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DEVELOPMENT AND EVALUATION OF TASTE MASKED FORMULATIONS OF FEXOFENADINE HYDROCHLORIDE

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ABSTRACT

Ease of administration and patient compliance is gaining significant importance in the design of dosage forms. Dysphagia (difficulty in swallowing) is a common difficulty among all age groups especially in elderly and pediatrics. Patients suffering from dysphagia show greater chances of being choked during consumption of liquid formulation. Thus, to mollify such a problem, liquid formulation of high viscosity was prepared. The objective of present research work was to design and develop pediatric taste masked formulations of Fexofenadine hydrochloride (FEH) with taste enhancement and improved bioavailability. The masking of bitter taste of the drug was a necessity to formulate it in a palatable form. Effective taste masking of Fexofenadine hydrochloride was achieved through complexation with selected cyclodextrin (2- Hydroxypropyl beta cyclodextrin/ Cavasolw7HP). Inclusion complex prepared by solid dispersion showing taste score 1 (tasteless) was selected and further subjected to in-vitro taste assessment study. Based on human panel studies, tasteless FEH-2HPBCD complex i.e., batch FE5 was selected as optimized batch and different child friendly taste masking technologies were screened for incorporation of the same. Oral flavored powder formulations were prepared by mixing drug-2HPBCD inclusion complex equivalent to 30mg of FEH with varying concentrations of sucralose and with different flavors like chocolate, lemon, cherry, pineapple, etc. Formulation batch contaning lemon flavor F4 was more acceptable to human volunteers. Effervescent granules were formulated by using different concentration of effervescent salts, sucralose and lemon flavor. Soft chewable lozenges were formulated by optimization for binder concentrations and various flavors like chocolate, cherry, lemon, pineapple, etc. Results conclusively demonstrated that successful taste masking of FEH was accomplished and that it could be formulated for oral administration with more acceptability to pediatrics and improved bioavailability.

Keywords *– Fexofenadine hydrochloride, 2-Hydroxypropyl-beta-cyclodextrin, Oral flavored powder, Soft chewable lozenges, Effervescent granules*

1. INTRODUCTION

Fexofenadine hydrochloride (FEH) is an intensely bitter tasting antihistaminic agent generally indicated for the treatment of sudden allergic attacks. The drug belongs to BCS class II and exhibits a poor water solubility (0.00266 mg/ml). It is used mainly for

relieving hay fever and allergy symptoms, such as sneezing and red, itchy, tearing eyes. The drug acts by blocking histamine, a substance in the human body responsible for causing allergic symptoms. It undergoes high first pass metabolism and thus exhibits low oral bioavailability of 33%. It does not readily pass through the blood brain barrier. It causes less drowsiness when compared to other antihistamines. Fexofenadine is known to be safe and effective for children 2–5 years old and 6–11 years old in treatment of seasonal allergic rhinitis. Marketed formulations of FEH include tablets, film-coated tablets and oral suspension. Pediatric patients find it difficult to swallow tablets and also sometimes they resist taking liquid medication.

Cyclodextrin (CD) is crystalline, cyclic oligosaccharide wherein the glucose units are linked by α- 1, 4 glycoside bonds and it is derived from starch. The characteristic arrangement of glucose units imparts the molecule a cone like structure, which makes the exterior of the cone hydrophilic and interior of the cone hydrophobic in nature. This pecularity of the polymer enables encapsulation of the drug in the cavity resulting in the improvement in the solubility, drug release as well as taste masking. Among the most commonly used forms are α-, β-, and γcyclodextrin, which have respectively 6, 7, and 8 glucose units. Molecular weight of β- cyclodextrin is 1135.3 β -Cyclodextrin is widely used in taste masking purpose; wherein β-Cyclodextrin make an inclusion complex with drug molecules and act as a host cavity so drugs make a complex in inert carrier matrix. Several applications of CDs in oral drug delivery include improvement of drug bioavailability due to increased solubility of drug, improvement of rate and extent of dissolution and /or stability of the drug at the absorption site, diminution of drug induced irritation, taste masking, etc. [1-14].

The taste masking of drug was carried out by forming drug-cyclodextrin inclusion complexes. This would not only improve taste of drug but it would also enhance drug solubility, *in vivo* thereby increasing its bioavailability. The taste masked formulations can be given orally with water and also along with solid/semisolid food. By masking the bitter taste of drug, patient acceptance and compliance can be improved.

2. MATERIALS AND METHOD

2.1 Materials

Fexofenadine Hydrochloride was a generous gift sample from Ami life sciences, Gujarat. All grades of cyclodextrin were gifted by Ashland, Mumbai. The flavors were procured from Firmenich, Mumbai. Sucralose was obtained as a gift sample from J. K. Sucralose, Delhi. All other chemicals used were of analytical grade.

2.2 Pre-Formulation Study

a) Organoleptic properties and description

The sample of Fexofenadine hydrochloride was studied for organoleptic characters and description.

b) Melting point determination

Melting point of drug was determined by capillary method. The capillary filled with drug powder was placed in Thiele's tube filled with liquid paraffin. The tube was heated and the melting point of drug powder was noted.

c) Drug-excipient compatibility:

The FTIR spectrum of drug and a physical mixture of drug and excipient were obtained using KBr press pellet method. The discs were prepared using manually operated KBr press model M-15. The scanning range was 4000-400cm^{-1}. The FTIR spectrum of the sample drug was compared with the standard FTIR spectrum of the pure drug to ascertain any significant changes in the sample drug.

d) Solubility Study of drug in different solvents

The aim of the solubility study was to decide or select solvent for drug extraction during percent drug content determination. Solubility of drug was determined in distilled water, methanol, and Phosphate buffer pH 6.8 and 0.001M HCL.

2.3 Development of UV spectroscopic method for FEH

2.3.1 Selection of analytical wavelength

Stock solution of fexofenadine hydrochloride was prepared by accurately weighing 10 mg of drug and dissolved in 10 ml of methanol to get concentration of 1000µg/ml. From the above stock solution, different concentrations of Fexofenadine hydrochloride ranging from 5-40 µg/ml were prepared in methanol, phosphate buffer pH 6.8 and 0.001M HCl. These solutions were scanned in UV range from 200-400nm using double beam UV-Vis spectrophotometer against respective blank. Wavelength scan from 400-200nm was done to find absorbance maxima.

2.3.2 Preparation of calibration curve of drug in methanol

Serial dilutions were done in Beer's range of 5-40 µg/ml from the above stock solution. The absorbance of each solution was recorded at λ_{max} 221nm using UV visible spectrophotometer. The graph of absorbance v/s concentration (µg/ml) was plotted.

2.3.3 Preparation of standard curve of drug in phosphate buffer pH 6.8

Stock solution of Fexofenadine hydrochloride was prepared by accurately weighing 100 mg of drug in 10 ml methanol; volume was made to 100 ml with phosphate buffer pH 6.8 to get solution of 1000 µg/ml. Serial dilutions were done in Beer's range of 5-40 µg/ml. The absorbencies were recorded at λ_{max} 221nm using UV visible spectrophotometer. The graph of absorbance v/s concentration (µg/ml) was plotted.

2.3.4 Preparation of standard curve of drug in 0.001M HCL

Stock solution of Fexofenadine hydrochloride was prepared by accurately weighing 100 mg of drug in 5 ml methanol; volume was made to 100 ml with 0.001M HCL to get solution of 1000 µg/ml. Serial dilutions were done in Beer's range of 5-40 µg/ml. The absorbencies were recorded at λ_{max} 221nm using UV visible spectrophotometer. The graph of absorbance v/s concentration (µg/ml) was plotted.

2.3.5 Effect of excipients on UV absorbance

UV spectral scanning of solutions containing same concentrations of drug and drug in presence of cyclodextrin was performed at wavelength range of 200-400 nm to detect any possible interference in analysis.

2.4 Development High Performance Liquid Chromatography for FEH

Chromatographic separation was achieved using HPLC System (Agilent technologies 1200 series) containing UV detector. The output signal was monitored and processed using Chem-station software. A Waters Reliant^{TM} C18 column (250 x 4.6 mm, particle size 5 micron) was used as the stationary phase. Mobile phase consisting of Mobile phase Acetonitrile: buffer pH 6.8 (40:60v/v) was delivered at a flow rate of 1.0 mL/min. The mobile phase was filtered through a 0.45 µ membrane filter and sonicate for 5 min. The column temperature was kept at 30°C. The detector was set at the wavelength of 254 nm. Injection volume kept was 20 µl.

2.4.1 Preparation of standard curve of drug

Stock solution of drug was prepared by accurately weighing 10mg of drug in 10ml acetonitrile to get solution of 1000 µg/ml. From the above stock different concentration ranging from 5-40 µg/ml were prepared by diluting with mobile phase and were injected on to the column. This method was found to be sensitive in the concentration range of 5-40ppm.

2.4.2 Method validation

i) System precision (injection repeatability): It was determined by performing six repeated analyses of working standard solution.

ii) Linearity: It was determined by building calibration curves. For the construction of calibration curve six calibration standard solutions were prepared at concentrations ranging from 5 to 40µg/ml of FEH. Each standard solution was injected once. Calibration curves of standard FEH were generated by plotting analyte peak area vs. concentration of the drugs.

iii) Limit of detection (LOD): It is the lowest concentration of an analyte that the procedure can reliably differentiate from background noise. It was determined by injecting the mobile phase three times into the system and the value with the highest peak area in the range of the retention time was determined. The concentration corresponding to three times the value of noise peak gave estimate of limit of detection.

iv) Limit of Quantification (LOQ): It is the lowest concentration that can be established with acceptable accuracy and precision. The noise of the instrument was determined as above. The concentration corresponding to 10 times the area of noise peak gave an estimate of limit of quantification.

v) **Accuracy:** Method accuracy was evaluated by injecting three consecutive injections of solutions of 10µg/ml, 20µg/ml and 40µg/ml.

2.5 Estimation of Bitterness Threshold concentration for Fexofenadine hydrochloride

Threshold for a taste is the minimum concentration of a substance that evokes perception of its taste. The threshold concentration of bitter taste of drug was checked by a sensory test on human volunteers. Aqueous solution of 5, 10, 20, 30, 40, 50 µg/ml of the drug was prepared. 1ml of each solution was placed on the center of the tongue of human volunteers for 10 second. They were then asked to spit out after 10 second and the mouth was thoroughly rinsed with water. A gap of 30 min was maintained in between testing two different solutions. Threshold value was selected on the basis of bitterness scale value. Bitterness level was noted using numerical scale and scored from 0-4. (0=good, 1= tasteless, 2= slightly bitter, 3= bitter and 4 = very bitter)

2.6 Preparation of taste masked drug inclusion complex

Complexation of drug was carried out using Cavasol W7HP and Cavitron W7HP7 (2-hydroxypropyl-beta cyclodextrin) derivative by different methods like Physical Mixture, Co-precipitation and Co-Evaporated dispersion technique in different molar ratio i.e, 1:1,1:2,1:3,1:4 and 1:5.

2.6.1 Physical Mixture

Drug and various grades of cyclodextrin were weighed and mixed in above said ratios by geometric dilution method. The mixture was triturated for 10 min to obtain a homogenous powder blend and it was further passed through sieve no. 80.

2.6.2 Co-precipitation method

2-Hydroxypropyl-beta cyclodextrin was dissolved in distilled water with the aid of heat. Drug powder was separately dissolved in minimum quantity of methanol. Drug solution in methanol was then added to aqueous solution of CD slowly under stirring at room temperature. After complete addition the mixture was maintained at 70°C while being stirred continuously with the help of mechanical stirrer for 1 hr. The co-precipitates were then filtered and dried at room temperature. The dried binary mixtures were passed through sieve no. 80 and stored in desiccator until further use.

2.6.3 Co-evaporation method

For the preparation of complex by co-evaporation method, methanol and water were used as solvents. The required quantity of drug and 2-hydroxypropyl-beta cyclodextrin was dissolved in methanol and water respectively. Both the solutions were mixed and solvents were evaporated by controlled heating at 45 - 50°C by vacuum rotary evaporator (Trident) at 100 RPM. The resultant solid was kept in desiccator, pulverized and then sieved through sieve #80 and stored in desiccators.

2.7 Characterization of Inclusion Complex

a) Human panel studies

Sensory analysis of tastants was carried out in trained healthy human volunteers. Sample equivalent to dose of drug (30mg) was held in mouth for 10 sec. Time interval between tasting two different samples was 10min. Bitterness level was recorded using numerical scale and scored from 0-4 (0=good, 1=tasteless, 2=slightly bitter, 3=bitter and 4=very bitter) Based on evaluation study, tasteless complex was selected as optimized complex. Optimized complex was further evaluated for DSC analysis, drug content, etc.

b) Drug content of inclusion complexes

For determination of drug content 10mg of complex was weighed and diluted with 10ml of methanol and sonicated for 15 min to dissolve. 1 ml of this solution was transferred into volumetric flask and volume was made to 10ml with methanol. This solution was subjected to HPLC analysis and the percent drug content was calculated.

***c) In vitro* taste evaluation**

Taste of drug inclusion complex was studied in vitro by determining drug release in simulated salivary fluid (SSF) (pH 6.8) to predict release in the human saliva. Drug inclusion complex equivalent to dose of API was placed in 10mL of SSF and shaken for 1 min. 1 ml of this solution was then transferred into volumetric flask and volume was made to 10ml with mobile phase. The amount of drug released was analyzed using HPLC.

d) Differential Scanning Calorimetric Analysis

The thermal analysis of FEH, 2HPBCD and durg-2HPBCD inclusion complex was carried out by employing DSC (Mettler Toledo DSC). Sample equivalent to 5 mg weight was heated in aluminum pans over a temperature range of 30°C to 300°C at a constant rate 10°C/min under nitrogen purge (40ml/min).

e) Powder characterization

Powder was characterized for angle of repose, Bulk density, tapped density, Hausner's ratio and Carr's index.

2.8 Formulation of oral flavored powder

Oral flavored powder was developed and optimized by mixing drug-2HPBCD inclusion complex equivalent to 30mg of FEH with varying concentrations of sucralose and with different flavors like chocolate, lemon, cherry, pineapple, etc.

2.8.1 Procedure

Drug-2HPBCD inclusion complex was transferred to mortar followed by addition of sucralose, different flavors and lactose. The mixture was triturated thoroughly for 5 min and passed through sieve no. 80. The composition of different batches of oral flavored powder of FEH are shown in table 1.

Table 1: Composition of different batches of oral flavored powder of FEH

Formulation Ingredient	F1	F2	F3	F4	F5	F6	F7	F8
Complex Equivalent to 30mg of drug	471	471	471	471	471	471	471	471
Sucralose (%)	0.5	0.5	0.5	0.5	1	1	1	1
Chocolate flavor (%)	0.5	-	-	-	0.5	-	-	-
cherry flavor (%)	-	0.5	-	-	-	0.5	-	-
Pineapple flavor (%)	-	-	0.5	-	-	-	0.5	-
Lemon flavor (%)	-	-	-	0.5	-	-	-	0.5
Lactose(mg)	24	24	24	24	21	21	21	21
Total (mg)	500	500	500	500	500	500	500	500

2.8.2 Evaluation of oral flavored powder

a) Gustatory sensation test:

Developed formulation was evaluated for taste masking using taste panel of six healthy human volunteers. About 10 mg of oral flavored powder of batch F1 to F8 given to the healthy human volunteers. The volunteers were asked to taste samples (10 mg) kept in the mouth for 10seconds and then asked to spit out and to give score. The numerical scale with following values: 0= Good , 1= Tasteless, 2= Slightly bitter, 3=Moderately bitter and 4= Extremely bitter. Based on evaluation study more acceptable formulation was selected as an optimized batch. The optimized batch was further evaluated for drug content, powder characterization, etc.

b) Drug content

For determination of drug content 10mg of oral flavored powder was diluted with 10ml of methanol and sonicated for 15 min to dissolve. 1 ml of this solution was transferred into volumetric flask and volume made to 10ml with mobile phase. This solution was subjected to HPLC analysis and the percent drug content was calculated.

c) Powder characterization

Oral flavored powder was characterized for angle of repose, Bulk density, tapped density, Hausner's ratio and Carr's index.

***d) In-vitro* evaluation**

In vitro dissolution study was carried out in USP apparatus I (basket type) using gastric pH (0.001M HCL) as a dissolution medium. The oral flavored powder was tied in a muslin cloth and placed in the basket of stirrer. A speed of 100 rpm and a temperature of 37±0.5° was set as the working condition for study. About 5 ml of aliquot was withdrawn at different time intervals of 5 to 45 min and filtered using a 0.2-µm nylon disc filter and the exact same volume was replaced with 5 ml of fresh dissolution medium. The filtered samples were suitably diluted and analyzed for the drug content using HPLC.

***e) In-vivo* evaluation**

Six male Wistar rats (200–250g) were employed for the study as per the animal protocol approved by the Institutional Animal Ethics Committee (IAEC), protocol no. 879/PO/Ere/S/05/CPCSEA. Animals were housed in standard conditions of temperature (22C), relative humidity (605%) and light (12h of light– dark cycles).

The Rat Behavioral Avoidance Taste Model is based on the principle that presentation of a bitter solution to water-deprived rats reduces the drinking frequency. Six male Wistar rats weighing from 200-250g were used for the study.

Procedure:

On first Day, the rats were deprived of water for overnight to motivate licking behavior but had access to food. On second day after the water deprivation period, 50 ml of water in a graduated siphon drinking bottles was provided to the rats for a period of 30 min, followed by removal of the bottles and recording of the volume consumed. After that on third and fourth day, rats were allowed for free access to water. At the end of fourth day, rats were again subjected to overnight water deprivation cycle. On fifth day after the water deprivation period, 50ml (1mg/ml) of drug solution was subjected to rats for a period of 30 min and the volume consumed was recorded. Then again for sixth and seventh day, rats were allowed for free access to water. At the end of seventh day, rats were again subjected to overnight water deprivation cycle. On eighth day, 50ml (1mg/ml) of Taste-masked formulation was subjected to rats for a period of 30min, followed by removal of the bottles and recording of the volume consumed. Temperature was kept constant throughout the experiment. Other avoidance responses such as jaw smacking, retreating was also observed.

Statistical Analysis

The differences between the groups were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's t-test or Tukey's multiple comparison test. A difference of $P<0.05$ was considered statistically significant as compared to the groups defined in the Figure legends.

2.9 Development of Effervescent Granules

2.9.1 Preparation of taste masked effervescent granules

Effervescent granules of Fexofenadine hydrochloride were prepared by wet granulation method. The drug- cyclodextrin inclusion complex equivalent to dose of drug was accurately weighed in mortar pestle. Citric acid, tartaric acid and sodium bicarbonate was added and mixed thoroughly. Then sufficient amount of isopropyl alcohol containing HPMC E15 was added to this blend and kneaded to form dough. To this, sucralose and lemon flavor was added. It was then left for solvent evaporation by air drying. Subsequently, the obtained solid mass was passed through sieve no. 80 and air dried for 15-20 minutes. This was further passed from sieve no. 16 and granules were retained on sieve no. 20. The composition of different batches of effervescent granules of FEH are given in table 2.

Table 2: Composition of different batches of effervescent granules of FEH

Formulation ingredient	E1 (mg)	E2 (mg)	E3 (mg)	E4(mg)
Complex Equivalent to 30mg of drug	471	471	471	471
Citric acid (%)	2.1	4.5	2.7	-
Tartaric acid (%)	4.5	-	3.6	4.5
Sodium bicarbonate (%)	7.2	8.9	7.0	8.9
Sucralose (%)	0.5	0.5	0.5	0.5
Lemon flavor (%)	0.5	0.5	0.5	0.5
Total (mg)	550	550	550	550

2.9.2 Evaluation of effervescent granules

a) Effervescence time

In vitro effervescence time was measured by pouring one dose of granules in a beaker containing 10 ml of Water. Effervescent Granules were allowed to disperse in water and the time required for complete dispersion was noted. Depending upon the effervescent time, optimized formulation was selected and further evaluated.

b) Gustatory sensation test:

The optimized batch E1 was evaluated for taste masking ability by using taste panel of six healthy human volunteers. About 10 mg of granules of batch E1 was given to the healthy human volunteers for taste perception. The volunteers were asked to taste samples (10 mg) kept in the mouth for 10 seconds and then asked to spit it out and give a score. The numerical scale with following values 0=Good, 1=Tasteless, 2=Slightly bitter, 3=Moderately bitter and 4= Extremely bitter.

c) Powder characterization

Effervescent granules were characterized for angle of repose, Bulk density, tapped density, Hausner's ratio and Carr's index.

d) Drug content

For determination of drug content 10mg of granules was diluted with 10ml of methanol and sonicated for 15 mins to dissolve. 1 ml of this solution was transferred into volumetric flask and was diluted to 10ml with mobile phase. This solution was subjected to HPLC analysis and the percent drug content was calculated.

e) In Vitro Drug Release Studies

In Vitro Drug release studies, the release rate of taste masked effervescent granules of FEH was determined using USP dissolution testing apparatus II (paddle method). The dissolution test was performed using 900 ml of 0.001M HCL, at 37 ±0.5°C and 50 rpm. Sampling was done at every one-minute interval. For each sample, five ml of the dissolution medium was withdrawn and the same amount was replenished with fresh dissolution medium. The sample withdrawn was filtered using a 0.2-µm nylon disc filter. The filtered samples were suitably diluted, if necessary and analyzed for the FFH content using HPLC.

2.10 Development of Soft Chewable Lozenges

2.10.1 Optimization of binder concentrations for preparation of soft chewable lozenges

Initially, different batches were taken for optimizing the binder concentration in the preparation of soft chewable lozenges. Soft chewable lozenge of Fexofenadine hydrochloride was prepared by molding method. The drug-cyclodextrin inclusion complex equivalent to 30 mg of FEH was weighed and to this acacia, HPMC E15, Sucralose, Citric acid and Coloring agent was added and mixed thoroughly. Then sufficient amount of isopropyl alcohol was added to get a pliable mass. The lozenge mass was then rolled out on a board with a roller to form a sheet of uniform thickness. The sheet was cut into round discs by means of a lozenge cutter. The resultant lozenges were air dried. The compositions of different batches of soft chewable lozenges of FEH are given in table 3.

Table 3: Composition of different batches of soft chewable lozenges of FEH

Formulation Ingredients	A1 (mg)	A2 (mg)	A3 (mg)
Complex Equivalent to 30mg of drug	471	471	471
Acacia	300	300	300
HPMC E15	2.3	4.7	7.1
Sucralose (%)	0.5	0.5	0.5
Citric acid (%)	1	1	1
Coloring agent	q.s	q.s	q.s
Isopropyl alcohol	q.s	q.s	q.s

2.10.2 Screening of flavors for optimized batch of soft chewable lozenges

From the above batches, the lozenge mass for batch A2 was non-sticky and lozenges obtained from it were smooth and shiny. So, the batch A2 was further screened for different flavors. The composition of different batches of soft chewable lozenges of FEH is depicted in table 4.

Table 4: Composition of different batches of soft chewable lozenges of FEH

Formulation Ingredient	L1 (mg)	L2 (mg)	L3 (mg)	L4 (mg)
Complex Equivalent to 30mg of drug	471	471	471	471
Acacia	300	300	300	300
HPMCE15	4.7	4.7	4.7	4.7
Sucralose (%)	0.5	0.5	0.5	0.5
Chocolate flavor (%)	0.5	-	-	-
Cherry flavor (%)	-	0.5	-	-
Pineapple flavor (%)	-	-	0.5	-
Lemon flavor (%)	-	-	-	0.5
Citric acid (%)	1	1	1	1
Coloring agent	q.s	q.s	q.s	q.s
Isopropyl alcohol	q.s	q.s	q.s	q.s

2.10.3 Evaluation of soft chewable lozenges

a) Gustatory sensation test

Developed formulation was evaluated for taste masking ability using taste panel of six healthy human volunteers. About 10 mg of sample of batch L1 to L4 was given to the healthy human volunteers. The volunteers were asked to taste the samples (10 mg) kept in the mouth for 10 seconds and then spit out and asked to give score with the aid of numerical scale with following values 0= Good, 1= Tasteless, 2= Slightly bitter, 3=Moderately bitter and 4= Extremely bitter. Based on evaluation study, pleasant mouth feeling formulation was selected as the optimized batch. The optimized batch was further evaluated.

b) Physical observation

The prepared lozenges were observed visually for appearance, texture and presence of any particles.

c) Weight Variation, Hardness and Thickness

The prepared Soft chewable lozenges were evaluated for Weight Variation, Hardness (using Monsanto hardness tester) and Thickness (using Vernier Caliper).

d) Drug content

For determination of drug content 10mg of lozenges was diluted with 10ml of methanol and sonicated for 15 min to dissolve. 1 ml of this solution was transferred into volumetric flask and volume made upto 10ml with mobile phase. This solution was subjected to HPLC analysis and the percent drug content was calculated.

***e) In Vitro* Drug Release Studies**

In Vitro Drug release studies the release rate of soft chewable lozenges of FEH was determined using USP dissolution testing apparatus II (paddle method). The dissolution test was performed using 900 ml of 0.001M HCL, at 37 ±0.5°C and 100 rpm. Sampling was done at 5, 10, 15, 20, 30, 45, 50-minute interval. For each sample 5 ml of the dissolution medium was withdrawn and the same amount of dissolution medium at 37±0.5 ºC was replenished to the dissolution medium. The sample withdrawn was filtered using a 0.2-µm nylon disc filter. The filtered samples were suitably diluted and analyzed for the drug content using HPLC.

2.11 Stability studies of oral flavoured powder, effervescent granules, soft chewable lozenges

The optimised batch were subjected for stability study as per international council for harmonization (ICH). Formulation was packed in *laminated aluminum packs and stored at different temperature for stability.*

Table 5: Stability studies of oral flavoured powder, effervescent granules, soft chewable lozenges

Condition	*Long Term Storage* 25ºc±2ºc/60±5%RH 3 Months			*Long Term Storage* 0±2-8°C 3 Months	Accelerated Conditions 40°C±2°C/75±5% RH 3 Months		
Formulation	Oral Flavoured Powder,	Effervescent Granules	Soft Chewable Lozenges	Soft Chewable Lozenges	Oral Flavoured Powder,	Effervescent Granules,	Soft Chewable Lozenges
Evaluation	Physical Appearance, Drug Content And *In-Vitro* Dissolution Study.	Drug Content, Effervescent Time And *In-Vitro* Dissolution Study.	Appearance, Drug Content And *In-Vitro* Dissolution Study.	Appearance, Drug Content And *In-Vitro* Dissolution Study.	Physical Appearance, Drug Content And *In-Vitro* Dissolution Study.	Drug Content, Effervescent Time And *In-Vitro* Dissolution Study.	Appearance, Drug Content And *In-Vitro* Dissolution Study.

3. RESULTS AND DISCUSSION

3.1 Preliminary study on drug

Table 6: Preliminary study on drug

Sr. No.	Parameter	Drug (FEH)
1	Color	White powder
2	Odor	Odorless
3	Taste	Intense bitter
4	Melting point	195-197°C

3.2 FTIR spectrum of fexofenadine hydrochloride

The IR spectrum of pure drug was found to be similar to the reference standard IR spectrum of Fexofenadine Hydrochloride. Compatibility of FEH and selected excipient to produce oral flavored powder, effervescent granules and soft chewable lozenges was assessed by placing mixture of FEH and each excipient in caped glass vials at room temperature for 30 days. Visual

observation of each mixture suggested that there was no change in color and appearance even after 30 days of the study. These results suggest that there is no chemical interaction between drug and excipient used for formulations.

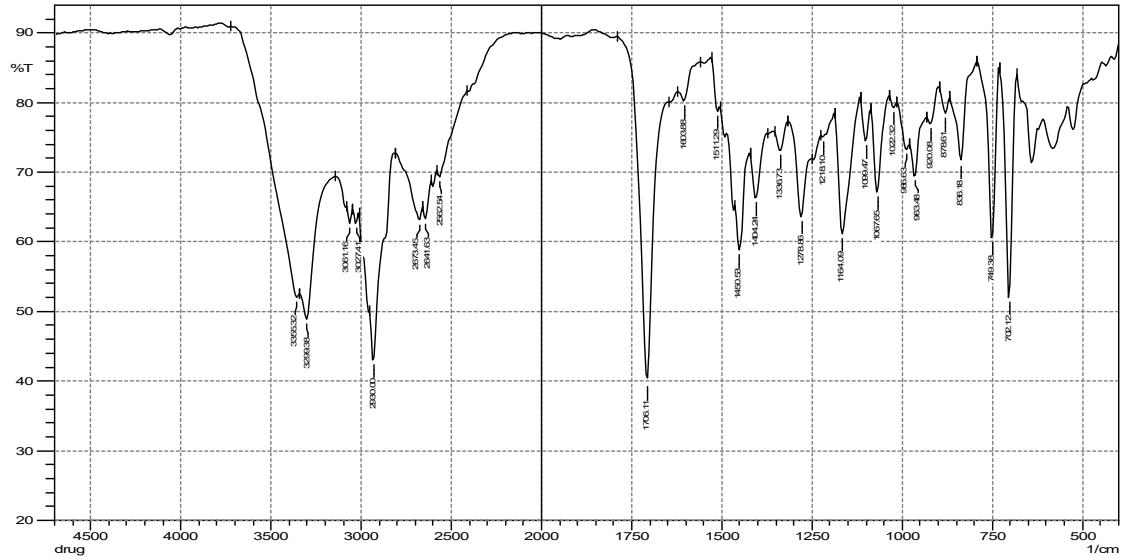

Fig. 1: FTIR spectrum of fexofenadine hydrochloride

Table 7: Interpretation of IR spectrum of FEH

Wave number cm^{-1}	Assignment
3355-3299	O-H stretching
2930	C-H stretching
1706	C-O stretching
1450	Aromatic C-C stretching
1278	C-N stretching
702.12	Aromatic rings

3.3 Solubility Study of drug in different solvents

Table 8: Solubility of FEH in various solvents

Vehicles	Solubility (mg/ml)
Methanol	Freely soluble
Water	0.0026
Phosphate buffer pH 6.8	0.5
0.001M HCL	1

From the above results it was concluded that Fexofenadine hydrochloride was freely soluble in methanol and soluble in 0.001 M HCL, slightly soluble in Phosphate buffer pH 6.8 and insoluble in water.

3.4 Analytical method development for estimation of FEH

Wavelength scan from 400-200 nm was performed to find absorption maxima. Maximum absorption was found at 221 nm in methanol, 0.001M HCl and phosphate buffer pH 6.8.

3.5 Calibration curve of drug in methanol

The concentration range of 5 to 40 µg/ml of drug was used for preparation of standard curve in methanol. The value of R^2 was found to be 0.9993 indicating that the relation of drug concentration and absorbance was linear.

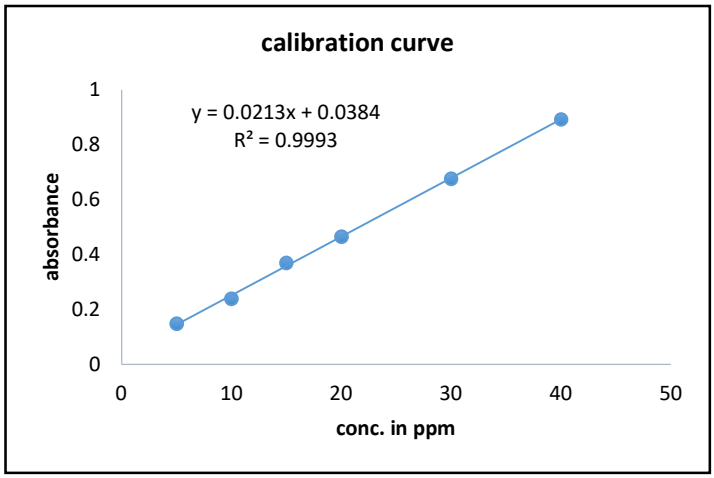

Fig. 2: Calibration curve of drug in methanol

3.6 Calibration curve of drug in phosphate buffer pH 6.8

The concentration range of 5 to 40µg/ml of drug was used for preparation of standard curve in methanol. The value of R^2 was found to be 0.9924 indicating that the relation of drug concentration and absorbance was linear.

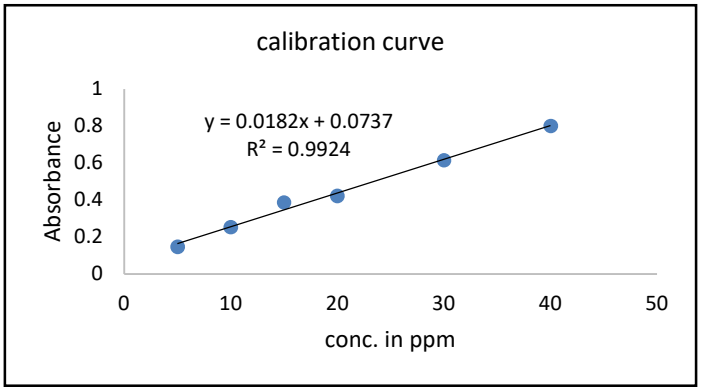

Fig. 3: Calibration curve of drug in phosphate buffer pH 6.8

3.7 Calibration curve of drug in 0.001M HCL

The concentration range of 5- 40 µg/ml of drug was selected for development of standard curve in methanol. The value of R^2 was found to be 0.9975 indicating that the relation of drug concentration and absorbance was linear.

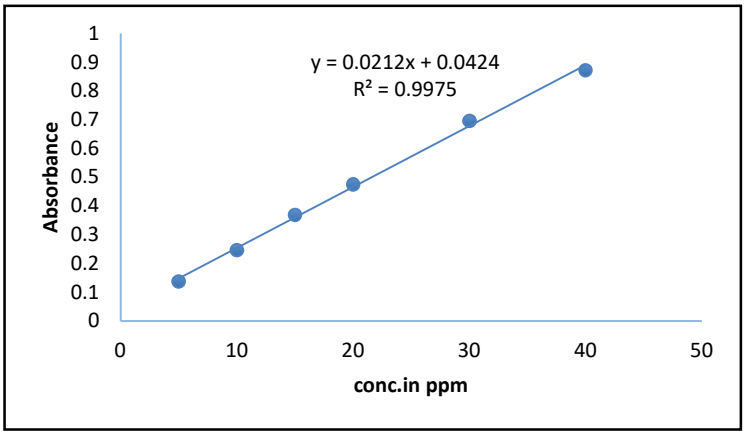

Fig. 4: Calibration curve of drug in 0.001M HCL

3.8 Effect of excipients on UV absorbance:

Direct spectrophotometry methods often suffer big disadvantages of their low selectivity and accuracy. UV scanning of pure drug and cyclodextrin were performed at wavelength range of 200-400 nm to detect any possible interference. There was significant interference in UV analysis as cyclodextrin was showing appreciable absorbance at 222 nm. So, for further analysis HPLC method was developed.

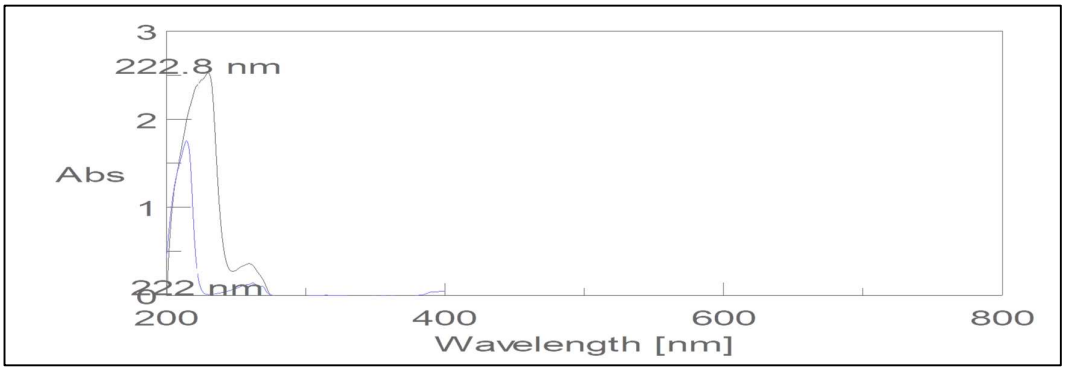

Fig. 5: Overlay UV spectrum of drug and excipient

3.9 High performance liquid chromatography for FEH

The retention time was found to be 4.22.

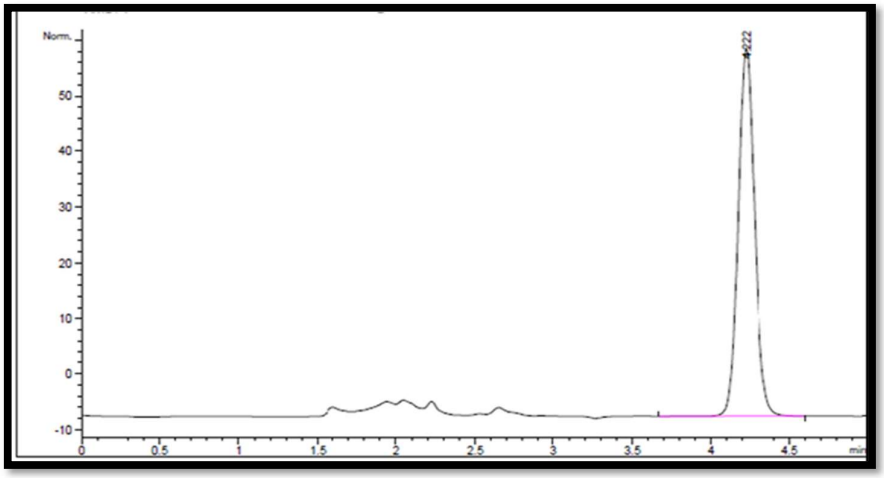

Fig.6: Chromatogram of FEH

The value of R^2 was found to be 0.9956 indicating that the relation of drug concentration and area was linear. The equation obtained was y = 46.361x, where y= absorbance and x= concentration.

Table 9: Data of calibration curve of FEH

Concentration (ppm)	Area
5	232.95
10	492.49
15	651.82
20	958.93
30	1438.30
40	1811.96

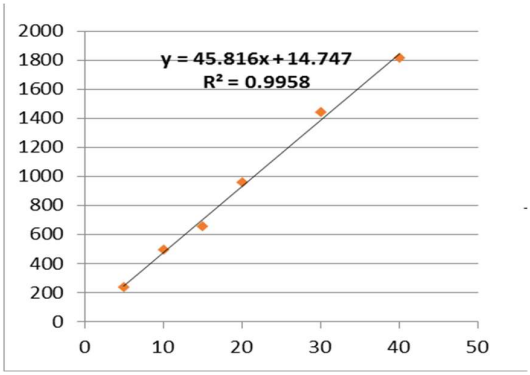

Fig 7: Calibration curve of FEH

3.10 Method validation

Table 10: Method validation data for FEH

Analytical parameter	FEH
Retention time	4.22
LOQ (µg/ml)	2
LOD (µg/ml)	0.5
Linearity	
Range (µg/ml)	5-40
Slope ± % RSD	46.361±0.862
System Precision	
Amount taken	20
Amount detected(µg/ml)	20.4
% RSD	0.06
R^2	0.9956

Table 11: Accuracy experiment using proposed method

Level	FEH		
	Amount taken (µg)	Amount detected (µg)	% recovery
1	10	10.05	105
2	20	20.04	100.2
3	40	39.5	98.75
Mean % recovery			101.3
%RSD			2.63

3.11 Estimation of Threshold concentration for fexofenadine hydrochloride

From the table below, it was concluded that the bitterness threshold of FEH is approximately 40µg/ml. No bitter taste was observed till a concentration less than 40 µg/ml by any of the six human volunteers.

Table 12: Determination of the Bitterness threshold concentration of FEH

Drug solution (ppm)	Human volunteers					
	1	2	3	4	5	6
5	1	1	1	1	1	1
10	1	1	1	1	1	1
20	1	1	1	1	1	1
30	1	1	1	1	1	1
40	2	2	2	2	2	2
50	3	3	3	3	3	3

3.12 Characterization of taste masked drug inclusion complex

When batches of drug-Cavitron W7HP7 complex evaluated by human volunteers, maximum formulations showed taste score of 3 and 4 which confirmed that bitter taste of FEH was not effectively masked using Cavitron W7HP7.

The results of taste evaluation of FEH-2HPBCD complex by human volunteers for Cavasol W7HP are depicted in table no 25. The batch FEH (1:5) showed taste score 1 which confirmed that bitter taste of FEH was successfully masked by using Cavasol W7HP.

Table 13: Taste evaluation of FEH-2HPBCD complex by volunteers for Cavasol W7HP

Batch no	HUMAN VOUNTEERS					
	1	**2**	**3**	**4**	**5**	**6**
FP1	4	4	4	4	4	4
FP2	4	4	4	4	4	4
FP3	4	4	4	4	4	4
FP4	3	3	3	3	3	3
FP5	2	2	2	2	2	2
FC1	4	4	4	4	4	4
FC2	4	4	4	4	4	4
FC3	3	3	3	3	3	3
FC4	4	4	4	4	4	4
FC5	3	3	3	3	3	3
FE1	4	4	4	4	4	4
FE2	3	3	3	3	3	3
FE3	2	2	2	2	2	2
FE4	2	2	2	2	2	2
FE5	**0**	**0**	**0**	**0**	**0**	**0**

(0=good, 1= tasteless, 2= slightly bitter, 3= bitter and 4 = very bitter)

3.12.1 Solubility study

Table 14: Solubility of drug and inclusion complex

Vehicle	Solubility mg/ml
Distilled Water (pure drug)	0.0026
Distilled Water (FE5-1:5 complex prepared by solid dispersion method)	40

3.12.2 Drug content assay

Drug content of inclusion complex prepared by solid dispersion method i.e FE5 1:5 was determined using HPLC and it was observed to be 99.8±0.14%.

***3.12.3 In vitro* taste evaluation**

The amount of drug released at salivary pH was found to be 0.08mg/10ml i.e 8 ppm in 60 seconds. The results of threshold response study of showed that threshold concentration of FEH is 40 ppm which is significantly lower than that obtained from *in-vitro* taste evaluation.

3.12.4 Differential Scanning Calorimetry analysis

When DSC thermogram of FEH and 2HPBCD are compared with that of drug-2HPBCD inclusion complex, it was observed that in thermogram of drug-2HPBCD inclusion complex the endothermic peak at 134.4°C corresponding to 2HPBCD is slightly shifted to 127°C and the endothermic peak at 204°C corresponding to FEH was not visible indicating complete encapsulation of drug in 2HPBCD.

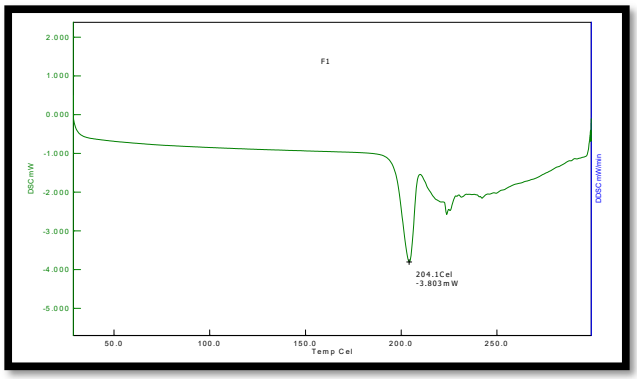

Figure 8: DSC thermogram of drug

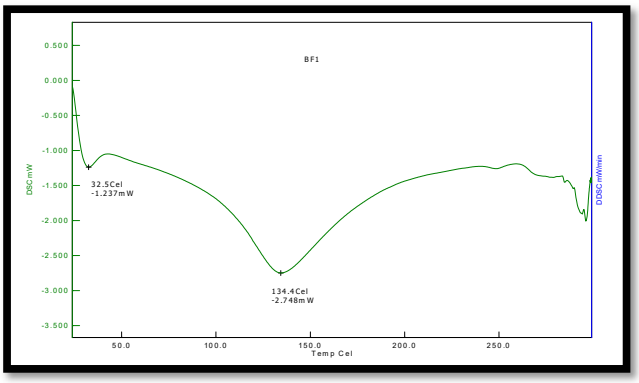

Figure 9: DSC thermogram of 2HPBCD

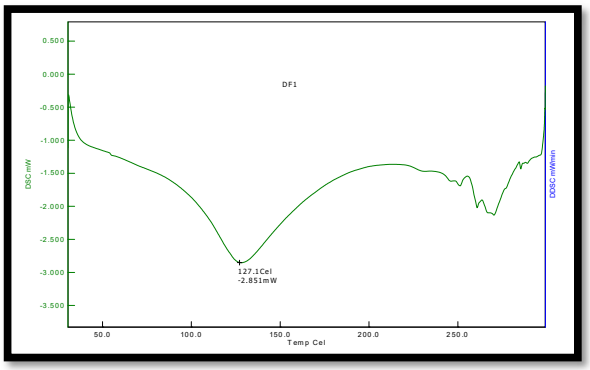

Figure 10: DSC thermogram of Drug-2HPBCD complex

3.12.5 Powder characterization

The results for evaluation of flow properties of optimized inclusion complex shows good flow property.

Table 15: Flow properties of inclusion complex

Batch	FE5- 1:5 (solid dispersion)
Angle of repose	32.2°±0.12
Bulk density	0.62±0.12gm/cm^3
Tapped density	0.71±0.14gm/cm^3
Carr's index	12%
Hausner's ratio	1.14

3.13 Formulation of oral flavored powder

The optimized batch was selected based on the basis of results obtained from various evaluation parameters.

3.13.1 Evaluation of oral flavored powder

a) Gustatory sensation test: All the developed formulations (F1-F8) showed acceptable palatability, but the batch F4 formulated using lemon flavor was more acceptable to volunteers, so batch F4 was selected as an optimized batch.

Table 16: Taste evaluation of formulations by volunteer

Batch no	Human volunteers					
	1	2	3	4	5	6
F1	0	0	0	0	0	0
F2	0	0	0	0	0	0
F3	0	0	0	0	0	0
F4	0	0	0	0	0	0
F5	0	0	0	0	0	0
F6	0	0	0	0	0	0
F7	0	0	0	0	0	0
F8	0	0	0	0	0	0

b) Drug content

Drug content of optimized batch F4 was found to be 100 ± 0.14 %

c) Powder characterization

The results for evaluation of flow properties of oral flavored powder batch show good flow property.

Table 17: Flow properties of oral flavored powder

Parameters	Batch F4
Angle of repose	32.2°±0.14
Bulk density	0.50±0.29 gm/cm^3
Tapped density	0.55±0.31 gm/cm^3
Carrs index	12%
Hausner's ratio	1.14

***d) In-vitro* evaluation**

In case of FEH 31.4% drug was released while as in case of oral flavored powder 100.16% drug was released. It is evident from observation that FEH oral flavored powder showed dramatic improvement in vitro dissolution profile compared to the pure FEH API in 0.001M HCL. The rate and extent of FEH release from oral flavored powder suggested that it may improve the oral bioavailability of FEH.

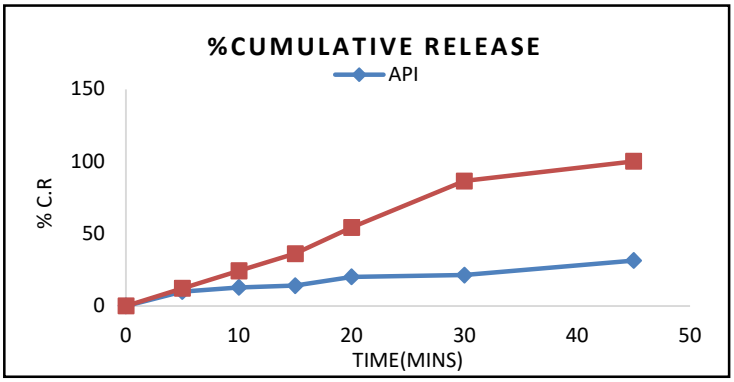

Figure 11: *In vitro* Drug release kinetics of FEH and batch F4

Table 18: In-vitro drug release study of FEH API and oral flavored powder

Sr. No.	Sampling time	% cumulative release	
		FEH API	oral flavored powder (F4 batch)
1	5	10	12.1
2	10	12.8	24.4
3	15	14.02	36.2
4	20	20.04	54.4
5	30	21.33	86.53
6	45	31.4	100.16

***e) In-vivo* evaluation**

The below observation for the volume consumed and the rats' behavior indicated that the bitter taste of FEH was effectively masked. This observation was further supported in this study by the higher consumption of the test solutions over that of pure drug solution by the rats, which indicated that the rats liked the taste of the test solutions.

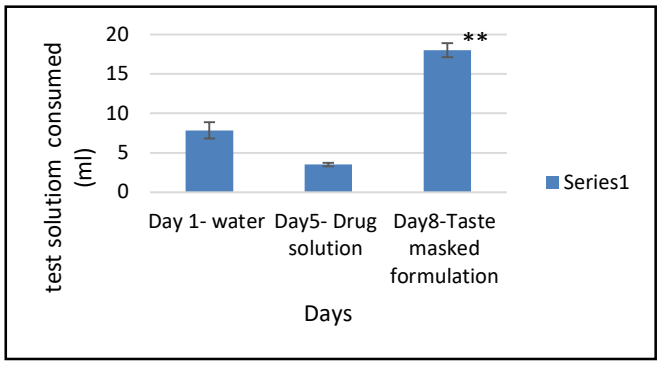

Figure12: In vivo taste assessment. *P<0.05, statistically significant difference in the volume of the test solution consumed (ml) as compared to that on day 8 taste masked formulation.

f) Stability studies

All the parameters were within the acceptable limits which showed that formulation was stable over the period of 3 months.

Table 19: Results of the parameters studied during Stability study of oral flavored powder

Parameters assessed	Sample to be performed		
	Physical appearance	Drug content %	In-vitro drug release in 45 min %
	25^0C ±2.0^0C/ 60% RH% ±5.0%		
0 days	White	100	100.16
30 days	White	99.98	100.14
60 days	White	99.80	100
90 days	White	99.85	99.99
	40^0C±2.0^0C/ 75% RH% ±5.0%		
0 days	White	100	100.14
30 days	White	99.86	100
60 days	White	99.80	99.9
90 days	White	99.76	99.82

3.14 Development of effervescent granules

Optimized batch was selected on the basis of the results of various evaluation parameters.

3.14.1 Evaluation of effervescent granules

a) Effervescence time

Among all the formulations E1 showed the least effervescence time (20 sec), so this was batch selected as a optimized batch and subjected to Gustatory sensation test.

Table 20: Result of Effervescence time

Formulation batch	Effervescence time (Seconds)
E1	20
E2	30
E3	35
E4	25

b) Gustatory sensation test

When batch E1 was subjected to human volunteers, it showed taste score 0 indicating that formulation E1 was more acceptable to the volunteers.

d) Powder characterization

Table 21: Flow properties of effervescent granules

Parameters	Batch F1
Angle of repose	33.4°±0.14
Bulk density	0.53±0.01 gm/cm^3
Tapped density	0.60±0.01gm/cm^3
Carrs index	12%
Hausners ratio	1.14

e) Drug content

Drug content of optimized batch E1 was found to be 102.2 ± 0.01%.

f) *In-Vitro* Drug Release Studies

The optimized batch (E1) showed more than 99 % release within 6 min. The optimized batch (E1) showed good bursting effect followed by rapid dissolution.

Table 22: In-vitro drug release study of FEH API and effervescent granules

Sr. No.	Sampling time (Minutes)	Effervescent granules (E1 batch)
1	1	21.3
2	2	33.1
3	3	44.4
4	4	67.73
5	5	91.5
6	6	100.12

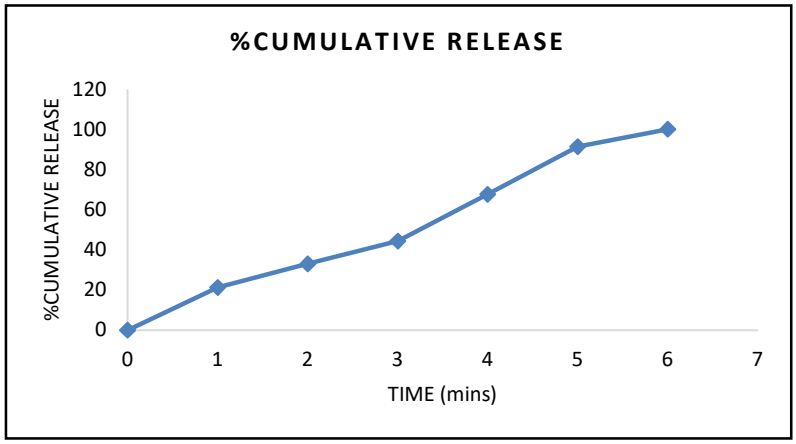

Figure 13: *In vitro* Drug release kinetics of FEH effervescent granules F1 batch

g) Stability studies

All the parameters were within the acceptable limits which showed that formulation was stable over the period of 3 months.

Table 23: Results of the parameters studied during Stability study of effervescent granules

Parameters assessed	Sample to be performed		
	Effervescent time (Seconds)	Drug content %	In-vitro drug release in 45 min %
	25^{0}C ± 2.0^{0}C/ 60% RH % ±5.0%		
0 days	20	102.1	100.12
30 days	20	102.1	100.10
60 days	19	101.1	100.1
90 days	19	100	99.9
	40^{0}C ± 2.0^{0}C/ 75% RH % ±5.0%		
0 days	20	100	100.13
30 days	20	99.86	100.12
60 days	18	99.80	100.10
90 days	17	99.76	99.82

13.15 Development of soft chewable lozenges

13.15.1 Optimization of binder concentration for preparation of soft chewable lozenges

Table 24: Selection of binder ratio on the basis of physical appearance

Binder (HPMC E15)		
Batch code	Binder concentration (%)	Nature of soft chewable lozenges form
A1	2.3	smooth, little sticky
A2	4.7	Smooth, shiny, easily removes from lozenges cutter, not sticky,
A3	7.1	Hard, little sticky

From the above table it could be concluded that batch containing 4.7% w/v of binder gives intact, smooth, non-sticky and shiny soft chewable lozenges, hence batch A2 was selected as an optimized for preparation of soft chewable lozenges.

13.15.2 Screening of flavors for optimized batch of soft chewable lozenges

The optimized batch was selected on basis of results obtained from various evaluation parameters.

13.15.3 Evaluation of soft chewable lozenges

a) Gustatory sensation test

The objective of this study is to conduct and evaluate the palatability of different formulations. All the batches formulated (L1-L4) showed acceptable palatability, but the batch L4 formulated using lemon flavor was more satisfactory, so this batch was selected and further evaluated.

Table 25: Taste evaluation of formulations by volunteers

Batch no	Human volunteers					
	1	2	3	4	5	6
L1	0	0	0	0	0	0
L2	0	0	0	0	0	0
L3	0	0	0	0	0	0
L4	0	0	0	0	0	0

13.3.2 Physical observation

The lozenges made out of L4 formulation batch appeared smooth, shiny and yellow in colour.

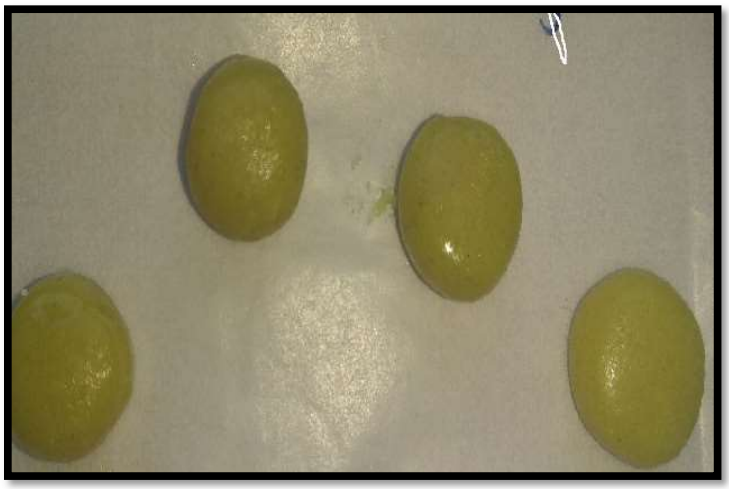

Figure 14: Shows the image of the optimized formulation L4

b) Drug content

Drug content of optimized batch L4 was found to be 100±0.01%

c) Physicochemical Characterization

The soft chewable lozenges made from optimized L4 batch showed thickness of 1.96mm with a diameter of 1mm. The lozenges showed a weight variation of 824±0.12 mg.

***d) In-Vitro* Drug Release Studies**

In vitro release studies were performed using USP Apparatus II (paddle type). The dissolution test was performed using 900 ml phosphate buffer (pH 6.8), 37 ± 0.5°C. Samples were collected at predetermined time intervals and replaced with equal volumes of fresh medium. The samples were analyzed using UV spectrophotometer λ= 224 nm. Drug concentration was calculated from a standard calibration plot and expressed as cumulative % drug release. FEH Soft chewable lozenges showed dramatic improvement in vitro dissolution profile within 50 mins. Percent cumulative release was found to be 94.2% after 50 mins.

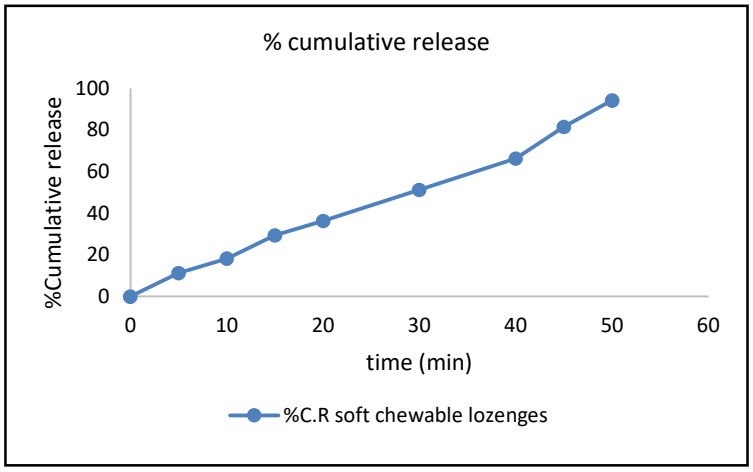

Figure 15: *In vitro* Drug release kinetics of Batch L4

e) Stability studies

All three formulations were subjected to stability study for three months at different temperature conditions and all formulations were found to be stable.

Table 26: Stability assessments of soft chewable lozenges

	Sample to be performed		
	0±2-8°C		
Parameter assessed	Physical appearance	Drug content	In-vitro drug release in 50 min %
0 day	Smooth	10.2.1	94.2
30 days	Smooth	102	94.2
60days	Smooth	100	94
90days	Smooth	100	94
	25^0C ±2.0^{0}C/ 60% RH%		
0 day	Smooth	10.2.1	94.3
30 days	Smooth	10.2	94.2
60days	Smooth	99.99	94.1
90days	Smooth	98.69	94
	40^0C±2.0^{0}C/ 75% RH%		
0 day	Smooth	102.1	94.3
30 days	Smooth	99.99	94.2
60days	Smooth	98.48	94.1
90days	Smooth	98.36	93.89

15. CONCLUSION

Effective taste masking of Fexofenadine hydrochloride was achieved through complexation with selected cyclodextrin (2-Hydroxypropyl beta cyclodextrin/ Cavasolw7HP). Results coclusively demonstrated that successful taste masking of FEH was accomplished and suggest that it could be formulated for oral cavity with more acceptability to pediatrics and improved bioavability.

14. ACKNOWLEDGEMENT

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