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Simultaneous determination of bambuterol and its two major metabolites in human plasma by hydrophilic interaction ultra-performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

In this study, a rapid and sensitive hydrophilic interaction ultra-performance liquid chromatography–tandem mass spectrometry (HILIC–UPLC–MS/MS) method was developed for simultaneous determination of bambuterol and its two major metabolites monocarbamate bambuterol and terbutaline in human plasma. All samples were simply precipitated using acetonitrile and separated on a UPLC-HILIC column under gradient elution with a mobile phase consisting of acetonitrile and water with the addition of 10 mm ammonium acetate and 0.1% formic acid at 0.4 mL/min. The analytes were detected by a Xevo TQ-S tandem mass spectrometer with positive electrospray ionization in multiple reaction monitoring mode. The established method was highly sensitive with the lower limit of quantification (LLOQ) of 10.00 pg/mL for each analyte, and the intra- and inter-day precisions were <12.8%. The analytical runtime within 4.0 min per sample made this method suitable for high throughput determination. The validated method was successfully applied to a clinical pharmacokinetic study of bambuterol in eight healthy volunteers. Furthermore, the effects of the chromatographic conditions on the retention of the analytes on HILIC were investigated, and the benefits of HILIC were evaluated by comparing with a C_{18} column. The results indicated that liquid–liquid partition and the electrostatic interactions played an important role in the retention of the analytes on HILIC in this study. And HILIC offered particular advantages over RPLC approach in the aspects of the peak symmetry, the column efficiency, and the column pressure.

1. Introduction

Bambuterol is a bis-dimethylcarbamate prodrug of the β_2-adrenoceptor agonist terbutaline and is widely used for the treatment of asthma and chronic obstructive pulmonary disease [1,2]. The bioconversion of bambuterol to its parent drug terbutaline involves, in the simplest case, a two-step hydrolysis catalyzed by butyrylcholinesterase (BChE) with monocarbamate bambuterol as an intermediate (Fig. 1) [3]. The previous studies showed that both bambuterol and monocarbamate bambuterol were BChE inhibitors, which inhibited their own hydrolysis and the first hydrolysis step of bambuterol to monocarbamate bambuterol was mainly responsible for the typical slow generation of terbutaline [4,5]. However, an in vitro study showed that the hydrolysis kinetics of monocarbamate bambuterol was much slower than that of bambuterol, resulting in a temporary accumulation of monocarbamate bambuterol [3]. That means monocarbamate bambuterol may play an important role in the slow release of terbutaline. The pharmacokinetics of bambuterol and its metabolite terbutaline has been investigated previously [6–8] while the major intermediate monocarbamate bambuterol has been excluded mainly due to the commercial unavailability of the standard. Investigation of the roles played by monocarbamate bambuterol can lead to a better understanding of the pharmacokinetics of bambuterol and the guidance

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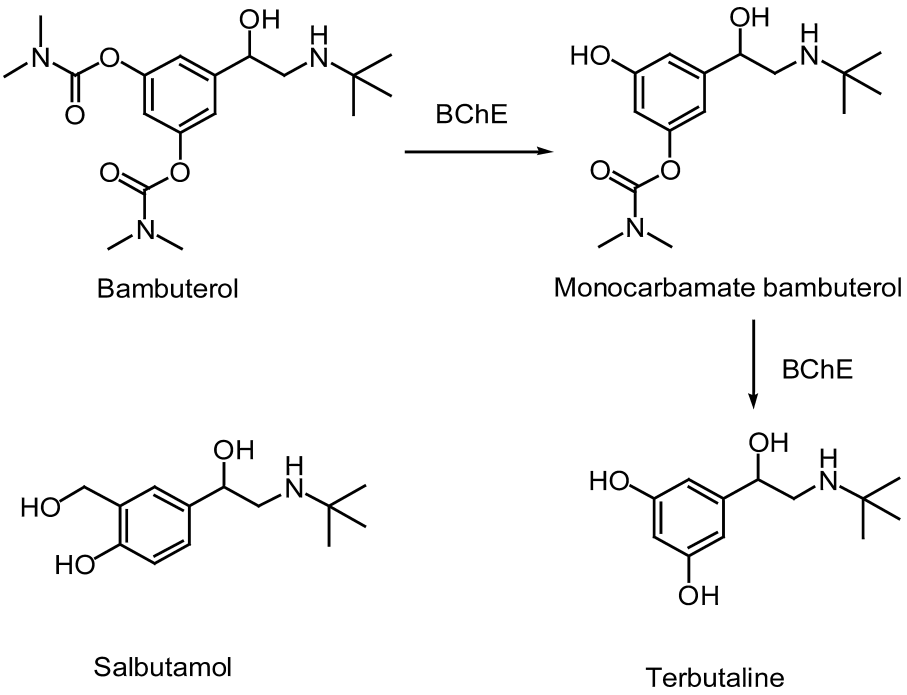

Fig. 1. Chemical structures of bambuterol, monocarbamate bambuterol, and terbutaline.

for its clinic use. Thus, it is of great importance to develop an analytical method able to simultaneously monitor the concentrations of monocarbamate bambuterol as well as bambuterol and terbutaline in plasma.

Several methods have been reported in literature for the simultaneous analysis of bambuterol and terbutaline. In our previous study, liquid–liquid extraction (LLE) coupled with RPLC–MS/MS was used [6]. The other analytical methods include dispersive solid-phase extraction coupled with LC–MS/MS [9], LLE coupled with chiral LC–MS/MS [7], enzyme hydrolysis/liquid–liquid extraction/derivatization coupled with gas chromatography/mass spectrometry (GC–MS) [10,11], solid-phase extraction (SPE) coupled with high-performance capillary zone electrophoresis [12], derivatization involving two steps coupled with GC–MS [13], SPE coupled with HPLC–MS [8] (Table S1). However, these methods are characterized by rather time-consuming sample preparation steps and analytical procedures. In addition, none of the reported analytical methods for bambuterol allow a simultaneous quantification of its major intermediate monocarbamate bambuterol.

In the reported methods, C_{18} column was the most common column used for simultaneous determination of bambuterol and terbutaline [6,8,9]. However, terbutaline is poorly retained on traditional reversed-phase liquid chromatography (RPLC) columns due to its relevant polarity, and it is usually eluted in close proximity of the dead time. In recent years, hydrophilic interaction liquid chromatography (HILIC) has become a popular alternative to RPLC and is also amenable to tandem mass spectrometry (MS/MS) detection, especially for the analysis of polar compounds [14–16]. This approach allows obtaining symmetrical peak shapes and acceptable retention factors for hydrophilic compounds that are poorly retained in RPLC. Furthermore, HILIC offers particular advantages over RPLC approaches, including lower back pressures and enhanced desolvation with electrospray ionization (ESI) owing to the large percentage of organic modifier in the mobile phase [17]. A lower back pressure allows the use of a faster flow rate, and the use of sub-2 μm particle materials with ultra-performance liquid chromatography (UPLC) system, which shorten the analysis time and increase the peak resolution. At the same time, the improved desolvation within the ESI mass source offers a better sensitivity and a lower limit of detection [18]. HILIC has been successfully applied to the analysis of several classes of compounds such as acrylamide [19], saponins [16], organophosphorus [20], and so on, but the application to the determination of phenethylamines, a main class of β_2-adrenoceptor agonists, has not been reported yet. In addition, unlike RPLC, so far the retention mechanisms of HILIC are still not well identified [21–23]. Therefore, this study using HILIC for the analysis of bambuterol and its metabolites, which are phenethylamines with high polarities, will provide a good reference for the analysis of other β_2-adrenoceptor agonists in this class.

In this study, a sensitive and rapid HILIC–UPLC–MS/MS method was developed for the first time to simultaneously determine bambuterol and its two major metabolites monocarbamate bambuterol and terbutaline in human plasma. This method was fully validated in terms of sensitivity, linearity, accuracy, precision, and stability and was further applied to a clinical pharmacokinetics study of bambuterol in eight healthy volunteers. In addition, the effects of the chromatographic conditions on the retention of the analytes on HILIC were investigated, including the organic phase proportion, the buffer concentration, the pH of mobile phase, and the column temperature. Moreover, the same bridged-ethylene hybrid (BEH) material functionalized with C_{18} ligands was used as a comparator technique to evaluate the benefits of HILIC over RPLC in the aspects of the peak symmetry, the column efficiency, and the column pressure.

2. Experimental

2.1. Materials and reagents

Bambuterol hydrochloride and terbutaline sulphate were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Salbutamol sulphate (IS) was purchased from Changzhou Yabang pharmaceutical Co., Ltd. (Jiangsu, China). Monocarbamate bambuterol hydrochloride (purity ≥ 98.9%, determined by HPLC analysis) was prepared in our