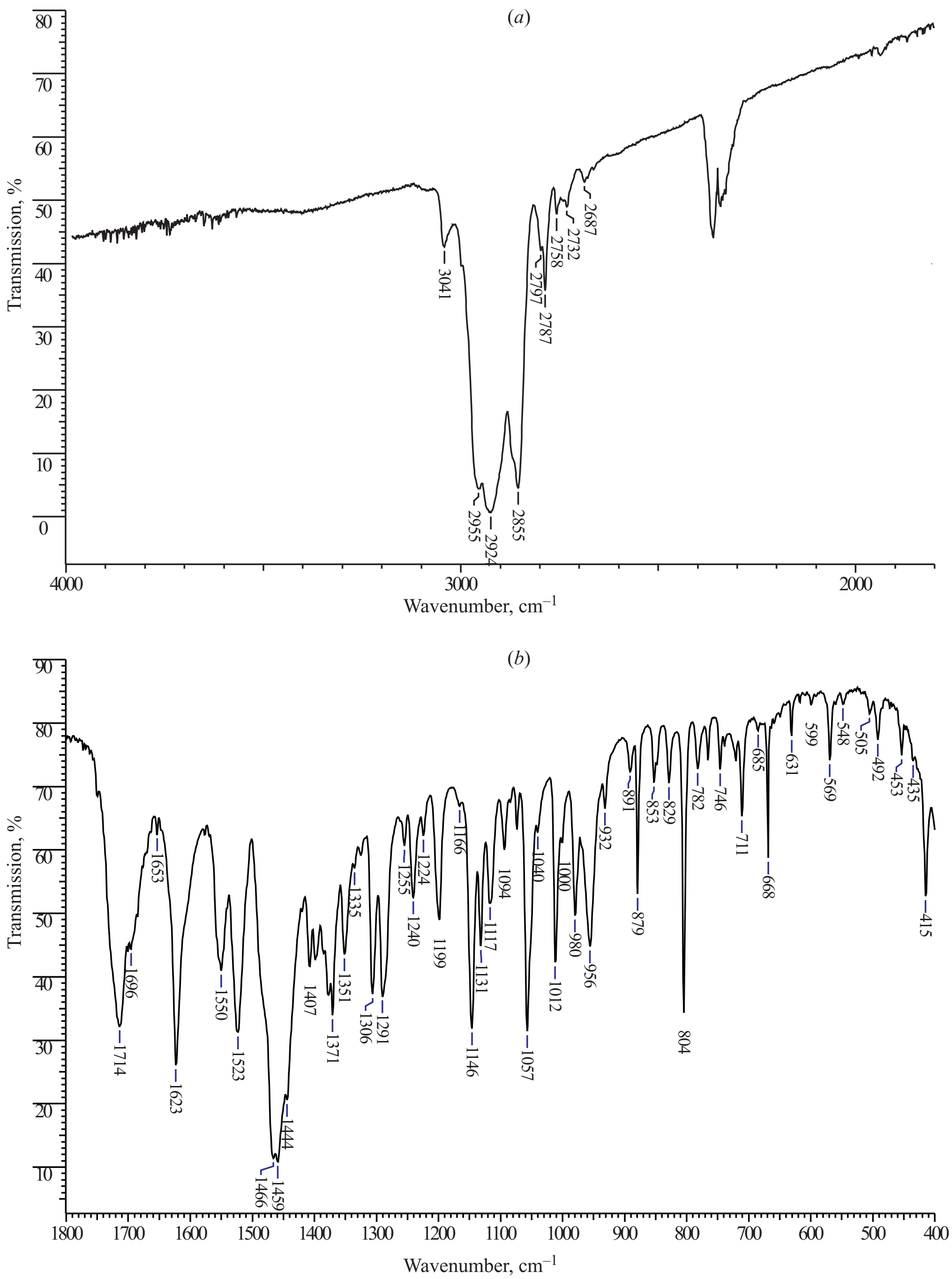


Fig. 1. The IR absorption spectrum of ofloxacin suspended in Vaseline oil: (*a*) 4000 – 1800 cm^{-1}; (*b*) 1800 – 400 cm^{-1}.

mation of a resonance betainelike structure. Carboxylate anions account for two intense bands in the spectrum of ofloxacin base: antisymmetric $\nu_{as(COO^-)}$ at 1591 cm^{-1} and symmetric $\nu_{s(COO^-)}$ at 1377 cm^{-1} (the latter band overlaps with the absorption band of Vaseline oil).

The presence of hydrogen atoms at a double bond in the aromatic nucleus is evidenced by a set of low-intensity $\nu_{C=H}$ bands in the region of 3100 – 3000 cm^{-1} (on the tail of the $\nu_{C=H}$ band of Vaseline oil). Some bands correspond to the nonplanar $\delta_{C=H}$ vibrations of aromatic hydrogen atoms. However, the molecule of sparfloxacin contains no such atoms and, accordingly, these bands are missing from the spectrum of this compound.

In the spectra of all fluoroquinolones prepared in the hydrochloride form, the bands in the range from 2800 to 2400 cm^{-1} correspond to the $\nu_{(NH_2^+)}$ stretching vibrations of protonated secondary aliphatic nitrogen atoms. The protonated tertiary nitrogen atoms also account for a set of absorption bands in this region. Pefloxacin mesylate is characterized by a broad band with a maximum approximately at 2729 cm^{-1}, which probably results from the superposition of bands related to the $\nu_{(NH^+)}$ and ν_{C-H} stretching vibrations in $N-CH_3$ groups.

The band corresponding to ν_{N-H} of a nonprotonated secondary aliphatic amino group is usually found in the vicinity of 3400 cm^{-1}. These vibrations are not manifested in the spectrum of sparfloxacin. The spectra of ciprofloxacin base and norfloxacin exhibit a broad band of relatively small intensity with a maximum about 3400 cm^{-1}, which is probably due to these vibrations. The primary aromatic amino group in the molecule of sparfloxacin accounts for the two bands: $\nu_{as(N-H)}$ at 3464 cm^{-1} and $\nu_{s(N-H)}$ at 3340 cm^{-1}.

The set of medium and weak bands at 2804 – 2688 cm^{-1} in the IR spectra of ofloxacin and levofloxacin correspond to the ν_{C-H} stretching vibrations of a methyl radical at the tertiary aliphatic nitrogen atom in the piperazinyl moiety and/or to the ν_{C-H} vibrations of methylene groups in R–O–Ar. In the IR spectrum of moxifloxacin hydrochloride, the ν_{C-H} bands of R–O–Ar are superimposed with the $\nu_{(NH_2^+)}$ bands related to the presence of protonated secondary aliphatic nitrogen atoms.

The IR spectrum of pefloxacin mesylate contains intense absorption bands at 1175 and 1055 cm^{-1}, which correspond to $\nu_{as(S=O)}$ $\nu_{s(S=O)}$ of the ionized sulfo groups of the methanesulfonic acid moiety. In the region of 1618 – 1485 cm^{-1}, this spectrum shows bands corresponding to $\nu_{C=C}$ of the aromatic nucleus, δ_{N-H} of piperazinyl radicals (protonated and nonprotonated), and δ_{N-H} of the primary aromatic amino groups.

In the IR spectra of ciprofloxacin hydrochloride, pefloxacin mesylate, moxifloxacin hydrochloride, and levofloxacin hemihydrate, the bands at 3550 – 3200 cm^{-1} either reflect the formation of intra- and intermolecular hydrogen bonds involving carboxy groups or represent overtones of the fundamental vibrations of some other functional groups (e.g., carbonyl in position 4). In the spectra of ciprofloxacin hydrochloride and moxifloxacin hydrochloride, the bands at 3526 and 3528 cm^{-1}, respectively, can be assigned to ν_{O-H} in COOH (monomer).

In the IR spectrum of ofloxacin, the $\nu_{C=O}$ band of carboxy carbonyl groups exhibits splitting, which can be explained by the Fermi resonance between this vibration and, probably, the $\nu_{C=C}$ band of the alkene moiety. The spectra of other fluoroquinolones studied exhibit no bands due to alkene $\nu_{C=C}$ vibrations. The spectra of ciprofloxacin hydrochloride, norfloxacin, and lomefloxacin hydrochloride also exhibit splitting of the $\nu_{C=O}$ bands of carbonyl groups in position 4, probably due to the Fermi resonance. In these cases, this can be due to the interaction between $\nu_{C=O}$ of the keto group and $\nu_{C=C}$ of the aromatic nucleus or δ_{N-H} ($\delta_{(NH_2^+)}$) of the piperazinyl radical.

The ν_{C-F} bands of the aromatic nucleus fall within the region of 1270 – 1100 cm^{-1}. These bands possess high or medium intensities and usually cannot be assigned because they overlap with the $\nu_{C=C}$ bands [11]. Moreover, in the case of fluoroquinolones, this region may also contain the bands of high and medium intensity due to the following vibrations: ν_{C-O} in COOH (1320 – 1210 cm^{-1} for dimer and 1190 – 1075 cm^{-1} for monomer); δ_{O-H} in COOH (1440 – 1395 cm^{-1} for dimer and 1380 – 1280 cm^{-1} for monomer); $\nu_{as(C-O-C)}$ (1275 – 1200 cm^{-1}) and $\nu_{s(C-O-C)}$ (1075 – 1020 cm^{-1}) in R–O–Ar; and ν_{C-N} (1350 – 1000 cm^{-1}). An analysis of the IR spectra of fluoroquinolones show that strong and medium absorption bands are not always present in the 1400 – 1100 cm^{-1} range, although in some cases there are several intense bands in this range. Taking into account this ambiguity, the bands due to ν_{C-O}, δ_{O-H}, $\nu_{as(C-O-C)}$, $\nu_{s(C-O-C)}$, and $\nu_{C=F}$ observed in this range cannot be reliably assigned.

As is known, the IR spectra of enantiomers are identical, but the spectra of a racemic mixture in the solid state may differ from the spectra of separate enantiomers [8]. In the case of fluoroquinolones, this behavior is characteristic of ofloxacin (racemate) and levofloxacin (left-hand isomer), which exhibit a number of differences. However, the main characteristic bands in the spectra of both ofloxacin and levofloxacin are retained.

The total collection of the IR spectra of fluoroquinolones is presented in monograph [12]. These IR spectra can be used as reference for the identification of parent compounds within the framework of the pharmacopoeial analysis.

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