

Development and Comparative Bioavailability Evaluation of Cefuroxime Axetil Oral Suspension by Single Dose Two Way Cross Over Study in Rabbits

Renju Prabhakaran^{*1}, K. Janakiraman², Jyoti Harindran³

¹ Sance Laboratories Pvt Ltd, Pala, Kottayam, Kerala, India- 686573.

² Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamilnadu, India-608002.

³ Department of Pharmaceutical Sciences, Mahatma Gandhi University, Kottayam, Kerala, India-686631.

Abstract

Cefuroxime axetil is a second generation cephalosporin antibiotic with activity against wide range of organisms. It is a prodrug that gets converted into Cefuroxime after oral absorption. Cefuroxime axetil 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, therefore the oral suspension and tablets cannot be substituted each other on mg/mg basis. The objective of this study was to develop an oral suspension of Cefuroxime axetil with improved oral bioavailability by inclusion complexation method using Hydroxy propyl betacyclodextrin. The complexation of Cefuroxime axetil and Hydroxy Propyl betacyclodextrin 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, redispersibility, pourability, Assay and invitro dissolution profile. Considering the invitro dissolution profile, formulation with 1:2.5 ratio of Cefuroxime axetil and Hydroxy propyl betacyclodextrin was selected as the optimized formulation. 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. From the results obtained it can be observed that there is significant improvement in the bioavailability of optimized formulation, compared to the marketed product. This demonstrate that the inclusion complexation method with Hydroxy Propyl betacyclodextrin can significantly improve the oral bioavailability of Cefuroxime axetil.

Keywords-Cefuroxime axetil, bioavailability, Betacyclodextrin, Inclusion complexation

1. 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. Cefuroxime axetil is available in the market as tablets and oral suspension [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 125mg using Hydroxy propyl betacyclodextrin. Cyclodextrins are having a 'bucket-like' structure, which allow them to accommodate guest molecules within its cavity, so forming an inclusion complexation, which helps in improving the solubility, oral bioavailability and stability of variety of drugs [7-15]. HP-betacyclodextrin 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 have been conducted for various in-vitro and in-vivo parameters.

2. MATERIALS AND METHODS

2.1 Materials

Cefuroxime axetil for the study was procured from Covalent Laboratories private limited, Hyderabad, India. Hp-Betacyclodextrin 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 K potassium was procured from Ningbo Hi Tech Biochemicals Co- Ltd China. Aspartame was received from Nutrasweet, China. Tutti frutti flavor and Peppermint flavor were procured from Firmenich, Switzerland.

The animals for the study were obtained from Albino Research and Training Institute, Hyderabad, India, where the study was carried out. The animal ethical committee approval was obtained for the study from CPCSEA with registration no: 1722/RO/ERe/S/13/CPCSEA.

Table 1: Combinations of Cefuroxime axetil and HP Betacyclodextrin for complexation

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

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

Sr. No	Ingredients	Ratio (mg/5ml)			
		1:1	1:2	1:2.5	1:3
1	Cefuroxime axetil :HP Betacyclodextrin 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 friuty 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

2.2 Preparation of Cefuroxime axetil: HP-Betacyclodextrin complex by inclusion complexation method

Cefuroxime axetil and HP-Betacyclodextrin were taken at 1:1, 1:2, 1:2.5 and 1:3 combinations at molecular weight ratio as shown in table-1.

Accurately weighed Cefuroxime axetil and HP-Betacyclodextrin were sifted through #30 mesh and mixed together to get uniform blend. The resulting mixture was slowly added to purified water in a beaker under stirring using mechanical stirrer. Stirring process continued for six hours to get a thick slurry of Cefuroxime axetil and HP-Betacyclodextrin 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 #60mesh and mixed thoroughly. The resulted Cefuroxime axetil: HP-Betacyclodextrin complex in different ratios were used for further processing to make dry suspension.

2.3 Preparation of dry suspension of Cefuroxime axetil 125mg/5ml

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

Cefuroxime axetil : HP betacyclodextrin 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 blend was filled in 30

ml HDPE bottle and closed with HDPE cap. Each bottle need to be reconstituted with water before administration to make the oral suspension.

2.4 Evaluation of Cefuroxime axetil oral suspension 125mg/5ml

2.4.1 Physicochemical properties of suspension

The physicochemical properties of suspension like colour, pH, redispersibility, Viscosity, Assay and pourability were evaluated.

2.4.2 Invitro dissolution studies

Invitro dissolution of all the combinations and market sample were tested using ELECTROLAB dissolution apparatus as per the method specified in United States Pharmacopoeia. 900ml of pH 7.0 Phosphate buffer was used as dissolution medium with USP apparatus 2 (Paddle), at 50 rpm. Temperature of the dissolution medium was maintained at 37 ±0.5 °C. The dry suspension was reconstituted with water and a quantity equivalent to 125mg 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 spectrophotometer. The limit for dissolution as per USP is not less than 60% (Q) in 30 min

2.4.3 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 the CPCSEA approval 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 months) were enrolled in the study. Rabbits were fasted for 12 hours 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 sequence of administration. Each animal received a single dose at a time.

Sampling procedure

The blood samples were collected through 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 minutes to separate the plasma for analysis

Quantitative drug analysis

The concentration of drug in plasma was determined by HPLC technique with ultraviolet detection at 279nm. 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 and statistical analysis

The pharmacokinetic characteristics such as C_{max} (ng/mL), T_{max} (h), K_{el} (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 (K_{el}) 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 k_{el} to the corresponding AUC_{0-t}, which is,

$$AUC_{0-\infty} = AUC_{0-t} + *C_t / K_{el}$$

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,

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

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

All values are expressed as the mean ± standard deviation (SD). The pharmacokinetic parameters obtained by following a single dose administration of the reference standard tablets and the floating tablets 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 FDA guidelines by using a software Bear v2.5.3

3. RESULTS AND DISCUSSION**3.1 Physiochemical properties of suspension**

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

Cefuroxime axetil dry suspension was reconstituted with adequate quantity of water. The color of the suspensions were observed to be white. The pH of all the formulations were 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 were meeting the specified limit of 90 to 110 % as per USP.

Table 3: Physiochemical properties of suspension

Sr. 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 %

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

3.2 Invitro dissolution studies

Invitro 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 invitro dissolution studies are given in figure 1.

From the dissolution studies, it is found that all the formulations and marketed sample are meeting the dissolution criteria of Not less than 60 % (Q) in 30 minutes. Among these formulation 1:2.5 is seem to have better release pattern than the marketed sample.

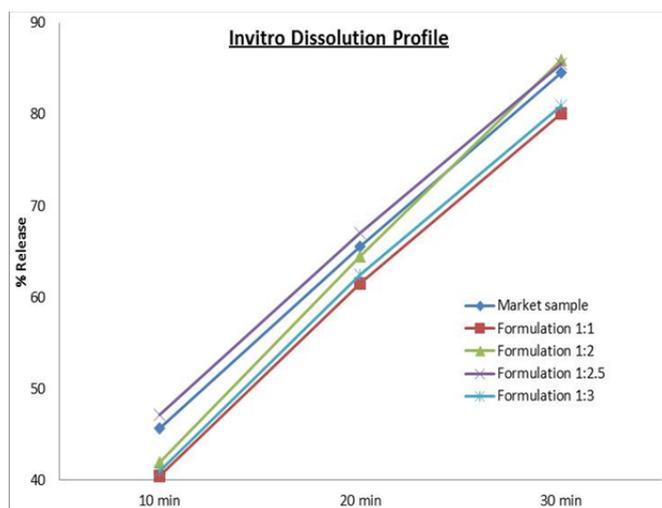


Figure. 1: Invitro dissolution profile

3.3 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 Figure 2 and the major pharmacokinetic parameters are presented in table 4.

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} 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.

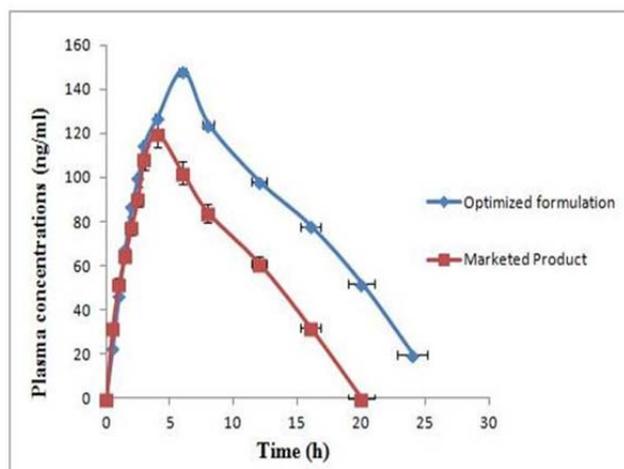


Figure. 2: Plasma drug concentrations at different time intervals for optimized formulation and Marketed Product

4. CONCLUSION

An oral suspension of Cefuroxime axetil was successfully developed by inclusion complexation method using Hydroxy propyl Betacyclodextrin. All the formulations developed were subjected to various quality control tests including physiochemical parameters and invitro dissolution, where all the formulations were meeting the quality parameters. Among the four formulations prepared, formulation with 1:2.5 ratio of Cefuroxime axetil and HP-Betacyclodextrin showed improved dissolution compared to the marketed product. So formulation with 1:2.5 ratio 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 demonstrate that the inclusion complexation method with Hydroxy Propyl betacyclodextrin can significantly improve the oral bioavailability of Cefuroxime axetil.

ACKNOWLEDGEMENT

The authors are thankful to the management of Sance Laboratories Pvt Ltd for providing the necessary facilities to carry out this work.

REFERENCES

1. Yuqian Du , Yinglei Zhai , Juhong Zhang , Chunnuan Wu, Cong Luo , Jin Sun ,Zhonggui He, *Asian journal of pharmaceutical Sciences*, 8 (2013), 287 -294.
2. J. Vinod and A. Chenthilnathan, , *Pelagia Research Library, Der Pharmacia Sinica*, 2013, 4(2):98-103
3. Nieves Ruiz-Balaguer, Amparo Nacher, Vicente G. Casabo, and Matilde Merino, *Antimicrobial Agents and Chemotherapy*, Feb. 1997, Vol. 41, no. 2, p. 445-448
4. Khan, Abdul Rehman., Kondawar, Kishan Vishwanth., Gosavi, Arun Shrinivas. Pharmaceutical Formulation WIPO Pat. Appl. No. 043707 to Wokhardt Research Centre(2002).
5. Nighute A. B., Bhise S. B.,*Int.J. PharmTech Res.*, 2009,1(3).
6. Patient information leaflet of Zinnat Suspension, GlaxoSmithKline Australia Pty Ltd, April 2011, Version 2.0
7. Birhade, S.T., Bankar, V.H., Gaikwad, P.D., Pawar, S.P., *IJPDR*,2010; 2, (3):199- 203
8. Ashok R. Pateli and Pradeep R. Vavia., *AAPS PharmSciTech*, 2008; 9:2
9. Arthur H. Kibbe, *Hand Book of Pharmaceutical Excipients*, American Pharmaceutical Association, USA, 2000, 3rd edition, 165-168
10. J. Szejtli, L. Szente, *Eur. J. Pharm. Biopharm*, 2005; 61:115-125.
11. Vishnumurthy Vummaneni and Dheeraj Nagpal, *International Journal of Research in Pharmaceutical and Biomedical Sciences*, Vol. 3 (2) Apr – Jun2012
12. Sharma, S., Lewis, S., *International Journal of Pharmacy and Pharmaceutical Sciences*, Vol 2(2), 2010.
13. Menjoje A.R, Kulkarni M.G. Pharmaceutical composition for improving palatability of drugs and process for preparation thereof. U.S Pat. No. 7378109 B2 to Council of Scientific and Industrial Research; 2008.
14. Soho H, Sultana Y and Khar R. K. *Drug Developments and Industrial Pharmacy*, 2004; 30(5):429-48.
15. Chatap V K., *Pharmainfo.net*, 2007;5.
16. Sarah Gould *, Robert C. Scott, *Food and Chemical Toxicology*, 43 (2005), 1451-1459
17. James et al. Pharmaceutical composition comprising cefuroxime axetil.U.S. Pat. No. 4865851 to Glaxo group ltd;1989.
18. Gedam Shweta S., Tapar K. K., Borse M. D. and Ghuge R. A., *IJPRD*, 2005; 1(12)03