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Sensitive inexpensive chromatographic determination of an antimicrobial combination in human plasma and its pharmacokinetic application

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ABSTRACT

This study represents simple inexpensive chromatographic determination of ciprofloxacin (CIP) and tinidazole (TIN) simultaneously in human plasma using HPLC-DAD followed by a pharmacokinetic application. C18 column was used as stationary phase with isocratic elution of a mobile phase composed of acetic acid solution (2%) and acetonitrile (85: 15, v/v) and ornidazole as internal standard (IS) with UV detection at 318 nm. The two drugs and the IS were separated at 6.55, 7.91 and 11.07 min for CIP, TIN and IS, respectively, with good selectivity and sensitivity for their analysis in presence of plasma matrix components and the drugs' metabolites. Sample preparation involved only protein precipitation without any complicated extraction procedures decreasing analysis time. For method validation, FDA regulations for analysis in biological fluids were followed. Pharmacokinetic (PK) study on six healthy volunteers was conducted after single oral dose administration of 500 and 600 mg of CIP and TIN, respectively. Dugs' plasma levels were followed for 12 or 72 h post dosing for CIP and TIN, respectively, and different PK data for the two drugs were calculated and they were comparable to the reported values demonstrating successful future application of the presented method in PK, bioequivalence and bioavailability studies.

1. Introduction

Ciprofloxacin (CIP), 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid, (Fig. 1a) is a fluoroquinolone-type antibiotic agent acting against many bacteria [1,2].

There are many works published regarding HPLC determination of CIP in biological fluids. Some of the reported methods about CIP quantitation in human plasma used switching devices [3], tedious extraction methods [4] and derivatization methods [5]. Meanwhile, many articles show the determination of CIP in human plasma after simple protein precipitation treatment [6–9].

Tinidazole (TIN), 1-[2-(Ethylsulfonyl) ethyl]-2-methyl-5-nitro-1H-imidazole (Fig. 1b) is a second-generation member of the 5-nitroimidazole group. It is used in Europe widely and in developing countries as well acting against bacteria and protozoa [1,2].

The literature survey revealed that several analytical methods were reported for TIN assay in human plasma by HPLC using protein precipitation treatment with 70% perchloric acid [10], liquid/solid extraction method [11] and liquid/liquid extraction method [12].

Meanwhile, CIP and TIN combination is marketed in several countries with the benefit of effectiveness against both protozoa and bacteria. The literature survey revealed that spectrophotometry [13], HPLC [14,15] and UPLC [16] methods have been reported for their analysis in combined dosage forms. However, the literature survey shows that there are no reported methods for simultaneous analysis of CIP and TIN in real human plasma. Monitoring plasma concentration of antibacterial drugs avoids either sub-therapeutic or excessive antibiotic levels thus avoiding toxicity and warranting efficacy. Therefore, the target of the present work is the development of an HPLC method for the in-vivo analysis of CIP and TIN binary mixture in human plasma and checking the applicability of this method in conducting a pharmacokinetic study for the studied drugs after one oral dose administration of their combined pharmaceutical formulation to healthy human volunteers.

For high throughput analysis which is an important requirement for a successful pharmacokinetic study, the proposed method involved very simple sample treatment (protein precipitation) and optimized chromatographic separation using only 15 min analysis time. The proposed method analyzed simultaneously the two drugs with the internal standard ornidazole (ORI), in presence of plasma interferences and the drugs metabolites at a single wavelength (318 nm). The method was also validated in accordance to the FDA [17].

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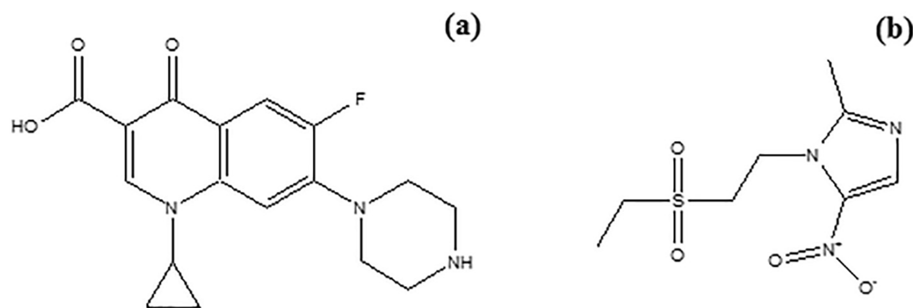

Fig. 1. Chemical structures of (a) ciprofloxacin and (b) tinidazole.

2. Experimental

2.1. Instrumentation and chromatographic conditions

An Agilent 1200 series (Santa Clara, USA), HPLC-DAD system was used with diode array detector which is connected to a computer with Agilent Chem Station Software. The HPLC system is supplied with automatic injector, quaternary pump and vacuum degasser. The separations were done on a reversed phase Agilent Zorbax SB-C18 (250 × 4.6 mm) column at 25 °C. Isocratic elution using acetic acid (2%) and acetonitrile (85: 15, *v*/v) was used. The mobile phase filtration was done using a 0.45 μm membrane filter and 1 mL.min^{-1} flow rate and 20 μL injection volumes were applied all over the runs. The chromatograms were extracted at 318 nm.

2.2. Materials and reagents

Pharmaceutical grades of CIP, TIN and ORI were kindly supplied by Pharco Pharmaceuticals (El Amriya, Alexandria, Egypt), Medical Union Pharmaceuticals-MUP (Abou Sultan – Ismailia, Egypt) and Pharaonia Pharmaceuticals (New Borg El-Arab City, Alexandria, Egypt), respectively, and certified to contain 99.99%. 99.95% and 99.98%, respectively. HPLC-grade acetonitrile and acetic acid (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) were used. High purity double distilled water was also used in the whole work. Pooled drug-free human plasma was obtained from the Alexandria blood bank. Tinifloxacin® capsules (labeled to contain 500 and 600 mg of CIP and TIN, respectively, per capsule) was purchased from the commercial market and is manufactured by Organo Pharma MS, El-Obour Industrial Zone, Egypt.

2.3. Stock solutions

A 1000 μg·mL^{-1} CIP stock solution was initially prepared in methanol with a minimum amount of HCl that allows CIP base to dissolve. During analysis, 2 peaks were noted for CIP. In response to this, double distilled water again with the least amount of HCl was used to prepare CIP stock solution. When the solution was analyzed, peak shouldering was still observed in the chromatogram. For this reason, the mobile phase was chosen to dissolve CIP which gave a very good symmetrical peak for CIP.

Standard solutions of CIP and TIN were prepared by transferring accurate weights of 25 mg of each drug substance separately into two 25-mL volumetric flasks, then dissolved in and diluted to volume using mobile phase and methanol for standard solutions of CIP and TIN, respectively, to obtain standard solutions of 1000 μg·mL^{-1}.

CIP standard solution of 1000 μg·mL^{-1} was further diluted by transferring an accurate volume of 0.25 mL of CIP standard solution into a 25-mL volumetric flask and diluted to volume using mobile phase to get a working solution of 10 μg·mL^{-1}. Meanwhile, TIN standard solution of 1000 μg·mL^{-1} was further diluted to get two working solutions by transferring separately two accurate volumes of 0.25 and 2.5 mL of TIN standard solution into two separate 25-mL volumetric flasks and then diluted to volume using mobile phase to get working solutions of 10 and 100 μg·mL^{-1}, respectively.

Also, ORI, the internal standard, solution was prepared by transferring accurate weight of 12.5 mg of ORI drug substance into a 25-mL volumetric flask, then dissolved in and diluted to volume using double distilled water to get standard solution of 500 μg·mL^{-1}.

The stock solutions of all drugs were stored in light protected vessels at 4 °C for at least 14 days with no changes.

2.4. Calibration and quality control (QC) standards

A 500 μL control human plasma in 5 mL centrifuge tubes was spiked with accurate volumes of 5–1000 μL of CIP working solution and 10 μL and 5–200 μL of 10 and 100 μg·mL^{-1} TIN working solutions, respectively, to achieve concentrations in the range of 0.1–20.0 and 0.2–40.0 μg·mL^{-1} plasma for CIP and TIN, respectively. Five microliters of ORI (IS) standard solution was added to each centrifuge tube followed by 750 μL of acetonitrile using a micropipette. The tubes were vortex mixed for 3 min. The samples were centrifuged for 15 min at 40,000 rpm. The supernatant was poured into another 5 mL centrifuge tubes and evaporated to dryness using a Christ rotational vacuum concentrator. The dried residue was reconstituted with 1000 μL mobile phase, vortex mixed for 3 min and re-centrifuged at 40,000 rpm for 5 min.

Initially, the supernatant layer was filtered by a 0.45 μm syringe adapter before injection into the HPLC but this caused great loss of CIP peak which is in compliance to CIP assay previous report [6]. Thus, a second centrifugation step was used before injection into the HPLC system to obtain a clearer supernatant layer and 20 μL volume of it was injected in triplicate and chromatographed with the previously stated conditions in Section 2.1. The ratios of analyte to IS peak areas versus the corresponding drug concentrations were used for calibration graphs construction.

Calibration standards were prepared at 0.1, 0.3, 0.4, 1, 4 and 20 μg·mL^{-1} plasma for CIP and 0.2, 1, 2, 5, 30 and 40 μg·mL^{-1} plasma for TIN. As per FDA guidelines [17] for selecting QCs, for accuracy and precision studies, QCs were prepared as six replicates at 4 concentration levels, including lower limit of quantitation (LLOQ), low (L: defined as three times the LLOQ), mid (M: defined as mid-range), and high (H: defined as high-range). While for other runs (during analysis of volunteers' samples), duplicates at only 3 concentration levels (LQC, MQC and HQC) were used. For CIP, QCs were prepared at 0.1, 0.3, 4 and 20 μg·mL^{-1} for LLOQ, LQC, MQC and HQC, respectively, while for TIN, 0.2 (LLOQ), 0.6 (LQC), 5 (MQC) and 40 μg·mL^{-1} (HQC) were prepared.

2.5. Pharmacokinetic study

2.5.1. Selection of subjects

Before conducting the study, ethical approval has been granted from the “Clinical studies ethics committee”, Faculty of pharmacy,

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