Comparative Bioavailability of Cefuroxime Axetil from Tablets and Self-Microemulsifying Drug Delivery Systems in Rats

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ABSTRACT

Cefuroxime axetil has poor bioavailability due to low solubility. This can be surmounted by preparing the drug by self-microemulsifying drug delivery system (SMEDDS). In this study the bioavailability of cefuroxime axetil from SMEDDS and tablets was evaluated in Wistar rats. The optimized SMEDDS formulation was prepared using Labrasol®, Gelucire® 44/14 and Lutrol®E400. The formulation was evaluated for micro-emulsification properties and percent in-vitro dissolution. The HPLC method was developed and optimized to estimate the drug content in the plasma. The method was clearly separating the cefuroxime A and B polymorphs and LOD and LOQ values are satisfactory. Rats were randomized in to two groups - one group of animals were administered with SMEDDS and another with tablet formulation. At frequent intervals the blood samples were withdrawn and analysed for drug content. The pharmacokinetic parameters were calculated using PK Solve software. The calculated bioavailability and tmax from SMEDDS was 1687.06 μg/mL.min and 50 min, respectively, whereas for tablet it was 1219.803 μg/mL.min and 62 minutes, respectively. The bioavailability of SMEDDS formulation was 1.5 times more than the marketed formulation, indicating a significant improvement in oral bioavailability. In conclusion, this study confirms that the SMEDDS formulation is a viable strategy for enhancing the oral bioavailability of cefuroxime axetil.

KEYWORDS: Self-emulsifying; Bioavailability; Pharmacokinetics; PK solver; SMEDDS; Cefuroxime; Antibiotic.

Introduction

Cefuroxime axetil is a selective second generation cephalosporin belonging to BCS class II used against different kinds of bacterial infections. It is reported to have bioavailability of 35 to 50%, maximum drug concentration occurs at 1 to 4 hours and elimination half-life is 1 to 2 hours (Ravindra et al., 2009) exhibiting poor, variable bioavailability. Hence it is difficult to establish optimal oral dosage schedule. Cefuroxime bioavailability after oral and IP administration was nearly 24% and 75%, respectively (Carretero et al., 2004).

Upon oral administration, cefuroxime axetil rapidly hydrolyses in intestinal mucosa with 37–52% of an oral dose reaching to systemic circulation as cefuroxime (Ravindra et al., 2009; Mandell GL and Perti WA, 1996). Peak serum levels occur within 2-3.6 hr following an oral dose; the reported area under the curve is 19.9 mg/mL.h in healthy subjects after administration of a single oral cefuroxime axetil 500 mg dose. Approximately 33–50% of the circulating cefuroxime is protein bound. It is distributed throughout the body tissues and fluids including gall bladder, liver, kidney, bones, uterus, ovary, sputum, bile, peritoneal, pleural and synovial fluids. It penetrates meninges during inflammation and reaches therapeutic levels within the CSF and crosses the placenta (Mandell GL and Perti WA, 1996). It is largely (52%) excreted unchanged in the urine and a small percentage is excreted in breast milk; most of the drug is recovered within the first 6 h after administration. Elimination half-life (T₁/2) is 1-2 hr in patients with normal renal functions and increases as renal function declines (Arora et al., 2010; Gudigennavar et al., 2013). The Cefuroxime exhibits good intestinal permeability with poor aqueous solubility. Therefore, absorption is dissolution rate- limited and hence increasing the dissolution of drug shall enhance the bioavailability. Therefore, enhancing the saturation solubility and effective surface area can greatly enhance the bioavailability of Cefuroxime (Kawabataa et al., 2011).

SMEDDS are isotropic and thermodynamically stable preparations consisting of oil, surfactants and co-surfactants forms oil-in-water micro emulsions when mixed with water under gentle stirring. On oral administration, digestive motility of stomach and intestine provides agitation required for self-emulsification (Reddy, 2011). SMEDDS technique was chosen for increasing the bioavailability because it provides smaller particle size.

with greater self-dispersability and maximum surface area for absorption of the drug (Pouton, 1997).

In this study, the bioavailability of cefuroxime axetil from SMEDDS and tablets was evaluated Wistar rats. Here, iloperidone was used as internal standard in the HPLC analysis as it is having similar analytical behavior and not found in cefuroxime compound. In this study HPLC method was optimized and used for estimating the plasma drug concentration of Cefuroxime, based on the data, pharmacokinetic parameters were calculated.

Materials and Methods

Cefuroxime axetil and Iloperidone were obtained as gift sample from Indoco Remedies (Mumbai, India). The marketed product of Cefuroxime 125 (Ceftin 125) was used in the study. All the surfactants and co surfactants were obtained as a gift sample from Gattefosse, Lutrol® E400 from BASF (Mumbai, India) and Oleic acid from Loba chemie Pvt. Ltd. (Mumbai, India). The mobile phase and reagents used in the HPLC analysis are of HPLC grade purchased from Merck and Co, Germany. HPLC water was prepared from Mili-Q (Merck Millipore, Darmstadt, Germany). All other cited chemicals used were of analytical grade. Double distilled water was freshly prepared and filtered through 0.22 μm filter and used whenever required. All other cited chemicals used were of analytical grade.

The UV-spectrophotometric analysis was carried out using UV-2401PC model (Shimadzu, Japan) double beam UV-spectrophotometer and results were processed by UV/PC Personal Spectroscopy Software Version 3.7 program. RP Kromasil C-8 (150*4.6 mm) column, 150mm x 4.6mm i.d used in the analysis. WTW P4 Universal pH-meter was used for pH measurements. The centrifugation of the solutions was performed by 1-6 model Sigma centrifuge (Mumbai, India). The solutions were degassed using a B-220 model ultrasonic bath (Branson, USA). Signals were detected by a UV-VIS detector by Shimadzu (model SPD-10A, Japan) and results were processed by a C-R7A Chromatopac integrator (Shimadzu, Japan). Particle size and zetapotential were determined by Malvern Zetasizer Nano ZS 170 version 7.02 (Malvern Instruments Ltd, Worceststershire, UK.)

Adult male Wistar rats weighing 200-240 g acquired from dedicated animal house of BLDEA’s College of Pharmacy, Bijapur after receiving approval from the Institutional animal ethics committee (IAEC) dated 20th Aug 2014 for studying the bioavailability of the formulation (Reg No. 1076/PO/ERs/S/07). All the experiments were performed as per the guidelines laid down by CPCSEA, India.

Saturation solubility study

Saturation solubility study of CA was performed with a variety of surfactants, cosurfactants and oils by shake flask method. The amount of drug dissolved in various vehicles was determined using UV spectroscopy analysis (Liu et al., 2012; Patel and Lalwani, 2011).

Pseudo ternary phase diagram

The pseudo ternary phase diagram of Water/ Labrasol: Gelucire14/Lutrol E400 was developed using aqueous titration method (Nabi et al., 2007; Balakrishnan et al., 2009). Phase diagrams were developed with five ratios of surfactant and co surfactant (1:1, 1:2, 1:3, 2:1 and 3:1). If clear and transparent mixtures were seen on stirring, the samples were considered to be monophasic (Zhang et al., 2008).

Preparation of liquid SMEDDS

The prototype formulations were prepared using labrasol as surfactant, Gelucire 44/14 as cosurfactant and Lutrol E400 as co-solvent. Accurately weighed cefuroxime axetil was taken and surfactant mixture was added and stirred. Lutrol E400 was added and thoroughly mixed by gentle stirring and sonicated for 15 minutes to form homogenous mixture (Puttachari et al., 2014).

Evaluation of liquid SMEDDS

In vitro release study: In vitro release of prototype formulations was performed by filling the liquid SMEDDS to hard gelatin capsules and testing in USP dissolution apparatus II (Lab India, Mumbai). 900 mL of 0.07 N HCl was used as dissolution media and paddle rpm of 100 were used as per USPharmacopoeia. 3 mL aliquots of sample were withdrawn every 5-15 min up to one hour and subsequently replenished with fresh buffer every time, diluted, filtered and analyzed for drug content by UV spectrometer (Shimadzu corporation, Japan. Model: 1700) at λmax 282 nm (Puttachari et al., 2013; Bhagwat and D'Souza, 2012).

Globule size and zeta potential: The globule size distribution and zeta potential were determined by dynamic light scattering using a globule size apparatus (Malvern Zetasizer nano ZS 170 version 7.02). Liquid SMEDDS (1 mL) was diluted to 250 times with 0.1 N HCl by distilled water under gentle stirring. A laser beam at 632 nm wavelength was used and light scattering was monitored at 25 ºC at a 90° angle. After achieving equilibrium, the emulsions were analyzed by zeta sizer (Ghosh et al., 2006).

Bioavailability Studies

Analytical procedures: Standard stock solution of CA was prepared by taking 100 mg of Cefuroxime axetil in 50 mL of acetonitrile: water (50:50) and made suitable dilution to get solution concentrations of 2, 5, 10, 20, 24, 50 and 100 ppm. These solutions were used for intra and inter day linearity calibration purposes. Standard internal solution was prepared by dissolving 25 mg of Iloperidonein 100 mL acetonitrile: water (50:50) and further diluted to get 400 mcg/mL.

The standard sample for animal study was prepared by taking 5 tablets of marketed Cefuroxime 125 mg, triturated well and prepared the dispersion in 25% PEG. From the dispersion the required dose was taken for oral administration (Gopinath et al., 2013). The bioavailability of the SMEDDS formulation was
compared with the commercial marketed formulation to assess the potential of SMEDDS over tablet formulation in enhancing oral bioavailability of cefuroxime axetil (Srinivasan et al., 2011).

**Development and optimization of HPLC method:**

**Preparation of mobile phase:** Prepared the degassed mixture of 0.05 M potassium dihydrogen phosphate and acetonitrile (68:32), adjusted the pH to 4.0 using phosphate buffer. The columns used, HPLC condition and other details are mentioned in Table 1.

**TABLE 1**

HPLC Chromatographic conditions.

<table>
<thead>
<tr>
<th>Column</th>
<th>RP Kromasil C-8(150*4.6 mm)5 μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pump mode</td>
<td>Isocratic</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1mL/min</td>
</tr>
<tr>
<td>Detection</td>
<td>UV, 281nm</td>
</tr>
<tr>
<td>Injection volume</td>
<td>10μL</td>
</tr>
<tr>
<td>Data acquisition time</td>
<td>25 min</td>
</tr>
<tr>
<td>Internal Standard</td>
<td>Iloperidone(25ppm)</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>5μL</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>0.05M Potassium dihydrogen Phosphate (pH 4.0): Acetonitrile (68:32)</td>
</tr>
<tr>
<td>Data acquisition time</td>
<td>16 minutes</td>
</tr>
<tr>
<td>Temperature</td>
<td>Ambient</td>
</tr>
</tbody>
</table>

Detailed search was made for finding the suitable internal standard (IS) to enhance the analytical capabilities of the method. Considering the structure, polarity, detectability and similarities to CF, Iloperidone was selected as internal standard (Gopinath et al., 2013).

**Animal study:** Rat has been used as a suitable model for assessing the intestinal absorption of drugs in humans. Wistar rats of either sex weighing 250–350 g were fasted for 20 hr but given free access to water. Anesthesia was induced 1 hr before surgery. All the studies were conducted adhered to ‘Principles of Laboratory Animal Care’.

The animals were randomly divided into two groups of six animals each. Group I known as reference group was administered with marketed tablet samples and group II known as test group was administered with SMEDDS. The administered dose was 50 mg/kg. The anesthesia solution was administered prior to intervention prepared by mixing 0.2 mL of a ketamine 50 mg/mL solution, 0.24 mL of a diazepam 5 mg/mL solution and 0.37 mL of an atropine 1 mg/mL solution. A total of 2.7 mg/mL of anesthesia solution was administered by an IP injection to each animal (Balaguer et al., 2002).

**Sample collection and analysis:** After mild anesthetization of animals, serial blood samples of 0.5 mL are withdrawn approximately at specified predetermined time intervals (0, 15, 45, 60, 120, 180 and 240 min) by retro-orbital puncture. The samples were collected and immediately transferred to tube containing disodium EDTA and shaken well. Plasma samples were centrifuged at 5000 rpm for 15 minutes to separate the plasma. The supernatant was centrifuged at 5000 rpm for 15 minutes. Collected the supernatant and evaporated to dryness in water bath (at 40 ± 2 °C) and reconstituted with mobile phase and injected to HPLC system (Szlagowska et al., 2010). The concentration of CA was varied in the range of 1.00×10⁻⁶ to 6.00×10⁻⁶ M and concentration of IS was kept constant at 1.00×10⁻⁵ M.

**Recovery studies:** Recovery experiments were carried out using standard addition method to study the accuracy and reproducibility of the proposed techniques. The recovery of the excipients to the analytical method was determined by adding known amounts of pure CA to the pre-analyzed tablet formulation and subsequent analysis with the proposed methods. The recovery results were calculated using the calibration equation after 10 repeated experiments (Szlagowska et al., 2010; Kaza et al., 2012).

**Analysis of tablet formulations:** Marketed cefuroxime 125 mg tablets of 5 Nos were weighed and average weight of the tablet was calculated. The amount of tablet powder corresponding to the average weight of a tablet was accurately weighed and transferred to a 100mL calibrated volumetric flask. After making up the volume with methanol, the solution was magnetically stirred for 10 min, transferred to centrifuge tubes and was centrifuged at 5000 rpm for 10 min. The supernatant solution was collected and absorbance values of UV spectrophotometry were recorded. The CA content was calculated using the linear regression equations obtained from the analysis of pure CA (Kumar et al., 2012).

CA comprises of two diastereoisomers, CA-A and CA-B. The ratio between the signals of these diastereoisomers (CA-A/(CA-A) + (CA-B)) is also a quality control parameter and should be between 0.48 and 0.55 according to the pharmacopoeias. The diastereoisomer ratio of the pure CA used in the analysis compromises the values stated in the pharmacopoeias. It is also stated that CA should be quantified using total signal responses of these diastereoisomers (Carretero et al., 2004).

**Pharmacokinetic parameters and statistical analysis:** The equations of the classic compartmental models were fitted to the experimental plasma concentrations of C versus time data obtained, using weighted least-squares non-linear regression by using PK Solver excel (PK solver 2.0 version 5.4.0.2). The data was fitted into various models and pharmacokinetic parameters were calculated.

The total area under the plasma drug concentration versus time curve (AUC) was calculated using two methods: a compartmental method in which the values were obtained from the equations used in the pharmacokinetic parameters obtained in the fit (AUCf); and a non-compartmental analysis in which the values
were calculated using linear trapezoidal rule with extrapolation to infinite. The plasma concentration versus time curve from 0 to the last measured concentration at time \( t \) (AUC\(_0^t\)) was calculated by trapezoidal integration. The AUC from the last experimental time to infinity (AUC\(_{\infty}^t\)) was calculated by dividing the last measurable plasma concentration value by the elimination rate constant (\( K_{el} \)). The total AUC\(_{\infty}^t\) was calculated as the sum of AUC\(_0^t\) and (AUC\(_{\infty}^t\)). \( K_{el} \) was calculated as the negative slope of the regression line of the terminal linear portion of the natural log serum concentration versus time curve. The AUC and areas at the first moment of the concentration–time curves (AUMC) were calculated by the linear trapezoidal method. One-way analysis of variance (ANOVA) was applied to compare the pharmacokinetic parameters obtained for theseroutes of administration and differences were significant (\( P > 0.05 \)) (Carretero et al., 2004; Wei et al., 2006).

Results and Discussion

Solubility studies

The saturation solubility of CA in various oils, surfactants, co-surfactants and co-solvents are depicted in Figure 1. Among surfactants, labrasol showed good solubility, among co-surfactant Gelucire 44/14 showed better solubility followed by Lurol 400.

In vitro release study

The in-vitro dissolution of prototype formulation was performed using USP II paddle type apparatus. The dissolution was rapid and complete. No marked difference in dissolution profile was observed between the formulations. More than 95% of drug released from the formulation within 15 minutes as shown in Figure 3. Based on self-emulsification property, drug loading capacity and in-vitro dissolution of prototype formulations, formulation code F-10 was selected as optimum formulation. This formula was used in globule size analysis and stability study. The drug release from the optimized formulation was compared with the marketed tablet formulation as well as plain drug suspension. From the results it was confirmed that the release of drug from SMEDDS formulation was more than 95% within 15 minutes whereas only 70% of drug was released from the conventional tablet formulation. SMEDDS, due to its miniscule particle size provides larger surface area which governs the faster dissolution of the drug in the medium.

Particle size and zeta potential

The particle size of optimized formulation on addition to water was determined using Zeta sizer Nano S90 instrument. The average droplet size range of the formulation composition is mentioned in Table 2.
optimized self-microemulsified system was found to be 74.03 nm as shown in Figure 4. The lowest mean particle size is attributed to the surfactant-cosurfactant mixture and cosolvent proportion due to stabilization of interfacial tension.

The marketed Cefuroxime tablet formulation was analyzed using optimized HPLC method. Ten independent experiments were performed and the content of the tablets was determined. Sharp peaks of CA appeared and no interference was observed originating from excipients or inactive ingredients (Al-Saida et al., 2000).

The blood was collected from rats by retro-orbital route at different time points and assayed for drug content by HPLC. Prior to that, calibration curves of cefuroxime axetil covering linear concentration ranges was obtained. Figure 5 and 6 A summary of calculated pharmacokinetic parameters on administration of SMEDDS and marketed tablet formulation are tabulated in Table 3. Figure 7 represents the plasma concentration – time curves in Wistar rats following single oral dose of cefuroxime loaded SMEDDS and marketed tablet formulation. At the end of 180 minutes, the plasma concentration of drug seemed to decline and stabilize with time. The curve of CA SMEDDS was found to be little greater than the CA tablet formulation. The $C_{\text{max}}$ of the SMEDDS formulation (11.98 μg/mL) was higher than the tablet formulation (8.03 μg/mL). The $T_{\text{max}}$ was 50.3 minutes for SMEDDS and whereas for tablet it was 62.22 minutes indicating faster onset of action. The $\text{AUC}_0^\infty$ value for SMEDDS was found to be 1885.5 μg/mL.min and tablet formulation was 1359.66 μg/mL.min, thus demonstrating improved bioavailability. The low bioavailability of tablet can be attributed to the poor aqueous solubility and extensive metabolism in liver. SMEDDS owing to its miniscule globule size after redispersion, enhances the surface area for absorption for drugs whose intestinal absorption is dissolution rate limited. The fine microemulsion in the GI tract helps in bypassing the first pass metabolism in liver by lymphatic transport of drug. In the current investigation, the content of surfactants is quite high which is assumed to play a key role in improving the bioavailability of the drug by various mechanisms by either disturbing the cell membrane and increasing the permeability across the membrane or able to partition well between the lipid and protein groups. SMEDDS can also interact with the polar heads in the lipid bilayer and alter the hydrogen bonding and ionic forces between the groups (Erpnjak et al., 2013). From the results of in vitro and in vivo correlation, it is clear that enhancement in the dissolution and release profile of Cefuroxime axetil can lead to improvement of bioavailability of Cefuroxime axetil.
Fig. 5 HPLC chromatogram of cefuroxime from plasma at 0 and 15 mins on administration of SMEDDS formulation.

Fig. 6. HPLC chromatogram of cefuroxime from plasma at 0 and 15 mins on administration of tablet formulation.
TABLE 3
Pharmacokinetic parameters calculated from Pk solver.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Pharmacokinetic parameters</th>
<th>SMEDDS formulation</th>
<th>Tablet formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>$AUC^0_\infty$</td>
<td>1687.06 µg/mL.min</td>
<td>1219.803 µg/mL.min</td>
</tr>
<tr>
<td>2.</td>
<td>$AUC^0_\infty$</td>
<td>1885.74 µg/mL.min</td>
<td>1359.754 µg/mL.min</td>
</tr>
<tr>
<td>3.</td>
<td>$K$ (Absorption rate constant)</td>
<td>0.0323 /min</td>
<td>0.017 /min</td>
</tr>
<tr>
<td>4.</td>
<td>$K_e$ (Elimination rate constant)</td>
<td>0.011 /min</td>
<td>0.015 /min</td>
</tr>
<tr>
<td>5.</td>
<td>Absorption half life</td>
<td>21.46 min</td>
<td>41.88 min</td>
</tr>
<tr>
<td>6.</td>
<td>Elimination half life</td>
<td>62.29 min</td>
<td>44.6 min</td>
</tr>
<tr>
<td>7.</td>
<td>$C_{max}$</td>
<td>11.98 µg/mL</td>
<td>8.034 µg/mL</td>
</tr>
<tr>
<td>8.</td>
<td>$t_{max}$</td>
<td>50.33 min</td>
<td>50.33 min</td>
</tr>
<tr>
<td>9.</td>
<td>$\text{AUMC}$</td>
<td>227850.66 µg/mL.min</td>
<td>166347.3 µg/mL.min</td>
</tr>
<tr>
<td>10.</td>
<td>Volume of distribution ($V_d$)</td>
<td>0.714 mL</td>
<td>0.71 mL</td>
</tr>
<tr>
<td>11.</td>
<td>Clearance</td>
<td>0.0079 (mL)/min</td>
<td>0.011 (mg/mL)/min</td>
</tr>
<tr>
<td>12.</td>
<td>Mean residence time (MRT)</td>
<td>120.83 min</td>
<td>124.54 min</td>
</tr>
</tbody>
</table>

Fig. 7. Mean plasma Cefuroxime content Vs time plot following administration of SMEDDS and tablet formulation to rats.

Conclusions

The cefuroxime SMEDDS was developed using optimum ratios of excipients based on saturation solubility and ternary phase diagram. The HPLC method was optimized to detect the cefuroxime in the plasma. The plasma concentration profile of cefuroxime axetil loaded SMEDDS represents significant improvement in drug absorption and bioavailability than the conventional tablet formulation. Both the $C_{max}$ and $AUC^0_\infty$ values of SMEDDS were approximately one-half fold greater than those of the tablet formulation indicating a remarkable improvement in the oral absorption of cefuroxime when administered in the form of SMEDDS. This was in agreement with the in-vitro dissolution results. Oral SMEDDS formulation was demonstrated for better bioavailability as compared to tablet formulation because of its higher dissolution rate owing to reduced particle size with increased surface area and reduced diffusion layer thickness. Moreover, immediate release of drug at the site of absorption avoids its passage to distal segments where it hydrolyzed into non-absorbable cefuroxime. Therefore the bioavailability of SMEDDS formulation was higher when compared to conventional tablet. The study proved SMEDDS as an efficient delivery system for hydrophobic drugs like cefuroxime axetil.

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References


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