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Simultaneous determination of phentermine and topiramate in human plasma by liquid chromatography–tandem mass spectrometry with positive/negative ion-switching electrospray ionization and its application in pharmacokinetic study

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ABSTRACT

A new method for simultaneous determination of phentermine and topiramate by liquid chromatography/electrospray tandem mass spectrometry (LC/MS/MS) operated in positive and negative ionization switching modes was developed and validated. Protein precipitation with acetonitrile was selected for sample preparation. Analyses were performed on a liquid chromatography system employing a Kromasil 60-5CN column (2.1 mm × 100 mm, 5 μm) and an isocratic elution with mixed solution of acetonitrile–20 mM ammonium formate containing 0.3% formic acid (40:60, v/v), at a flow rate of 0.35 mL/min. Doxazosin mesylate and pioglitazone were used as the internal standard (IS) respectively for quantification. The determination was carried out on an API 4000 triple-quadrupole mass spectrometer operated in multiple reaction monitoring (MRM) mode using the following transitions monitored simultaneously: positive *m/z* 150.0/91.0 for phentermine, *m/z* 452.1/344.3 for doxazosin, and negative *m/z* 338.3/77.9 for topiramate, *m/z* 355.0/41.9 for pioglitazone. The method was validated to be linear over the concentration range of 1–800 ng mL^{-1} for phentermine, 1–1000 ng mL^{-1} for topiramate. Within- and between-day accuracy and precision of the validated method at three different concentration levels were within the acceptable limits of <15% at all concentrations. Blood samples were collected into heparinized tubes before and after administration. The simple and robust LC/MS/MS method was successfully applied for the simultaneous determination of phentermine and topiramate in a pharmacokinetic study in healthy male Chinese volunteers.

1. Introduction

Phentermine, a central norepinephrine-releasing drug, approved by the U.S. Food and Drug Administration (FDA) in 1959 for short-term obesity treatment, remains the most widely prescribed antiobesity drug in the USA [1]. Safety concerns for phentermine relate primarily to its cardiovascular risk factor profile, which is associated with phentermine's stimulant properties, and are dose-dependent [2]. Topiramate is a sulfamate-substituted monosaccharide approved by the FDA in 1996 for the treatment of seizure disorders, and for migraine prophylaxis in 2004 [3]. It is associated with significant weight loss and maintenance, however, it is not approved for obesity monotherapy owing to its adverse effects profile, which is dose related [4]. Combination phentermine/topiramate extended release (ER) was approved by the FDA in July 2012 for the long-term treatment of obesity in obese or overweight individuals with obesity related comorbidities at a recommended dose of 7.5/46 mg daily [3,5]. This combination works synergistically to cause weight loss at lower doses compared with the individual products used alone, thereby reducing adverse effects. Phentermine and topiramate extended release capsules thus currently demonstrate greater efficacy than monotherapy through its synergistic potential and multiple mechanisms of

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action, while its improved tolerability reinforces the role of combination pharmacotherapy in long-term weight loss maintenance [6–9].

A variety of methods have been reported for the quantitative determination of phentermine in biosamples including gas chromatography/mass spectrometry (GC/MS) [10], high-performance liquid chromatography (HPLC) with fluorescence detection [11]. And also for topiramate including high-performance liquid chromatography (HPLC) [12], capillary electrophoresis with indirect UV detection [13], and liquid chromatography/electrospray tandem mass spectrometry (LC–MS/MS) [14]. However, no report has been mentioned in the literature for the simultaneous determination of phentermine and topiramate in human plasma. In view of the wide use of the phentermine and topiramate combination preparation, a sensitive and robust method should be developed for the simultaneous determination of phentermine and topiramate in biosamples for the pharmacokinetic and bioequivalent study.

In the present study, a simple, rapid and specific LC–MS/MS method operated in positive and negative ionization switching modes was developed for the simultaneous determination of phentermine and topiramate in human plasma with the limit of quantification of 1 ng mL^{-1}. Advantages of this method included simple sample preparation procedure, simultaneous determination and shorter run time. It has been fully validated and successfully applied to the pharmacokinetic study of phentermine and topiramate extended release capsules in healthy male Chinese volunteers.

2. Experimental

2.1. Chemicals and reagents

Phentermine hydrochloride (97.8% purity), topiramate (99.5% purity), doxazosin mesylate (98.5% purity, I.S.) and pioglitazone hydrochloride (99.2% purity, I.S.) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade methanol, acetonitrile and ammonium formate were all purchased from Merck KGaA (Darmstadt, Germany). Analytical grade formic acid and ammonium acetate were purchased from Dima Technology Inc. (Guangzhou, China). Purified water used throughout the study was commercially available (Hangzhou Wahaha Co., Ltd., China). Fresh frozen healthy human plasma was collected from Union Hospital Affiliated to Tongji Medical College, Huazhong University of Science & Technology (Wuhan, PR China). Other chemicals and reagents were of analytical grade and obtained commercially. The chemical structures of phentermine, topiramate and IS are shown in Fig. 1.

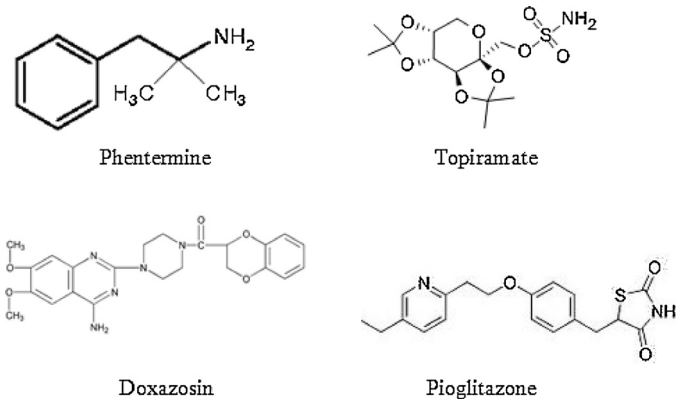

Fig. 1. Structures of phentermine, topiramate, doxazosin and pioglitazone.

2.2. Chromatographic and mass spectrometric conditions

An Agilent 1200 High Performance Liquid Chromatography System (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump, a degasser, an autosampler and a column oven was used in this study. Chromatographic separation was accomplished by employing a Kromasil 60-5CN column (2.1 mm × 100 mm, 5 μm) from GL Sciences Inc. (Torrance, CA, USA) and an isocratic elution with mixed solution of acetonitrile–20 mM ammonium formate containing 0.3% formic acid (40:60, v/v), at a flow rate of 0.35 mL/min. The injection volume was 5 μL and the run time was 7 min. The temperatures of the analytical column and autosampler were set at 35 °C and 4 °C, respectively. Under these conditions, the retention time for topiramate, pioglitazone, phentermine and doxazosin were 1.87 min, 2.12 min, 4.47 min and 4.61 min, respectively.

Detection was performed on an API 4000 triple-quadrupole mass spectrometer (AB Sciex, Framingham, MA, USA), equipped with an electrospray ionization (ESI) interface used in the positive/negative ion-switching scan mode and operated with Analyst software version 1.5.1 (Applied Biosystems). Typically, the source conditions of topiramate and pioglitazone were set as follows: collision activated dissociation gas (CAD), 10 psi; curtain gas (CUR), 25 psi; nebulizer gas (GS1), 20 psi; heater gas (GS2), 40 psi; capillary voltage, −4500 V; vaporizer temperature, 500 °C. The source conditions of phentermine and doxazosin were set as follows: collision activated dissociation gas (CAD), 10 psi; curtain gas (CUR), 25 psi; nebulizer gas (GS1), 35 psi; heater gas (GS2), 60 psi; capillary voltage, 5500 V; vaporizer temperature, 500 °C. All mass spectrometry (MS) investigation were carried out with turbo ion spray source that operate in both positive and negative ion modes under multiple reaction monitoring (MRM) conditions. Positive and negative ion-switching scan modes with the dwell time set to 200 ms and settling time set to 0.7 s for positive ion mode and 0.8 s for negative ion mode. Detection of topiramate and internal standard (pioglitazone) was performed in negative ion mode (ESI−) from 0 to 2.8 min, while the detection of phentermine and internal standard (doxazosin) was performed in positive ion mode (ESI+) from 2.8 to 7 min. The product ion chromatograms are shown in Fig. 2., and the mass spectrometric parameters for each analyte are shown in Table 1.

2.3. Preparation of calibration standards and quality control (QC) samples

Standard stock solutions of phentermine, topiramate, pioglitazone and doxazosin were individually prepared in methanol at 434.2, 483.5, 505.6 and 465.4 μg mL^{-1}, respectively. These solutions of the standards were serially diluted with methanol to produce combined standard working solutions at concentrations of 10, 25, 60, 150, 500, 2000 and 8000 ng mL^{-1} for phentermine, and 10, 25, 100, 300, 1000, 3000 and 10,000 ng mL^{-1} for topiramate. The internal standard working solution was diluted with methanol to 500 ng mL^{-1}. Calibration samples were freshly prepared by spiking appropriate amount of the working solutions into 180 μL blank plasma to provide the final concentrations in the range of 1–800 ng mL^{-1} for phentermine, and 1–1000 ng mL^{-1} for topiramate. The quality control (QC) samples were also prepared with blank plasma at low, middle and high concentrations of 3, 80, 650 ng mL^{-1} for phentermine, and 3, 50, 800 ng mL^{-1} for topiramate. All solutions described above were stored at 4 °C.

2.4. Sample preparation

A 200 μL plasma sample was transferred into a 1.5 mL Eppendorf tube together with 20 μL of IS working solution (500 ng mL^{-1}). 1 mL acetonitrile was added to precipitate plasma proteins, the