IJBPAS, November, 2014, 3(11): 2548-2560

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

QUALITATIVE AND QUANTITATIVE ANALYSIS ON CIPROFLOXACIN HYDROCHLORIDE

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ABSTRACT

Modern spectroscopic methods are very effective and sensitive tool for the qualitative and quantitative analysis of many drugs and the results are well employed in the quality control laboratories of pharmaceutical firm. In the present work, qualitative and quantitative analysis were made on an antibacterial drug namely ciprofloxacin Hydrochloride through the means of FTIR and FT Raman and UV-Visible spectroscopy. A qualitative analysis of this drug has been carried out by making a satisfactory vibrational band assignment for the fundamental modes of vibration observed in the FTIR and FT Raman spectra. The stability of the drug under different environmental conditions is one of the quality assurance method undertaken in the pharmaceutical laboratory. The UV-Visible spectra have been recorded for the drug kept in suitable storage condition and for that exposed to various environmental hazards. The assay of tablet of this drug was done using UV-Visible spectroscopy and compared with the labelled amount.

Keywords: FTIR, FT Raman, UV-Visible Spectroscopy, Antibacterial Drug, Stability, Drug Assay

INTRODUCTION

Ciprofloxacin HCl, fluoroquinoline antibacterial agent is widely used to treat a number of infections including:endocarditis, gastroenteritis, malignant otitis externa, respiratory tract infections, tuberculosis. urinary tract infections etc **[1]**. It is also used before certain types of surgery to prevent infections which may occur after these procedures and corneal ulcers and eye infections. Ciprofloxacin hydrochloride

works by killing the bacteria that have caused the infection. Ciprofloxacin hydrochloride is basically 1-cyclopropyl-6-fluro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid hydrochloride. Its empirical formula is $C_{17}H_{18}FN_3O_3$. HCl . The molecular weight of which is 367.8 **[2]**.

Pharmaceutical science deals with the identification, selection, presentation and standardization of various drugs. It is very essential to design pharmaceutical products that consistently deliver the intended performance, which demands monitoring of their quality incessantly. Quality of drug plays a very vital role indicating the suitability of drug product for its intended use. To investigate the structure and analysis of some pharmaceutical and biological active compounds spectroscopic techniques have been widely

used in the recent past. Gunasekaran *et al.*, **[3, 4]** have done the qualitative analysis and structural conformation on some sample of pharmaceutical and biological importance using IR and UV-Visible spectral measurements. By keeping all these factors, in the present investigation a qualitative and quantitative analysis have been made on an antibacterial drug Ciprofloxacin Hydrochloride. Though investigations on Ciprofloxacin Hydrochloride have been made by many but not much work is done on this said drug spectroscopically. Hence the present study aims to make use of FTIR, FT Raman and UV-Visible spectroscopic methods in the qualitative and quantitative analysis of this drug.

In this work, a quality analysis of Ciprofloxacin Hydrochloride has been carried out by employing FTIR and FT-Raman spectroscopic techniques. The change in quality of this drug when stored under various conditions has been studied by UV-Visible spectroscopy.

The molecular structure is shown in **Figure 1**.

EXPERIMENTAL

The spectroscopic pure sample of Ciprofloxacin Hydrochloride was procured from a reputed pharmaceutical firm, Uttaranchal, India and used as such. The FTIR and FT Raman spectra were measured using Perkin Elmer Spectrum1 spectrometer over the region 4000-400 cm^{-1} and The FT Raman spectra were measured using 1064 nm line of Nd: YAG Laser operating at 200 mW on BRUKER RFS 27 spectrometer in the region 4000-50 cm^{-1}, respectively at Sophisticated Analytical Instrumentation Facility (SAIF), IIT, Chennai, India. The UV-Visible spectral measurements have been made using UV-1700 Series in the wavelength region 200-800 nm at pharma analytical Lab, Puduchery. The FTIR and

FT Raman spectra of Ciprofloxacin Hydrochloride are presented in **Figure 2 and 3** respectively.

RESULTS AND DISCUSSION

Vibrational Spectral Analysis

Fourier transform infrared (FTIR) and Raman spectroscopic methods are being extensively used to identify the structural groups present in a compound. The vibrational band assignments of Ciprofloxacin Hydrochloride have been made in accordance with their position, shape and relative intensity. Also the assignments have been made in analogy with the structurally related molecules. The qualitative investigation on the vibrational band assignments derived from FTIR and FT Raman spectra for Ciprofloxacin Hydrochloride are presented in **Table 1**.

O-H stretching vibrations

Alcohols and phenols, in vapour state or in dilute solution in non-polar solvents exhibit a sharp rather weak O-H stretching absorption due to non-bonded or free OH groups. These non-bonded O-H stretching bands appear near 3650 cm-1 in alcohols and near 3600 cm-1 in phenols. Inter - molecular hydrogen bonding increases as the concentration of the solute in solution increases and additional bands start to appear at lower frequencies near 3550-3200 cm-1 at the expense of the free hydroxyl band. In case, where intra molecular bonding occurs the hydroxyl group (O-H) band appears at 3590 - 3400 cm-1 **[5, 6]**. Based on this, in the present investigation, the band observed at 3529 cm-1 is assigned to O-H stretching vibrations.

N-H stretching vibrations

N-H stretching frequencies corresponding to the symmetrical and asymmetrical NH stretching vibrations for dilute solutions are occur near 3520 cm^{-1} to 3480 cm^{-1}. In the spectra of solid samples are observed near 3350 cm^{-1} to 3180 cm^{-1} because of Hydrogen bonding **[7]**. Based on this, in the present investigation, N-H symmetric stretching vibrations are observed at 3379 $cm^{-1}$and N-H symmetric stretching occurred at 3204 cm^{-1}.

C-H stretching vibrations

The hetero aromatic compounds and its derivatives are structurally very close to benzene. The C-H stretching vibrations **[8-11]** of hetero aromatic structure occur in the region 3100-3000cm^{-1} for asymmetric stretching and 2990-2900cm^{-1} for symmetric stretching modes of vibration. Further, in this region, the bands are not much affected due to the nature and position of the substituents. Heterocyclic compounds C-H vibration absorption bands are usually weak for detection. Hence, in the present work, the FTIR bands observed at 3084,3034 and 3012 cm^{-1} have been assigned to C-H asymmetric and the symmetric stretching

vibrations observed at 2926,2841 cm^{-1}. Also the corresponding Raman bands are observed at 3085, 3016, 2987 and 2845 cm^{-1}.

C=O stretching vibrations

The carbonyl group exhibits a strong absorption band due to C=O stretching vibration and is observed in the region 1850-1550 cm^{-1}. In fluorouracil, the bands observed at 1621 cm^{-1} in IR and 1617 cm^{-1} in Raman are assigned to C=O symmetry stretching. The band observed at 1657 cm-1 in IR and 1660 cm^{-1} in Raman assigned to C=O asymmetry stretching vibration **[12, 13]**. A strong band at 1712 cm^{-1} is assigned for C=O carbonyl stretching of nalidixic acid **[14]**. The band at 1684 cm^{-1}, which is close to the literature range with due characteristics is assigned for C=O stretching **[15]** in benzocaine. Keeping this in mind, the sharp band present in the expected region being at 1899,1708,1624 and 1552 cm^{-1} in the FTIR spectrum are allotted to be due to C=O stretching vibrations. Also the corresponding Raman bands are identified at 1707 ,1624 and 1548 cm^{-1}.

C=C stretching vibrations

Benzene has two doubly degenerate modes and two non-degenerate modes of vibrations due to stretching of C=C bonds. The C=C stretching vibrations occur in the region 1625-1430 cm^{-1}. In the present work, the FTIR bands observed at 1494, 1448 and 1399 cm^{-1} have been assigned to C=C stretching vibrations. Also the corresponding Raman band is identified at 1493 and 1464 cm^{-1}.

C-N stretching vibrations

Medium to weak absorption bands for the unconjugated C-N linkage in primary, secondary and tertiary aliphatic amines appear in the region of 1250-1020 cm^{-1}. Aromatic amines display strong C-N stretching absorption in the 1342-1266 cm^{-1} region [16]. Hence in the present case the FTIR bands observed at 1383,1343,1308 and 1271 cm^{-1} and corresponding Raman bands bserved at 1385,1345,1298 and 1272 cm^{-1} are assigned to C-N stretching vibrations.

C-F Vibrations: In the vibrational spectra of related compounds, the band due to the C-F stretching vibration **[17]** may be found over a wide frequency range 1360-1000 cm^{-1}, since the vibration is easily affected by adjacent atoms or groups. Monofluorinated compounds have a strong band in the frequency range 1110-1000 cm^{-1} due to C-F stretching vibration. In the present work, the bands observed at 1091,1045,1024 cm^{-1} in the FTIR spectrum and at 1050, 1024 cm^{-1} in the FT Raman spectrum is assigned to C-F stretching vibrations of Ciprofloxacin Hydrochloride.

Deformation vibrations.

The C-C-C bending bands always occur below 600 cm^{-1}. Isopropyl benzenes **[18]** have a medium intensity absorption band in the region 560-480 $cm^{-1.}$ The bands at 564,557,539,494,479 cm^{-1} are due to C-C-C bending. The C-C-H asymmetric and symmetric bending vibrations are observed at 1220, 1192 cm^{-1} and 1143, 1106, 852, 837 cm^{-1} in the FTIR spectrum. The corresponding FT Raman bands identified at 1234, 1191 and 1145, 1106 cm^{-1} . The IR bands observed at 987, 943, 921 and 889 are assigned to C-H in plane bending vibrations. The corresponding FT Raman bands identified at 947, 894 cm^{-1}. The C-H out of plane bending bands occurs at 636, 666, 703, 716, 750, 774, 785, 804 and 828 cm^{-1} in the FTIR spectrum. The corresponding FT Raman bands identified at 637, 666, 716, 752, 785 and 829 cm^{-1}.

Thus, a satisfactory vibrational band assignment has been made by observing the position, shape and intensity of the vibrational bands both in IR and Raman spectra of the chosen drug and hence studied their quality.

Qualitative Analysis using UV-Visible spectroscopy

Quality assurance plays a central role in determining the safety and efficacy of medicines. Maintaining proper storage conditions for health commodities and essential medicines is vital to ensuring the quality. UV-Visible spectrophotometry is a satisfactory method employed in the quality analysis of drugs. The method is most often used in a quantitative way to determine concentrations of an absorbing species in solution, using the Beer-Lambert law:

$$\mathbf{A = - \log_{10}(I\ o/I) = \varepsilon.\ c.\ L}$$

where A is the measured absorbance, Io is the intensity of the incident light at a given wavelength, I is the transmitted intensity, L the path length through the sample, and c the concentration of the absorbing species.

Beer-Lambert law

The UV-Visible spectral measurements are carried out on Ciprofloxacin Hydrochloride and by checking the sample obeying Beer's law, the absorption peaks are identified. The sample shows absorption peaks at 320 and 270 nm. The spectral measurements were carried out for different concentrations (2.5, 5, 7.5, 10, 12.5 mcg/mL) of the drug. The UV-Visible spectra of Ciprofloxacin Hydrochloride at different concentrations are shown in the **Figure 4**. It is found that as the concentration increases, the absorption level increases satisfying Beer's law. The linearity curve, verifying Beer's law for the drug Ciprofloxacin Hydrochloride is also shown in **Figure 4** and the linearity coefficient found to be 0.9929, which is used as a parameter to identify the quality of the drug Ciprofloxacin Hydrochloride.

Study of storage condition

Among the different quality assurance measures used in the control of manufacturing and formulation of drugs, two parameters are important which is the check on the shelf life of the drugs, stability under different storage conditions which has to be tested at every stage. Hence during the fabrication of the drugs, the various raw materials that are used in the fabrication of the drugs should undergo a rigorous qualitative test. Spectroscopic technique, UV-Visible bands were made to study the quality of the drugs under different storage conditions.

The ideal storage condition (ISC) for the drugs used in the present study should be stored in airtight containers, at temperatures between 59 and 86 ºF (15 and 30 ºC) is permitted. Store away from heat and light. Do not store in moisture place.

Three sets of equal amount of Ciprofloxacin Hydrochloride in the powder form have been taken for the investigation. One set of the drug was stored at room temperature *i.e.*, at ideal storage condition (ISC), while another was stored at cold condition (ice point), the third set of the drug was exposed to sunlight continuously for a stipulated period of 4 h to make an internal standard calculation on the compound Ciprofloxacin Hydrochloride.

UV-Visible spectral investigation has been carried out to study the variations in the absorbance of λ_{max} in Ciprofloxacin Hydrochloride at different storage conditions. The samples of Ciprofloxacin Hydrochloride are exposed to different storage conditions *viz.*, ideal storage condition, cold condition and sunlight. Overlaid UV-Visible spectra of Ciprofloxacin Hydrochloride (at 7.5 mcg/mL concentration) at different storage condition and bar chart shows the variance of λ_{max} with absorbance at different storage condition for Ciprofloxacin Hydrochloride is shown in **Figure 5**. From this figure, it is observed that the absorbance of the drug kept at ideal storage condition varies with the absorbance of the drug placed in the sunlight and at the ice point. Hence it can be concluded that the drug under study is to be stored in ideal storage condition to retain its pharmaceutical properties.

The internal standard ratio among the absorbance of wavelength maxima are calculated and the sets of internal standards of these drugs stored under different storage conditions are compared with that of the drugs stored under ideal storage condition to check whether any change in the light absorption characteristics of the drugs has taken place. From the **Tables 2**, it is observed that the internal standard calculation for the various storage conditions showed significant change with the drug kept in ideal storage condition. It is

observed that the drug activity changes more significantly due to improper storage of the drugs.

Assay of Drug– UV-Visible spectroscopy

Assay is the estimation of potency of an active principle in a unit quantity of the reparation. The potency is the measure of the biological activity of a drug. In present study, UV-visible spectrophotometric technique is used to study the assay of drugs. It is popular among patients for accuracy of dosage, compactness, portability, blandness of taste and ease of administration **[19]**. Quantitative spectrometry is an extension of calorimetry and many

pharmacopical substances are assayed spectrophotometrically **[20]**. In the present work, medicine of Ciprofloxacin Hydrochloride in the form of tablet is subjected for the quantitative estimation of the drug substance in the tablet. According to Indian Pharamacopoeia the method of assay is by different methods in each of the above tablet. As a model experiment to understand how UV-Visible spectroscopic technique is employed in the assay, this method is applied in Ciprofloxacin Hydrochloride to estimate the active substance in the tablet

The tablets containing Ciprofloxacin Hydrochloride as the active ingredient is obtained from a leading pharmaceutical company labeled, as Ciprolan-500mg. The UV-visible spectra are recorded for all the sample in the pure form and for the tablet. By comparing the absorbance in the pure and tablet form of the sample, the quantitative estimation of the drug can be estimated. A solution of the test substance is made at a known concentration in a suitable solvent. The absorbance is noted at a selected wavelength which is preferably that of wavelength maxima having a fairly broad, flat-topped peak. Then by making a parallel determination of a solution prepared from a pure reference sample for the same concentration, the amount of active ingredient in the test substance is calculated. All the measurements are based on the fundamental law of spectrophotometry (*i.e.*) Beer-Lambert's law which is stated as $A = \log (I/I_0) = \alpha bc$ where, I_0 is the intensity of the incident monochromatic beam which emerges with intensity I through a solution of path length of 'b' having concentration 'c' and 'α' is the absorbance. In drug analysis, the determination of the drug content is carried out by preparing a stock solution of the test sample and the solution is diluted to the same concentration as that of the standard sample and the absorbance of the resulting solution is measured. The drug content of the tablet is calculated from Beer's law. (See Below).

In the present work, a single component system is chosen for the assay. From the spectroscopic point of view, a single component system is the one for which, at the wavelength selected for the measurement, the determination of the analyte is not influenced either by another substance or by background absorption.

To calculate the assay of the Ciprofloxacin Hydrochloride tablets, the tablets containing the strength of the pure drug is employed in the work which is Ciprolan-500 mg manufactured by a leading pharmaceutical company. The UV-Visible spectrum of the sample exhibits wavelength maximum at 270 and 321 nm. The average weight of one tablet is found to be : 782.5 mg mg. The UV-visible spectra are recorded and absorbance is noted **(Figure 6)**. The quantitative estimation or assay of the active substance Ciprofloxacin Hydrochloride is estimated in tablet (ciprolan -500 mg) and is found to be 499.72 mg.

$$\text{Drug content of the tablet or assay} = \frac{\text{Test absorption}}{\text{Standard absorption}} \times \frac{\text{Standard weight}}{\text{Test weight}} \times \text{Average weight of the tablet}$$

Table 1: Vibrational band assignments of Ciprofloxacin Hydrochloride

Frequency (cm-1) FTIR	Frequency (cm-1) FT Raman	Vibrational band assignment
3529(vs)		OH stretching
3379(s)		N-H Asym.stretching
3204(m)		N-H Sym.stretching
3084(s)	3085(w)	C-H Asym.stretching
3034(s)		C-H Asym.stretching
3012(s)	3016(m)	C-H Asym.stretching
2926(s)	2987(w)	C-H sym.stretching
2841(m)	2845(vw)	C-H sym.stretching
2763(s)		C-H stretching
2691(s)		C-H stretching
2620(s)		C-H stretching
2501(s)		C-H stretching
2463(s)		C-H stretching
1899(vw)		C=O stretching
1708(vs)	1707(w)	C=O stretching
1624(vs)	1624(s)	C=O stretching
1552(s)	1548(w)	C=O stretching
1494(vs)	1493(vw)	C=C stretching
1448(vs)	1464(w)	C=C stretching
1399(s)		C=C stretching
1383(vs)	1385(s)	C-N stretching
1343(s)	1345(w)	C-N stretching
1308(s)	1298(vw)	C-N stretching
1271(vs)	1272(w)	C-N stretching
1220(s)	1234(vw)	C-C-H Asym.bending
1192(s)	1191(vw)	C-C-H Asym.bending
1143(s)	1145(vw)	C-C-H Sym plane bending
1106(m)	1106(vw)	C-C-H Sym plane bending
1091(m)		C-F in plane bending
1045(m)	1050(vw)	C-F stretching
1024(s)	1024(w)	C-F stretching
987(s)		C-H in plane bending
943(s)	947(vw)	C-H in plane bending
921(m)		C-H in plane bending
889(m)	894(vw)	C-H in plane bending
852(m)		C-C-H sym.bending
837(m)		C-C-H sym.bending
828(m)	829(w)	C-H out of plane bending
804(m)		C-H out of plane bending

785(w)	785(vw)	C-H out of plane bending
774(w)		C-H out of plane bending
750(w)	752(w)	C-H out of plane bending
716(vw)	716(vw)	C-H out of plane bending
703(vw)		C-H out of plane bending
666(vw)	666(vw)	C-H out of plane bending
636(vw)	637(w)	C-H out of plane bending
564(w)		C-C-C bending
557(w)		C-C-C bending
539(w)		C-C-C bending
494(w)	497(vw)	C-C-C bending
479(w)	472(vw)	C-C-C bending

vs-very strong, s-strong, m-medium, w-weak, vw-very weak

Table 2: Internal Standard Calculation Of Ciprofloxacin Hydrochloride

Storage conditions	A321/A270
Ideal storage condition	0.4491
Sunlight	0.4248
Cold condition	0.4379

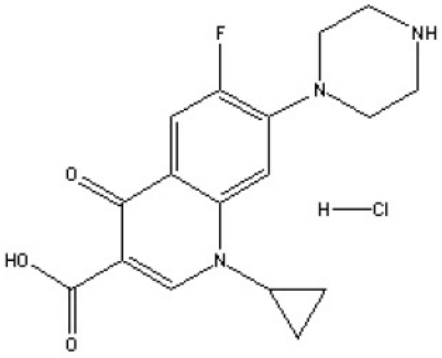

Figure 1: Molecular structures of Ciprofloxacin Hydrochloride

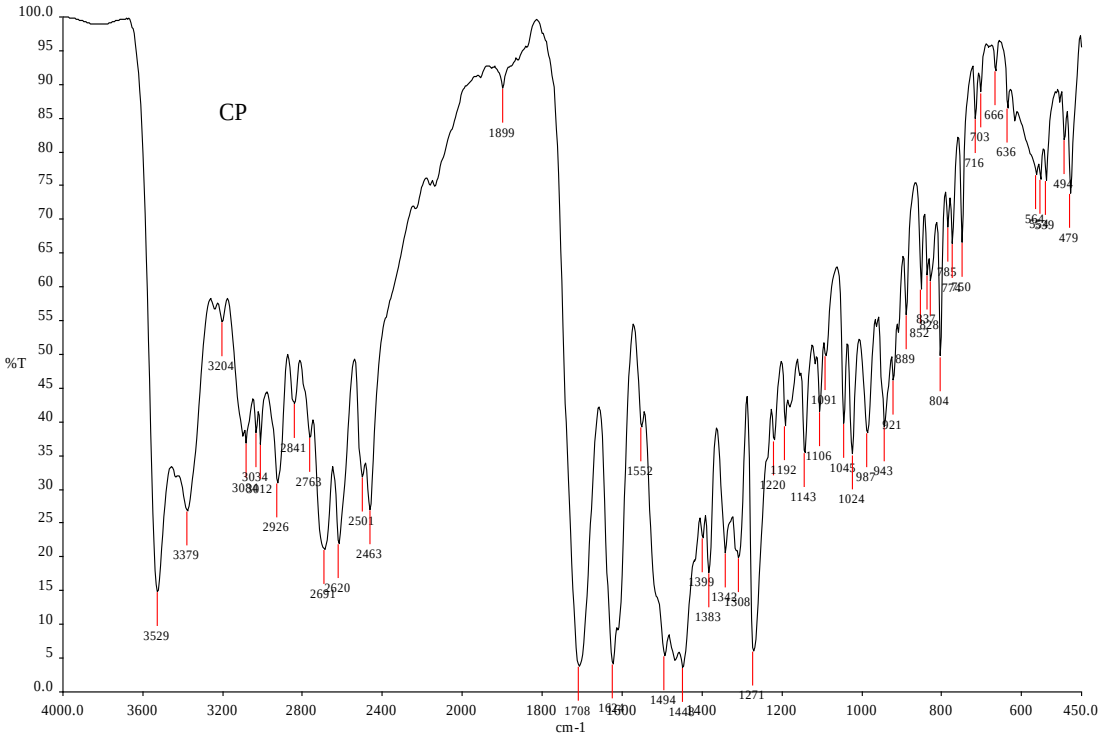

Figure 2: FTIR spectrum of Ciprofloxacin Hydrochloride

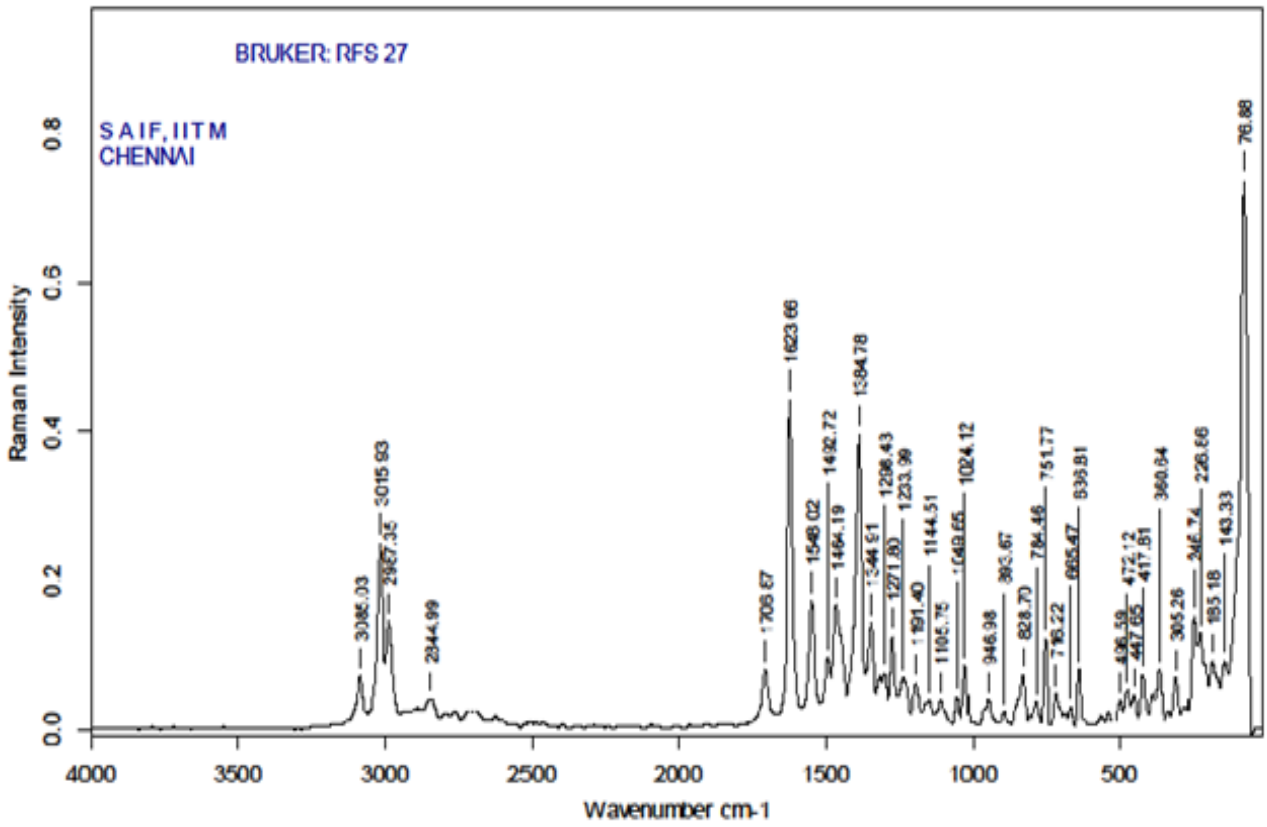

Figure 3: FT-Raman spectrum of Ciprofloxacin Hydrochloride

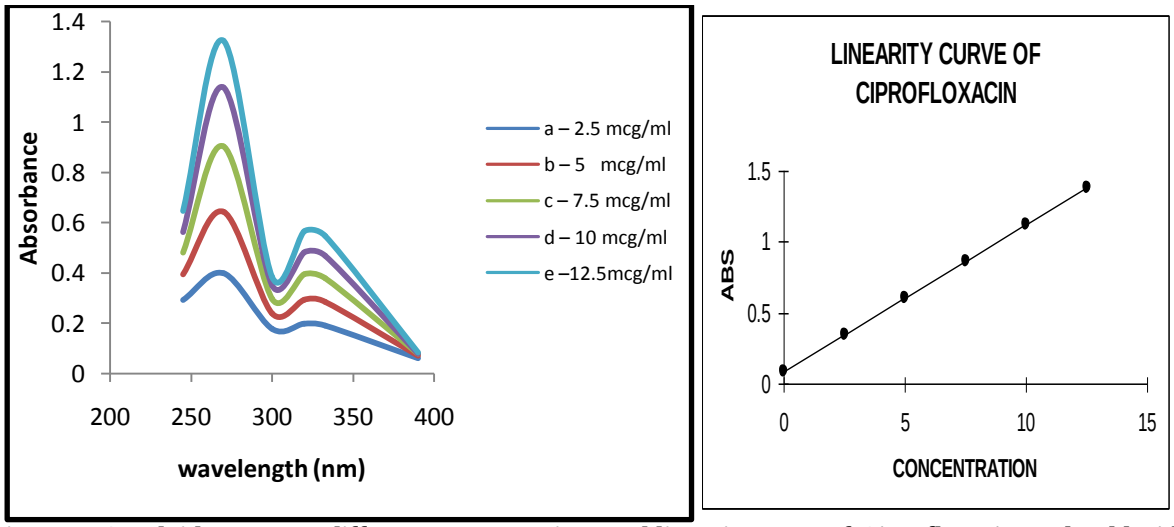

Figure 4: Overlaid spectra at different concentrations and linearity curve of Ciprofloxacin Hydrochloride

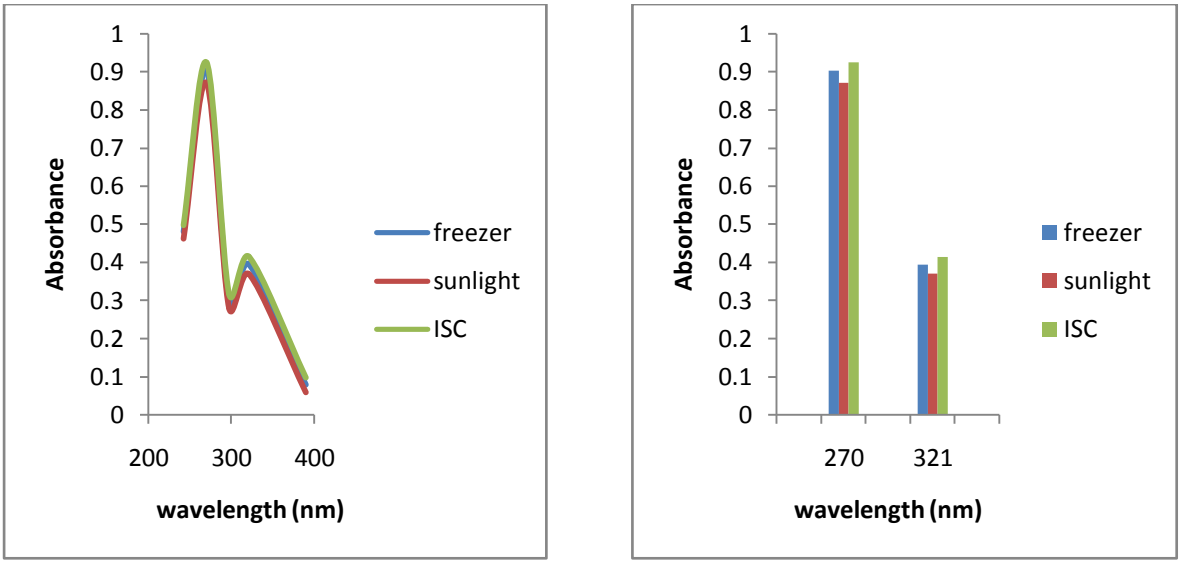

Figure 5: Overlaid spectra at different storage condition and bar chart for the variance of λmax with absorbance at different storage condition for Ciprofloxacin Hydrochloride

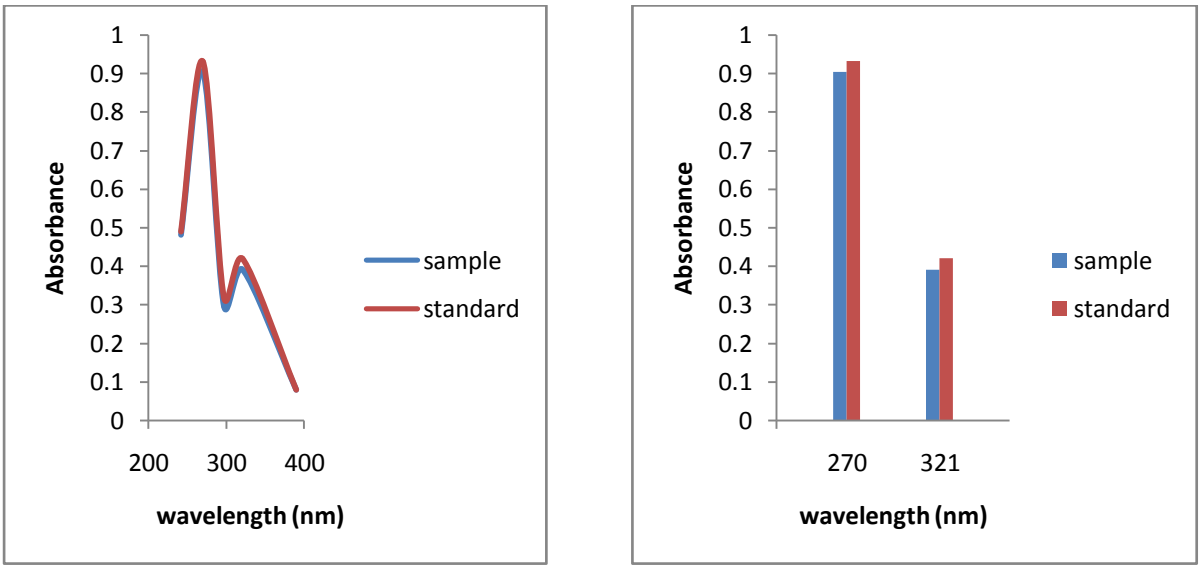

Figure 6: Overlaid spectra and bar chart for the variance of λmax with absorbance of pure Ciprofloxacin Hydrochloride and ciprolan tablet

CONCLUSION

FTIR, FT-Raman and UV-Visible spectroscopic techniques have been employed for the qualitative analysis of the antibacterial drug Ciprofloxacin Hydrochloride. A satisfactory vibrational assignment of the drug has been done from the FTIR and FT-Raman spectra of the drug. They confirm the basic functional groups present in the compound. The internal standard calculation has been made for different absorbance value for the drug exposed to different environment conditions using UV-visible spectral measurements.The method of assay has been employed in ciprolan tablet. In

ciprolan 500 mg, the active substance present is calculated as 499.72 mg.

REFERENCES

[1] Sean C Sweetman, Martindale, Thirty sixth edition, Royal Pharmaceutical Society of Great Britain (RPS) Publishing, UK, **2009**, p-243.

[2] Medicine and Healthcare products Regulatory Agency, ciprofloxacin hydrochloride, British pharmacopoeia (B.P), volume 1, UK, **2010**, pp 511-512.

[3] S. Gunasekaran, T.S. Renuga Devi and P.S. Sakthivel, *Asian J. Chem.*, **20**, 4249 (2008).

[4] S. Gunaskaran, S. Srinivasan and U. Ponnambalam, *Asian J. Chem.*, **16**, 23 (2004).

[5] Thilak Kumar R and Umamaheswari S, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2001,685-693.

[6] Robert M Silverstein, Clayton Bassler G and Terence C Morrill. Spectrometric identification of organic compounds, 4th Edition, John Wiley, New York 1997; 111.

[7] Gunasekaran S, Natarajan RK, Syamala D and Rathika R. Ind J Pure Appl & Phys 2006 ; 44:315.

[8] Gunasekaran S and sailatha.E; Ind J Pure & Appl Phys,2009;259-264.

[9] Gunasekaran S, Ponnambalam U & Muthu S, Acta Ciencia Indica, xxx (2004) 015.

[10] Mohd Chanan & Varma P K, Indian J Pure & Appl Phys,77B (3) (2003) 315.

[11] Srinivasan S, Gunasekaran S, Ponnambalam U, savarianandam A , Gnanaprakasam S & Natarajan S, Indian J Pure & Appl Phys, 43 (2005) 459.

[12] Renuga devi T S and Gayathri S, Volume 2, May-June 2010; Article 019.

[13] Gunasekaran S, Ponnambalam U, Muthu S, Kumaresan S, *Indian J. Physics*(10),1141 (2004).

[14] Gunasekaran S, Rathika R, *Indian J. Physics* 41,503(2005).

[15] Gunasekaran S and Sankari G, *Spectrochim Acta* Part A, 6, 117 (2005).

[16] Arulmozhichelvan P. Spectroscopic and Microwave Measurements of Some Crystalline, Ceramic, Polymeric and Pharmaceutical Materials, Ph.D. Thesis, University of Madras, Chennai, India 1993.

[17] S. Gunasekaran and J. Marshall, *Indian J. Phys.*, **64**, 367 (1991).

[18] Gilbert A S, Spectrochim Acta, 32A (1976) 931.

[19] Atherden L.M., Bentley and Driver's Text Book of Pharmaceutical Chemistry, Oxford university Press, New Delhi,, edn.8(1998).

[20] Gorog S. Ultravoilet-Visible Spectrometry in Pharmaceutical Analysis, CRC Press, Inc. (1995).