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Comparative in Vivo Evaluation of Delayed Release Pellets and Oral Solution Containing Fenofibric Acid

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***Abstract*—** *Fenofibrate is a third-generation fibric acid derivative indicated for the treartment of primary hyper-lipidemia or mixed dyslipidemia. Fenofibric acid delayed release (DR) pellets were successfully developed and optimized using wurster process. The present investigation aimed to evaluate the pharmacokinetic properties of Fenofibric acid DR pellets using rabbits as animal model. Blood samples were collected at various time intervals. The plasma concentration of Fenofibric acid was estimated by ultra-high performance liquid chromatography (UHPLC-UV) method. The pharmacokinetic parameters were calculated from the plasma concentration of Fenofibric acid vs. time profile. The sustained Tmax, lower Cmax and prolonged Mean residence time (MRT) indicate an delayed extended release of Fenofibric acid DR pellets compared to Fenofibric acid (as choline salt) API. From the obtained results, it is concluded that the test formulation was provided extend the delivery of Fenofibric acid in desired rate*.

***Keywords*—** *Delayed release; Fenofibric acid; formulation variables; Pellets; Pharmacokinetics; process parameters.*

I. INTRODUCTION

Pharmaceutical invention and research are increasingly focusing on delivery systems which enhance desirable therapeutic objectives while minimizing side effects. Now a days, the multiparticulate drug delivery systems are notably relevant for attaining controlled or delayed release oral formulations with reduced risk of local irritation, low risk of dose dumping, increased bioavailability as well as reproducible and short gastric residence time.

Multiple-dose units have many kinetic and therapeutic advantages over single-dose sustained release units, such as improved bioavailability, easy administration, reproducible gastric residence time, low risk of dose dumping, low intra and inter subject variability, flexibility of blending of different release profile and divided into various dose strengths without formulation changes. The most commonly used pelletization techniques are Suspension/solution layering, extrusion spheronization and powder layering. However, suspension/solution layering (Wurster) technique is most preferable in the pharmaceutical industry owing to its advantages like continuous process, less manual interruption and batch to batch reproducibility [1-3].

Fenofibrate is a third-generation fibric acid derivative indicated for the treartment of primary hyper-lipidemia or mixed dyslipidemia. Fenofibrate is a prodrug and requires enzymatic cleavage via first pass metabolism, hydrolysis at the ester bond to form fenofibric acid, which is the active metabolite. Insolubility of fenofibrate in water was negatively impact the in vivo performance of the product. Hence, novel fenofibrate formulations were developed with different approaches to overcome the challenges with solubility, to prevent the recrystallization of drug in acidic pH and to improve bioavailability. Choline fenofibrate is a newly developed choline salt of fenofibric acid and is more hydrophilic than fenofibrate. It does not require first pass hepatic metabolism to become active, as it dissociates to free fenofibric acid within the gastrointestinal tract and rapidly absorbed throughout the gastrointestinal tract [4].

Impact of various formulation variables and critical process parameters on Fenofibric acid DR pellets were statistically interpreted and significant variables were optimized in our earlier investigation [5-6]. The present study aimed to evaluate the pharmacokinetic parameters of optimized formulation of Fenofibric acid DR pellets.

II. MATERIALS AND METHODS

Materials

Choline fenofibrate was obtained from RA CHEM Pharma Ltd., Hyderabad as gift sample, Sugar spheres (Arun pharma, Hyderabad), Povidone (BASF, Mumbai), Polyethylene glycol (Clariant, Hyderabad), Hypromellose (Dow chemical's, Mumbai), Ethocel 45 cps (Colorcon, Goa), Eudragit L 30 D55 (Evonik), Triethyl citrate (Merck, Mumbai), Talc (Luzenac, Mumbai), Isopropyl alcohol (Avantor, Hyderabad), Purified water and empty hard gelatin capsule shells size 0 (ACG, Hyderabad) were used as received.

Methods

Preparation of Fenofibric acid DR Pellets

Fenofibric acid DR Pellets were prepared by employing bottom – spray fluid bed (Wuster) coating process (Glatt GPCG 1.1). The dosage form was designed to obtain the delayed extended release. Drug loaded pellets were prepared by spraying the aqueous drug dispersion over non pariel seeds (Sugar spheres (20#- 25# ASTM)) employing wurster process (Bottom spray fluid bed coating technology). The drug dispersion was coated on to sugar spheres using 1.0 mm of spray nozzle with a spray rate of 2-6 g/min, 0.8-1.2 Kg/cm^2 of atomization air pressure, 50-65 cfm of air volume and product

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temperature 37-43°C. The drug dispersion was sprayed until get desired weight gain. The drug loaded pellets were dried for 10 minutes at 37-43°C. Hydro alcoholic (IPA : Water 80:20) ER coating solution was coated over the drug loaded pellets using wurster process at a spray rate of 4-8g/min & 34-38°C as product temperature. The ER coated pellets were dried for 15 minutes at 34-38°C. Further, the aqueous enteric coating dispersion was coated on to the ER coated pellets at 28-32°C as product temperature and at a spray rate of 2-6g/min. Enteric coated pellets were subjected for drying at 35°C for 15 minutes. Final pellets were sifted through #14-#18 ASTM mesh to separate the fines and agglomerates and collect the desired portion. The composition of the optimized formula described in Table I.

TABLE I. Composition of the Fenofibric acid DR Pellets.

S.No.	Composition	mg/ Capsule
I	**Core**	
1	Sugar Spheres (#25-#30)	139.7
II	**Drug loading**	
2	Choline Fenofibrate	178.53
3	PVP K 30	13.97
4	Polyethylene glycol 6000	1.4
5	Purified water	Q.S
III	**Extended release coating**	
6	Ethylcellulose	8.38
7	Polyethylene glycol 6000	1.68
8	Hypromellose	0.83
9	Isopropyl alcohol	Q.S
10	Purified Water	Q.S
IV	**Enteric coating**	
11	Methacrylic acid copolymer (Eudragit L 30 D 55)	93.10
12	Triethyl citrate	18.62
13	Talc	9.31
14	Purified Water	Q.S
	Total	**465.50**

Evaluation of Fenofibric acid DR Pellets

Assay

Fenofibric acid drug loaded pellets equivalent to 135mg of Fenofibric acid were transferred into 100mL volumetric flask, added 70mL of methanolic NaOH and sonicated for 15minutes with intermittent shaking. Made up the volume with methanolic NaOH. The solution was filtered through 0.45µ nylon membrane filter. Transfer 5mL of this solution into a 50mL volumetric flask and made up the volume with diluent (Acetonitrile:pH 2.5 buffer = 700:300). The solution was filtered through 0.45µ nylon membrane filter.

The following chromatographic conditions were employed for analysis:

Column: Kromosil 100, C18, 250 x 4.6 rnm, 5 pm or equivalent.

Injection volume: 20µL

Flow rate: 1.0 mL/min.

Detector: UV, 286nm

Run time: 10 minutes

Calculations:

Assay of Fenofibric acid:

$$= \frac{A_T}{A_S} \times \frac{W_S}{25} \times \frac{5}{50} \times \frac{100}{W_T} \times \frac{50}{5} \times \frac{100}{LC} \times P \times 0.756$$

Where,

A_T = Peak area of Choline fenofibrate obtained from the Sample Solution.

A_S = Average Peak area of Choline fenofibrate obtained from the standard Solution

W_S = Weight of Choline fenofibrate working standard taken in mg

W_T = Weight of sample taken in mg

P= Potency of Choline fenofibrate working standard used (on as is basis)

LC = Label claim

0.756 = Mol. Wt of fenofibric acid/ Mol. Wt of Choline Fenofibrate

In vitro drug release studies

The Fenofibric acid DR pellets equivalent to 135mg Fenofibric acid were accurately filled into size 0 hard gelatin capsules and evaluated for in vitro drug release studies, which were performed using USP Type II dissolution test apparatus. The stirring speed of 50 rpm, and the temperature was maintained at 37°C±0.5°C. These conditions were kept constant for all dissolution studies. The study was carried out in 500 mL of 0.05M sodium phosphate buffer pH 3.5 for 120min followed by 900 mL of 0.05M sodium phosphate buffer pH 6.8 for 30, 60, 90, 120, 240, 360 and 480min. 10ml of sample was withdrawn periodically and replaced with equal volume of fresh dissolution medium [7]. The collected samples were filtered through 0.45µ nylon membrane filter and analyzed to assess the % drug dissolved by employing same chromatographic conditions as that of assay.

The % labeled amount of Choline fenofibrate dissolved at respective time intervals (Dn) was estimated from following formulae:

Acid stage:

$$= \frac{A_T}{A_S} \times \frac{W_S}{50} \times \frac{3}{100} \times \frac{500}{W_T} \times \frac{100}{LC} \times P \times 0.756$$

Buffer stage:

$$= \frac{A_T}{A_S} \times \frac{W_S}{50} \times \frac{3}{100} \times \frac{900}{W_T} \times \frac{25}{5} \times \frac{100}{LC} \times P \times 0.756$$

Where,

A_T = Peak area of Choline fenofibrate obtained from the Sample Solution.

A_S = Average Peak area of Choline fenofibrate obtained from the standard Solution

W_S = Weight of Choline fenofibrate working standard taken in mg

W_T = Weight of sample taken in mg

P = Potency of Choline fenofibrate working standard used (on as is basis)

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0.756 = Mol. Wt of fenofibric acid/ Mol. Wt of Choline Fenofibrate

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Drug release kinetics

The drug release kinetics and mechanism from the formulations were studied by fitting the data obtained from the in vitro drug release study into several mathematical equations [8].

Stability studies

The optimized frmulation of Fenofibric acid DR pellets were filled into hard gelatin capsules and subjected for stability studies according to guidelines of international conference of harmonization (ICH), at an accelerated (40ºC/75%RH) and long term (25ºC/60%RH) stability conditions [9].

In-vivo evaluation of Fenofibric acid DR capsules

Pharmacokinetic parameters

Parallel design was selected for the study. Healthy albino male rabbits of weighing (2.0-2.5Kg) were divided into 2 groups, each consisting of 6 animals. Reference (Fenofibric acid (as choline salt) API, as solution) and Test formulation (Fenofibric acid DR pellets, which were filled into capsules of around 17mg to mimic the actual dosageform) were given orally via silicone rubber gastric intubation tube to the first group and second group respectively. All the rabbits were housed in individual cages at room temperature, fasted prior to the 12 hours of drug administration and have access to water and food after 4 hours of dosing throughout the study period. The study was approved by the Institute Animal Ethical Committee (IAEC) with IAEC.1032/PO/Re/s/07/CPCSEA/002/2015.

Blood samples of approximately 2 mL were collected through marginal ear vein at Pre dose, 0.5, 1, 2, 4, 6, 8, 10,12, 18, 24 and 28 hours.All the blood samples were collected into K_2EDTA coated tubes. Samples were centrifuged at 4000 rpm for 5 minutes. A simple protein precipitation method was used for the extraction of Fenofibric acid from the rabbit plasma samples. Ultra-high performance liquid chromatography (UHPLC-UV) method method was used for estimation of Fenofibric acid form plasma [10]. Pharmacokinetic parameters such as T_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and Mean residence time (MRT) of Fenofibric acid were estimated by non-compartmental method using Win-Nonlin version 6.2 (Pharsight Corporation, Mountain View, CA).

III. Results and Discussion

Preparation of pellets

Fenofibric acid DR pellets were prepared by employing wurster process. The prepared pellets were evaluated for stability stuides and in vivo studies.

Evaluation of pellets

The results obtained from the micromeritic properties indicated that optimized formulation shown good flow properties according to USP limits, which are crucial to attain uniformity of dosage units while capsule filling. Assay of the formulation was observed as 98.7%. Drug release profile of the optimized formulation was comparable with the marketed formulation (Fig. 1) and the similarity factor (F_2) was found as 71. The dissolution data fitted into various kinetic models, the obtained results concluded that the drug release followed the first order kinetics as r^2 values were higher for first order model (0.954) than zero order model (0.847). The n value is greater than 0.45 (0.580); hence the mechanism of drug release was non-fickian diffusion.

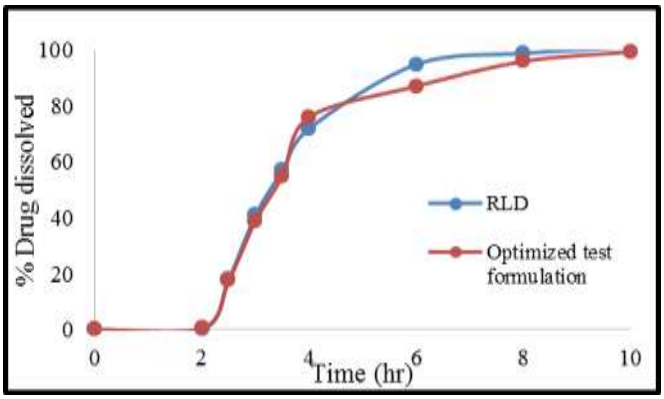

Fig. 1. Dissolution profile of the Reference listed drug (RLD) vs. Optimized test formulation.

Stability studies

Results obtained from the stability studies (Table II & III) shown that there was no significant change in description, assay, and dissolution profile at both accelerated and real time stability conditions for 6 months

TABLE II. Stability results of optimized formulation at Accelerated (40°C ± 2° C & 75%RH) storage conditions.

Parameter	Testing Frequency				
	Initial	1 Month	2 Month	3 Month	6 Month
Description	White to off-white spherical pellets filled in capsule				
Assay	98.7	98.9	98.5	98.2	98.0
Dissolution profile					
2	0.5	0.6	0.5	0.5	0.6
2.5	17.5 ± 2.4	17.8 ± 2.1	17.1 ± 2.6	17.7 ± 2.7	17.6 ± 2.5
3.5	55.0 ± 1.9	56.2 ± 1.4	55.8 ± 1.7	56.1 ± 1.2	56.3 ± 1.4
6	87.2 ± 1.8	87.8 ± 1.1	88.3 ± 0.9	88.1 ± 0.7	87.9 ± 0.9

TABLE III. Stability results of optimized formulation at real time (25°C ± 2°C & 60%RH) storage conditions.

Parameter	Testing Frequency		
	Initial	3 Month	6 Month
Description	White to off-white spherical pellets filled in capsule		
Assay	98.7	99.1	98.9
Dissolution profile			
2	0.5	0.5	0.5
2.5	17.5 ± 2.4	17.2 ± 2.7	17.9 ± 2.1
3.5	55.0 ± 1.9	56.1 ± 1.7	55.8 ± 1.3
6	87.2 ± 1.8	87.4 ± 0.9	88.1 ± 1.1

Pharmacokinetic parameters

The plasma concentration time profile of Fenofibric acid solution and Fenofibric acid DR capsules is depicted in Fig. 2. The pharmacokinetic parameters observed from test formulation and reference is represented in Table IV. The pharmacokinetic data subjected t-test and very significant

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differences ($p<0.05$) were noticed for T_{max}, C_{max}, AUC and MRT. From the obtained results, it was concluded that the test formulation exhibits better extension of the plasma concentration compared to drug solution (Reference).

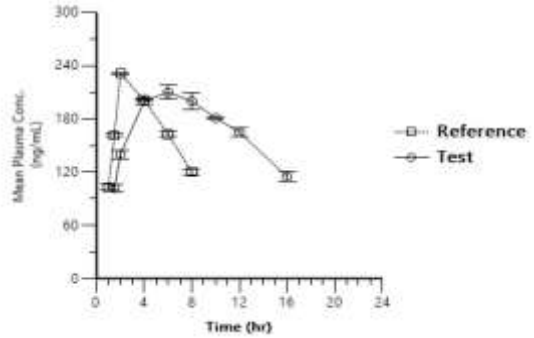

Fig. 2. Plasma concentration vs. Time profiles of Reference (Fenofibric acid solution) and (Test) Fenofibroc acid DR Capsules.

TABLE IV. Summary of pharmacokinetic parameters of Reference (Fenofibric acid solution) and Test (Fenofibroc acid DR Capsules).

S. No.	Pharmacokinetic Parameters	Reference	Test
1	C_{max} (ng/ml)	230 ± 3.03	210 ± 5.22
2	T_{max} (h)	2.00 ± 0.0	6.00 ± 0.0
3	AUC_{0-t} (h*ng/ml)	1300 ± 13.44	2600 ± 70.24
4	$AUC_{0-\infty}$ (h*ng/ml)	2200 ± 93.24	4200 ± 273.90
5	MRT (hr)	4.10 ± 0.05	8.15 ± 0.00

IV. CONCLUSION

Fenofibric acid delayed release pellets were successfully fabricated by fluid bed coating technology. The physico chemical properties are satisfactory. The drug releasse profile is similar to the marketed formulation. The pharmacokinetic parameters shown that the optimized formulation exhibits extended plasma concentration. As the optimized formulation is stable and biavailable, it can be used as an alternative to the marketed formulation.

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