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A sensitive and reliable method for therapeutic monitoring of α_1-blockers in rabbit plasma by ion-pair chromatography with enhanced fluorescence detection

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ABSTRACT

A sensitive, reliable and reproducible ion-pair chromatographic (IPC) method with enhanced fluorescent detection suitable for therapeutic drug monitoring was developed and validated for the determination of α_1-blockers. The chromatographic and fluorescence enhancement conditions were optimized for maximum sensitivity and selectivity. Sodium dodecyl sulfate (SDS) has a dual role in the newly developed IPC method as an ion pair reagent as well as enhancement of the native fluorescence of the studied drugs. Additionally, a simple isocratic separation mode with mobile phase containing acetonitrile and 20 mM phosphate buffer (pH 6.3) (60:40, v/v) containing 25 mM SDS was the best for separation. The flow rate was set at 0.8 mL min^{-1} and the effluent was monitored by a fluorescence detector and the wavelengths at emission wavelength of 389 nm after excitation at =250 nm. The developed IPC method provided rapid separation of α_1-blockers with good peak resolution compared with conventional reversed phase (RP-C_{18}) methods. The method was validated in accordance with the requirements of USP-FDA guidelines and shown to be suitable for intended applications. The linearity range for the developed IPC method in rabbit plasma obtained was 0.5–20 ng mL^{-1} with correlation coefficients of more than 0.9985. The limits of detection (S/N = 3) and quantification (S/N = 10) were 0.16–0.71 ng mL^{-1} and 0.53–2.14 ng mL^{-1}, respectively. The inter-day and intra-day precision % RSD was 1.14–3.91 and accuracy of 9.06–102.3%. The proposed IPC–FL method was successfully applied for the bioequivalence study of two formulations for terazosin from different brands in rabbits by a randomized, two-way, single-dose, crossover study and proved to be bioequivalent.

1. Introduction

Benign prostatic hyperplasia (BPH), a condition characterized by hyperplastic nodules in the urethral region and transition zone of the prostate, overall prostatic enlargement, and lower urinary tract symptoms, is highly prevalent among middle-aged and elderly men [1]. Although primarily introduced for the management of hypertension, α_1-adrenergic-receptor antagonists (α_1-blockers) have become the standard of care for the medical management of BPH related lower urinary tract symptoms (LUTS) [2]. α_1-Blockers relax the smooth muscles in the prostate and are indicated for the symptomatic treatment of BPH due to evidence of their positive and rapid effect on LUTS [3,4]. However, these agents have the potential to produce orthostatic hypotension and other blood pressure-related adverse effects in normotensive patients and in those receiving concurrent treatment with other antihypertensive agents. As a result, more uroselective, less vasoactive α_1-blockers have been developed such as tamsulosin and alfuzosin [5]. Chemically, selective α_1-adrenergic receptor antagonists are 6,7-dimethoxyquinazolin-4-amine derivatives including; prazosin (PRZ), terazosin (TER), doxazosin (DOX) and alfuzosin (ALF) as shown in Fig. 1 [6].

Development of analytical method for the assay of the studied α_1-blockers in biological samples is essential. The blood and tissue levels of α_1-blockers are difficult to measure due to low dosages used (1–5 mg) [9]. Therefore, the therapeutic drug monitoring (TDM) study of α_1-blockers requires highly sensitive and selective methods to determine the plasma levels after oral administration of the drug. In addition, it is essential to employ well characterized and fully validated analytical methods to yield reliable results which are extremely beneficial in therapeutic drug monitoring (TDM).

On the other hand, several reports were found in the literature for the analysis of α_1-blockers in pharmaceutical formulations as well as in biological fluids. These methods included UV–visible spectrophotometry [7–11], spectrofluorimetry [12–14], flow injection analysis (FIA) [15–17], voltammetry [18,19], potentiometry [20,21], high-performance thin layer chromatography (HPTLC) [22–27], high-performance liquid

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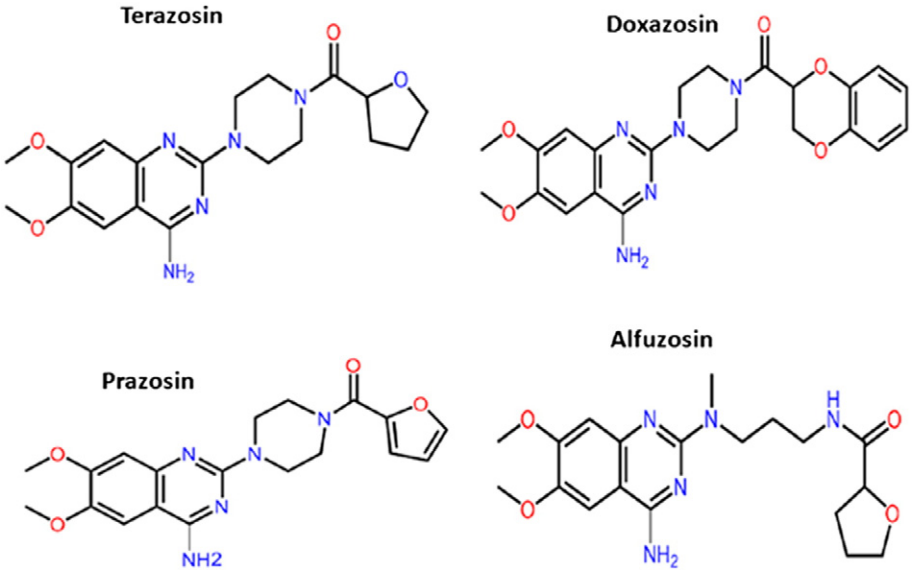

Fig. 1. Chemical structures of α_1-blockers.

chromatography (HPLC) with UV detection [28–33], fluorescence detection [34–37] and mass detection [38–41]. Although a variety of analytical techniques existed, many of these techniques lack enough sensitivity and selectivity which enables the determination of small concentrations of α_1-blockers in complex matrices [7–11]. Some of the reported methods have some practical complications for routine laboratory use or required relatively expensive instruments [38–41]. A major problem encountered during the analysis of these drugs was attributed to the strong spectral overlapping of similar drugs such as PRZ, TER, and DOX in their respective real samples [28]. Additionally, the bad resolutions obtained for some chromatographic methods and laborious extraction procedures limit the use of the others [28–34].

One of the biggest challenges facing chromatography users is the separation of ionic species for which the use of RP-HPLC is somewhat restricted because they are barely retained on non- polar stationary phases. In RP-HPLC separation, polar and ionic compounds that have higher affinity to the polar mobile phase elute first with low and poor retention. The development of ion-pair chromatography (IPC) allows the separation of complex mixtures of polar or ionic species. IPC technique is now an established and valuable separation strategy [42]. To achieve the adequate retention pre-requisite to good resolution, the mobile phase was supplemented with a specific IPR. These are large number of organic ionic compounds, oppositely charged to the analyte of interest [43,44]. A suitable IPR should have a hydrophobic moiety to interact with the stationary phase and ionic moiety with opposite charge status to the analyte [45,46].

In this research, we aimed to develop a simple, sensitive, reliable, and cost effective HPLC method using IPC with fluorescence detection for the determination of α_1-blockers for TER, DOX, PRZ and ALF to be convenient for their monitoring in plasma and BE studies. The separation mechanism was based on ion-pair interactions between basic drugs and ion-pair reagent (IPR) on non-polar stationary phase. The performance results of IPC were compared with those of RP-C_{18} column results, using the same mobile phase without IPR. The method was optimized and validated in accordance with USP-FDA guidelines [47]. In addition, the dual role played by the ion pairing reagents such as SDS in separation mechanism as well as to enhance the native fluorescence of the studied drugs hence, increases method sensitivity [48]. Finally, this method was applied for the determination of TER in rabbit plasma using PRZ as internal standard to study BE of two formulations containing TER and all pharmacokinetic parameters were assessed.

2. Experimental

2.1. Chemicals and reagents

PRZ, TER, DOX and ALF were purchased from Egyptian Pharmaceuticals Industries (Cairo, Egypt). It was used without further purification and certified to contain 98% for TER and ALF, 97% for DOX and 99% for PRZ (w/w) on dry weight basis. Sodium dodecyl sulfate (SDS) and its analogues (C7–C12) were from Novartis Pharma AG, Basel, Switzerland. Sodium dihydrogen phosphate, sodium hydroxide, and phosphoric acid for pH adjustment were from Cairo Pharmaceuticals Co., Cairo, Egypt. Double distilled water was obtained through WSC-4D water purification system (Hamilton Laboratory Milton Glass Ltd., Kent, USA). All chemicals were of analytical or HPLC grade and were supplied from Sigma Aldrich (Seelze, Germany). Pharmaceutical formulations were purchased from the local market. Itrin® tablets (Kahira/Abbott Co., Cairo, Egypt) were labeled to contain 2 mg TER. Terazin® tablets (Pharaonia Co., Alexandria, Egypt) were labeled to contain 2 mg TER.

Blank rabbit plasma used herein was supplied from Animal house, Taibah University (Al Madinah Al Munawarah, Kingdom of Saudi Arabia) and they were stored in deep-freezer at −30 °C until analysis.

2.2. Instrumentations and chromatographic conditions

The HPLC system used in this study was a Shimadzu Prominence system equipped with LC-20AD quaternary gradient pump, Prominence RF-20A fluorescence detector, CBM-20A communication bus module, CTO-20A column oven, SIL-20AP autosampler and Shimadzu LC solution software (ver. 1.21 SP1) from Shimadzu, Japan. The chromatographic separation was carried out on Cosmosil column, 5C18-MS II (150 × 4.6 mm, 5 μm i.d.) (Nacalai, Japan). Security guard cartridge (C_{18}) (4 mm × 3 mm, i.d.) was from Phenomenex (Torrance, USA). The separations were conducted isocratically using a mobile phase composed of a mixture of acetonitrile and 20 mM phosphate buffer (pH 6.3) (60:40, v/v) containing 25 mM SDS at a flow rate of 0.8 mL min^{-1}. The mobile phase was freshly prepared, filtered and degassed by sonication before use. The column condition was maintained at ambient temperature. The effluent was monitored by a fluorescence detector and the wavelengths were set at λ_{ex} = 250 nm and λ_{em} = 389 nm. In addition, ultrasonic cleaner (Cole-Parmer, Chicago, USA), pH meter, model 3305 (Jenway, London, UK), Sartorius handy balance H51 (Hanover, Germany) and oil-less vacuum pump (Rocker, Taiwan) were used in this study.