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Determination of tulobuterol in rat plasma using a liquid chromatography–tandem mass spectrometry method and its application to a pharmacokinetic study of tulobuterol patch

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Abbreviations:
LC–MS/MS, liquid chromatography–tandem mass spectrometry
MR, multiple reaction monitoring
IS, internal standard
HPLC, high performance liquid chromatography
ESI, electrospray ionization
LLE, liquid–liquid extraction
LLOQ, lower limit of quantification
QC, quality control
RE, relative error
RSD, relative standard deviation
MF, matrix factor

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Tulobuterol patch
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ABSTRACT

A sensitive and accurate liquid chromatography–tandem mass spectrometry (LC–MS/MS) method has been developed and validated for determination of tulobuterol in rat plasma for the first time. Plasma samples were extracted by liquid–liquid extraction method with methyl *tert*-butyl ether and the analyte and clenbuterol (IS) were separated on a Venusil MP C_{18} column (100 mm × 2.1 mm, 3 μm) using 0.1% formic acid–water–methanol as mobile phase, with a runtime of 5 min. The analyte was detected in multiple reaction monitoring (MRM) mode with positive electrospray ionization. Transitions of m/z 228.2 → 154.0 for tulobuterol and m/z 277.1 → 203.0 for the clenbuterol were monitored. The linear range was 0.5–100 ng/ml (r = 0.9967) for tulobuterol with the lower limit of quantitation of 0.5 ng/ml. The intra-day and inter-day precisions were less than 10.3% for the analyte and the accuracy was less than −8.6%. The RSD of matrix effect and recovery yield were within ±15% of nominal concentrations and tulobuterol was stable during stability studies. The validated method has been successfully applied to a pharmacokinetic study of three doses of tulobuterol patch in rats for the first time.

1. Introduction

Tulobuterol is the third generation of β2-adrenoceptor agonist widely used in the treatment of bronchial asthma and chronic obstructive pulmonary disease (COPD) [1–3]. Transdermal tulobuterol was designed to maintain drug level at a constant effective concentration over a 24 h period [4,5]. The formulation of tulobuterol patch could effectively avoid the first-pass effect and prevent excessive increase of drug concentration in blood, thereby reducing adverse reactions [6–8].

In order to control the maximum blood concentration and get a better therapeutic effect during the "morning dip" [4,9], a "crystal reservoir system" was designed and different dosages were applied [4,10]. Based on the relative lower blood concentration compared to oral or inhalation formulations [6], a sensitive analytical method to determinate the pharmacokinetic profile of transdermal tulobuterol was expected.

Based on the previous papers, determination of tulobuterol by gas chromatographic coupled with electron capture detector or mass spectrometry has been developed [11–15], and the lower limit of quantification (LLOQ) of tulobuterol in serum

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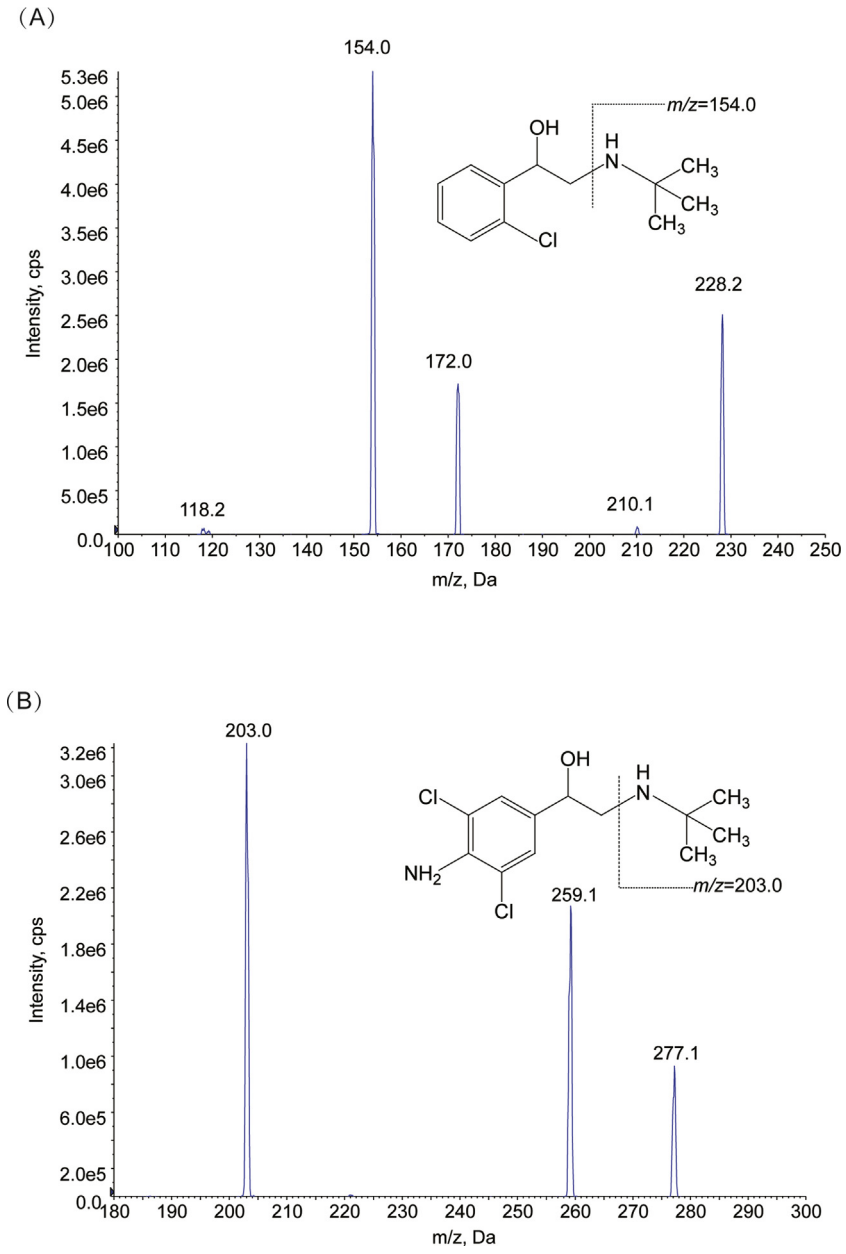

Fig. 1. Chemical structures of tulobuterol hydrochloride (A) and clenbuterol hydrochloride (B). Product ion spectra of (A) tulobuterol (228.2 → 154.0) and IS (277.1 → 203.0).

ranged from 0.2 to 170 ng/ml, however, the procedure for sample preparation was complicated and time-consuming. Xu et al. [16] established a convenient detecting method using liquid chromatography–electrospray ionization mass spectrometry, but only one dose was studied. Our present study has established and validated a selective and sensitive quantification method to determine three different doses of transdermal tulobuterol patch (0.5, 1.0 and 2.0 mg) in rat plasma for the first time, using liquid chromatography–tandem mass spectrometry (LC–MS/MS). The analytical period is shorter and the plasma volume is smaller compared to other methods mentioned above [11–16], and the range of detection (0.5–100 ng/ml) is the widest so far.

The method was applied to study the pharmacokinetics of a new generic transdermal tulobuterol patch developed by our pharmaceutical laboratory. This tulobuterol patch possessed a simple preparation and lower cost compared to branded tulobuterol patch (Amiaid, Japan). There were three different doses (0.5, 1.0 and 2.0 mg designed for babies, children and adults) commercially available for branded tulobuterol patch (Amiaid, Japan). Therefore, our study focused on a pharmacokinetic research of three doses of generic transdermal tulobuterol patch, and the linearity among doses was also analyzed, providing a reference for subsequent research and clinical application.

2. Experiment

2.1. Chemicals and reagents

Tulobuterol hydrochloride and clenbuterol hydrochloride (internal standard, IS, Fig. 1) were obtained from National Institutes for Food and Drug Control (Beijing, China), the purity of these reference standards were all more than 99.9%.

Methanol and methyl *tert*-butyl ether of HPLC grade were from Fisher Scientific (Fair Lawn, NJ, USA). Distilled water prepared with demineralized water was employed throughout the experiment. HPLC grade reagents such as acetic acid and ammonia hydroxide