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POLYMORPHS OF LOMEFLOXACIN: PREPARATION, CHARACTERISATION & EVALUATION OF ITS ANTI-MICROBIAL ACTIVITY

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ABSTRACT

The present work was undertaken with the aim to study crystal forms of Lomefloxacin HCl. The objective of the project was to make different crystal forms of Lomefloxacin HCl. It was planned to prepare crystal forms using solvents of varying polarity and by change of phase, to characterize the crystal forms using techniques like optical microscopy, IR spectroscopy, differential scanning calorimetry and X-ray diffractometry and to study the dissolution profile of the crystal forms of Lomefloxacin HCl. Since tablet is the most common formulation available for this Lomefloxacin HCl, it was also planned to evaluate the impact of the differing crystal morphology on the formulation behavior of the Lomefloxacin HCl & its anti-microbial activity.

KEY WORDS

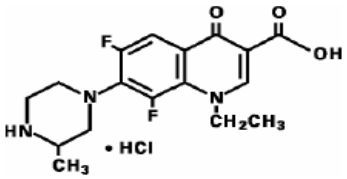
polymorphism; preformulation; dissolution rate; Differential scanning calorimeter (DSC); FTIR; thermal analysis; X-ray powder diffractometer.

INTRODUCTION

Polymorphism is the phenomena of the ability of a compound to exist in various crystalline forms[1], in which the arrangement of molecules has different crystal lattice. So the each state of polymorphic form of crystal shows different in structure and properties in the same manner as the crystals of two different compounds. Melting point, compressibility, hygroscope, Solubility, Density, Hardness, crystal habit, color, and bioavailability finally change in the efficacy of the Drugs[2].Therefore it is important to evaluate each active pharmaceutical ingredient with regard to Polymorphs and select most stable polymorphs[3].

So discovery and characterization of polymorphs are essential preformulation step in pharmaceutical research and development. Currently one of the most widely used methods for preparation of polymorphs is recrystalizations from solvents change method. This Lomefloxacin HCl is a chemically new class of [1- Ethyl - 6, 8 – difluoro -1, 4–dihydro –7 – (3 methyl -1- piprazinyl) –oxoquinoline -3-carboxylicacid]. It is a widely used fluoroquinolone broad spectrum antimicrobial agent, which are highly effective in the treatment of a wide variety of clinical infections. Showed in **Figure: 1**

Figure 1: Lomefloxacin



MATERIAL & METHODS

Lomefloxacin HCl was obtained from IPCA Laboratories. Bhopal, India. The various solvents used for preparation of crystals were Acetone, Acetonitrile, Acetic acid, Ammonia, Benzene, Chloroform, Di-methyl sulfoxide, (Chempure Laboratories, Chennai), Dimethylformamide, Dichloromethane, Distilled water, Ethanol, Isopropanol, and Methanol (Reachem Laboratories Chem.Pvt.Ltd.Chennai).

Preparation of Crystal Forms from Different Solvents

The Pure Lomefloxacin HCl (**Figure: I**) (0.5g) was dissolved in respective solvents (20ml) at its boiling point to check its solubility. To this solution, another weighed amount (2g) of Lomefloxacin HCl was added and refluxed with Distilled water, Methanol & Ethanol (120ml) for 20 minutes. The solution was filtered through Whatmann filter paper and concentrated by recovery of the solvent to one third of its original volume and kept for crystallization at room temperature to afford well defined crystals of Lomefloxacin HCl (Figure: 2).The crystals obtained were collected by filtration, dried under vacuum for 24hours and stored in well container. The yield was found to be 99%.

Characterization of Crystals[4]

Optical Microscopy[5]

All the crystals so prepared were viewed under optical microscope for their physical characterization. The samples were prepared by placing a small amount of respective crystal powder (previously passed through No.100 sieve) on the slide, dispersed in a drop of mineral oil (liquid paraffin) and covered with cover slip. The slides were visualized by means of binocular polarizing microscope under 10X/0.25 Ph1 & 40X/0.45 Ph 2.When Polarized transmitted light was used to illuminate the sample, the background of the image appeared dark and the sample appeared bright. Samples were observed at a magnification of 100X also. Photomicrographs were taken by using Kodak film roll.

Differential scanning calorimetry[6]:

The instrument was calibrated using Indium as standard. The sample (2-10mg) was weighed accurately in aluminums pan and sealed hermetically using a crimper. Thermo grams were obtained by heating the encapsulated samples at a constant heating rate of 5^{o}C/min with chart speed of 5 mm/min under an atmosphere of nitrogen. The exact peak temperatures, melting point and heat of fusion were determined. The temperature range for the scan was 30^{o}C to 450^{o}C for all the samples.

IR spectroscopy[7]:

The crystal samples (2-2.5 mg) were triturated with dried potassium bromide (100 mg) using agate mortar and pestle. These quantities were usually sufficient to give a disc of 13 mm diameter and a spectrum of suitable intensity. The mixture after grinding into a fine powder was spread uniformly in a suitable die and compressed into a pellet form at a pressure of about 10kg/cm^{1} for three minutes by using hydraulic press. The resultant pellet was mounted in a suitable holder in the FT-IR spectrophotometer and full range spectra of all crystals were recorded form 4000 cm^{-1} to 400 cm^{-1}.

Powder x-ray diffraction spectroscopy[8]:

All crystal samples were ground and screened through 100 mesh. The x-ray diffraction pattern was recorded using Phillips analytical automatic powder diffractometer at 30mA, 40KV. The samples were scanned at a temperature 25^{0}C at the diffraction angle 2 θ over the range of 5-40 θ.

Formulation & Evaluation of Pre and Post Compressional Properties[9]:

Selected polymorphs previously passed through 100 mesh were mixed with sufficient quantity of microcrystalline cellulose, sodium starch glycolate, polyvinylpyrrolidone, magnesium stearate and talc by geometrical dilution. The powder mixture was compressed in an electrically driven Tablet punching machine (Cadmach, Ahmadabad) using 9 mm punch to obtain 300mg of tablets and evaluated all the official Pre, Post Compression Properties of respective tablets according to I.P. These results were shown in **Table: 01**

Table: 01 Comparison of Pre & Post Compressional properties of all crystal forms of prepared Tablets

Solubility after 4hrs	Form I	Form II	Form III	Lomefloxacin
Absorbance (285 nm)	0.687	0.823	0.854	0.450

Formulation	Thickness (mm)	Hardness (Kg/cm^2)	Friability (%)	Weight variation (mg)	Disintegration time (sec)	True density (g/ml)
Form-I	3.4±0.02	4.33±0.25	0.18	300.2±0.15	140±4	1.0674
Form-II	3.3±0.02	4.18±0.06	0.15	299.2±0.15	115±5	1.0507
Form-III	3.4±0.02	4.20±0.08	0.13	297.4±0.02	110±5	1.0415
Lomeflo-xacinpure	3.3±0.02	4.43±0.062	0.22	300.4±0.52	180±5	1.0876

In-Vitro Dissolution studies[10]:

The in vitro dissolution of tablets made for certain of the crystal forms was performed using USP dissolution apparatus. 6.8pH phosphate buffer (900ml) was used as the dissolution medium and paddles rotating at 50r.p.m. as stirring devices. The dissolution was carried out for 60 min. Samples (5ml) were withdrawn at 5, 10, 15, 30, 45 and 60 minutes intervals. The same volume of medium was replaced immediately to maintain the sink condition. The absorbance of the resulting solutions (after filtering through whatman filter paper) was taken at 285nm after suitable dilutions of the solutions using the same medium. All the above results were shown in **Figure: 6**

Determination of the antimicrobial activity[10]:

Antibacterial Screening

Antibacterial activities of the test Lomefloxacin crystals (Form I, II & III) complexes have been carried out against various Gram-positive and Gram negative bacteria by agar well diffusion method. Each selective medium was inoculated with the microorganism suspended in soybean casein broth (SBCB). The test solutions were prepared in dimethylsulfoxide (DMSO).Once the agar was solidified, it was punched with a 5 mm diameter wells and filled with 25L of 1 mg/mL test compounds(Form-I,II&III) and positive controls (tetracycline, streptomycin and rifampicin). The plates were incubated at 35±2°C for 24 hr. The antimicrobial activity was calculated by measuring the zones of inhibition surrounding the disc[12]. Clear inhibition zones

around the discs indicate the presence of antimicrobial activity. All data on antimicrobial activity are the average of triplicate analyses and results were shown in **Table: 3**

RESU LTS & DISCUSSION

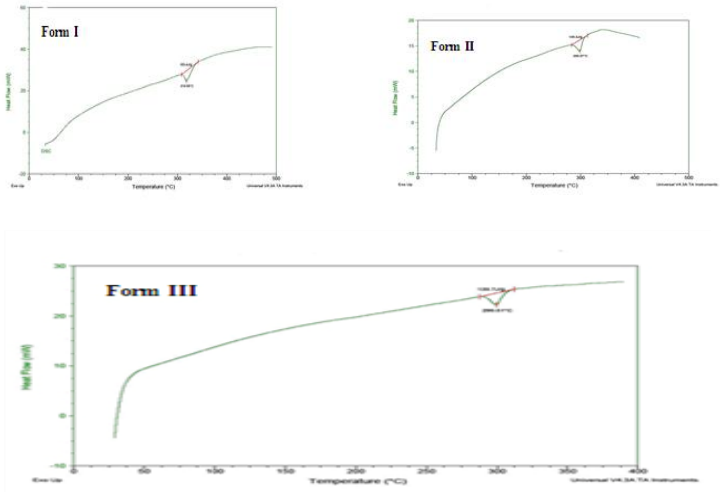
The free base of Pure Lomefloxacin showed appreciable solubility only in Methanol, Ethanol & Distilled water to be used as solvent for Re-crystallization. From DSC data, it was observed that the shown spectra are not provided significant variation in between Lomefloxacin-pure and obtained crystal forms i.e., Onset, endset & peak fusion of prepared crystals were similar to Lomefloxacin-pure form shown in figure: 2&3.However unlike DSC the SEM Photographs showed a significant variation in their shape between Form-I, well as Lomefloxacin-Pure but except Form-II and Form-III were same shown in **Figure: 2**.

Figure: 2 Photomicrographs of Form I, Form II, and Form III

Form I (Distilled Water) **Form II (Methanol)**

Form III (Ethanol)

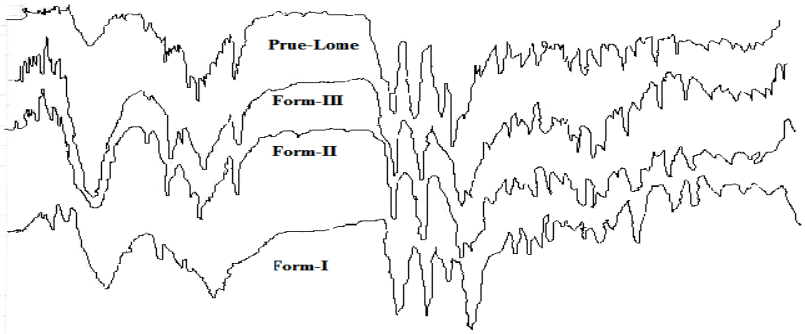
Figure: 3 DSC thermographs of Form I, Form II, and Form III



IR spectra of the crystals were recorded using potassium bromide disc method as discussed earlier. On comparing these spectra, differences in the park pattern were observed in the region

of 1400 cm^{-1} to 500 cm^{-1}**. Form-II** and **Form-III** afforded super imposable spectra in this region while the IR spectrum of **Form-I** offered differences in this region. So on this basis of (From figure: 4) the hydrochloride crystal forms could be categorized into two different forms. **Form-I** belonging to one category and **Form-II** and **Form-III** together belonging to a different category.

Figure: 4 FT-IR Comparison spectra of Form I, Form II, and Form III



All the samples were submitted for XRD analysis. Comparison of the XRD Spectra of the three hydrochloride crystal forms showed same peak pattern in **Form-II** and **Form-III** while, a different pattern was observed in **Form-I**. Considering XRD analysis to be the final parameter in deciding the existence of polymorphs in a compound, it could be said that **Form-II** and **Form-III** crystal forms belonged to same category of polymorphs. **Form-I** might be a different crystal habit of this polymorph. (From figure: 5& Table: 2) From the Dissolution studies Form-III has shown the fastest rate of dissolution and highest solubility after 4 hrs compared to Form-I, Form-II, & Lomefloxacin pure(Figure: 6). This study was extended to, 12 different bacterial strains were used to screen for the antimicrobial activity.

Figure: 5 XRD spectrums of Form I, Form II, and Form III

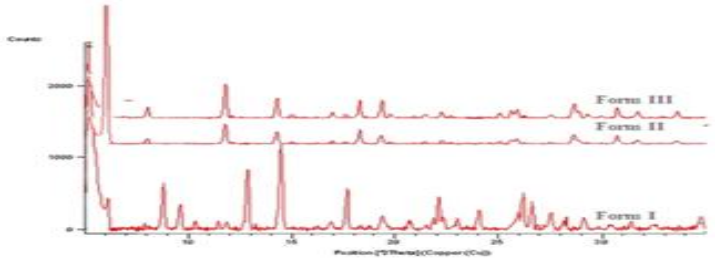


Table: 2 Peak (2θ) values with intensities of various crystal forms of Lomefloxacin

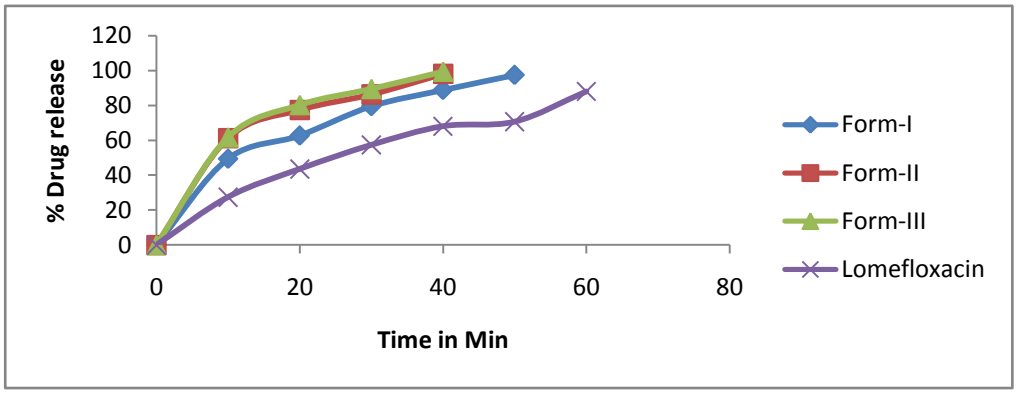
Crystal Form	2θ Value	Intensity	2θ Value	Intensity	2θ value	Intensity
	5.2	**100**	12.8	36.7	24.2	11.1
Form-I	**6.1**	**17.4**	**14.4**	**51.6**	26.1	20.6
	8.8	26.2	17.6	22.5	**27.6**	**14.51**
	9.6	14.1	22.1	18.3	27.4	9.39
	5.1	**19.2**	**14.3**	**10.31**	25.9	4.33
Form-II&III	**6.07**	**100**	18.3	9.7	**28.6**	**7.32**
	8.08	5.26	19.4	9.11	30.7	4.95
	11.8	17.6	22.2	3.08	**33.6**	**3.2**

The test compounds were found to have antibacterial activity against various bacterial species, as it is shown in Table: 3 of the prepared crystals were tested, Form III & Form II showed strong antibacterial activity against *Escherichiacoli, Proteusvulgaris, Staphylococcus pyrogenes* and *Bacillus subtilis* with zone of inhibition 20 mm and moderate activity was shown with other bacterial species. Mild antibacterial activity was seen with Form I compound against *Salmonella paratyphi, Klebsiella pneumonia, Proteus mirabilis, Staphylococcus pyrogenes and Bacillusmegatarium* with zones of inhibition ranging from6-8 mm. The compound Form I was found to have no activity against *Lactobacillus*, but the compound clearly active against the remaining bacterial species These results are favored the optical microscopy, IR spectroscopy & XRD results indicating Form-I to be a different crystal habit than Form-II, Form-III & as well as pure Lomefloxacin drug.

Table: 3 Antibacterial activity of Form-I, II and III complexes along with reference drugs, Tetracycline (T), Rifampicin (R) &Streptomycin(S)

S.No	Test organism	Zone of inhibition (mm)			Standards(mm)		
		Form-I	Form-II	Form-III	T	R	S
1	Pseudomonas aeruginosa	10	17	18	32	22	28
2	Escherichia coli	15	20	20	25	28	32
3	Proteus vulgaris	18	20	20	15	25	30
4	Enterobacter aerogenes	18	15	17	26	35	35
5	Salmonella paratyphi	8	14	15	20	21	30
6	Klebsiella pneumonia	6	14	16	20	19	30
7	Proteus mirabilis	8	16	16	19	20	33
8	Lactobacillus	_	8	10	15	8	25
9	Staphylococcus pyrogenes	6	12	14	25	21	30
10	Bacillus subtilis	15	20	20	30	25	40
11	Staphylococcus aureus	17	7	9	25	28	31
12	Bacillus megatarium	18	9	11	25	30	35

Figure: 6 Dissolution dissolution profiles of prepared tablets from selected crystals of Lomefloxacin



CONCLUSION

Lomefloxacin crystal forms were prepared by crystallization from methanol, ethanol, and distilled water (polar, protic).It was planned to characterize the crystal forms using techniques like optical microscopy, IR spectroscopy, differential scanning calorimetry and X-ray diffractometer. In this characterization melting point or DSC data did not help. But Optical microscopy, IR Spectroscopy & X-RD spectra have given spectacular insight in the characterization of these crystal forms Such as Form **II** and Form **III** were same size and shape but Form **I** was different in size and shape. It included dissolution profile of prepared crystal forms of Lomefloxacin HCl and evaluated its anti microbial activity. The dissolution study of the crystals afforded very interesting results. Form **II** and Form **III** offered fastest dissolution & greater antimicrobial activity than Form **I** and Lomefloxacin pure.

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