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Formulation development and *In Vivo* evaluation of Fexofenadine HCl solid dispersions by spray drying technique

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ABSTRACT

The applicability of the solid dispersion technique as a method for enhancing the GI absorption of a drug has been explored in order to achieve better dissolution characteristics and better bioavailability for poorly soluble drugs. Fexofenadine hydrochloride is an anti-histaminic agent used in the treatment of rashes and other allergic reactions. The objective of the present work is to improve the oral bioavailability of the poorly permeable Fexofenadine hydrochloride by solid dispersions using spray drying technique. Three formulations (F1, F2, F3) were prepared using Pluronics (Poloxamer188, Poloxamer407 and Cremophor RH 40) as solubilizers, HPMC 5CPS and ethanol as a co-solvent. The prepared formulations were evaluated for compatibility studies by X-ray diffraction, Differential Scanning Calorimetry and Polarized light microscopy. They were then evaluated for drug content, in vitro dissolution studies and in vivo studies were conducted to evaluate the relative bio availability of the drug. XRD studies showed no incompatibility, DSC and PLM studies confirmed the conversion of the drug from crystalline to amorphous form. From the above formulations F1 showed the drug content of 102.4% which complied with the assay limits and a percentage cumulative drug release of 99% which was found the best from all the formulations. In vivo studies revealed that F1 showed 6 fold increases in the relative bioavailability when compared with the pure drug and hence it was considered as the optimized formulation.

Key words: Fexofenadine HCl, Solid dispersions, Pluronics, Spray drying, in vivo bioavailability studies.

INTRODUCTION

The improvement of the bioavailability of poorly water-soluble drugs is one of the greatest challenges of drug development [1]. Oral bioavailability of a poorly water-soluble drug was greatly enhanced by using its solid dispersion in a surface-active carrier [2]. Solid dispersions have been explored as potential delivery systems for many poorly water soluble drugs [3]. Solid dispersion systems have been realized as extremely useful tool in improving the solubility of poorly water-soluble drugs [4]**.** The use of solid dispersions to increase the dissolution rate and the bioavailability of poorly water-soluble drugs is now well established. Solid dispersions represent a useful pharmaceutical technique for increasing the dissolution, absorption and therapeutic efficacy of drugs in dosage forms [5]**.**

Fexofenadine hydrochloride is an anti-histaminic agent used in the treatment of rashes, hay fever, sneezing, rhinorrhea, urticaria, allergic rhinitis and hypersensitivity reactions with manifestations such as angioedema, dyspnea, flushing and systemic anaphylaxis [6].

The extents of absorption for poorly water-soluble drugs are affected by these efflux pathways [7]. Among the efflux transporters, the most well known and widely studied is the P-glycoprotein (P-gp) efflux transporters [8]. Pgp is a 170-kDa membrane transporter which is part of the ATP-binding cassette (ABC) [9]. However, hydrophobic drugs can be released from the micelles and are more likely to be transported by the efflux pumps [10]. The ABC transporters may reduce the amount of drug absorbed and limit bioavailability in a dose-dependent, inhibitable, and saturable manner [11]. Due to its ability to expel therapeutics, the presence of intestinal P-gp is associated with a decrease in oral bioavailability and is thought to be one of the most significant causes for decreased permeability and therefore oral bioavailability [12].

The aim of the present study was, therefore, to investigate the physical state of the drug in solid dispersions with various solubilizers by spray drying technique and to perform in vivo studies to analyse the improvement in the bioavailability of the optimized formulation.

MATERIALS AND METHODS

Fexofenadine HCl and HPMC 5 CPS was obtained from Dr. Reddy's laboratories, Hyderabad. Poloxamer 188, poloxamer 407, LutrolF 108, Cremophor RH 40 and Soluplus were obtained from BASF, Maharashtra, Gelucire 44/14 and 50/13 was obtained from Gattefose SAS, France. Ethanol was obtained from Hong Yang Chepael Coep, China. Methanol and isopropyl alcohol were procured from Merck Specialities Pvt .Ltd, Mumbai, India and other excipients used were analytical grade.

2.1 Animals

Male Wistar rats (weighing approximately 250±25 g) were procured from institutional animal house. The animals were maintained at a temperature of 25^0C and humidity 60% and were supplied with food and water. The study protocol was approved by the institutional animal ethics committee (IAEC), No: **P21/VCP/IAEC/2012/3/VVR/AE4/RARS.**

2.2 METHODS:

2.2.1 Preliminary Solubility Studies Fexofenadine HCl:

The solubility studies were conducted by using various solubilizers (Polaxomer-188, Polaxomer-407, Cremophor-RH-40, Soluplus, Gelucire-44/14, Gelucire-50/13, Lutrol-F108), co-solvents (Ethanol: water (1:2), Methanol: water (1:2), Isopropyl alcohol: water (1:2) were shown in **Table 1**. 1 gm of solubilizer was accurately weighed and taken in a conical flask and to this 100 ml water is added. 100 mg Fexofenadine hydrochloride pure drug was added to this solution and kept on rotary shaker for 48 hours at 150 rpm speed. After 48 hours of shaking the solution is filtered, 1ml of the filtrate is taken and diluted to 100 ml in volumetric flask with distilled water. This solution was filled in vials and was analyzed by HPLC.

Table 1: List of solubilizers and co-solvents used for study:

PURE DRUG	SOLUBILIZERS	CO SOLVENTS
Fexofenadine HCl	Polaxomer-188	Ethanol: water (1:2),
	Polaxomer-407	Methanol: water(1:2),
	Cremophor-RH-40	Isopropyl alcohol: water (1:2).
	Soluplus	
	Gelucire-44/14	
	Gelucire-50/13	
	Lutrol-F108	

2.2.2 Preparation of Fexofenadine HCl Solid Dispersions:

Two parts of solubilizer was melted at its melting point and 1 part of drug was added to it and mixed. To this ethanol as a cosolvent was added till the clear solution appears. Then to the above mixture 100 mg/ml of HPMC 5 CPS solution was added and stir continuously for half an hour using mechanical stirrer until the mixture was clear and homogenous. Then the resultant solution (F1, F2 & F3) was processed for spray drying. The parameters of spray drying and composition of the formulations were depicted in **Table 2 & Table 3** respectively**.**

Table 2: Parameters are used for spray drying.

Sr. No	Parameters	Set value
1.	Inlet temperature	45 ºC
2.	Outlet temperature	30ºC
3.	Inlet High temperature	65 ºC
4.	Outlet high temperature	38 ºC
5.	Cool temperature	75ºC
6.	Aspiration speed	080m^3/hr
7.	Cycle time	245 min
8.	Oxygen	21.0%

Table 3: Composition for the different formulations of Fexofenadine HCl.

Ingredients (units)	F1 (%w/w)	F2 (%w/w)	F3 (%w/w)
Fexofenadine (gm)	20	20	20
Polaxomer-188 (gm)	40	-	20
Polaxomer-407 (gm)	-	40	-
Cremophor RH 40 (gm)	-	-	20
HPMC 5Cps (gm)	40	40	40
Ethanol (mL)	q.s	q.s	q.s
Distilled water	q.s	q.s	q.s

2.2.3 Drug content
The amount of drug present in 100 mg equivalent amount of solid dispersion was determined by using HPLC method and drug concentration was determined from standard graph.

2.2.4 Preparation of buffer solution:
Dissolve 1.0 g of monobasic sodium phosphate, 0.5 g of sodium per chlorate, and 0.3 mL of phosphoric acid in 300 mL of water with vortexing and is sonicated for 10 min.

2.2.5 Preparation of standard solution:
Dissolve an accurately weighed quantity of Fexofenadine HCl in water to obtain a solution having a known concentration.

2.2.6 Preparation of mobile phase:
Mixture of acetonitrile and buffer solution in the ratio (7:3), were taken and degassed using sonicator.

2.2.7 Preparation of test sample:
Accurately weighed 100 mg of Fexofenadine HCl Solid dispersion formulation was taken and dissolved in mobile phase in a beaker. This is taken in a volumetric flask and the volume is made to 100 ml with the mobile phase. From this 3.5 ml of solution is taken in a 10 ml volumetric flask and is made up to 10 ml with mobile phase. This solution was transferred into vials and injected into HPLC with optimized chromatographic conditions.

2.2.8 Chromatographic conditions:
The liquid chromatograph is equipped with a 257-nm detector and a 4.6mm × 10cm column that contains packing L_1 (C_{18} Column). The flow rate is 1.5 ml per minute.

2.2.9 *In Vitro* Drug Release Studies:
The in vitro dissolution studies were performed for filled capsules of pure drug and solid dispersion formulations by using Electro lab-USP type-II dissolution test apparatus, 0.001 HCl as dissolution medium, temperature was maintained at 37±0.5ºC and RPM was adjusted at 50.

The samples are drawn at specified time intervals like 5, 10, 20, 30, 45, 60 minutes and the obtained samples were analyzed by using Waters HPLC at 257 nm. The cumulative percentage drug release was calculated.

2.2.10 X-RAY Diffraction Studies:
Initially (500 mg) each of Fexofenadine HCl pure drug, HPMC5CPS , poloxamer 188 and poloxamer 407 were placed in the crucible and analyzed by Bruker A6 advance PXRD instrument.

2.2.11 Differential Scanning Calorimetry:
A differential scanning calorimeter (Model DSCQ1000) was used to obtain the DSC curves representing the rates of heat uptake with respect to temperatures (40 to 200°C). About 3 mg of sample was weighed and placed in a standard open aluminium pan. An empty pan of the same type was utilized as the reference. Samples were heated from 40 to 200°c at a heating rate of 5°c /min, under dry nitrogen atmosphere.

2.2.12 Pharmacokinetic Study:
The pharmacokinetic characteristics for pure drug and solid dispersion of Fexofenadine were evaluated using twenty four healthy Wistar rats weighing 250±10 g used in the study. All rats were dosed following an overnight fast, food was returned 4 h after dosing. Rats were divided into four groups at random. First group was administered with Fexofenadine (as such) suspension which was prepared in 5% methocel, second group was administered with solid dispersion suspension formulation 1. Third group was administered solid dispersion suspension formulation 2. Fourth group was administered solid dispersion suspension formulation 3. Each animal received dose equivalent to 30 mg/kg of Fexofenadine in humans. Blood samples (approximately 0.5 ml) were obtained with syringes for every formulation and for pure drug at 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 7.00, 24.00, 52.00 hrs post dose. Plasma was separated by centrifugation of the blood at 5000 rpm in cooling centrifuge for 5minutes and stored at −20°C until analysis.

2.2.12.1 Preparation of Plasma Samples for HPLC Analysis:
Rat plasma (0.5 ml) was processed for chromatography by precipitating proteins with 2.5 ml of ice-cold absolute ethanol for each 0.5 ml of plasma. After centrifugation the ethanol was transferred into a clean tube. The precipitate was resuspended with 1 ml of acetonitrile by vortexing for 1 min. After centrifugation (5000 – 6000 rpm for 10 min), the acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a stream of nitrogen at room temperature. Samples were reconstituted in 200 µ1 of mobile phase was injected for HPLC analysis.

2.2.12.2 Pharmacokinetic data analysis for solid dispersions and pure drug:
The area under the drug concentration-time curve from zero to 52 h (AUC) was calculated using the trapezoidal rule. The maximum plasma concentration of the drug (C_{max}) and the time to reach C_{max} (T_{max}) were obtained directly from the plasma profiles.

The relative bioavailability (BA) of the solid dispersion to the reference (pure drug suspension) was calculated as follows:

$$\text{Relative Bio Availability (\%)} = \frac{AUC_{test}}{AUC_{reference}} \times \frac{Dose_{reference}}{Dose_{test}}$$

Where, AUC_{test} and $AUC_{reference}$ are AUCs obtained after the oral administration of the solid dispersion formulation and the reference (pure drug suspension), respectively. $Dose_{test}$ and $Dose_{reference}$ are the doses of the two products.
The pharmacokinetic parameters were analyzed by a non compartmental analysis using Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA). All values are expressed as the mean±SD. Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Statistical parameter $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

3.1 Preliminary Solubility Studies Fexofenadine HCl:
The solubility studies for the solid dispersion samples were carried out in Waters HPLC and the results are shown in **Table 4 & Figure 1.** Solubility studies were conducted for all the solubilizers mentioned and all the solubilizers except Poloxamer 188, Poloxamer 407 and Cremophor RH 40 as they gave turbid solutions on adding HPMC 5 CPS (10%). So these solubilizers were chosen for further studies.

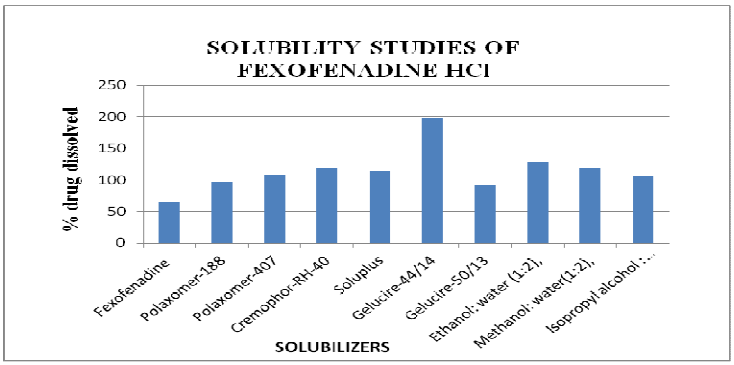

Figure 1: Comparison of solubility of Fexofenadine HCl in different solubilizers

Table 4: Solubility studies of Fexofenadine HCl in different polymers

Solvents used	Amount of drug dissolved (%w/v)
Pure Drug	65.2%
Polaxomer-188	97.25
Polaxomer-407	108.35
Cremophor-RH-40	118.52
Soluplus	113.50
Gelucire-44/14	198.60
Gelucire-50/13	92.34
Ethanol: water (1:2),	128.17
Methanol: water(1:2),	118.85
Isopropyl alcohol: water (1:2).	106.83

3.2 Evaluation of Fexofenadine HCl Solid Dispersions:

Table 5: Results of the assay of the formulations F1, F2, F3

S. No	Trials	Ingredients	% assay
1	I	Fexo+pola188+HPMC	102.5
	II	Fexo+pola188+HPMC	102.4
2	I	Fexo+pola407+HPMC	108.8
	II	Fexo+pola407+HPMC	108.8
3	I	Fexo+Cremo RH 40+HPMC	104.4
	II	Fexo+Cremo RH 40+HPMC	104.3

The percentage purity of the Formulations F1, F2, F3 were found to be 102.45, 108.8 and 104.35 respectively, results are depicted in **Table 5**.

3.2.1 *In vitro* Dissolution Studies Of Fexofenadine HCl Pure Drug and Solid Dispersions:

The samples are drawn at specified time intervals and the obtained samples were analyzed by using Waters HPLC at 257 nm. The cumulative percentage drug release of Fexofenadine HCl pure drug, formulation 1, 2 and 3 was shown in **Table 6, 7, 8 & 9** respectively.

Table 6: In vitro dissolution profile of Fexofenadine HCl Pure drug

Unit	Time in minutes					
	5	10	20	30	45	60
1	66	81	88	95	93	95
2	62	75	84	91	95	94
3	56	68	77	81	85	87
Average	61	75	83	89	91	92
SD	5.0	6.5	5.6	7.2	5.3	4.4
Min	56.0	68.0	77.0	81.0	85.0	87.0
Max	66.0	81.0	88.0	95.0	95.0	95.0
%RSD	8.2	8.7	6.7	8.9	5.8	4.7

Table 7: *In Vitro* dissolution profile of formulation 1

Unit	Time in minutes					
	5	10	20	30	45	60
1	68	82	89	93	97	100
2	70	80	90	94	99	100
3	69	83	90	96	98	98
Average	69	82	90	94	98	99
SD	1.0	1.5	0.6	1.5	1.0	1.2
Min	68.0	80.0	89.0	93.0	97.0	98.0
Max	70.0	83.0	90.0	96.0	99.0	100.0
% RSD	1.4	1.9	0.6	1.6	1.0	1.2

Table 8: *In Vitro* dissolution profile of formulation 2

Unit	Time in minutes					
	5	**10**	**20**	**30**	**45**	**60**
1	31	49	72	82	90	94
2	30	41	61	74	86	93
3	42	57	79	92	98	98
Average	34	49	71	83	91	95
SD	6.7	8.0	9.1	9.0	6.1	2.7
Min	30.0	41.0	61.0	74.0	86.0	93.0
Max	42.0	57.0	79.0	92.0	98.0	98.0
% RSD	19.7	16.3	12.8	10.9	6.7	2.8

Table 9: *In Vitro* dissolution profile of formulation 3

Unit	Time in minutes					
	5	**10**	**20**	**30**	**45**	**60**
1	29	40	64	82	93	95
2	39	53	74	84	90	90
3	39	54	75	86	92	93
Average	36	49	71	84	92	93
SD	5.8	7.8	6.1	2.0	1.5	2.5
Min	29.0	40.0	64.0	82.0	90.0	90.0
Max	39.0	54.0	75.0	86.0	93.0	95.0
% RSD	16.1	15.9	8.6	2.4	9.7	2.7

In vitro drug release study of formulations F1, F2, F3 prepared with Poloxamer-188, Poloxamer 407 and Cremophor RH 40 and the percent of drug release from the formulations F1, F2, F3 in the 60th min was found to be 99%, 95% and 93% respectively.

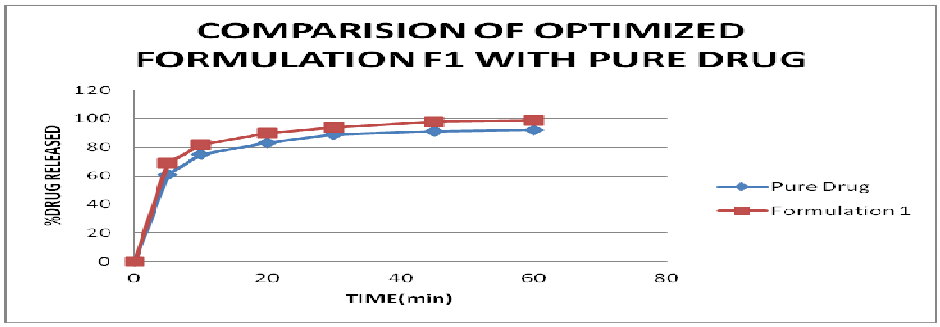

Figure 2: Comparison of *in vitro* drug release of F1 and the Fexofenadine HCl (pure drug)

From **Figure 2**, the better drug release was observed in formulation F1 than the pure drug.

3.3 X-RAY Diffraction Studies:

The Fexofenadine HCl solid dispersions were analysed in Bruker A6 advance PXRD instrument to find out whether the solid dispersions of various drug polymer ratios are crystalline or amorphous.

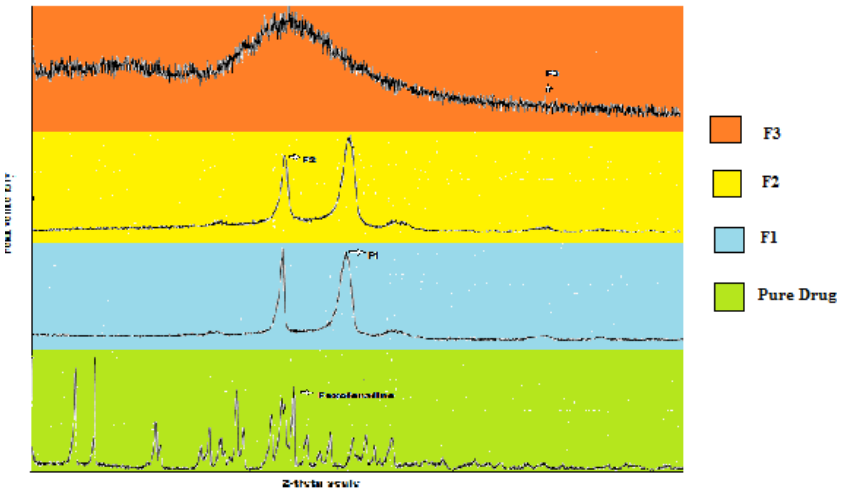

Figure 3: Powder X-ray diffraction patterns of pure drug and different formulations

From the above overlay shown in **Figure 3** it is evident that the drug is in crystalline form and so sharp peaks were observed. It can also be said that the drug has got converted to amorphous form but the peaks observed are due to the instability of Poloxamer 188 which was used in Formulation F1. It can be said that the drug has got converted to amorphous form but the peaks observed are due to the instability of Poloxamer 407 that was used in formulation F2. It is also observed that the drug has not got converted to amorphous form so no peaks were observed with Cremophor RH 40.

3.4 Differential Scanning Calorimetry (DSC):

Table 10: Glass transition temperature (Tg) of the polymer and formulations

Ingredient	Glass transition temperature
HPMC 5 CPS	149.20°C
Formulation F1	144.82°C
Formulation F2	148.29°C
Formulation F3	140.09°C

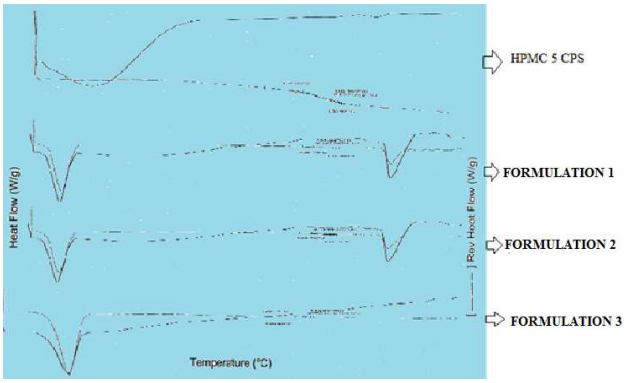

Figure 4: DSC thermo grams of pure drug and different formulations

On performing DSC for all the formulations of solid dispersions of fexofenadine HCl and the polymer the glass transition temperatures obtained were 149.20,144.82,148.29,140.09°C for the HPMC 5CPS, Formulation 1, Formulation 2, Formulation 3 respectively. So it can be concluded that all the formulations were converted to amorphous state, results are shown in **Table 10** & **Figure 4**.

3.5 Polarized Light Microscopy:
PLM photographic pictures

Figure 5: Fexofenadine

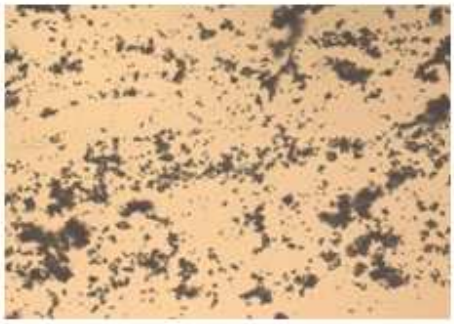

Figure 6: Formulation 1 HCl pure drug

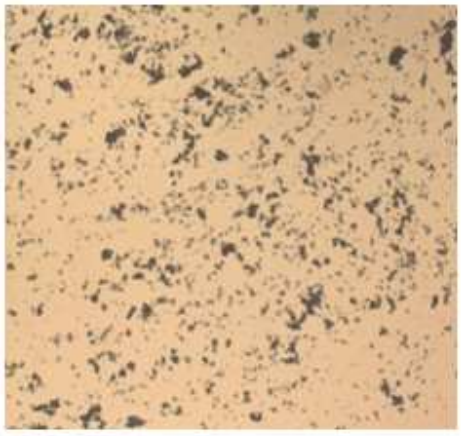

Figure 7: Formulation2.

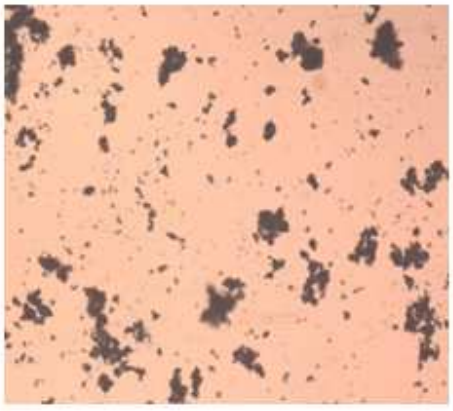

Figure 8: formulation 3

The photographs showed that the pure drug was crystalline in nature and the formulations (F1, F2, F3) were amorphous in nature shown in **Figure 5, 6, 7 & 8** of pure drug and the formulations 1, 2 & 3 respectively.

3.6 *In Vivo* Studies:

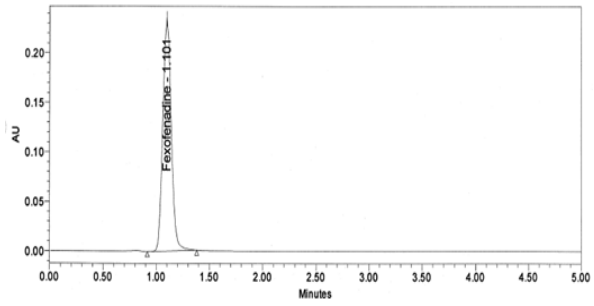

Figure 9: Standard HPLC chromatogram of Fexofenadine HCl

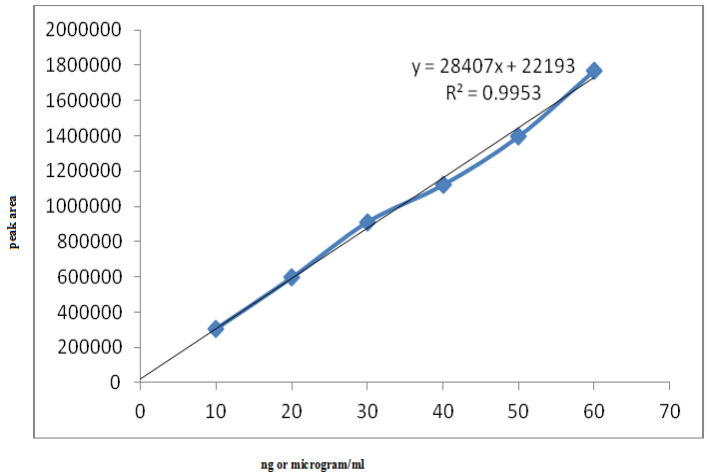

Figure 10: Standard graph

The Fexofenadine plasma concentrations in rats treated with solid dispersion formulation was significantly higher than those treated with pure drug suspension. Plasma pharmacokinetic parameters of Fexofenadine after oral administration of the three formulations to Wistar rats are shown in **Table 11**. The HPLC chromatogram of Fexofenadine HCl and standard graphs are shown in **Figure 9 & 10** respectively. Comparing of all three formulations with pure drug Fexofenadine, formulation 1 has showed significant values. Cmax of the solid dispersion formulation 1, 1.75 µg mL^{-1} was significant ($p<0.05$) as compared to the pure drug suspension formulation 0.52 µg mL^{-1}. Tmax of both solid dispersion formulation 1 and pure drug suspension was 2.83 and 1.50 h, respectively. AUC is an important parameter in evaluating bioavailability of drug from dosage form, as it represents the total integrated area under the blood concentration time profile and represents the total amount of drug reaching the systemic circulation after oral administration. $AUC_{0-\infty}$ for solid dispersion formulation 1 was higher (12.14µg hmL^{-1}) than the pure drug suspension formulation 2.65 µg hmL^{-1}. Statistically, $AUC_{0-\infty}$ of the solid dispersion formulation 1 was significantly higher ($p<0.05$) as compared to pure drug suspension. Higher amount of drug concentration in blood indicated better systemic absorption of Fexofenadine from solid dispersion formulation 1 as compared to the pure drug suspension**.**

Table 11: Pharmacokinetic Parameters of Fexofenadine pure drug and different formulations

Pharmacokinetic parameters	Fexofenadine Pure drug	Formulation 1	Formulation 2	Formulation 3
Dose (mg/kg)	30	30	30	30
C_{max} (µg/ml)	0.52	1.75	1.16	1.64
AUC_{0-t} (µg.hr/ml)	2.62	11.97	7.94	11.21
AUC_{0-inf} (µg.hr/ml)	2.65	12.14	8.05	11.38
T max (hr)	1.50	2.83	1.88	2.65
$t_{1/2}$ (hr)	4.22	9.52	8.72	9.32
K el (hr^{-1})	0.164	0.081	0.035	0.062

CONCLUSION

In the present investigation, three formulations were prepared by using different polymers like poloxamer 188, poloxamer 407 and cremophor RH 40 by spray drying technique with different ratios cosolvents. Based on the evaluation parameters formulation F1 was found to be optimized formulation. From DSC thermograms there was no evidence of interactions between drug and the used excipients. XRD studies revealed that the conversion of the drug from crystalline to amorphous form.

After oral administration of different formulations (F1, F2 & F3) and pure drug suspensions of Fexofenadine HCl (30 mg kg^{-1}) in male Wistar rats, formulation F1 showed superior absorption profile. The relative bioavailability of F1 solid dispersion formulation was also enhanced in comparison with pure drug and other formulations. It can be

concluded that the present study successfully illustrates the potential utility of solid dispersion formulation for the delivery of poor water-soluble compounds such as Fexofenadine.

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