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Research paper

# Investigation on physicochemical and biological differences of cefpodoxime proxetil enantiomers

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**Abstract**

Cefpodoxime proxetil (CP) is a prodrug of cefpodoxime acid (CA), and is supplied as racemic mixture of *R*- and *S*-enantiomers. CP has only 50% absolute bioavailability, and the reasons responsible for low bioavailability remain poorly understood. The present work ascertains physicochemical and biological properties of individual isomers of CP and explores their capacity to optimize delivery of CP. Both isomers showed similar pH stability behavior, but *R*-isomer was more susceptible to enzymatic metabolism compared to *S*-isomer, when incubated with enzymes collected from various segments of GIT. Based on the in vitro and in vivo results, use of *S*-isomer for development of a dosage form such as gastro-retentive dosage form can improve oral bioavailability of CP.


*Keywords:* Cefpodoxime proxetil; Enantiomers; Differences; Enzymes; Segments; Regional metabolism; GIT; Physicochemical; Biological

## 1. Introduction

Cefpodoxime proxetil (CP) is a prodrug of cefpodoxime acid (CA), and a third generation cephem type broad spectrum antibiotic administered orally. CP is a non-crystalline, slightly basic compound and is absorbed from the gastro-intestinal tract after oral administration and hydrolyzed to its parent moiety cefpodoxime acid (CA) by non-specific esterases in the intestinal wall/plasma [1–3]. The drug possesses an asymmetric carbon atom in the ester group (Fig. 1) and is supplied as a racemic mixture of *R*-isomer and *S*-isomer [4–7]. These *R*- and *S*-isomers of CP are equivalent in biological action and therapeutic efficacy, but differ in few physico-chemical properties [8]. Hamamura et al. reported that the two isomers exhibit different solubility values and *R*-isomer shows pH-dependent solubility and exhibits a typical micro-crystallization or gelation phenomenon in the acidic pH values [5].

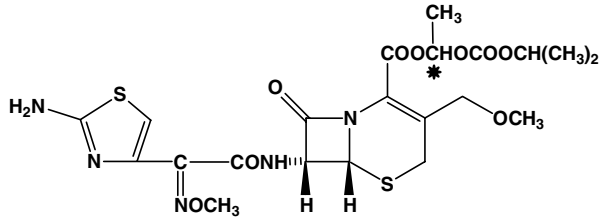


Fig. 1. Structure of Cefpodoxime proxetil (* – asymmetric carbon).

Although CP is designed to improve the permeability and thus bioavailability of the parent molecule CA, it still has only 50% oral bioavailability. The reasons for poor bioavailability of CP remain poorly investigated. Reported studies have pointed possible reasons of low bioavailability as, the low solubility and a typical gelation behavior of CP particularly in acidic environments [5,9,10], and pre-absorption luminal metabolism into CA by the action of digestive enzymes [1]. The detailed study of the properties and behavior of isomers can provide

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opportunity of applying rational formulation strategies, to improving absorption and bioavailability of CP. The objective of the present exercise is to study physicochemical and biological differences of enantiomers of CP and to suggest possibility of developing formulation containing pure isomers, with a view to improve the oral bioavailability of CP.

## 2. Materials and methods

### 2.1. Materials

The reference standards of CP and CA were obtained from Ranbaxy Research Laboratories Ltd, Gurgaon, India. The *R*- and *S*-isomers of CP were prepared in-house by preparative chromatography of CP. The isomers were separated on a reversed-phase preparative column chromatograph, and racemic CP was eluted slowly at a flow rate of 15–20 ml/h with a mobile phase consisting of water and acetonitrile in 32:68 composition. Fractions were collected periodically and analyzed by HPLC for their content. Fractions containing similar isomer in major proportion were mixed, concentrated in rotary evaporator and freeze dried. The purity of each isomer used was about 90%, as analyzed by HPLC. All reagents used were of analytical grade and materials used for preparation of mobile phase and HPLC analyses were of chromatography grade.

### 2.2. Analytical methods

In-house developed and validated HPLC analytical methods were utilized for detecting quantities of CP and CA in the biological samples. The detection of CP and CA was performed by two separate HPLC methods based on reversed-phase columns. A HPLC system (Shimadzu Corporation, Japan) equipped with a UV–vis spectrophotometric detector, and data acquisition software (CLASS-VP, version 6.14 SP1) was utilized for the purpose. The HPLC method for CP employed acetonitrile: ammonium acetate buffer (pH 5.0) pumped at a flow rate of 1 ml/min in 36:64 composition, and analysis was carried at a temperature of 30 °C and a detection of 235 nm. The HPLC method for determination of CA from plasma employed acetonitrile:phosphate buffer (pH 3.0) in 10:90, flow rate of 1 ml/min, and detection at 269 nm.

### 2.3. Animal studies

All animal studies were done according to the guidelines of the Institutional Animal Ethics Committee (IAEC) of National Institute of Pharmaceutical Education and Research (NIPER), Punjab, India. Male Sprague–Dawley (SD) rats in the weight range 250–275 g were used in various experiments. The rats were housed under standard laboratory conditions and fasted overnight with water allowed ad libitum before conducting any experiment.

### 2.4. Differential scanning calorimetry

The separated and purified enantiomers of CP were analyzed under 80 ml/min of dry nitrogen purge with a heating rate of 5 °C/min, using Mettler Toledo DSC 821e (Greifensee, Switzerland) operating with Star software version Solaris 5.1, equipped with automated cooling accessory. The DSC instrument was calibrated for temperature and heat flow with indium. Samples of about 8–12 mg were taken in standard aluminum pans, sealed with a pin-hole and then thermograms were obtained in the range of 25–200 °C.

### 2.5. Powder X-ray diffractometry (PXRD)

A Philips PW1729 powder X-ray diffractometer (Philips, Holland) was used to analyze CP and its enantiomers. The radiation used was generated by a copper K$\alpha$ source fitted with Ni filter at 0.154 nm wavelength at 20 mA and 35 kV. Samples were scanned over a range of $2\theta$ values from 5° to 40° at a scan rate of 1°/min.

### 2.6. Optical rotation studies

The optical rotation and specific rotation of 0.2% methanolic solutions of each enantiomer were measured using AUTOPOL™ IV, an automatic polarimeter (RUDOLPH Research, Flanders, NJ, USA), at ambient temperature.

### 2.7. In vitro studies

#### 2.7.1. pH-stability

The stability of enantiomers in various buffers was assessed. Enantiomers were incubated in buffers of pH values – 1.2, 4.5, 5.4, and 6.8 for 24 h at 37 °C. These pH values represent the local environments of stomach, duodenum, jejunum and ileum, respectively. At specified time points, samples were withdrawn and immediately analyzed by HPLC.

#### 2.7.2. Enzyme incubation studies

*2.7.2.1. Intestinal homogenate preparation.* Enzyme fractions were prepared individually from four regions of the upper GIT of rats namely – stomach, duodenum, jejunum and ileum. Immediately after sacrificing the animals ($n = 3$), their abdominal portion was exposed and stomach, duodenum, jejunum and ileum sections of GIT were isolated, perfused with ice-cold saline and maintained at 0 °C. The intestinal segments were cut along the axis and the apical portion of individual region was scrapped with the help of a glass slide. The tissue scrapings were homogenized in Potter–Elvejhem tube with Teflon pestle at 0 °C in 0.25 M sucrose solution, filtered through nylon cloth and centrifuged at 4000*g* for 10 min at 4 °C preliminarily to separate macrosized tissue debris. The precipitate is discarded and supernatant was further