Research Article
ISSN: 0974-6943

Raman spectroscopy as an analytical tool for characterization of fluoroquinolones

Subhashree Sahoo[1]*, Chandra Kanti Chakraborti[1], Subash Chandra Mishra[2], Upendra Nath Nanda[3], Sharmistha Naik[1]
[1]*Department of Pharmaceutics, Kanak Manjari Institute of Pharmaceutical Sciences, Rourkela ,769015,Orissa, India.*
[2]*Metallurgical and Materials Engg. Dept., National Institute of Technology, Rourkela 769 008,Orissa,India.*
[3]*Department of Pharmaceutics, Seemanta Institute of Pharmaceutical Sciences, Jharpokharia ,757086,Orissa,India.*

Received on: 05-12-2010; Revised on: 14-01-2011; Accepted on:09-03-2011

ABSTRACT

The aim of this paper is to produce the basis for the Raman spectroscopy as a promising new official method for identification of fluoroquinolone antibacterials. Raman spectroscopic analysis data for three pure fluoroquinolone antibacterials like Norfloxacin, Ciprofloxacin and Ofloxacin, have been performed by a low resolution portable Raman Spectrometer (Raman system R-3000) using 785 nm solid state diode laser and having fibre optic sampling probe. The Raman spectra are collected over the wave number range from 140 to 2500 cm^{-1} at room temperature. From our overall Raman spectroscopic analysis, it has been found that those fluoroquinolones show prominent characteristic peaks for pyridone nucleus, piperazinyl ring, carboxylic acid and fluorine substituent. In addition, the characteristic peaks at 872.7, 771.47 and 797.5 cm^{-1} for Norfloxacin, Ciprofloxacin and Ofloxacin, respectively, are assigned to fully symmetric vibrational normal mode of the molecule containing the C-F bond. Since the important characteristic groups are available in all the cases, their Raman spectral pattern is more or less similar. From the above-mentioned information, it can be concluded that Raman spectroscopic analysis, a non-destructive method with minimal water interference, may also be used as a useful tool for the identification of compounds, specially the fluoroquinolones.

Key words: Norfloxacin, Ciprofloxacin, Ofloxacin, Raman spectroscopy

INTRODUCTION

Fluoroquinolones are widely used synthetic broad-spectrum antibacterial agents. They form a group of antimicrobial agents with different chemical structures and spectra of activity. The general molecular structure of fluoroquinolones like Norfloxacin, Ciprofloxacin and Ofloxacin consists of 1-substituted-1,4-dihydro-4-oxopyridine-3-carboxylic moiety combined with an aromatic or heteroaromatic ring (Fig. 1). The benzopyridone nucleus (quinolone) proved to be more responsive to chemical manipulation in order to enhance antibacterial potency. The subsequent discovery of fluorine atom and piperazinyl ring on the quinolone nucleus revolutionized the chemistry and clinical importance of fluoroquinolones [1,2].

While Raman spectroscopy has long been recognized as a valuable research technique, only very recently this technique has emerged as an important analytical tool across a number of industries and applications. Due to its sensitivity, high information content, and non-destructive nature, Raman spectroscopy is now used in many applications across the fields of chemistry, biology, geology, pharmacology, forensics, pharmaceuticals, materials science, and failure analysis. Moreover, FTIR and Raman spectroscopy are used as complementary techniques, because each method looks at different aspects of a given sample [3]. Since dispersive Raman spectroscopy operates at visible wavelengths, its signal is much stronger. In addition, the technique is more sensitive and it delivers higher spatial resolution. This makes dispersive Raman as the best choice for small particles and minor component analysis [4].

It has also been found that Raman spectroscopy can identify fluoroorganic compounds in liquid, gaseous, crystalline or amorphous solid states. The method is more sensitive than other methods for detecting the C-F bond of fluoroorganic compounds, permitting detection of fluoroorganic compounds at 10^{-3}-10^{-6} g/L concentrations level. Raman emission for C-F bond is centered on 500-800 cm^{-1}, which is characteristic for fluoroorganic compounds. On the other hand, the frequency range for C-F bond, FTIR absorption is from 1000 cm^{-1} to 1400 cm^{-1}. But these FTIR absorptions are subject to interference by other functional groups present in respect to fluoroorganic compounds. On the other hand, in case of Raman spectroscopy, the C-F bond vibration assigned as follows: 710-785 cm^{-1} for trifluoromethyl groups; 726 cm^{-1} for 1-bromoperfluorooctane; 730 cm^{-1} for perfluorodecanoic acid; 750 cm^{-1} for triperfluoropropylamine; and 730 cm^{-1} for 1,3,5-tris-(trifluoromethylbenzene). Most of these compounds have an aromatic ring, so resonance Raman can be observed [5]. As fluoroquinolones are aromatic fluoroorganic compounds having C-F groups as a part of molecular structure, this type of C-F bond can also be identified by Raman spectroscopy.

Till now, Norfloxacin, Ciprofloxacin and Ofloxacin, and their formulations are included in USP XXIV [6]. Moreover, Norfloxacin and Ciprofloxacin, and their formulations are included in BP [7] and IP [8]. However, the Raman spectroscopic analysis 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, polarographic methods, etc. have been recomended. A review of the literature reveals that very little attention has been paid to the Raman spectroscopic analysis for the identification and determination of fluoroquinolone antibacterials. While FTIR is sensitive to functional groups and highly polar bonds, Raman spectroscopy is more sensitive to backbone structures and symmetric bonds.[9] Taking into consideration of above positive aspects of Raman spectroscopic analysis, we have used this as an analytical tool for the identification of fluoroquinolones like Norfloxacin, Ciprofloxacin and Ofloxacin.

***Corresponding author.**
Mrs. Subhashree Sahoo
Department of Pharmaceutics,
Kanak Manjari Institute of Pharmaceutical Sciences,
Rourkela ,769015, Orissa, India
***Tel.:** +91-9861376820*
Fax. +91-661-2480752
***E-mail:** subha77t@yahoo.co.in*

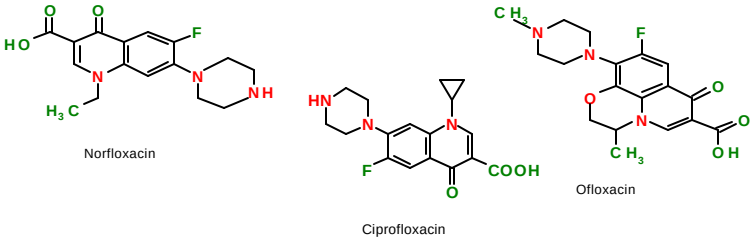

Figure 1. Molecular Structures of some Fluoroquinolones

MATERIALS AND METHODS

Materials:

Pure samples of Norfloxacin (C16H18FN3O3), Ciprofloxacin (C17H18FN3O3) and Ofloxacin (C18H20FN3O4) were provided as gift samples by Dr. Reddy's Research Foundation, Hyderabad, India. The drugs were of 99.8% to 98.0% purity.

Methods:

The Raman system R-3000 instrument, manufactured by Raman systems INC.USA, is a low resolution potable Raman Spectrometer using a 785 nm solid state diode laser adjusted to deliver 250 mw to the sample having spectral resolution 10 $cm^{-1}$and 12 v dc/5A power supplies and USB connectivity. This Raman Spectrometer is having fibre optic sampling probe with safety shutter and automatic focusing caps for both solid and liquid samples. The solid powder samples i.e., pure drugs were enclosed in plastic poly bags and tested directly at room temperature. The interference of the outside light was also prohibited to prevent photon shot noise. The spectra were collected over the wave number range from 140 to 2500 cm^{-1}.

RESULTS

By Raman spectroscopy of Norfloxacin, the prominent Raman shifts have been observed at 485.6, 872.7, 1418.5 and 1655.1 cm^{-1} (Fig. 2). The Raman shifts at 485.6 cm^{-1} indicates strong bending vibration of C-C of the aliphatic chain and C-N stretching vibration of piperazinyl group [10,11,12]. The band at 872.7 cm^{-1} represents the symmetric stretching vibration of C-F group [13]. The peak at 1418.5 cm^{-1} is due to symmetric stretching vibration of O-C-O group of carboxylic acid and methylene deformation mode of the piperazinyl group [14]. A band at 1655.1 cm^{-1} is due to symmetric stretching of the carbonyl group $\upsilon_{C=O}$ of the pyridone moiety, the stretching vibration of (C-C) aromatic ring chain. In addition, it (peak at1655.1 cm^{-1}) also indicates the $N^{+}H_2$ scissoring of piperzinyl group [10,14,15,16,17] (Table 1a).

In case of Ciprofloxacin, the prominent Raman shifts have been observed at 484.22, 771.47, 1411.63 and 1655.11 cm^{-1} (Fig. 3). The Raman shifts at 484.22 cm^{-1} indicates strong bending vibration of C-C of the aliphatic chain of cyclopropyl group and C-N stretching vibration of piperazinyl group [10,11,12]. The band at 771.47 cm^{-1} represents the symmetric stretching vibration of C-F group [5]. The peak at 1411.63 cm^{-1} is due to symmetric stretching vibration of O-C-O group of carboxylic acid and methylene deformation mode of the piperazinyl group [14]. A band at 1655.11 cm^{-1} is due to symmetric stretching of the carbonyl group $\upsilon_{C=O}$ of the pyridone moiety, the stretching vibration of (C-C) aromatic ring chain [18]. In addition, it (peak at1655.11 cm^{-1}) also indicates the $N^{+}H_2$ scissoring of piperzinyl group [10,14,15,16,17,18] (Table 1b).

By Raman spectroscopy of Ofloxacin, the prominent Raman shifts have been observed at 518.4, 797.5, 1419.8 and 1649.6 cm^{-1} (Fig. 4). The Raman shift at 518.4 cm^{-1} represents the bending vibration of aliphatic carbon atom, C-N stretching vibration of piperazinyl group and O-H torsional vibration of carboxylic acid [10,11,12,14]. The band at 797.5 cm^{-1} represents the symmetric stretching vibration of C-F group [5].The peak at 1419.8 cm^{-1} is due to symmetric stretching vibration of O-C-O group of carboxylic acid and methylene deformation mode of the piperazinyl group [14]. A band at 1649.6 cm^{-1} is due to symmetric stretching of the carbonyl group $\upsilon_{C=O}$ of the pyridone moiety, the stretching vibration of (C-C) aromatic ring chain. In addition, it (peak at1649.6cm^{-1}) also indicates the $N^{+}H_2$ scissoring of piperzinyl group [10,14,15,16,17] (Table 1c).

Table 1. Prominent Raman Shifts of Some Fluoroquinolones

a)Prominent Raman Shifts of Norfloxacin

Raman Shifts(cm^{-1})	Functional Groups / Vibrations
485.6	Strong δ(CC) aliphatic chain and C-N stretching vibration
872.7	Symmetric vibration of C-F bond
1418.5	$\upsilon_{s\ O\text{-}C\text{-}O}$ and methylene deformation of the piperazinyl group
1655.1	υ_s of C=O group of pyridone moiety and $N^{+}H_2$ scissoring of piperzinyl group

b) Prominent Raman Shifts of Ciprofloxacin

Raman Shifts(cm^{-1})	Functional Groups / Vibrations
484.22	Strong δ(CC) aliphatic chain and C-N stretching vibration
771.47	Symmetric vibration of C-F bond
1411.63	υ_sO-C-O and methylene deformation of the piperazinyl group
1655.11	υs of C=O group of pyridone moiety and $N^{+}H_2$ scissoring of piperzinyl group

c) Prominent Raman Shifts of Ofloxacin

Raman Shifts(cm^{-1})	Functional Groups / Vibrations
518.4	Strong δ(CC) aliphatic chain, C-N stretching vibration and O-H torsional vibration
797.5	Symmetric vibration of C-F bond
1419.8	υ_s O-C-O and methylene deformation of the piperazinyl group
1649.6	υs of C=O group of pyridone moiety and $N^{+}H_2$ scissoring of piperzinyl group

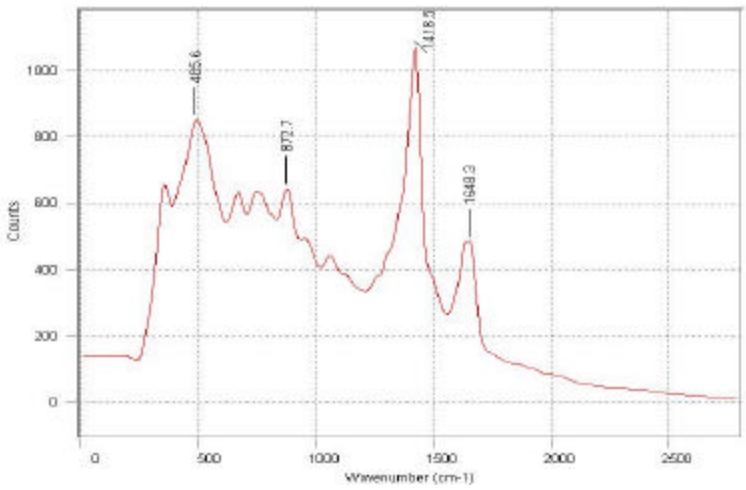

Figure. 2: Raman Shifts of Norfloxacin

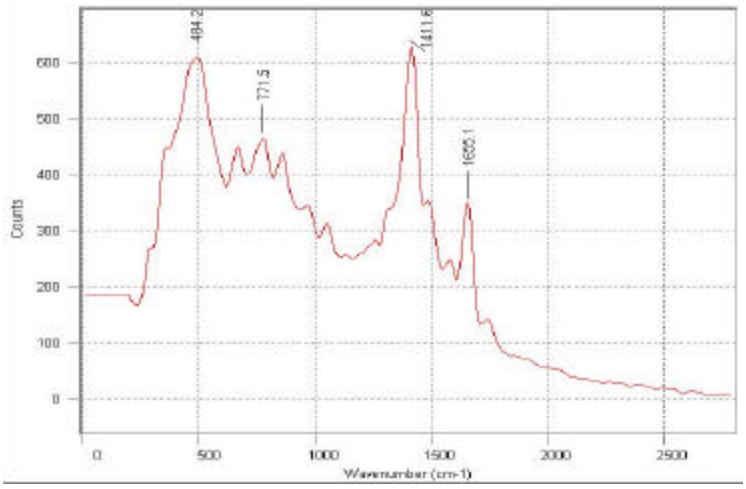

Figure. 3: Raman Shifts of Ciprofloxacin

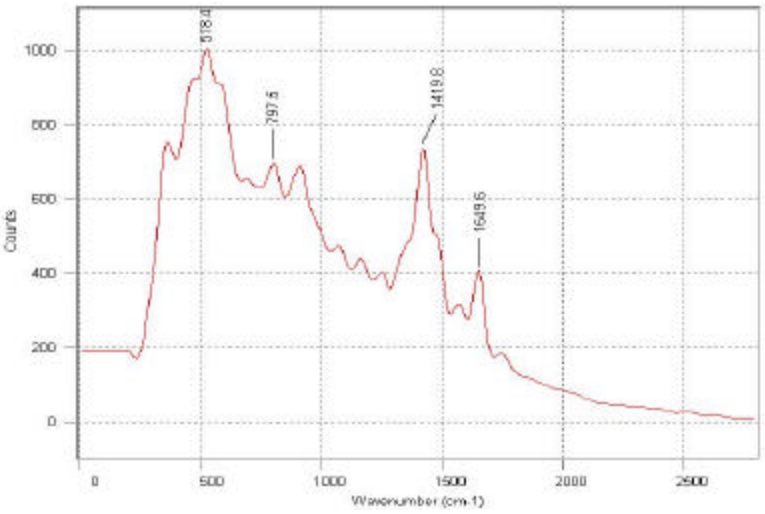

Figure. 4: Raman Shifts of Ofloxacin

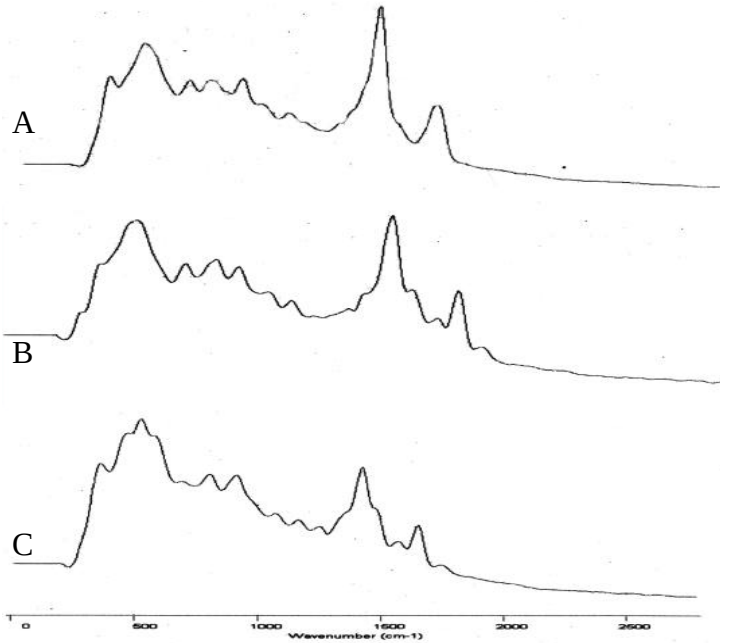

Figure. 5: Comparative Raman Shifts of Fluoroquinolones

For our convenience, the comparative Raman spectra have also been represented in Fig. 5.

DISCUSSION

It is known that the backbone of fluoroquinolone antibacterials consists of a 1-substituted-1,4-dihydro-4-oxopyridine-3-carboxylic moiety combined with an aromatic or heteroaromatic ring. In addition, as has been mentioned earlier, the quinolone nucleus as well as fluorine atom and piperazinyl ring on the quinolone nucleus are proved to be more responsive to their antibacterial potency [1]. From our overall Raman spectroscopic analysis, it has been found that Norfloxacin, Ciprofloxacin and Ofloxacin also show prominent characteristic peaks for pyridone nucleus, piperazinyl ring and fluorine substituent. Moreover, the presence of carboxylic acid moiety indicates the symmetric stretching vibration of O-C-O group. The piperazinyl group characterized by methylene deformation and $N^{+}H_2$ scissoring due to resonating structure of the aromatic ring has commonly been detected in our study [14] (Table 1).

Several scientists have suggested that the compounds having C-F group along with resonating aromatic ring structure can be characterized by Raman spectroscopy, when Raman spectra are within 500-800 cm^{-1}[5]. From our investigation, the characteristic peaks at 872.7, 771.47 and 797.5 cm^{-1} for Norfloxacin, Ciprofloxacin and Ofloxacin, respectively, are assigned to fully symmetric vibrational normal mode of the molecule containing the C-F bond. Since the important charateristic groups are available in cases of all the drugs, their Raman spectral pattern is more or less similar (Fig. 5).

Considering the above-mentioned information, it can be concluded that Raman spectroscopic analysis, a non-destructive method with minimal water interference, may also be used as a useful tool for the identification of compounds, specially the fluoroquinolones. So, this study

may serve as the basis for the Raman spectroscopy to be considered as a promising new official method for identification of fluoroquinolone antibacterials.

REFERENCES

1. Martin AR. Anti-infective agents, In; Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry (Delgado JN, Remers WA; Eds.) New York, USA; Lippincott – Raven Publishers. (1998): 197-202.
2. Petri WA (Jr). Antimicrobial agents, In; Goodman and Gilman's the Pharmacological Basis of Therapeutics (Hardman JG, Limbird LE, Gilman AG; Eds.) New York, USA; Mc Graw-Hill Medical Publishing Division. (2001): 1179-1183.
3. Venkeirsbilck T, Vercauteren A, Baeyens W, Weken GVD, Verpoort F,Vergote G, Remon JP. Applications of Raman Spectroscopy in pharmaceutical analysis. Trends. Anal. Chem. (2002) 21(12): 869-877.
4. Clarke RH, Londhe S, Premasiri WR, Womble ME. Low-Resolution Raman Spectroscopy: Instrumentation and Application in Chemical Analysis. J. Raman Spectrosc. (1999) 30: 827-832.
5. Sharts DOSHCM, Gorelik VS. Method and apparatus for determination of carbon-halogen compounds and applications thereof. United States Patent 6445449; Available from http://www.freepatentsonline.com/6307625.html, accessed on 20.01.2011.
6. The United States Pharmacopeia 31, NF 26, Vol. 2 and 3. Rockville: US Pharmacopeial Convention; 2008.
7. British Pharmacopoeia, Vol. I and II. London: The Stationary Office; 2008.
8. Indian Pharmacopoeia, Vol. 1 and 2. New Delhi: The Controller of Publication, Ministry of Health and Family Welfare, Government of India; 2007.
9. Silverstein RM, Webster FX. Spectrometric Identification of Organic Compounds, 6th edn, New York, USA; Jhon Wiley and Sons, (2002): 71-109.
10. Raman Data and Analysis; Available from http://www.horiba.com/fileadmin/uploads/scintific/Documents/Raman/bands.pdf, accessed on 20.01.2010.
11. Tua Q , Eisenb J, Changa C. Band Shifts in Surface Enhanced Raman Spectra of Indolic Molecules Adsorbed on Gold Colloids; Available from http://www.icors2010.org/abstractfiles/ICORS20101040.5375VER.5.pdf,accessed on 2.01.2010.
12. Xu J, Stangel I, Butler IS, Gilson DFR. An FT-Raman Spectroscopic Investigation of Dentin and Collagen Surfaces Modified by 2-Hydroxyethylmethacrylate. J. Dent. Res.(1997) 76: 596-601.
13. Gruodis A, Alkasa V, Powell DL, Nielsen CJ, Guirgis GA, Durig JR. Vibrational spectroscopic studies, conformations and ab initio calculations of 1,1,1 trifluoropropyltrifluorosilane. J. Raman Spectrosc. (2003) 34: 711-724.
14. Bright A, Devi TSR, Gunasekaran S. Spectroscopical Vibrational Band Assignment and Qualitative Analysis of Biomedical Compounds with Cardiovascular Activity. Int. J. Chem. Tech. Res. (2010) 2(1): 379-388.
15. Skoulika SG, Georgiou CA. Rapid Quantitative Determination of Ciprofloxacin in Pharmaceuticals by Use of Solid-State FT-Raman Spectroscopy. Appl. Spectrosc.(2001) 55(9): 1259-1265.
16. Lawrence BA, Lei Z, Liling Z, Christopher LE, Andrew RB. Solid-State NMR Analysis of Fluorinated Single - Carbon Nanotubes: Assessing the extent of Fluorination. Chem. Mater. (2007) 19(4): 735-744.
17. Agarwal UP, Reiner RS, Pandey AK, Ralpha SA, Hirth KC, Atalla RH. Raman Spectra of Liginin Model Compounds; Available from http://www.treesearch.fs.fed.us/pubs/20194, accessed on 20.01.2010.
18. Tom RT, Suryanarayana V, Reddy PG, Baskaran S, Pradeep T: Ciprofloxacin protected gold nanoparticles. Langmuir (2004) 20(5): 1909-1914.

Source of support: Nil, Conflict of interest: None Declared