

Original Article

IMPROVEMENT OF BIOAVAILABILITY OF CEFUROXIME AXETIL ORAL SUSPENSION BY INCLUSION COMPLEXATION METHOD

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ABSTRACT

Objective: Cefuroxime axetil, a prodrug of Cefuroxime, is a poorly water soluble drug, thus it has got only limited solubility and dissolution rate in gastric fluids. Also, the bioavailability of Cefuroxime axetil oral suspension is only 40-45% when compared to the 60% bioavailability of tablets. The objective of this study was to develop an oral suspension of Cefuroxime axetil with improved oral bioavailability by inclusion complexation method using Hydroxypropyl beta cyclodextrin [HP-Beta-cyclodextrin]

Methods: The complexation of Cefuroxime axetil and HP-Beta cyclodextrin was carried out at 1:1, 1:2, 1:2.5 and 1:3 ratios respectively. The prepared suspensions were evaluated for various parameters like pH, viscosity, re-dispersibility, pourability, and assay and *in-vitro* dissolution profile. A leading marketed product and the optimized formulation were evaluated for the pharmacokinetic parameters like C_{max}, AUC_{0-t}, AUC_{0-∞} and T_{max} in healthy adult male rabbits.

Results: Considering the *in-vitro* dissolution profile, formulation with 1:2.5 ratios of Cefuroxime axetil and HP-Beta cyclodextrin was selected as the optimized formulation. The C_{max} of Optimized formulation and Marketed product were 148±1.26ng/ml and 126±1.52 ng/ml respectively, and the AUC_{0-t} of Optimized formulation and Marketed product were 989±16.42 ng. h/ml and 613±24.26 ng. h/ml respectively, which shows a significant improvement in the bioavailability of optimized formulation

Conclusion: From the results obtained it can be observed that there is a significant improvement in the bioavailability of optimized formulation compared to the marketed product. This demonstrates that the inclusion complexation method with HP-Beta cyclodextrin can significantly improve the oral bioavailability of Cefuroxime axetil.

Keywords: Cefuroxime axetil, Bioavailability, Beta cyclodextrin, Inclusion complexation

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INTRODUCTION

Cefuroxime axetil is a prodrug of Cefuroxime, which is a second-generation cephalosporin antibiotic with activity against wide range of organisms. The bactericidal activity of cefuroxime axetil is due to Cefuroxime's binding to essential target proteins and the resultant inhibition of bacterial cell wall synthesis. After oral administration, Cefuroxime axetil is absorbed and then rapidly hydrolyzed by the nonspecific esterases which is distributed in the intestinal mucosa and portal blood, and ultimately transformed into the pharmacologically active Cefuroxime [1-3].

Cefuroxime axetil is a poorly water soluble drug; thus, it has got only limited solubility and dissolution rate in gastric fluids. The bioavailability of oral suspension is only 40-45% when compared to the 60% bioavailability of tablets. Therefore, the oral suspension and tablets cannot be substituted each other on mg/mg basis [4-6].

In this study attempt has been made to improve the oral bioavailability of Cefuroxime axetil oral suspension 125 mg by inclusion complexation method using HP-Beta cyclodextrin. Cyclodextrins are having a 'bucket-like' structure, which allows them to accommodate guest molecules within its cavity, so forming an inclusion complexation, which helps in improving the solubility, oral bioavailability, and stability of a variety of drugs [7-15]. In this study, HP-beta-cyclodextrin is selected due to its improved water

solubility and safety compared to other cyclodextrins [16]. A leading marketed sample is used in this study as a competitor product, which has used spray drying technology with Stearic acid to mask the taste [17, 18]. A comparative evaluation of prepared oral suspensions and marketed product has been conducted for various *in-vitro* and *in-vivo* parameters.

MATERIALS AND METHODS

Materials

Cefuroxime axetil for the study was procured from Covalent Laboratories private limited, Hyderabad, India. HP-Beta cyclodextrin was purchased from Signet Chemical Corporation Pvt ltd, India (mfg. by: Roquette). Sucrose was received from EID Parry Ltd, India; Xanthan was procured from Deosen, China. Acesulfame potassium was procured from Ningbo Hi-Tech Biochemicals Co-Ltd China. Aspartame was received from NutraSweet, China. Tutti fruity flavor and Peppermint flavor were procured from Firmenich, Switzerland.

Methods

Preparation of cefuroxime axetil: HP-beta-cyclodextrin complex by inclusion complexation method

Cefuroxime axetil and HP-Beta cyclodextrin were taken at 1:1, 1:2, 1:2.5 and 1:3 combinations at molecular weight ratio (shown in table-1).

Table 1: Combinations of cefuroxime axetil and HP-beta cyclodextrin for complexation

Ingredients	Ratio (mg/5 ml)			
	1:1	1:2	1:2.5	1:3
Cefuroxime axetil	158.250	158.250	158.250	158.250
HP-Beta-cyclodextrin	477.915	955.830	1194.790	1433.750
Total weight	636.165	1114.080	1353.040	1592.000

Accurately weighed Cefuroxime axetil and HP-Beta cyclodextrin were sifted through #30 mesh and mixed together to get a uniform blend. The resulting mixture was slowly added to purified water in a beaker under stirring using mechanical stirrer. The stirring process continued for six hours to get thick slurry of Cefuroxime axetil and HP-Beta-cyclodextrin complex.

The slurry was transferred to a tray and dried in hot air oven at 45 °C until the complex is adequately dried. The dried complex was passed through #60 mesh and mixed thoroughly. The resulted

Cefuroxime axetil: HP-Beta-cyclodextrin complex in different ratios were used for further processing to make dry suspension.

Preparation of taste masked dry suspension of cefuroxime axetil 125 mg/5 ml

Cefuroxime axetil taste masked dry suspension was formulated by mixing Cefuroxime axetil: HP Beta-cyclodextrin complex along with other inactive ingredients as shown in table 2. Formulations were prepared with each combination of Cefuroxime axetil: HP Beta-cyclodextrin complexes (1:1, 1:2, 1:2.5 and 1:3).

Table 2: Formula for cefuroxime axetil taste masked dry suspension 125 mg/5 ml

S. No.	Ingredients	Ratio (mg/5 ml)			
		1:1	1:2	1:2.5	1:3
1	Cefuroxime axetil: HP Beta-cyclodextrin complex	636.165	1176.468	1454.518	1634.984
2	Sucrose (#40 mesh grade)	1691.876	1286.649	1078.111	942.762
3	Sucrose (#80 mesh grade)	563.959	428.883	359.371	314.254
4	Acesulfame K	25.000	25.000	25.000	25.000
5	Aspartame	30.000	30.000	30.000	30.000
6	Xanthan gum	8.000	8.000	8.000	8.000
7	Tutti fruity premium flavour	35.000	35.000	35.000	35.000
8	Peppermint premium flavour	10.000	10.000	10.000	10.000
	Average weight:	3000.000	3000.000	3000.000	3000.000

Cefuroxime axetil: HP-Beta-cyclodextrin complex, Sucrose (#80 mesh), Acesulfame K, Aspartame, Xanthan gum, Tutti fruity flavor and Peppermint premium flavor were sifted through mesh # 40 and mixed together. Sucrose (# 40 mesh) was sifted through mesh #30 and added to above blend and mixed well. 18g of the blend was filled in 30 ml High-density polyethylene [HDPE] bottle and closed with HDPE cap. Each bottle needs to be reconstituted with water before administration to make the oral suspension.

Evaluation of cefuroxime axetil oral suspension 125 mg/5 ml

Physicochemical properties of suspension

The physicochemical properties of suspension like color, pH, dispersibility, Viscosity, Assay and pourability were evaluated.

In-vitro dissolution studies

In-vitro dissolution of all the combinations and market sample were tested using ELECTROLAB dissolution apparatus as per the method specified in United States Pharmacopoeia [USP]. 900 ml of pH 7.0 Phosphate buffer was used as dissolution medium with USP apparatus 2 (Paddle), at 50 rotations per minute [rpm]. The temperature of the medium was maintained at 37±0.5 °C. The dry suspension was reconstituted with water and a quantity equivalent to 125 mg of Cefuroxime axetil were used for the dissolution study. During the dissolution study, 5 ml samples were withdrawn at 10 min, 20 min and 30 min intervals. The samples were filtered through 0.22 µm filter, and the concentration of Cefuroxime axetil in the filtrate was tested using a spectrophotometer. The limit for dissolution as per USP is not less than 60% (Q) in 30 min.

Pharmacokinetic study in rabbits

The bioavailability evaluation of optimized formulation and marketed product was carried out at Albino Research and Training Institute, Hyderabad, India with approval from the Committee for the Purpose of Control And Supervision of Experiments on Animals (CPCSEA) for the study with registration no: 1722/ RO/ ERe/S/13/CPCSEA.

Animals and study design

Six healthy adult male rabbits (Weighed: 1.5-2 Kg, aged: 8-10 mo) were enrolled in the study. Rabbits fasted for 12 h with free access to water before the study started. A single dose two-way crossover design study was conducted on rabbits. There was a washout period of one week between the two doses. The rabbits were divided into two groups

Protocol of study

Administration of the two products (optimized formulation and Marketed product) to the animals was carried by means of a two-way crossover design. The subjects were randomly divided into two

equal groups and assigned to one of the two sequences of administration. Each animal received a single dose at a time.

Sampling procedure

The blood samples were collected from the ear marginal vein of the rabbits, which were held in wooden cages, in heparinized glass centrifuge tubes with the aid of sterilized disposable plastic syringes just before and at 1, 2, 4, 6, 8, 10, 12, 15, 18, 21 and 24h after the drug administration. The blood samples were centrifuged at 3000 rpm for 10 min to separate the plasma for analysis

Quantitative drug analysis

The concentration of drug in plasma was determined by HPLC technique with ultraviolet detection at 279 nm. Estimation of drug concentration was carried out by interpolating the peak area of best formulation on a calibration curve of spiked the blank plasma over the range assayed

Pharmacokinetic analysis

The pharmacokinetic characteristics such as C_{max} (ng/ml), T_{max} (h), Kel (h⁻¹), t_{1/2el} (h), V_d (ng/ml), AUC₀₋₂₄ (ng. h/ml), AUC_{0-∞} (ng. h/ml), AUMC₀₋₂₄ (ng. h²/ml), AUMC_{0-∞} (ng. h²/ml), MRT₀₋₂₄ (h) and MRT_{0-∞} (h) of drugs were determined from the plasma concentration-time profile. The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}) were obtained directly from the plasma concentration-time data. The area under the plasma concentration-time curve up to the last time (t) showing a measurable concentration (C_t) of the analyte (AUC_{0-t}) was determined by applying the linear trapezoidal rule. The apparent elimination rate constant (Kel) was calculated by the log-linear regression of the data points of describing a terminal log-linear decaying phase. The AUC_{0-∞} values (express the magnitude of absorption) were determined by adding the quotient of *C_t and the appropriate kel to the corresponding AUC_{0-t}, which is,

$$AUC_{0-\infty} = AUC_{0-t} + C_t / Kel$$

Where *C_t is the last detectable plasma concentration.

The sampling period covered more than 96% of the total AUCs for both reference and test. The apparent elimination half-life (t_{1/2}) of drug and in plasma was calculated by using the following equation,

$$t_{1/2} = (\ln 2) / Kel$$

The ratio of $C_{max}/AUC_{0-\infty}$ was also computed and used as a measure for the rate of absorption.

Statistical analysis

All values are expressed as the mean±standard deviation (SD). The pharmacokinetic parameters obtained by following a single dose administration of the marketed product and the optimized formulation to normal Rabbits were compared using paired 't' test, considering a probability of $P<0.05$ to be significant.

Bioavailability and statistical analysis were performed according to the Food and Drug Administration (FDA) guidelines by using a software Bear v2.5.3

RESULTS

Physiochemical properties of suspension

Physiochemical properties of reconstituted suspension were tested as part of quality control tests, the results of which are shown in table 3.

Table 3: Physiochemical properties of suspension

S. No.	Tests	Formulations			
		1:1	1:2	1:2.5	1:3
1	Color	White	White	White	White
2	pH (Limit: 3.5 to 7)	5.98	6.01	5.92	6.02
3	Viscosity	319cps	340cps	395cps	410cps
4	Redispersibility	Easy	Easy	Easy	Easy
5	Pourability	Easily Pourable	Easily Pourable	Easily Pourable	Easily Pourable
6	Assay (Limit: 90 to 110 %)	97.85 %	96.89 %	99.50 %	97.68 %

Cefuroxime axetil dry suspension was reconstituted with an adequate quantity of water. The color of the suspensions was observed to be white. The pH of all the formulations was within the specified limit of 3.5 to 7 as in USP. Adequate viscosity was observed in all the formulations, providing sufficient stability and pourability of suspension. All the formulations were easy to redisperse with water by shaking by hand for some time. All the suspensions were easily pourable making it easy to dispense. The Assay of all the formulations was meeting the specified limit of 90 to 110 % as per USP.

In-vitro dissolution studies

In-vitro dissolution of all the combinations and market sample were tested using ELECTROLAB dissolution apparatus as per the method specified in United States Pharmacopoeia. The results of in-vitro dissolution studies are given in fig. 1.

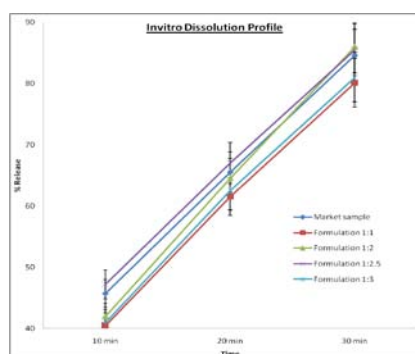


Fig. 1: In-vitro dissolution studies-All values are represented as mean, n=6

From the dissolution studies, it was found that all the formulations and marketed sample were meeting the dissolution criteria of not less than 60 % (Q) in 30 min. Among these formulation 1:2.5 seemed to have better release pattern than the marketed sample.

Pharmacokinetic study

The pharmacokinetic evaluation of optimized formulation and marketed product was carried out using a single dose, two-way

crossover design study on six healthy male rabbits. The Plasma drug concentrations at different time intervals for optimized formulation and Marketed Product are presented in fig. 2 and the major pharmacokinetic parameters are presented in table 4.

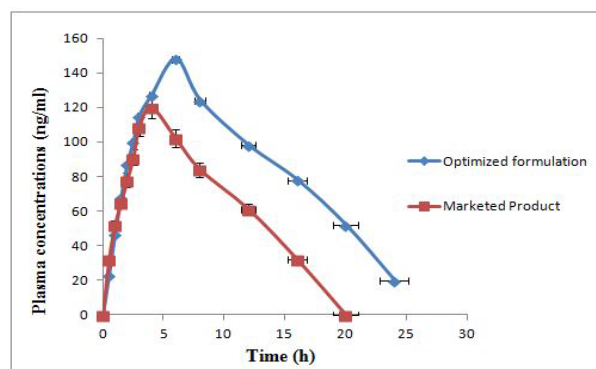


Fig. 2: Plasma drug concentrations at different time intervals for optimized formulation and marketed product

Various pharmacokinetic parameters were calculated during the study, from which the C_{max} of optimized formulation and marketed product were found to be 148 ± 1.26 ng/ml and 126 ± 1.52 ng/ml respectively, and the AUC_{0-t} optimized formulation and marketed product were 989 ± 16.42 ng. h/ml and 613 ± 24.26 ng. h/ml respectively, which shows a significant improvement in the bioavailability of optimized formulation, compared to the marketed product.

Table 4: Comparison of pharmacokinetic parameters of optimized formulation and Marketed Product

Parameters	Optimized formulation	Marketed product
C_{max} (ng/ml)	148 ± 1.26	126 ± 1.52
AUC_{0-t} (ng. h/ml)	989 ± 16.42	613 ± 24.26
$AUC_{0-\infty}$ (ng. h/ml)	1225 ± 38.54	1004 ± 35.14
T_{max} (h)	6.00 ± 1.23	4.50 ± 0.24
$t_{1/2}$ (h)	3.05 ± 0.519	1.56 ± 0.01
K_{el} (h^{-1})	2.807 ± 0.11	2.189 ± 0.33

Each value is mean±SEM of 6 rabbits in each group

DISCUSSION

In this study attempt has been made to improve the oral bioavailability of Cefuroxime axetil oral suspension 125 mg by inclusion complexation method using HP-Beta-cyclodextrin. The optimized formulation is better in taste, dissolution and bioavailability compared to the marketed product. Many studies are available for the improvement of bioavailability of Cefuroxime axetil with plain Beta-cyclodextrin [1, 2]. In this study, HP-Beta-cyclodextrin is selected instead of plain Beta-cyclodextrin due to its improved water solubility and safety profile [16]. So it is an additional advantage to the patients to have tastier, efficient and safer medicine. Some other studies are available for masking the bitter taste of Cefuroxime axetil, like spray drying technique with Stearic acid, complexation with ion exchange resins, etc, but non of them claim to have improved the bioavailability of oral suspension[18].

CONCLUSION

An oral suspension of Cefuroxime axetil was successfully developed by inclusion complexation method using HP-Beta-cyclodextrin. All the formulations developed were subjected to various quality control tests including physicochemical parameters and *in-vitro* dissolution, where all the formulations were meeting the quality parameters. Among the four formulations prepared formulation with 1:2.5 ratios of Cefuroxime axetil and HP-Beta-cyclodextrin showed improved dissolution compared to the marketed product. So formulation with 1:2.5 ratios was selected as the optimized formulation. The optimized formulation and the marketed product were subjected to pharmacokinetic study using healthy male rabbits, which the results shows a significant improvement in the bioavailability of optimized formulation compared to the marketed product. This demonstrates that the inclusion complexation method with HP-Beta-cyclodextrin can significantly improve the oral bioavailability of Cefuroxime axetil.

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CONFLICT OF INTERESTS

Declare none

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