https://doi.org/10.48048/tis.2023.4271

Formulation Development and Evaluation of Once Daily Fexofenadine Hydrochloride Microsponge Tablets

Ramani Gade[1,2,*], Sreekanth Nama[1] and Prameela Rani Avula[3]

[1]Department of Pharmaceutics, Priyadashini Institute of Pharmaceutical Education and Research, Guntur, Andhra Pradesh, India
[2]School of Pharmacy, Jawaharlal Nehru Technological University, Kakinada, Andhra Pradesh, India
[3]Department of Pharmaceutics, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India

([*]Corresponding author's e-mail: ramanigade169@gmail.com)

Received: 19 May 2022, Revised: 27 June 2022, Accepted: 19 July 2022, Published: 28 November 2022

Abstract

The objective of present study was to formulate and develop once daily Fexofenadine Hydrochloride tablets to improve the aqueous solubility by microsponge technology. Fexofenadine Hydrochloride is an antihistamine and belongs to BCS class-III with low permeability and poor bioavailability about 30 - 35 %. The microsponges were prepared by Quashi emulsion solvent diffusion method with Quality by design approach. The study of effect of independent variables Eudragit EPO (500 to 1,000 mg), internal phase volume (DM: ETH) (5 to 10 mL) and RPM (500 to 1,000) on responses were analyzed to optimize the formulation with desirable results by using central composite design and response surface method. Optimized formulation F22 showed percent production yield (99.10 %), percent drug entrapment (99.45 %), particle size (94.12 µm) and percent drug release of prepared tablets at 5, 10, 15 and 30 min were 60.6, 80.75, 87.47 and 92.85 %, respectively. It showed Higuchi mechanism of release kinetics by diffusion. *In vivo* pharmacokinetics of the prepared tablets was studied in rabbits (IAEC/NRML/2022–2023/09) with and without permeation enhancer to find the rate of absorption. when compared to Marketed product -Allegra 24 (F21) with (t1/2 absorption) 0.192711±0.00278 h and MRT 19.10608±0.257571 h and formulation without poloxamer 407 (F22) (t1/2 absorption) 0.165013±0.024164 h and MRT 19.32228±0.764531 h, maximum absorption was observed for the Formulation F23 with poloxamer 407 (15 %) with (t1/2 absorption) of 0.144662±0.006787 h and Mean residence time (MRT) of 24.10796±1.01232 h. Microsponge technique improved the aqueous solubility of the fexofenadine Hydrochloride and Eudragit EPO extended the mean residence time up to 24 h along with improved permeability in presence of Poloxamer 407 to full fill the needs of BCS class-III drug.

Keywords: Microsponge technology, BCS class-III drug, Eudragit EPO, Once daily, Quality by design, Central composite design, Response surface method

Introduction

Micro particulate drug delivery system offers incorporation of wide variety of drugs and feasibility to develop various dosage forms. So, micro particulate drug delivery gaining importance in the future pharmaceutical market. The loading, maintenance, control and extent of drug release is more effective with modified micro particulate drug delivery systems. Microsponge drug delivery system is a highly cross-linked, porous, non-collapsible, polymeric system consisting of porous microspheres of a myriad of interconnecting voids with improved thermal, physical and chemical stability [1].

The microsponges ranges in size from 5 - 300 µm in diameter. A typical 25 µm sphere can have up to 250,000 pores and an internal pore structure of 10 ft in length. It provides a total pore volume of about 1 ml/g for extensive drug retention. Microcapsules have the problem of burst drug release due to rupture of polymer coat. Liposome are difficult to formulate, lower payload to leaching, limited chemical stability and microbial instability due to fatty nature. We can overcome these problems with Microsponge drug delivery systems. [2,3]

Fexofenadine Hydrochloride is a 2nd generation, partially selective, peripheral H1 blocker used in allergic rhinitis and urticaria. The elimination half- life of the drug is (14.4 h) and 60 - 70 % undergo protein binding [4]. So, the oral bioavailability is 30 - 41 %. It belongs to class III of the biopharmaceutical classification system (BCS). Fexofenadine Hydrochloride has poor aqueous solubility

and low passive permeability. P-Glycoprotein efflux induced intestinal secretion is the reason for the incomplete absorption (35 %) following oral absorption. So, there is a need to increase bioavailability and maintenance of steady state plasma concentrations. Microsponge drug delivery system can fulfill the requirements by increasing the permeability and modifying the drug release from dosage form. [5,6]

Formulation development and optimization of pharmaceutical products is a time consuming and cost affective process. So, industries adopting Quality by design (QBD) approach as per ICH Q8 guidelines to resolve these problems. Quality by design (QBD) enables product development and optimization of experimental variables based on predetermined objectives to enhance product quality and understanding the process. RSM (response surface methodology) offers assessment of multi variants and their interaction on responses by using polynomial mathematical models. Central composite design, 3-level factorial design, Box-Behnken design and D-optimal designs are some of the kinds of RSM to optimize the experimental trails. Among them, Central composite design provides complete assessment and understanding of the process at each level of the variable. So, the present research work focused on the formulation and development of modified oral drug delivery system of Fexofenadine Hydrochloride microsponges by central composite design with enhanced bioavailability [7].

Materials and methods

Fexofenadine Hydrochloride, Eudragit EPO provided by Aurabindo pharmaceuticals, Hyderabad as gift sample. Ethanol, Poly vinyl alcohol, Poloxamer 407, Magnesium stearate, Avicel PH 102, cross povidone, Sod. Steryl fumerate and talc from SD fine chemicals, Mumbai, India.

Analytical method for the estimation of fexofenadine hydrochloride [8]

A reversed-phase high-performance liquid chromatographic (HPLC) method was developed and validated using the Schimadzu system for the determination of Fexofenadine Hydrochloride in pharmaceutical dosage forms and in rabbit plasma for pharmacokinetic studies. The analysis was performed on a Kromosil C 18 column with dimensions (250×4.6 mm, 5 µm particle size). The mobile phase consisted of a mixture of phosphate buffer solution (pH 2), acetonitrile and triethyl amine in the ratio of 65:35:0.3 v/v pumped at a flow rate of 1 mL/min. The ultraviolet detector was operated at 220 nm and sample run time was fixed at 10 min for data collection.

Table 1 Independent and dependent variables chosen in Central composite design by Response surface method.

S.NO	Independent variable	Level (–1) Low	Level (0) Medium	Level (+1) High
1	Eudragit EPO (mg)	500	1,000	1,500
2	Internal phase volume (DM:ME) mL	5	7.5	10
3	RPM	500	750	1,000
	Dependent variables	**Criteria**		
1	% Production yield	maximize		
2	% Drug entrapment	maximize		
3	Particle size (µm)	minimize		
4	% drug release @ 5 min	In the range		
5	% drug release @ 10 min	In the range		
6	% drug release @ 15 min	In the range		
7	% drug release @ 30 min	In the range		

Preparation of fexofenadine hydrochloride microsponges [9,10]

Fexofenadine Hydrochloride microsponges with Eudragit EPO were prepared by the quasi-emulsion solvent diffusion method. Eudragit EPO dissolved in the internal phase of Dichloromethane and ethanol (DCM: ethanol 1:1). 5 mL glycerin was added as plasticizer. 500 mg of Fexofenadine Hydrochloride was added to the above mixture and subjected to ultrasonication at 35 °C. 1 g of Poly vinyl alcohol (PVA) was dissolved in 100 mL of distilled water at 60 °C and cool down to room temperature which acts as external phase. The internal phase added drop wise to the external phase under stirring at RPM (Rotations per

min) range 500 - 1,000 for 4 h. Diffusion of internal phase into external phase and its evaporation leads to formation of microsponges. Then the microsponges were washed with distilled water for 3 times, filtered and dried in hot air oven at 40 °C for 12 h. The microsponges stored in airtight containers. The amount of drug, external phase volume and plasticizer concentration kept constant throughout the study based on preliminary studies. The compositions of Fexofenadine Hydrochloride microsponge are given in **Tables 1** and **2**.

Optimization of microsponge formulations [11]

The preparation of microsponges was systematically optimized by using Design Expert 11.0.1 software (Stat-Ease Inc., USA). The independent variables and responses were selected based on the extensive literature survey and preliminary experiments. The central composite design of Response surface method was used for the optimization of the independent variables such as the amount of Eudragit EPO, Internal phase volume (DM:ETH) in 1:1 ratio and Rotations per min (RPM) used for preparation of microsponge formulations. The independent variables were studied at 3 different levels such as low (−1), intermediate (0) and high (+1) and a total of 20 experimental trials were conducted. The obtained microsponges were evaluated for dependent variables such as % production yield, % drug entrapment, particle size and % cumulative drug release of compressed tablets at 5, 10, 15 and 30 min. The obtained results were analyzed statistically by Response surface method (RSM). The linear, quadratic and polynomial regression equation models were used to fit the data. The models were statistically analyzed by ANOVA, lack of fit and correlation coefficient. The optimized formulation was selected with desired goals for response variables by numerical optimization.

Evaluation of Fexofenadine Hydrochloride microsponges [12,13]

Differential scanning calorimetric (DSC) analysis

Thermal analysis by DSC was carried out on Fexofenadine Hydrochloride and optimized formulation. Samples were subjected to DSC analysis using Differential scanning calorimeter (Universal V4. 5A, TA Instruments, Netherlands). Indium was used to calibrate cell constant and temperature axis. An accurately weighed 4 mg samples were sealed in standard aluminum pans and scanned at a rate of 10 °C /min using dry nitrogen flow of 50 mL/min. Each sample was scanned between 100 and 300 °C.

Powder X-ray Diffraction (PXRD) analysis

The PXRD analysis of Fexofenadine Hydrochloride pure and microsponges tablet formulation blend obtained by XPERTPRO x-ray difractometer (PAN analytical Empyrean, Almelo, Netherlands) with Cu Kα radiation of wavelength 1.541585 A°. The divergence slit and anti-scattering slit were set at 0.5° for the illumination on 10 mm sample and the samples were analyzed at 2 θ angle over 5 - 50 °C/min, voltage of 45 kV and 45 mA current.

Production yield

Production yield of the microsponges measured in triplicates by the following formula

$$\% \text{ Production yield} = \frac{Practical\ yield}{Theorotical\ yield} \times 100$$

Entrapment efficiency

10 mg of dried drug loaded microsponges samples were crushed in mortar and pestle was dissolved in mixture of phosphate buffer solution (pH2), acetonitrile (65:35). Then the solution was added into 100 mL volumetric flask and made up the volume. The solution was filter through whatman filter paper and was analyzed by RP-HPLC method at 220 nm. The entrapment efficiency of loaded microsponges were calculated in triplicates by the following formula.

$$\% \text{ Drug entrapment efficiency} = \frac{Drug\ content\ of\ microsponges(Actual)}{Drug\ content\ of\ microsponges\ (Theorotical)} \times 100$$

Determination of particle size and porosity of microsponges

The particle sizes of produced microsponges were analyzed by optical microscopy. The instrument was calibrated to find out the value of 1 unit of eye piece. Sizes of 100 microsponges were analyzed in ×10. Mercury intrusion porosimetry (Autoscan 60, Quanta chrome, USA) was used to determine porous properties of optimized formulation in the pressure range 0 - 4,000 kg/cm^2. The sample of microsponges was placed in volume calibrated cell in vacuum chamber and submerged under pool of mercury. The

mercury was progressively forced into small pores of the microsponges with increased pressure. So that, the apparent volume of mercury was decreased. From the results of intrusion volume, total pore surface area and pore diameter was determined. Porosity was calculated from apparent and true volumes of mercury levels.

Formulation of tablets of optimized batch

The optimized trail of microsponges equivalent to 180 mg dose were compressed into tablets by direct compression technique using excipients Avicel PH 102: 50 %, Cross povidone: 4 % Sod. Steryl fumerate: 2 % and Talc: 2 %. Total weight of the tablet: 500 mg. Marketed product Allegra 24, optimized formulation without poloxamer 407 and optimized formulation with poloxamer 407(15 %) as permeation enhancer were considered as formulation F21, F22 and F23, respectively.

Evaluation of tablets [14]

Formulation blends of tablets were evaluated for flow properties. Compressed tablets were tested for thickness, weight variation, friability, and drug content, hardness and disintegration time in triplicates.

***In-vitro* drug release profile [15]**

In vitro-dissolution studies were conducted in 900 mL of 0.001N Hydrochloride by using USP type-II (paddle) dissolution apparatus. A temperature of 37±0.5 °C and 50 RPM were maintained throughout the study. 5 mL of aliquots were withdrawn at time intervals of 5, 10, 15, 20, 25, 30, 35, 40 and 45 min and replaced with fresh dissolution medium. After suitable dilutions, the samples were analyzed by RP-HPLC at 220 nm.

***In-vivo* Animal studies [16,17]**

The *in vivo* animal experiments were performed in male Albino rabbits (2 - 2.5 kg) provided by the animal house facility of the Nirmala college of Pharmacy, Andhra Pradesh, India. The animals in polypropylene cages were freely accessed to water and food. CPCSEA guidelines were followed for care and maintenance of the animals. The protocols were approved by the Institutional Animal Ethics Committee (IAEC/NRML/2022–2023/09). *In-vitro* drug release data was analyzed to study the drug release pattern by fitting into mathematical equations.

Pharmacokinetic evaluation

The pharmacokinetic evaluation was carried out by randomly dividing rabbits into 3 groups with 6 animals in each group. The animals were kept for 12 h fasting prior to the study. The treatment formulations were given to the animals by the oral route with the help of feeding cannula. The animals in group I received the suspension of marketed product Allegra 180 mg (Aventis) once daily tablet, animals in group II received the suspension of optimized microsponge tablet of Fexofenadine Hydrochloride and animals in group III received Optimized formulation with poloxamer 407 equivalent to animal dose in mg/kg, respectively. After administration of the treatments to animals, blood samples (approx. 0.5 mL) were withdrawn at time points 0, 0.5, 1, 1.5, 2, 4, 8, 12, 24, 36 and 48 h from the marginal ear vein and stored in heparinized tubes. From the samples, plasma was separated by centrifugation and dissolved in acetonitrile to separate drug by protein precipitation of liquid-liquid extraction. The nylon filters of 0.45 µm size used for filtration of samples and subjected to analysis using the previously developed HPLC method of the drug in rabbit plasma. The pharmacokinetic parameters shown in **Table 5** were estimated from plasma concentration versus time plot.

Animal dose in (mg/kg) = Human dose in (mg/kg) $\times \frac{Human\ Km}{Animal\ Km}$

Correction factor (Km) = $\frac{Body\ weight\ Kg}{Body\ surface\ area\ m^2}$

Body surface area of Rabbit (m^2) = $Body\ weight\ in\ g^{2/3} \times \frac{9.9}{10000}$ = 0.182 of 2.5 kg rabbit

Results and discussion

The fexofenadine Hydrochloride microsponges in oil/water emulsion were prepared by Quashi emulsion diffusion method by using Eudragit EPO as polymer, Dichloromethane and ethanol (1:1) as internal phase at RPM ranges from 500 to 1,000. The produced microsponges were of porous and spherical in shape [19].

Evaluation of fexofenadine hydrochloride microsponges and tablets

The optimized formulations were subjected to evaluation of characteristic parameters like pore volume (2.04 mL/g), total pore surface area (20.05 m^2/g), pore diameter (0.4 µm), bulk density (0.32 g/mL), Tapped density (0.41 g/mL) and % porosity (21.96). The pre compressed optimized microsponge tablet blend shown bulk density (0.304 g/mL), tapped density (0.353 g/mL), Hausner ratio (1.161), carr's index (16.11) and angle of repose (22°). The compressed tablet of optimized microsponges shown thickness (3.12±0.05 mm), hardness (4.12±0.25 kg/cm^2), friability (0.12±0.01 %), drug content (0.51±0.2 %) and disintegration time (30±2 s). The effect of independent variables on responses was shown in **Table 2** [20].

Table 2 The effect of independent variables on the responses of microsponges and compressed tablets.

S.NO	RUN	Eudragit EPO (mg)	DM:ME (mL)	RPM	% PY	% DE	Particle size (µm)	% DR @ 5 min	% DR @ 10 min	% DR @ 15min	% DR @ 30 min
F1	10	500	5	500	70.1	80.23	98.46	59.24	67.12	75.78	81.23
F2	19	1,500	5	500	92.7	94.74	123.67	48.24	55.78	66.48	74.82
F3	13	500	10	500	83.5	84.04	98.45	60.88	63.05	74.22	79.73
F4	15	1,500	10	500	93.2	95.74	110.32	50.56	57.23	68.56	75.18
F5	7	500	5	1,000	76	86.16	99.89	57.42	69.12	79.47	83.17
F6	1	1,500	5	1,000	93.7	94.24	115.45	54.02	68.93	78.78	78.52
F7	11	500	10	1,000	86.3	95.04	95.33	63.89	74.56	84.01	92.03
F8	3	1,500	10	1,000	98.6	99.79	97.86	57.05	80.34	86.56	93.24
F 9	17	159.1035847	7.5	750	63.2	77.23	92.42	61.2	71.34	81.12	88.06
F 10	20	1840.896415	7.5	750	90.3	95.54	113.54	47.74	63.56	72.26	80.23
F 11	9	1,000	3.2955	750	84.6	89.86	118.23	58.63	72.86	80.23	85.03
F 12	6	1,000	11.704	750	98.4	98.3	101.42	64.67	78.12	86.34	90.42
F 13	2	1,000	7.5	330	87.1	91.23	103.23	50.41	53.89	65.78	74.85
F 14	18	1,000	7.5	1,170	95	97.05	94.78	59.04	72.45	81.12	87.62
F 15	16	1,000	7.5	750	97.2	95.78	96.89	63.05	81.21	85.23	93.12
F16	5	1,000	7.5	750	94	95.41	95.83	63.12	78.34	86.04	90.02
F 17	8	1,000	7.5	750	93.1	95.3	95.81	63.85	78.67	85.11	89.54
F 18	14	1,000	7.5	750	95	93.1	95.78	62.36	78.01	85.02	91.45
F 19	4	1,000	7.5	750	92	96.58	95.12	61.34	79.19	84.67	91.62
F 20	12	1,000	7.5	750	96.4	97	95.33	62.14	80.45	87.11	91.78

Statistical evaluation of response variables by DOE

The significance of coefficients of variables was analyzed by analysis of variance (ANOVA) with a range of P values ($p \leq 0.0001$ to $p \leq 0.05$) and obtained R-square values (0.90418 to 0.989) were shown in **Table 3**. The polynomial regression equations were generated to establish the relationship between dependent and independent variables by adopting response surface method. The positive and negative sign of coefficients represents positive and negative effects on responses respectively. All the independent variables followed 2nd order quadratic polynomial models. The difference between predicted and adjusted regression values were less than 2 for all the equations. So, the experimental design was continued to find out Numerical optimized formulation [21].

$$Y = \beta_0 + \beta_1 X_1 + \beta_1 X_2 + \beta_3 X_3 + + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 ++ \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$$

Table 3 β coefficient values of independent variables based on ANOVA results for predicting the responses.

	% Yield	% DE	particle size (µm)	% DR @ 5 min	% DR @ 10 min	% DR @ 15 min	% DR @ 30 min
Model	1,702.76	697.13	1,580.79425	551.869	1,417.6	904.863	833.147
Intercept	94.5786	95.53	95.7772501	62.6495	79.345	85.5496	91.3102
X_1-Eudragit EPO	7.91013***	5.1134***	6.6405823***	–3.9685***	–1.8053**	–2.0503*	–2.45799***
X_2-DM: ME	3.82093***	2.4482***	–4.670251***	1.72939***	1.6897**	1.69261**	1.86755**
X_3-DRPM	2.08073*	2.2163***	–2.6785927***	2.04834***	5.9299***	5.09478*	4.06217***
$X_1 X_2$	–2.28875*	–0.767	–3.29625***	–0.345	1.4363*	0.86*	1.215*
$X_1 X_3$	–0.30625	–1.673*	–2.37375***	1.385*	2.8438***	2.1025**	1.69*
$X_2 X_3$	0.16875	1.2025*	–1.09875*	0.6925	2.4338**	1.475*	3.84***
X_1^2	–6.1363***	–3.242***	2.645983***	–2.9299***	–4.414*	–3.2536***	–2.87878***
X_2^2	–0.91608	–0.522	5.06605596***	–0.3914	–1.5714***	–0.9219*	–1.61305*
X_3^2	–1.07695*	–0.5	1.24060827***	–2.8397***	–5.9272*	–4.3991***	–3.90762***
Lack of Fit	0.7309	0.8525	0.2674	0.4042	0.5097	0.2602	0.5682
R-Squared	0.98261	0.9821	0.996695	0.98438	0.989	0.98754	0.97768
Adj R-Squared	0.96695	0.966	0.99372051	0.97032	0.9791	0.97633	0.9576
Pred R-Squared	0.93107	0.9446	0.98217588	0.91803	0.9508	0.93242	0.90418

$p \leq 0.05$ * $p \leq 0.01$** $p \leq 0.0001$***

Regression equation for fitted model % yield (Y_1): +94.5786 +7.91013X_1 +3.82093X_2 +2.28875 X_3 – 2.288750 X_1 X_2 –6.1363 X_1^2 –1.07695 X_3^2

Increase in percentage yield was observed with increased polymer concentration, internal phase volume and RPM due to decrease in viscosity of polymer and reduced surface tension between internal and external phases. The proportions of polymer, internal phase and RPM affect the yield. Formulation F8 showed highest percent yield (98.6 %).

Regression equation for fitted model % Drug entrapment (Y_2): +95.53 +5.1134 X_1 +2.4482 X_2 +2.2163 X_3 –1.673 X_1 X_3 +1.2025 X_2 X_3 –3.242 X_1^2
Percent drug entrapment increased with increase in polymer amount, internal phase volume and RPM. We can observe from **Table 2** that F9 showed lowest drug entrapment (77.23 %)

Regression equation for fitted model % Particle size (µm) (Y_3): + 95.7772501 +6.6405823X_1 –4.670251 X_2 –2.6785927 X_3 –3.29625 X_1 X_2 –2.37375 X_1 X_3 –1.09875 X_2 X_3 +2.645983 X_1^2 +5.06605596 X_2^2 +1.24060827 X_3^2
Particle size increases with increase in polymer concentration and decrease with increase in internal phase volume and RPM due to decrease in adhesive forces of polymer at high RPM and breakdown of polymer chain due to increased volume of internal phase which facilitate complete solubility of polymer.

Regression equation for fitted model % Drug release @ 5 min (Y_4): 62.6495-3.9685X_1 +1.72939X_2 +2.04834X_3 +1.385 X_1 X_3 –2.9299 X_1^2 –2.8397 X_3^2
Formulation F10 released 47.74 % drug with high amount of polymer (Eudragit EPO 1,840 mg) and F12 released 64.67 % drug with high amount of internal phase volume (DM: ETH 11.704 mL).

Regression equation for fitted model % Drug release @ 10 min (Y_5): 79.345-1.8053X_1 +1.72939 X_2 2.04834 X_3 +1.4363 X_1 X_2 +2.8438 X_1 X_3 +2.4338 X_2 X_3 –4.414 X_1^2 –1.5714 X_2^2 –5.9272 X_3^2
Formulation F2 and F16 released 55.78 and 78.34 % drug respectively due to the effect of internal phase and RPM on polymer amounts.

Regression equation for fitted model % Drug release @ 15 min (Y_6): 85.5496-2.0503 X_1 +1.69261 X_2 +5.09478 X_3 +0.86 X_1 X_2 +2.1025 X_1 X_3 +1.475 X_2 X_3 –3.2536 X_1^2 –0.9219 X_2^2 –4.3991 X_3^2
At 15 min, Formulation F2 and F20 released 66.48 % and 87.11 % of drug respectively

Regression equation for fitted model % Drug release @ 30 min (Y_7): 91.3102-2.45799 X_1 +1.86755 X_2 +4.06217 X_3 +1.215 X_1 X_2 +1.69 X_1 X_3 +3.84 X_2 X_3 –2.87878 X_1^2 –1.61305X_2^2 –3.90762 X_3^2

At 30 min, Formulation F2 and F8 released 74.82 and 93.24 % of drug, respectively

Decrease in drug release observed with rate retardant ability of polymer and the release increased with internal phase volume and RPM due to size reduction of microsponges which facilitate increased surface area at different time intervals. As the time proceeds drug release rate decreased due to increase in the path length of dosage form.

Graphical illustration of influence of independent variable on responses by DOE

The 2 D contour plots and 3D response surface plots illustrate the effect of 2 independent variables on responses by keeping the 3rd independent variable constant.

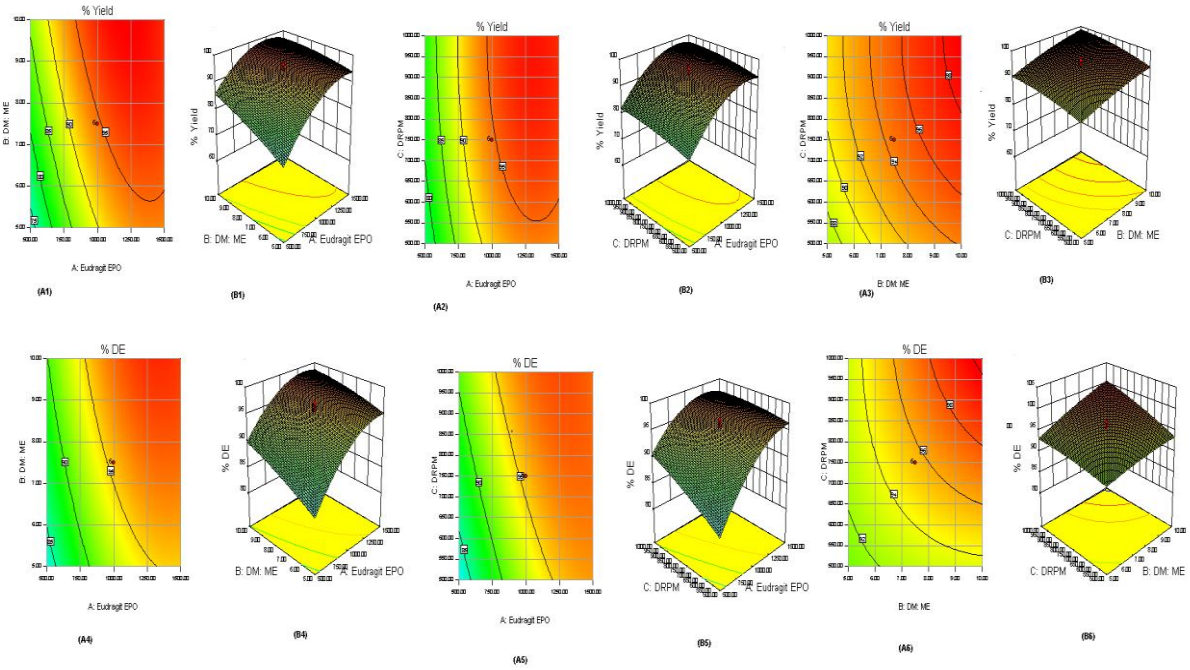


Figure 2 Contour plots (A1, A2, A3, A4, A5 and A6) and response surface plots (B1, B2, B3, B4, B5, B6 and B7) showing effect of Eudragit EPO (X_1), Internal phase volume (DM:ETH) (X_2), and RPM (X_3), on % yield and % DE.

The results of percent production yield and drug entrapment supported by the previous study S. Tamizharasi *et al.* (2008) that the production yield and drug entrapment of microsponges were increased along with increased polymer amount at constant amounts of drug [10].

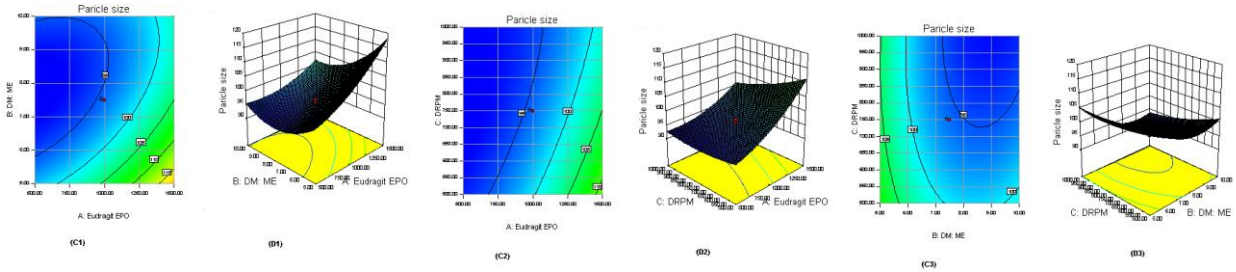


Figure 3 Contour plots (C1, C2 and C3) and response surface plots (D1, D2 and D3) showing effect of Eudragit EPO (X_1), Internal phase volume (DM: ETH) (X_2), and RPM (X_3), on particle size (µm).

Effect of internal phase volume on particle size was supported by the previous study Rishabh Srivastava *et al.* (2012) that increase in internal phase volume, decreased the viscosity of polymer and formed microsponges with reduced size [23].

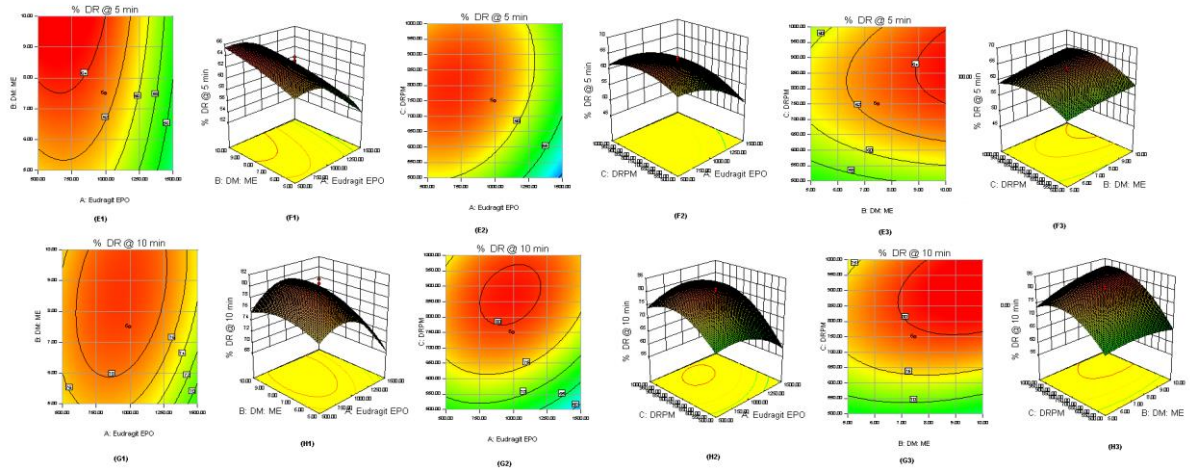


Figure 4 Contour plots (E1, E2, E3, G1, G2 and G3) and response surface plots (F1, F2, F3, H1, H2, and H3) showing effect of Eudragit EPO (X_1), Internal phase volume (DM:ETH) (X_2), and RPM (X_3), on % Drug release at 5 and 10 min.

Desavathu *et al*. (2017) studied that increase in stirring rate (RPM), increased the production yield, reduced the particle size and increase in surface area, fasten the drug release rate. The results were comparable to the present study [24].

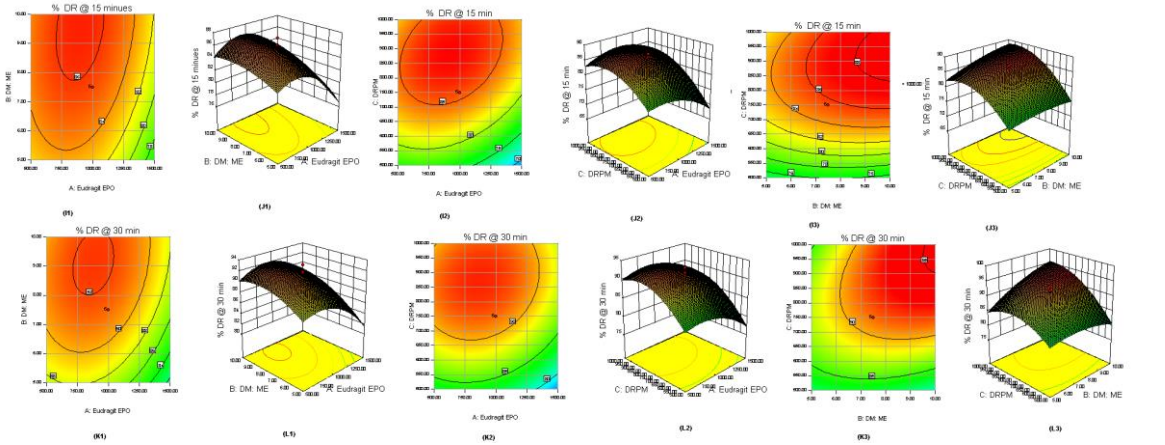


Figure 5 Contour plots (I1,I2,I3,K1,K52and K3) and response surface plots (J1,J2,J3,L1,L2, and L3) showing effect of Eudragit EPO (X_1), Internal phase volume (DM:ETH) (X_2), and RPM (X_3), on % Drug release at 15 and 30 min.

Md. Mofizur Rahman *et al.* studied that crosspovidone showed faster disintegration due to high water wicking ability. Crosspovidones are densely cross-linked homopolymers of N-vinyl 2-pyrrolidones. Their porous particle morphology enables them to rapidly absorb liquids into the tablet by capillary action and to generate rapid volume expansion and hydrostatic pressures that result in tablet disintegration [25,26].

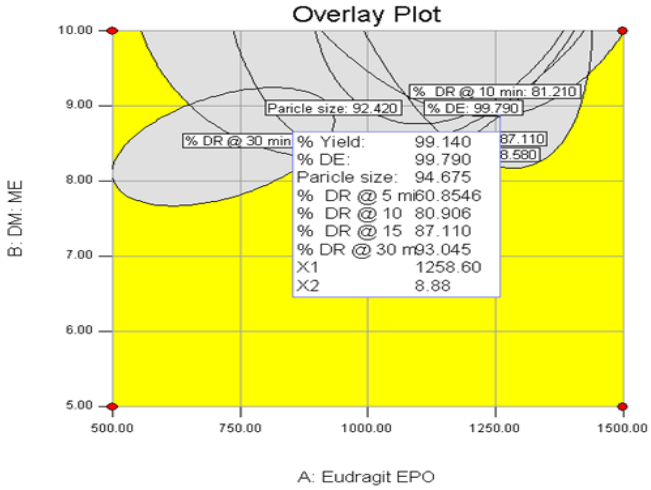


Figure 6 Overlay plot of Fexofenadine Microsponges optimized formulation.

Numerical formulation optimization by response surface method

The optimized batch of Fexofenadine Hydrochloride microsponges were prepared and compressed into tablets by direct compression technique. The obtained results were comparable to marketed Fexofenadine Hydrochloride tablet (Allegra 24 h) and optimized formulation was showed in **Tables 4** and **5** [27].

Table 4 Validation of optimized formulation of Fexofenadine Hydrochloride microsponges.

Independent variable	Name	Level	Response	Predicted value	Observed value	% Error
A	Eudragit EPO (mg)	1,258.60	% Yield	99.13	99.10±0.1	0.03 %
B	DM:ETH (mL)	8.88	% DE	99.78	99.45±0.065	0.33 %
C	RPM	1,000	Particle size(um)	94.67	94.12±0.062	0.58 %
			% DR 5 min	60.85	60.6±0.045	0.41 %
			% DR 10 min	80.90	80.75±0.041	0.18 %
			% DR 15 min	87.11	87.47±0.10	0.4 %
			% DR 30 min	93.04	92.85±0.02	0.2 %

DM: ETH: Dichloromethane: ethanol Eudragit EPO: Eudragit EPO RPM: Rotations per min Desirability: 0.975

The drug release kinetics was best fitted with 1st order with Higuchi diffusion mechanism as shown in **Table 4**.

Table 5 Cumulative % drug release profiles of optimized and marketed formulation of immediate release tablets of Fexofenadine Hydrochloride microsponges.

Time (min)	Marketed brand (Allegra 24)	Optimized formulation	Desirability as per USP
1	35.74	36.31	
2	52.85	54.19	
5	60.60	63. 14	Not less than 30 %
10	80.75	80.47	
15	84.47	85.56	

Time (min)	Marketed brand (Allegra 24)	Optimized formulation	Desirability as per USP
20	88.49	88.12	
25	90.24	90.36	
30	92.85	92.24	Not less than 85 %
35	94.45	94.47	
40	96.34	95.12	
45	99.27	97.45	

Evaluation of drug release kinetics

Table 6 Drug relaese kinetics of optimised formulation of Fexofenadine hydochloride microsponge tablets.

						Korsemeyer- peppas constants	
Formulation	**0 order**	**1st order**	**Higuchi**	**Hixon-crowell**	**Peppas**	**n**	**k**
Marketed	–0.6582	0.9288	0.9078	0.4266	0.6724	0.0083	1.71981.6108
Optimized	–0.6326	0.9439	0.9465	0.3995	0.6401	0.0069	1.74161.6234

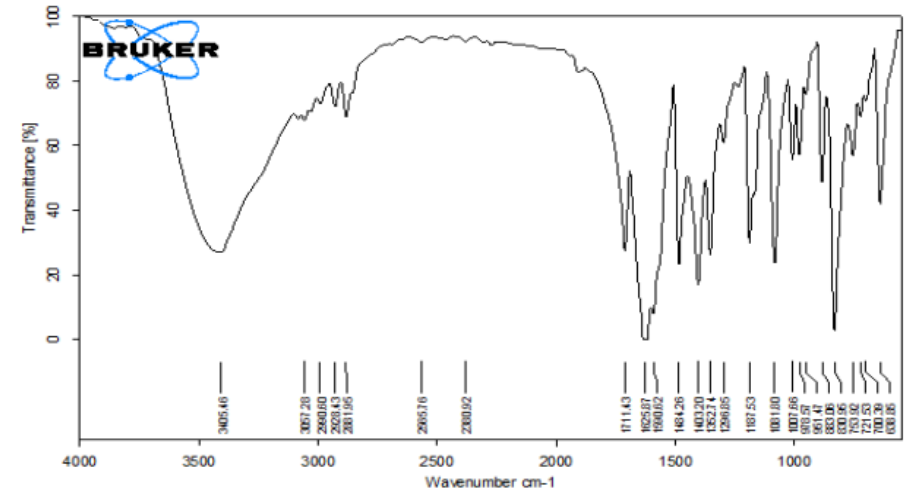
Stability study of prepared fexofenadine hydrochloride microsponge tablets

Table 7 Stability study data of optimized formulation.

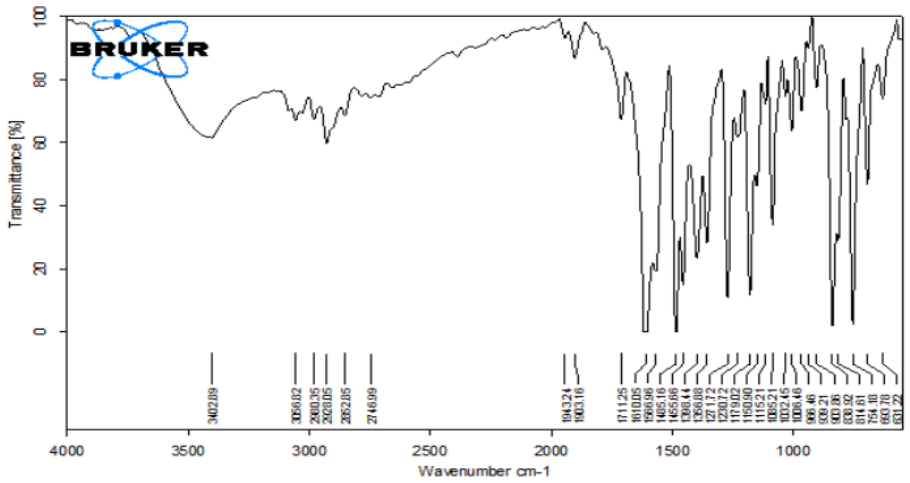
	Cumulative % drug release at 40±2 °C and 75±5 % Relative humidity			
Time (min)	**0 days**	**30 days**	**90 days**	**180 days**
0	0	0	0	0
1	35.74	36.72	37.42	37.81
2	52.85	52.84	52.89	53.01
5	60.60	60.62	60.64	60.87
10	80.75	80.85	80.92	81.00
15	84.47	85.21	85.34	85.47
20	88.49	88.92	89.03	89.23
25	90.24	90.24	90.45	90.64
30	92.85	92.93	93.01	93.24
35	94.45	94.56	94.78	94.56
40	96.34	96.45	97.02	97.44
45	99.27	99.34	99.24	100.1

FTIR study of optimized formulation

The FTIR spectra showed characteristic peaks of the pure drug and formulation blend of optimized Fexofenadine Hydrochloride microsponge tablet in **Figure 1**. The FTIR spectra of pure Fexofenadine hydrochloride displayed a peak characteristic of O-H stretching vibration at 3,405.46 cm^{-1}, C-H aromatic stretching at 2,990.60 cm^{-1}, C-H aliphatic stretching at 2,881.95 cm^{-1}, C=O stretching at 1,711.43 cm^{-1}, and C=C stretching at 1,625.87 cm^{-1}. The spectra of optimized formulation of Fexofenadine Hydrochloride microsponge tablets showed the characteristic peaks without any change. FTIR study revealed no interaction between drug and polymer. This study was supported by the literature Arefin *et al*. (2016) [28].

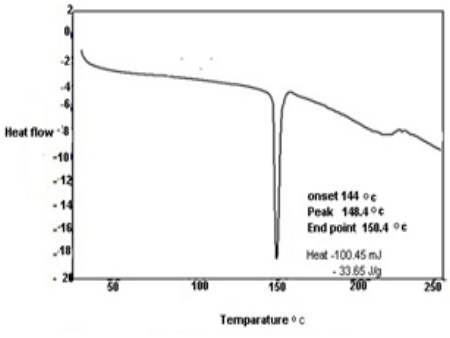


(a)

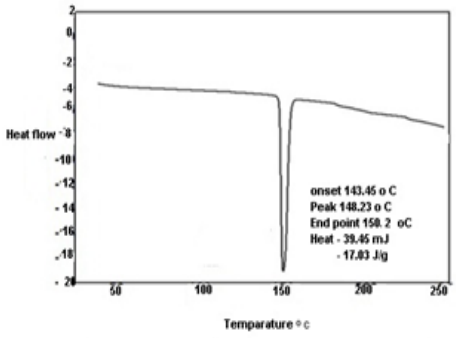


(b)

Figure 7 FTIR spectra of (a) pure drug Fexofenadine Hydrochloride (b) Formulation blend of Fexofenadine Hydrochloride microsponges tablets (F23).



(a) DSC of pure drug of Fexofenadine hydrochloride



(b) DSC of pure optimized formulation of Fexofenadine hydrochloride microsponge tablet

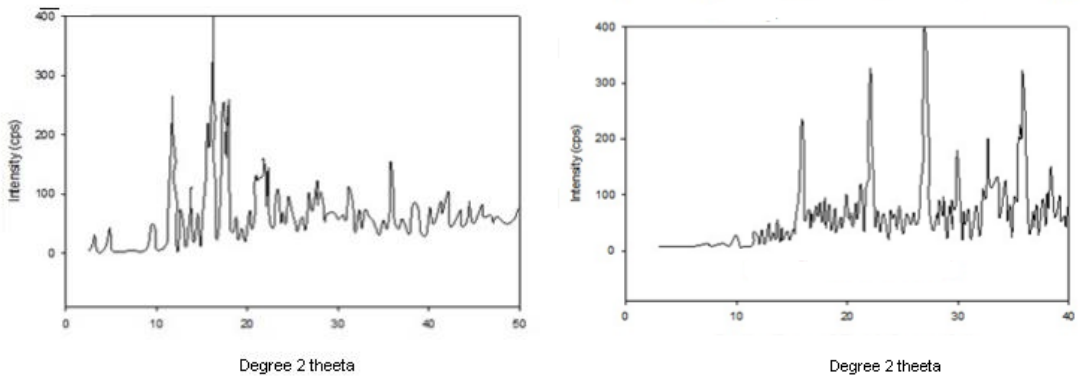
Figure 8 DSC spectra of (a) pure drug Fexofenadine Hydrochloride (b) Formulation of Fexofenadine Hydrochloride microsponges tablets (F23).

DSC of optimized formulation

The DSC thermo gram of Fexofenadine Hydrochloride showed endotherm in the range of 143.45 to 150.4 °C in pure drug and formulation blend as shown in **Figure 2**. It revealed that the compatibility was existed between polymer and drug.

PXRD analysis of optimized microsponges

The FXD at 2° theta represents sharp peaks at 14.02°, 17.7°, 18.23°, 19.5°, 19.72° and 22.68° have indicated crystalline nature of the drug. Microsponge tablet showed reduced peak number and intensity in comparison with pure drug in which the Fexofenadine peak number decreased from 25° to 8.5°, and peaks at 14.02°, 17.7°, 18.23° and 22.68° had been disappeared. The diffraction pattern of the microsponge tablet indicated that a reduction in crystallinity and an amorphous form in microsponge tablets which increases solubility and dissolution.



(a) (b)

Figure 9 PXRD spectra of (a) pure drug Fexofenadine Hydrochloride (b) Formulation blend of Fexofenadine Hydrochloride microsponges tablets (F23).

The previous study by Eedara *et.al*. (2021) supported the results of DSC and PXRD studies of Fexofenadine Hydrochloride. [29]

SEM analysis of optimized formulation of microsponges

SEM analysis images revealed that all the microsponges are spherical and porous in nature. No intact drug particles observed on rough surface.

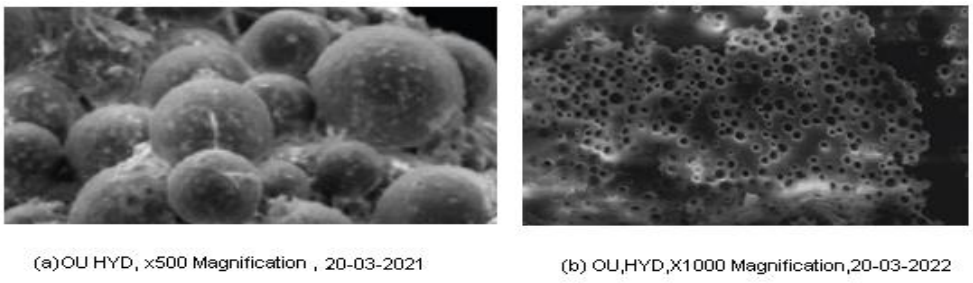


Figure 10 SEM analysis of Fexofenadine Hydrochloride microsponge tablets F23 (a) X500 µm (b) X1000 µm.

***In-vivo* pharmacokinetic evaluation**

One compartment kinetics with modified Wagner-nelson method was used to study the pharmacokinetic profile of Fexofenadine Hydrochloride marketed product (Reference), Microsponge tablets of formulation F22 and F23 have been depicted in **Figure 10**. A significant difference was observed in kinetics of developed microsponges tablet formulation when compared to marketed product

and developed formulation without poloxamer 407 which acts as permeation enhancer in GI absorption. All the results were calculated for 6 times.

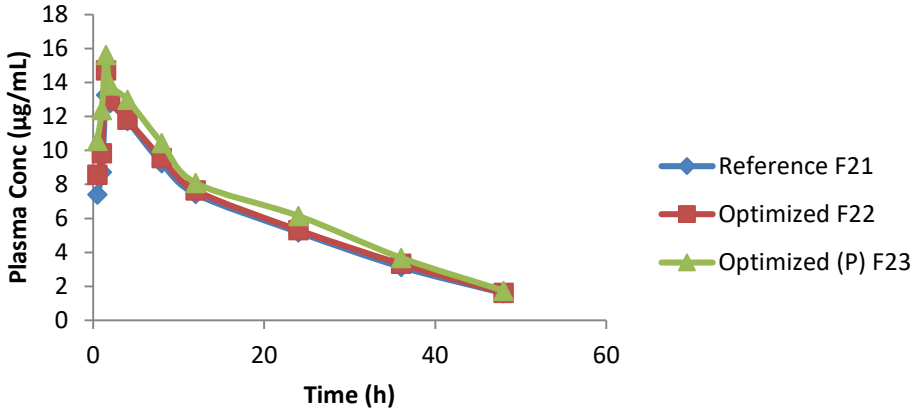


Figure 11 *In-vivo* Plasma drug concentration versus time profile of marketed product/Reference Allegra 24 (F21), optimized formulation (F22) and optimized formulation with poloxamer 407 (F23).

Table 8 Pharmacokinetic parameters of marketed and optimized formulations.

Pharmaco kinetic parameter	Units	Marketed product (Allegra 24)	Optimized formulation F22	Optimized formulation with Poloxamer 407(15 %) F23
$t1/2k_a$	h	0.192711±0.00278	0.165013±0.024164	0.144662±0.006787
$t1/2k_e$	h	13.05061±0.17922	13.22817±0.600737	16.5657±0.725262
V/F	(mg)/(µg/mL)	1.859154±0.002806	1.682309±0.034078	1.441617±0.07062
CL/F	(mg)/(µg/mL)/h	0.098744±0.00108	0.088152±0.001463	0.060321±0.00143
Tmax	h	1.189546±0.003213	1.056874±0.14102	0.998111±0.04821
Cmax	µg/mL	9.907066±0.062554	11.03424±0.214843	13.05303±0.243768
AUC 0-48h	µg/mL*h	182.9393±4.546075	204.3486±4.844524	281.2276±5.720046
AUC 0-inf	µg/mL*h	198.6959±3.857114	222.5706±7.852865	325.262±10.10543
AUMC	µg/mL*h^2	3796.3±169.5264	4300.572±382.565	7841.403±615.1413
MRT	h	19.10608±0.257571	19.32228±0.764531	24.10796±1.01232
A	µg/mL	10.71135±0.023569	11.80987±0.218527	13.72961±0.257802
k_a	1/h	3.596814±0.004204	4.200554±0.145213	4.791504±0.025762
k_e	1/h	0.053112±0.000632	0.052399±0.001041	0.041842±0.001516
% Relative bioavailability			111.70 %	153.72 %

$p < 0.05$ Mean±S.D n = 6

The results in **Table 8** had shown *In-vivo* pharmacokinetics of F23, formulation with poloxamer 407 (15 %) showed absorption half-life of 0.144662±0.006787 (ug/mL) and MRT of 24.10796±1.01232 h with significant difference ($p < 0.05$) when compared to F21 and F22. However, T max of all the formulations were nearly same [16].

Conclusions

From the above study, it was concluded that Microsponge technology offers a better approach to increase the dissolution rate of poor aqueous soluble drugs and at the same time can control the release rate based on drug to Eudragit EPO ratio (500 mg:1,258.60 mg). Fexofenadine Hydrochloride permeability was improved by poloxamer 407 (15 %) with AUC_{0-48h} (ug/mL.h−1) 281.2276±5.720046 and AUC 0-∞ (ug/mL.h−1) 325.262±10.10543. The percent relative bio availability of F23 was 153.72 % which was higher than Formulation F22 (111.70 %). Quashi emulsion diffusion method was suitable for the preparation of once daily Fexofenadine Hydrochloride microsponge tablets.

Acknowledgements

Special thanks to Priyadarshini Institute of Pharmaceutical Education and Research and Nirmala College of Pharmacy for providing the facilities to carry out the experimental study and *In vivo* studies.

Special thanks to Dr. Ajit Kumar Acharya, School of Pharmacy, Department of Pharmaceutics, College of Health Sciences, Mekelle University, Mekelle, Ethiopia.

References

[1] S Kaity, S Maiti, AK Ghosh, DK Pal, A Ghosh and S Banerjee. Microsponges: A novel strategy for drug delivery system. *J. Adv. Pharm. Technol. Res*. 2010; **1**, 28390.

[2] PS Rawat, A Dhyani, V Singh and D Juyal. A brief review of microsponges: An update. *J. Pharm. Innov.* 2017; **6**, 134-9.

[3] R Srivastava and K Pathak. Microsponges: A futuristic approach for oral drug delivery. *Expert Opin. Drug Deliv.* 2012; **9**, 863-78.

[4] D Axelrod and L Bielory. Fexofenadine hydrochloride in the treatment of allergic disease: A review. *J. Asthma Allergy* 2008; **19**, 19-29.

[5] KV Godse, NJ Nadkarni, G Jani and S Ghate. Fexofenadine in higher doses in chronic spontaneous urticaria. *Indian Dermatol. Online J.* 2010; **1**, 45-6.

[6] S Sachdeva, V Gupta, SS Amin and M Tahseen. Chronic urticaria. *Indian J. Dermatol.* 2011; **56**, 622- 8.

[7] J Malakar, AK Nayak and S Goswami. Use of response surface methodology in the formulation and optimization of bisoprolol fumarate matrix tablets for sustained drug release. *ISRN Pharm.* 2012; **2012**, 730624.

[8] S Dey, HB Samal, SA Srinivas, GS Reddy, P Mounika, R Gujrati, S Preetika, K Choudhary and P Prashanth. RP-HPLC method for the estimation of fexofenadine in bulk and in dosage forms. *J. Pharm. Res.* 2011; **4**, **7**.

[9] S Shah, S Madan and S Agrawal. Formulation and evaluation of microsphere based Oro dispersible tablets of itopride hydrochloride. *Daru* 2012; **20**, 24.

[10] AR Pawar, NA Shete, PV Jadhav, VK Deshmukh and JS Mehetre. Enhancement of aqueous solubility, dissolution profile, and oral bioavailability of pentoxifylline by microsponges. *Pharmaceut. Fronts* 2021; **3**, e200-e207.

[11] LX Yu, G Amidon, MA Khan, SW Hoag, J Polli, GK Raju and J Woodcock. Understanding pharmaceutical quality by design. *AAPS J.* 2014; **16**, 771-83.

[12] L Kumar, MS Alam, CL Meena, R Jain and AK Bansal. Chapter 4 fexofenadine hydrochloride. *Profiles Drug Subst. Excip. Relat. Methodol.* 2009; **34**, 153-92.

[13] J Mohammed, S Mohammed, MSA Khan, Y Alshehary, NR Alrwaili, YA Alzahrani, SI Syed and S Alsheri. Preparation and *In vitro-In vivo* evaluation of lutiolin loaded gastroretentive microsponge for the eradication of *helicobacter pylori* infections. Pharmaceutics. 2021; **13**, 2094.

[14] M Preis, L Grother, P Axe, J Breitkreutz, J Breitkreutz. *In-vitro* and *in-vivo* evaluation of taste-masked cetirizine hydrochloride formulated in oral lyophilisates. *Int. J. Pharm.* 2015; **491**, 8-16.

[15] S Nagadani, V Maravajhala. Formulation, *In vitro* and *In vivo* evaluation of taste-masked oral disintegrating tablets of fexofenadine hydrochloride using semi synthetic and natural superdisintegrants. *Int. J. Appl. Pharm.* 2021; **13**, 99-108.

[16] AM Nasr, MK Qushawy, MM Elkhoudary, AY Gawish, SS Elhady and SA Swidan. Quality by design for the development and analysis of enhanced *In-Situ* forming vesicles for the improvement of the bioavailability of fexofenadine hydrochloride *In vitro* and *In vivo*. *Pharmaceutics* 2020; **12**, 409.

[17] S Maher, DJ Brayden, L Casettari and L Illum. Application of permeation enhancers in oral delivery of macromolecules: An update. *Pharmaceutics* 2019; **11**, 41.
[18] SNA Alhammid. Enhancement of the solubility and the dissolution rate of candesartan cilexetil using microsponge technology. *Asian J. Pharm. Clin. Res.* 2018; **11**, 385-90.
[19] A Moin, TK Deb, RA Osmani, RR Bhosale and U Hani. Fabrication, characterization, and evaluation of microsponge delivery system for facilitated fungal therapy. *J. Basic Clin. Pharm.* 2016; **7**, 39-48.
[20] NA Rajab and MS Jawad. Formulation and *In vitro* evaluation of piroxicam microsponge as a tablet. *Int. J. Pharm. Sci.* 2016; **8**, 104-14.
[21] D Patel, DH Patel, D Gohil, H Sheth, S Patel, K Pandya and CH Shah. Formulation and evaluation of floating microsponges of allopurinol. *Pharma Sci. Monit.* 2016; **7**, 135-54.
[22] W Chen, A Palazzo, WE Hennink and RJ kok. Effect of particle size on drug loading and release kinetics of gefitinib-loaded PLGA microspheres. *Mol. Pharm.* 2017; **14**, 459-67.
[23] R Srivastava, D Kumar and K Pathak. Colonic luminal surface retention of meloxicam microsponges delivered by erosion based colon-targeted matrix tablet. *Int. J. Pharm.* 2012; **427**, 153-62.
[24] M Desavathu, R Pathuri and M Chunduru. Design, development and characterization of valsartan microsponges by quasi emulsion technique and the impact of stirring rate on microsponge formation. *J. Appl. Pharm. Sci.* 2017, **7**, 193-8.
[25] MDM Rahman, S Roy, S Hasan, MDA Alam, MK Jha, MDQ Ahsan and MDJ Ferdaus. Effect of mode of addition of disintegrants on dissolution of model drug from wet granulation tablets. *Int. J. Pharm. Sci. Res*. 2011; **2**, 84-92.
[26] SK Swamy, G Arun, S Bethi and AB Goud. Effect of various super disintegrants on the drug release profile of orally disintegrating tablets. *Asian J. Pharm. Tech.* 2016; **6**, 99-105.
[27] HP *Patel*, P Karwa, B Rama and Patel NJ. Formulation and evaluation of immediate release tablets of zolpidem tartrate by direct compression. *Int. J. Pharm. Sci. Rev. Res.* 2011; **7**, 80-6.
[28] P Arefin, I Hasan and MS Reza. Design, characterization and *In vitro* evaluation of HPMC K100 M CR loaded fexofenadine hydrochloride microspheres. *Springerplus* 2016; **5**, 691.
[29] BB Eedara, D Nyavanandi, S Narala, PR Veerareddy and S Bandari. Improved dissolution rate and intestinal absorption of fexofenadine hydrochloride by the preparation of solid dispersions: *In vitro* and *In Situ* evaluation. *Pharmaceutics* 2021; **13**, 310.