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Highly sensitive and selective high-performance liquid chromatography method for bioequivalence study of cefpodoxime proxetil in rabbit plasma via fluorescence labeling of its active metabolite

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ABSTRACT

Cefpodoxime proxetil (CFP), a broad-spectrum third-generation cephalosporin, has been used most widely in the treatment of respiratory and urinary tract infections. For bioequivalence study of CFP in rabbit plasma, it was necessary to develop a highly sensitive and selective high-performance liquid chromatographic (HPLC) method with fluorescence (FL) detection. The pre-column labeling of cefpodoxime acid (CFA) (active metabolite) with an efficient benzofurazan type fluorogenic reagent, 4-*N*,*N*-dimethyl aminosulfonyl-7-fluoro-2,1,3-benzoxadiazole (DBD-F) was carried out in the present study in 100 mM borate buffer (pH = 8.5) at 50 °C for 15 min. The obtained fluorescent products were separated on C_{18} column with an isocratic elution of the mobile phase, which consists of 10 mM phosphate buffer (pH = 3.5)/CH_3CN (70:30, v/v). The fluorescent product (DBD-CFA) was detected fluorimetrically at 556 nm with an excitation wavelength of 430 nm. Cefotaxime sodium was used as internal standard. The method was validated according to the requirements of US-FDA guidelines. The correlation coefficient of 0.999 was obtained in the concentration ranges of 10–1000 ng mL^{-1}. The limits of detection and quantification (S/N = 3) were 3 and 10 ng mL^{-1}, respectively. Plasma CFA levels were successfully determined in rabbit with satisfactory precision and accuracy. The proposed HPLC-FL method was successfully applied to study bioequivalence in rabbits for two formulations of different brands contained CFP (prodrug) in a randomized, two-way, single-dose, crossover study and all pharmacokinetic parameters for the two formulations were assessed.

1. Introduction

Cefpodoxime proxetil (CFP), an oral, broad spectrum, third-generation cephalosporin, is an ester prodrug that de-esterified *in vivo* and converted to its active metabolite, cefpodoxime acid (CFA) (Fig. 1). It demonstrates strong activity against pathogens frequently associated with the respiratory tract, urinary tract, and skin and soft tissue infections [1,2]. CFA (pK_a = 2.20) exists predominantly in the ionic form at intestinal pH and thus exhibits poor permeability [3]. To overcome the poor oral bioavailability of the parent drug, the free carboxylic acid moiety was esterified with an isopropyloxycarbonyloxyethyl group. Thus, the lipophilicity was improved enabling the compound to be absorbed by passive diffusion after oral administration [4]. Although CFP was designed to improve the permeability and thus bioavailability of CFA, it still has only 50% oral bioavailability, when administered orally. Reasons responsible for low bioavailability of CFP remained poorly understood. Studies have pointed possible reasons of low bioavailability as the low solubility, gelation behavior of CFP in acidic media and pre-absorption luminal metabolism into CFA by the action of digestive enzymes [5,6]. There is a need to understand the mechanistic aspects, to enable improving the delivery of the drug. CFP is absorbed from the intestinal tract after oral administration and hydrolyzed to its parent moiety cefpodoxime acid (CFA) by nonspecific esterases enzyme in the intestinal wall/plasma [7,8]. The blood and tissue levels of CFA are difficult to measure as low dosages used from CFP (100–200 mg) [9]. Therefore, the bioequivalence studies of CFP require highly sensitive and selective method to determine the plasma levels of CFA after oral administration of the prodrug.

On the other hand, very few methods were reported for the analysis of the active metabolite CFA in biological fluids using

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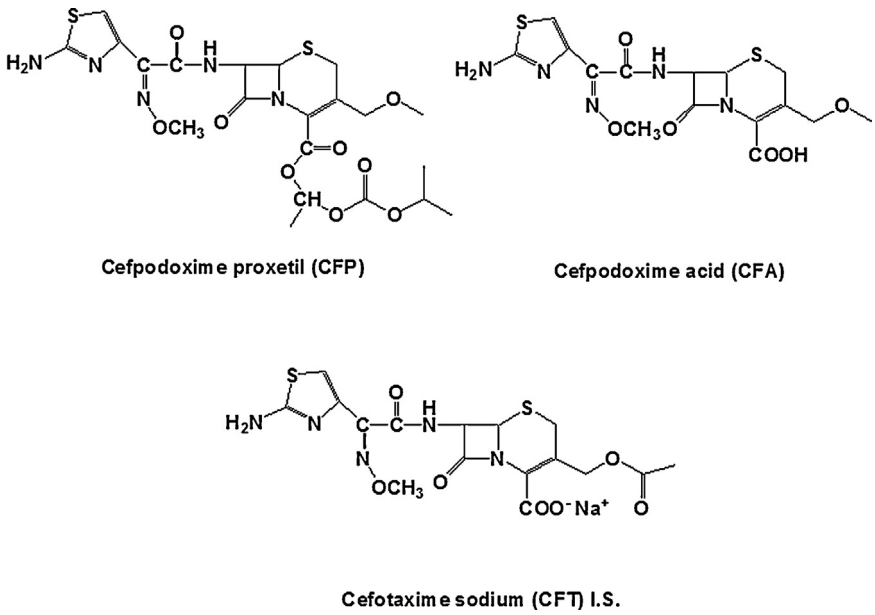

Fig. 1. Chemical structures of cefpodoxime proxetil (CFP), cefpodoxime acid (CFA) and cefotaxime sodium (CFT, internal standard).

HPLC-UV detection [10–12] and spectrofluorimetric method [13]. The HPLC-UV methods lack sufficient sensitivity and selectivity for the determination of the low plasma levels. Although, the spectrofluorimetric method was sensitive enough for the determination of CFA in plasma samples, the interferences from endogenous plasma components hindered its applications in bioequivalence studies. Therefore, it was necessary to develop a highly sensitive and selective method for analysis of the active form CFA in biological fluids. HPLC with fluorescence (HPLC-FL) detection has been widely used especially in the field of bioanalysis, because of its sensitivity and selectivity. Since CFA does not fluoresce, a pre-column labeling step with fluorogenic reagents has to be carried out to make this detection method more useful [14]. Until now, HPLC-FL detection was not reported for the determination of CFA in biological samples. CFA possessing primary amino functional group that could be derivatized with some fluorescence labeling reagents such as benzofurazan (2,1,3-benzoxadiazole) compounds. Recently, many fluorescent labeling reagents with the benzofurazan skeleton have been developed and some of them are available in the market such as NBD-F (7-fluoro-4-nitro-2,1,3-benzoxadiazole), ABD-F (4-aminosulfonyl-7-fluoro-2,1,3-benzoxadiazole), SBD-F (7-fluoro-2,1,3-benzoxadiazole-4-sulfonate) and DBD-F (4-*N*,*N*-dimethylaminosulfonyl-7-fluoro-2,1,3-benzoxadiazole). In this study, DBD-F was chosen as a fluorescent labeling reagents for its low fluorescence back ground, large quantum yield of the resultant fluorescent derivatives, high reactivity to amine compounds and high excitation and emission wavelengths of the resultant derivatives, thus avoids the interference derived from the bio-matrixes [15].

This investigation aimed to develop a highly sensitive and selective HPLC-FL detection for determination of CFA in rabbit plasma suitable for bioequivalence study of CFP. The method involved a pre-column fluorescence labeling reaction of CFA with DBD-F reagent. The obtained fluorescent products were separated on C_{18} column and the fluorescent products were detected online with a fluorescence detector at 556 nm after excitation at 430 nm. In order to improve method accuracy, cefotaxime sodium (CFT) was used as internal standard. The method was validated according to the requirements of US-Food and drug administration (FDA) guidelines. The developed HPLC method was applied to study bioequivalence of CFP (prodrug) tablets in rabbit plasma for two formulations in a randomized, two-way, single-dose, crossover study by tracing the plasma levels of the active metabolite, CFA and all pharmacokinetic parameters were assessed.

2. Materials and methods

2.1. Reagents and chemicals

CFP (%purity 99.2 ± 0.36) was obtained from Roussel Uclaf (Romainville, France). CFA (%purity 98.3 ± 0.25) was obtained from Toronto Research Chemicals, Inc. (Toronto, Ontario, Canada). 4-*N*,*N*-dimethylaminosulfonyl-7-fluoro-2,1,3-benzoxadiazole (DBD-F) was purchased from Tokyo Kasei Co. (Tokyo, Japan). Orelox® 100 mg CFP tablets (Sanofi-Aventis, France) and Vantin® 100 mg CFP tablets (Pfizer, USA) were obtained from local market. All solvents and other chemicals used throughout this study were of HPLC analytical grade. Ultra-pure water was obtained from a Millipore system (Bedford, MA, USA). The study protocol was approved by the Ethics Committee of Assiut University.

2.2. Instrumentations and chromatographic conditions

The HPLC system used in the study was a Younglin Autochro-3000 HPLC system (Younglin, Korea) with fluorescence detector (FP, Jasco, Japan) and a Rheodyne injection valve with a 20-μL loop. The chromatographic separation was carried out on Cosmosil column, 5C18-MS II (150×4.6 mm, 5 μm i.d.) (Nacalai, Japan). The separations were conducted isocratically using a mobile phase composed of a mixture of CH_3CN/10 mM phosphate buffer pH 3.5 (30:70, v/v) at a flow rate of 1.0 mL min^{-1}. The mobile phase was prepared weekly, filtered and degassed by sonication before use. The column condition was maintained at ambient temperature. The effluent was monitored by a Jasco fluorescence detector and the wavelengths were set at 430 nm (excitation) and 556 nm (emission). The peak areas obtained from the FL detector were calculated using a Younglin Autochro-3000 chromatography software. For the protection of the analytical column during working with plasma samples C_{18} ($4 \text{ mm} \times 3$ mm i.d.) security guard cartridge system (Phenomenex, Torrance, USA) was used. In addition,