Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 7, 2015

Original Article

PREPARATION AND CHARACTERIZATION OF CRYSTALS OF PRULIFLOXACIN

GANESHAN V, ETHIRAJ T

Department of Pharmaceutics, Erode College of Pharmacy, Erode
Email: ethirajecp@gmail.com

Received: 16 Mar 2015 Revised and Accepted: 30 May 2015

ABSTRACT

Objective: Aim of this present study was to prepare and characterize the different polymorphs of Prulifloxacin using Powder X-Ray Diffraction analysis (PXRD), Scanning Electron Microscopy (SEM), Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectrometry (FTIR) and dissolution studies.

Methods: Polymorphs of Prulifloxacin were prepared by cooling crystallization method using various solvents and they exhibited three different polymorphic forms that polymorph 1 (P-1), polymorph 2 (P-2) and polymorph 3 (P-3) with acetonitrile, acetone and dichloromethane.

Results: Rod shaped crystal obtained from acetone (P-2) showed the highest percentage drug release in dissolution study than other crystals due to smaller particle size. P-1 form showed higher melting point in thermogram due to more stability. Melting temperatures obtained from DSC graph indicate the existence of different crystal forms. Significant differences were found in FTIR studies. Appearance and disappearance of specific peaks on the PXRD pattern reveal that the formation of newer crystal forms.

Conclusion: It was concluded that the Prulifloxacin existed three polymorphic forms and they showed highest solubility and percentage drug release than amorphous form.

Keywords: Prulifloxacin, Cooling crystallization

INTRODUCTION

Prulifloxacin (fig. 1) is chemically, 6-Fluoro-1-methyl-7-[4[(methyl-2-oxo-1,3-dioxol-4-yl)methyl]-1-piperazinyl]-4-oxo-1-H,4H-[1, 3]. thiazeto (3,2-a) quinoline-3-carboxylic acid. Fluoro quinolones are synthetic chemotherapeutic agents that have a broad spectrum of antimicrobial activity as well as a unique mechanism of action, resulting in inhibition of bacterial DNA gyrase and topoisomerase IV. Mostly Prulifloxacin is used in uncomplicated and complicated urinary tract infection (UTI), community acquired respiratory tract infections, gastroenteritis including infectious diarrhea.

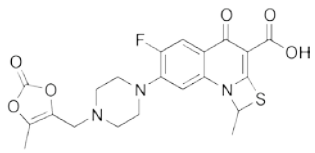

Fig. 1: Chemical structure for Prulifloxacin

Polymorphism comes from the greek word, Polus=many and morph=shape. Thus it is defined as the ability of a substance to exist as two or more crystalline phases that have different arrangements or conformations of the molecules in crystal lattice.[1] It is well recognized that polymorphism and solvate formation affect the various pharmaceutically important physicochemical properties, such as stability, solubility, dissolution rate, crystal habit (shape), tableting behaviour.

Six polymorphic forms of Pefloxacin were prepared from various solvents like methanol, distilled water, ethanol, acetonitrile, isopropanol, and DMF [2]. Norfloxacin exist in three polymorphs, it was obtained by solvents like acetonitrile, isopropanol and acetone [3]. Lomefloxacin showed three polymorphs from solvents like water, methanol, and ethanol [4]. Sparfloxacin have two polymorphs from water [5]. Levofloxacin exist one pseudopolymorph from water [6]. The main objective of this work is to increase the aqueous solubility to enhance the absorption, bioavailability and stability of the selected drug. In this present work, it was planned to prepare different crystal forms of Prulifloxacin using various solvents and to characterize them using various instrumental techniques such as powder X-ray diffractometry (PXRD), thermal study by differential scanning calorimetry (DSC), infra red (IR) spectroscopy, scanning electron microscopy (SEM). Since crystal forms may differ in their dissolution behavior, it was also planned to study the dissolution profile of the prepared crystal forms.

MATERIALS AND METHODS

Materials

Prulifloxacin was obtained from Hetero Drugs, Hyderabad, India. The solvents used for crystallization are acetonitrile, acetone, dichloromethane and distilled water, which were purchased from S. D. Fine Chemicals Ltd, Mumbai, India.

Preparation of polymorphs by cooling crystallization method

Solubility of Prulifloxacin drug was checked by adding 5 ml of acetonitrile to 0.5 g of drug and known amount of Prulifloxacin (2.5 g) was added to the above solution. Then 25 ml of acetonitrile was added and kept over water bath to reflux for 2 h. After the solubilization, the hot solution was slowly cooled to room temperature. The solution was filtered and the filtrate was kept at room temperature until to form well defined crystals of Prulifloxacin (P-1). The obtained crystals were collected by vacuum filtration, dried for 24 h in desiccators and stored.

Other polymorphic forms of Prulifloxacin were prepared by the similar method using acetone (P-2) and dichloromethane (P-3).

Characterization of crystal forms

Differential scanning calorimetry (DSC)

The DSC thermo gram was obtained using a DSC-6300SSI Nano Tech and the temperature range of scan was set from 100 to 400 °C at a rate of 10 °C/min. The sample (50-100 mg) was purged under a flow of nitrogen at a flow rate of 50 ml/min. The exact peak temperature, melting point and heat of fusion was determined.

Powder x-ray diffraction (PXRD)

The powder x-ray diffraction patterns of the samples were recorded using a Ricnaku Miniflex 2C. The operating condition was as follows:

Target, cu, voltage 40kV, current 30 mA, receiving slit,0.3 mm, preset time, 0.60 s, scan speed 10 (deg/min), sampling pitch 0.1 °. The divergent slit and scatter slit 1° and auto slit were not used.

Fourier transforms infrared spectrometry (FTIR spectra)

The FTIR spectra of prepared crystals was recorded on a double beam IR spectrometer (Shimadzu) using the potassium bromide disk technique in the range of 400-4000 cm^{-1}. No polymorphic changes were observed to be induced by grinding or compressing Prulifloxacin raw material for sample preparation.

Solubility measurement

Each prepared crystal was weighed individually about 100 mg and placed in a 100 ml Erlenmeyer flask with a stopper. Phosphate buffer pH 6.8 was added in each flask and mechanically shaken at a rate of 80 strokes min^{-1} for 20 h. An aliquot (1 ml) of each solution was withdrawn and filtered through a 0.45 m Millipore filter. The solubility of each polymorphic form was determined by measurement of the absorbance at 272 nm using UV spectrophotometer.

Scanning electron microscopy (SEM)

Scanning electron microscopy study was performed to characterize the surface morphology or the crystals. The morphology of various crystals was investigated by the use of a Jeol JSM-6100 instrument. The samples were mounted on a metal stub with an adhesive and coated under vacuum with gold.

Dissolution study

The *in-vitro* dissolution study was carried out using eight stations of LAB INDIA DISSO 2000 dissolution test apparatus. The samples of crystals (100 mg) was prepared by direct compression and will be placed in the dissolution medium of phosphate buffer (pH 6.8) at 37±2 °C with a constant speed of agitation at 100 rpm using USP type II (paddle). The fixed volume (1 ml) of the sample was withdrawn (with replacement) at 15, 30, 45, 60, 120 and 240 min time intervals and diluted approximately. The percentage drug release was determined by VU spectroscopy method using 272 nm.

Stability studies

Accelerated stability study of these three crystals was carried out as per the ICH guidelines [7]. The stability samples were (n=3) kept at 40±2 °C and 75±5% RH in stability testing chamber (LabTop Mumbai, India) for a period of 30, 60, 90 d. After completion of above said period, samples were taken out and analyzed for drug content.

RESULTS AND DISCUSSION

Differential scanning calorimetry

The DSC curves (fig. 2) of all prepared crystal form showed endothermic peaks at 224.01 °C, 226.43 °C, 222.33 °C and 220.91 °C for amorphous form of drug P-0, crystals of P-1, P-2 and P-3 respectively. The exothermic peaks were obtained at 268.37 °C, 266.78 °C, 267.74 °C, 265.33 °C for P-0, P-1, P-2 and P-3 respectively. From endothermic peaks obtained in the DSC curves of crystalline forms of Prulifloxacin, they differ in the melting point from an amorphous form which indicated that each form exhibited different crystalline form with characteristic crystal surface. Higher melting point (from endothermic peak) of the P-1 form of polymorph indicated that it was more stable than amorphous form and other crystalline forms.

Fig. 2: DSC graphs for Prulifloxacin (P-0) and its polymorphs (P-1, P-2, P-3)

Table 1: X-ray diffraction data for Prulifloxacin (P-0) and its polymorphs (P-1, P-2, P-3)

P-0		P-1		P-2		P-3	
2θ	**I%**	**2θ**	**I%**	**2θ**	**I%**	**2θ**	**I%**
6.99	218	11.90	303	11.20	40	11.30	82
8.65	40	12.40	301	11.50	84	11.70	193
13.80	68	14.11	163	14.50	89	14.06	216
15.29	160	15.18	52	15.10	57	15.16	57
18.66	198	18.60	48	19.24	17	18.57	80
22.46	44	22.90	273	22.78	179	22.90	415
27.76	212	27.00	189	27.30	73	27.60	117
30.63	171	31.00	43	30.33	59	31.20	25
33.96	173	33.89	127	33.00	26	33.77	112
37.80	73	37.82	114	37.56	38	38.92	25
39.18	45	40.43	47	38.00	18	40.21	44
43.70	44	44.82	43	43.40	23	43.61	44
45.07	55	45.78	82	45.78	24	45.61	36
49.86	40	51.01	104	49.03	18	49.75	31
55.42	40	55.32	46	54.73	19	50.95	25
58.42	59	58.46	66	58.19	23	58.24	41
77.50	64	77.44	55	77.38	119	77.44	94

X-ray powder diffraction

It showed different distinction in the position of the peak (table 1), clearly indicating different crystal lattice. The presence of new peaks at 11.90 °, 31.00 ° and 51.01 ° in P-1 made it different from the commercial sample. A few additional peak also appeared in P-2 (at 11.20 °, 19.24 ° and 38.00 °) and in P-3 (at 31.20 °, 38.92 ° and 50.95 °) suggesting them to be new crystalline forms. Besides the difference in the position of 2θ values, the peak intensity counts were also different in all the forms. All these forms showed well resolved diffraction patterns with various characteristic peaks, hence it confirmed that the polymorph of Prulifloxacin P-1, P-2 and P-3 (fig.

3) were properly obtained. Appearance and disappearance of some peaks on the XRD spectrum represented the complete formation of crystals of P-1, P-2, P-3 with different crystal lattice from amorphous form which was successively achieved by cooling crystallization method.

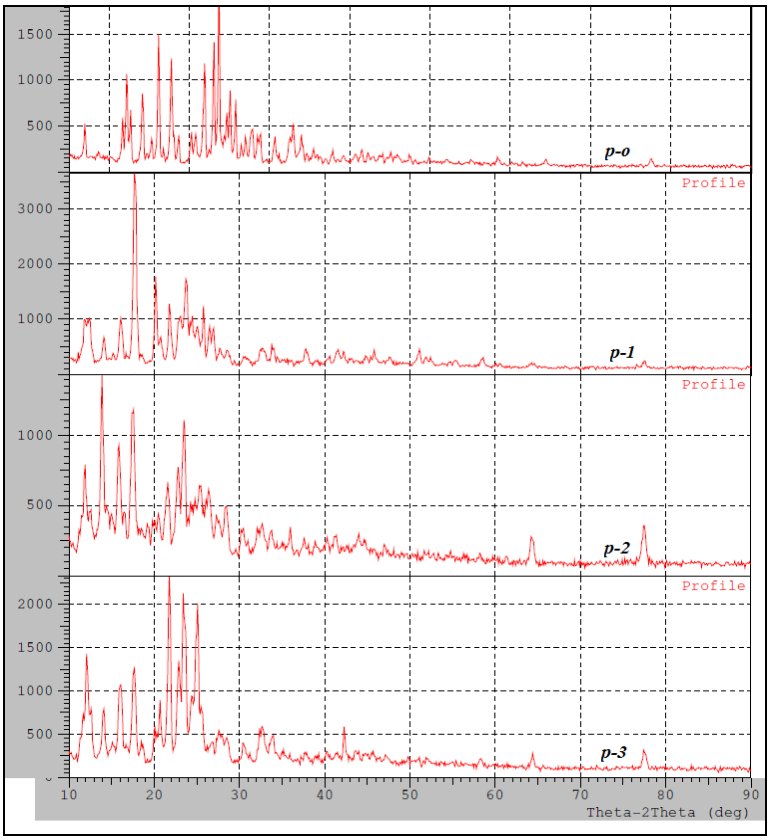

Fig. 3: X-ray patterns for Prulifloxacin (P-0) and its polymorphs (P-1, P-2, P-3)

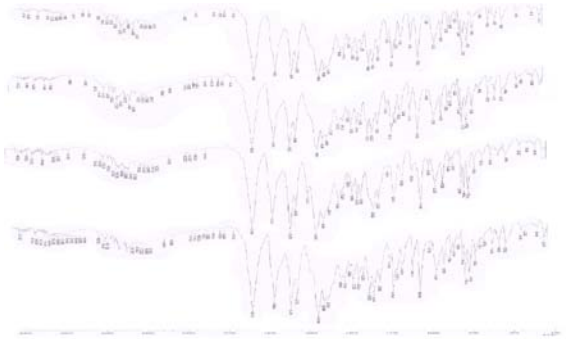

Fig. 4: Comparison of IR spectrum of crystals (P-1, P-2, P-3) with amorphous form P-0

Fourier transforms infrared spectrometry (FT-IR spectra)

The IR Spectra of the three crystal forms were presented in fig. 4. The strong bands due to C-F stretching were appeared between 1250 and 1100 cm^{-1}. The peaks of crystals of P-1, P-2, P-3 showed at 1157.33, 1130.32, 1118.75 cm^{-1} respectively. The N-H stretching showed the peaks ranges from 1400-1300 cm^{-1} and P-1, P-2, P-3 showed peaks at 1348.29, 1377.72, 1386.16 cm^{-1}. There was not much significant difference in the absorption band due to N-H stretching vibration. The broad bands of COOH group have peaks in range of 3000-2800 $cm^{-1}$and peaks for P-1, P-2, P-3 appeared at 2976.26, 2972.40, 2762.16 cm^{-1}. From IR spectrum of amorphous and polymorphs, it revealed that there was no alteration in the characteristic peaks of functional groups which indicated there was no interaction between the drug and solvents during the crystallization process.

Solubility

The solubility values of the three forms of P-1, P-2, P-3 were 0.65, 0.85, 0.50 mg/ml respectively. The differences in solubility of the three forms were due to their different polymorphic nature and this study revealed that P-2 form has more solubility.

Scanning electron microscopy

SEM photographs of three polymorphic forms of Prulifloxacin were shown in fig. 5. These clearly demonstrated the difference in the morphology of each crystalline form. P-1, P-2 and P-3 crystals were plate, rod or needle and prismatic shaped particles respectively which clearly indicating the formation of different typed crystals. The particles present in the pure drug have irregular shape and also have smaller particle size. Based on the photographs obtained from scanning electron microscopy, homogeneity has observed in the surface of each crystalline form with different particle size. The formation of regularly shaped particles of each crystalline form indicated that the amorphous form has been converted in to polymorphs.

Dissolution studies

Fig. 6 showed the dissolution profile of all the three crystalline forms and amorphous form in pH 6.8 buffer solution. After 240 min, the cumulative percentage drug release of each form was calculated by the use of calibraion graph obtained at 272 nm (fig. 7). Increasing order of drug release was as follows P0<P3<P1<P2, the values of % drug release were 51.06%, 58.40%, 72.80%, and 89.40% respectively. Due to more solubility of P-2 form showed highest percentage drug release. From solubility and dissolution studies,

polymorph (P-2) obtained from acetone showed highest cumulative percentage drug release when compared to others.

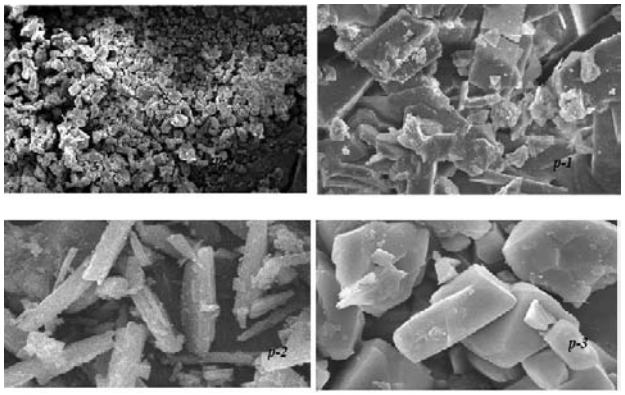

Fig. 5: SEM photographs of Prulifloxacin (P-0) and its polymorphs (P-1, P-2, P-3)

Stability studies

All the crystals were screened for accelerated stability studies and did not show any physical changes during the study period. The drug content were observed (n=3) for all the crystals and results were tabulated in table 2, hence they were quite stable at accelerated storage conditions. The stability of each crystalline form was proved by determining the percentage content under the above said an accelerated storage condition. Values nearly 100 % indicated that all the polymorphic forms were stable without any alteration on the physical characters.

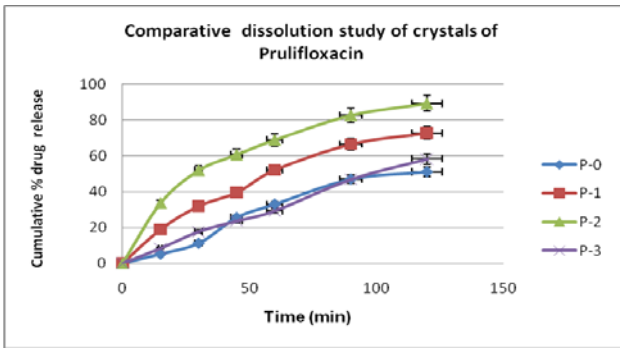

Fig. 6: Graph for comparative dissolution study data (P-0 =amorphous form, P-1 = polymorph 1, P-2 = polymorph 2, P-3 = polymorph-3) (*n=4)

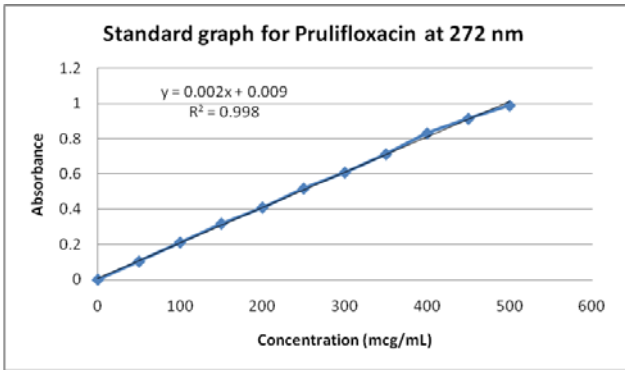

Fig. 7: Standard graph for Prulifloxacin (λ max at 272 nm)

Table 2: Stability studies data

Crystal forms	Percentage drug content* (%)		
	After 30 days	After 60 days	After 90 days
P-1	99.35±0.2291	98.94±0.4007	98.69±0.2651
P-2	99.42±0.1553	98.86±0.1652	98.38±0.1997
P-3	99.52±0.2502	98.88±0.4804	98.63±0.2022

(*n=3)

CONCLUSION

The drug was found to exist in three forms depending upon the crystallizing solvents. All the results obtained from different studies can be very well correlated for all forms prepared. The Prulifloxacin crystals obtained from solutions with acetonitrile, acetone, and dichloromethane have the polymorphic form of P-1, P-2, and P-3. The solubility, PXRD, IR, DSC, SEM and dissolution rates of the different polymorphic forms were differed in their studies. It was concluded that all the polymorphic forms were stable and they had more solubility and percentage drug release than pure form, hence it may leads to enhance the absorption and bioavailability of drug. If this process can be scaled up to pre-clinical study for further to develop in to an invaluable technology in future.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Raul P, Venugopalan P. Polymorphism: an overview. J Sci Edu Resonance 2009;14(9):882-983.
2. Mange RY, Anwar RS, Ganesan V, Rajani G, Renu C. Studies on the crystal forms of Pefloxacin: Preparation, characterization and dissolution profile. J Pharm Sci 2008;97(7):2637-48.
3. Barbas R, Proens R, Cristina P. A new polymorph of Norfloxacin completes characterization and relative stability of its trimorphic system. J Therm Anal Calorim 2007;89(3):687-92.
4. Veerendra K, Nanjwade, Manvi FV, Shamrez AM, Meenaxi MM, Basavaraj K, *et al*. Development and characterization of novel pharmaceutical crystalline complex of Lomefloxacin. Int J Drug Dev Res 2012;4(1):227-33.
5. Antinio L, Jonathan C, Burley, Timothy J, Prior, Robert C, *et al*. Concomitant hydrate polymorphism in the precipitation of Sparfloxacin from aqueous solution. J Crystal Growth Design 2008;8(1):114-8.
6. Hiroaki K, Chisa W, Reimei M, Hideo H. Effect of dehydration on the formation of Levofloxacin pseudopolymorphs. Chem Pharm Bull 1995;43(4):649-53.
7. Nighute AB, Bhise SB. Preparation and evaluation of microcrystals of Cefuroxime axetil. Int J Pharm Tech Res 2009;1(3):424-30.