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Short communication

Development and validation a LC–MS/MS method for the simultaneous determination of agomelatine and its metabolites, 7-desmethyl-agomelatine and 3-hydroxy-agomelatine in human plasma: Application to a bioequivalence study

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ABSTRACT

A novel sensitive and selective LC–MS/MS method for the determination of agomelatine, 7-desmethyl-agomelatine and 3-hydroxy-agomelatine in human plasma was developed and validated. After simple protein precipitation, the analytes were separated on a Phenomenex ODS3 column (4.6 × 150 mm, 5 μm, Phenomenex, USA) with mobile phase consisted of methanol and 5 mM ammonium formate solution (containing 0.2% formic acid) at a ratio of 70:30 (v/v) with a flow rate of 0.8 mL/min. The MS acquisition was performed in multiple reactions monitoring (MRM) mode with a positive electrospray ionization source. The mass transitions monitored were *m/z* 244.1 → 185.1, *m/z* 230.1 → 171.1, *m/z* 260.1 → 201.1 and *m/z* 180.1 → 110.1 for agomelatine, 7-desmethyl-agomelatine, 3-hydroxy-agomelatine and internal standard (phenacetin), respectively. The method was validated for specificity, linearity and lower limit of quantification, precision and accuracy, extraction recovery, matrix effect and stability. The calibration curves for agomelatine, 7-desmethyl-agomelatine and 3-hydroxy-agomelatine in human plasma were linear over concentration ranges of 0.0457–100 ng/mL, 0.1372–300 ng/mL and 0.4572–1000 ng/mL, respectively. Intra- and inter-day precisions and accuracies data met the acceptance criteria of FDA guideline for bioanalytical method validation. The developed method has been successfully applied to a bioequivalence study in healthy Chinese volunteers.

1. Introduction

Agomelatine is a specific agonist of MT1 and MT2, and a selective antagonist of 5-HT_{2c} receptors [1,2]. It is a napthalenic compound chemically designated as *N*-[2-(7-methoxy-1-naphthyl) ethyl] acetamide (Fig. 1) with a selectivity of large than 100-fold for MT1 and MT2 receptors with no significant affinities to muscarinic, adrenergic, dopaminergic or histaminergic receptors, which has strong effects on improving depression and fewer adverse reactions [3,4].

Agomelatine has a short half-life of about 2 h in human beings. It is rapidly absorbed from the gastrointestinal tract and immediately transported to the liver [5], where it is extensively metabolized by the P450 (CYP) isoenzymes namely CYPA1, CYPA2, and CYP2C9. 7-desmethyl-, 3-hydroxy-, and 3-hydroxy-7-desmethyl-agomelatine (Fig. 1) were identified as the three metabolites of agomelatine, which have less activity than the parent drug [6–8].

For new generic agomelatine products with clinical trial permission of China Food and Drug Administration (CFDA), bioequivalence (BE) evaluation is one of the pivotal clinical studies required in support of its marketing application [9]. Our preliminary BE experiment indicated that the within-subject variability of AUC and C_{max} were 53% and 70% for agomelatine, 21.2% and 37.8% for 3-hydroxy-agomelagtine, 42.6% and 61.4% for 7-desmethyl-agomelatine. In

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addition, the AUC in plasma of 3-hydroxy-agomelagtine and 7-desmethyl-agomelatine were found about 15-fold and 3/4 of that of agomelagtine. Due to the smaller within-subject variability and high in vivo concentrations of 3-hydroxy-agomelagtine and 7-desmethyl-agomelatine, these two metabolites were selected as auxiliary index for BE assessment in the present study, which can provide ample information for the BE study, thus the in vivo data of these two metabolites are needed.

To our knowledge, several LC–MS/MS methods for the determination of agomelatine in human plasma have been published [10–12]. Two of the reported bioanalytical methods [10,11] employed a liquid–liquid extraction procedure and a time-costing evaporation process, and the linear ranges were relatively narrow, which are not adequate for the determination of this high variability drug. In addition, only the parent drug agomelatine was determined in these two reported LC–MS/MS methods. Ogawa et al. [12] reported a UPLC–MS/MS method for determination of ramelteon, agomelatine, and melatonin in human plasma, and it employed tedious and costly sample preparation technique (solid-phase extraction) and time-consuming evaporating procedure. Up to date, there were no reports described a method for the simultaneous determination of agomelatine and its major metabolites using LC–MS/MS. In the present study, we developed and fully validated a simple, rapid and sensitive enough LC–MS/MS method for the simultaneous determination of agomelatine, 7-desmethyl-agomelatine and 3-hydroxy-agomelatine in human plasma for the first time. This assay employed a simple protein precipitation procedure and obtained high extraction recoveries and wide linear ranges, which was successfully applied to a bioequivalence study in healthy Chinese volunteers.

2. Experimental

2.1. Chemicals and reagents

Reference standards of agomelatine (purity 99.5%), 7-desmethyl-agomelatine (purity 98.2%) and 3-hydroxy-agomelatine (purity 98.0%) were all provided by Chongqing Fuke pharmaceutical group Co., Ltd (Chongqing, China). Reference standard of phenacetin (internal standard, IS, purity 99.8%) was purchased from National Institutes for Food and Drug Control (Beijing, China). HPLC grade methanol and formic acid were supplied from Merck (Darmstadt, Germany) and Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China), respectively. Ammonium formate (analytical grade) and hydrochloric acid (analytical grade) were obtained from Sinopharm Chemcial Reagent Co., Ltd. (Shanghai, China). Sodium metabisulphite (analytical grade) was purchased from Tianjin Fengchuan Chemical Reagent Science And Technology Co., Ltd. (Tianjin, China). Blank human plasma was obtained from healthy volunteers with heparin used as anticoagulant. Purified water was made by AHL-2001-P trace and analysis type of ultra-pure water machine (Echo pu, Shanghai, China).

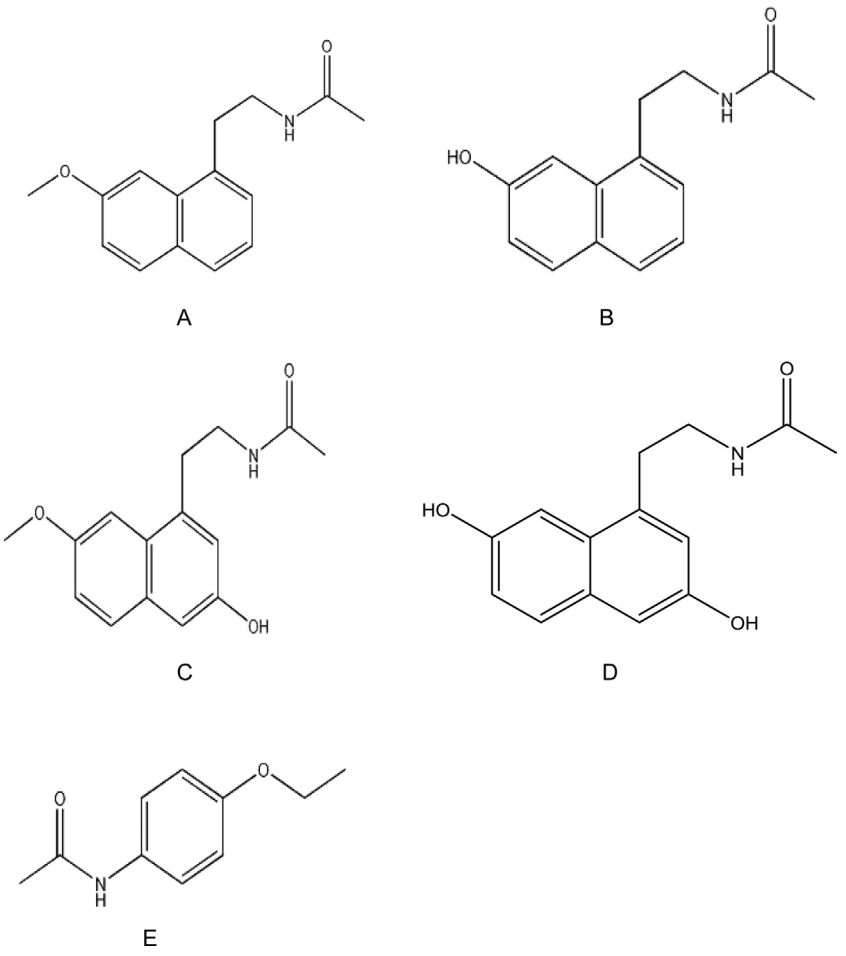

Fig. 1. Chemical structures of agomelatine (A), 7-desmethyl-agomelatine (B), 3-hydroxy-agomelatine (C), 3-hydroxy-7-desmethyl-agomelatine (D) and phenacetin (IS) (E).