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Development and full validation of a liquid chromatography-tandem mass spectrometry method for determination of carbinoxamine in beagle plasma and its application to a pharmacokinetic study

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ABSTRACT

Carbinoxamine maleate is an antihistamine drug with mild sedation effects, which is used to treat seasonal and perennial allergic rhinitis clinically. In order to optimize drug therapy, reduce drug accumulation, lessen the frequency of adverse effects and facilitate clinical research, a high performance liquid chromatography tandem mass spectrometry assay was firstly established and fully validated for the quantitative admeasurements of carbinoxamine. After extraction with ethyl acetate, the chromatographic separation was implemented on a C18 column (Hypersil GOLD, 100 mm × 2.1 mm, 3.0 μm) using gradient elution with water (containing 0.1% formic acid) and methanol at the flow rate of 0.4 mL/min. The analytes were measured under multiple reactions monitoring (MRM) mode with *m/z* 291.2 → 202.1 for carbinoxamine and *m/z* 285.0 → 193.2 for diazepam (IS) using electrospray ionization source (ESI) in the positive ion mode. A satisfactory linearity was obtained over the wide extent of 0.1–100.0 ng/mL ($r > 0.99$). Inter- and intra-day precision and accuracy of the assay were favorably accorded with the currently recognized limits (< 8.9%). The mean extraction recoveries for carbinoxamine ranged from 74.00% to 86.4%. The pharmacokinetic characteristics of carbinoxamine were subsequently evaluated in beagles. Following intragastric administration (0.534 mL/kg), carbinoxamine possessed a large apparent volume of distribution of the central compartment ($Vc = 1005.7 \pm 945.9$ L/kg), oral clearance ($Cl = 112.446 \pm 53.249$ L/h/kg), and a relatively long absorption time ($T_{max} = 2.38 \pm 1.00$ h). This analytical method with adequate sensitivity was applicable to pharmacokinetic study and could monitor concentrations of carbinoxamine in beagle plasma. Moreover, the methodology could be used for further bioequivalence determination and addressing metabolism associated with the drug.

1. Introduction

Carbinoxamine is an oral antihistaminic which takes effect basically by blocking the histamine-H_1 receptor [1–4]. It has been remedyed for hayfever and allergic conjunctivitis as mono-therapy or in combination with pseudoephedrine and paracetamol [5,6]. Because of its anticholinergic properties, carbinoxamine is also used to cure of Parkinson's disease [7]. It is available as the maleic acid salt on the market and the chemical structure of carbinoxamine maleate was presented in Fig. 1.

Carbinoxamine maleate is an antihistamine drug with mild sedation effects, which is prescribed to treat anaphylactic rhinitis clinically. However, the improper dose of the antihistamines may bring about serious sedative effects, such as fatigue, hypotension and dyspnea [8]. Complete pharmacokinetic characteristics for carbinoxamine could provide theoretical basis of safety and effectiveness in the clinical. Moreover, due to the easy taken and the durable action, the controlled release formulation of carbinoxamine is more suitable for children allergic disease treatment. It is worth pointing out that the amount of chemical drugs exceeds than 3500 kinds at present in China, however, the percentage of pediatric formulations is < 10% and child-specific medicines are fewer than 100 categories. The lack of drug and appropriate dosage form suitable for children is a big short board for the pharmaceutical industry development. With the purpose of optimizing drug therapy, reducing drug accumulation, lessening the frequency of adverse effects and facilitating clinical research, it is essential to develop reliable, rapid and sensitive analysis methods *in vivo*. Over these years, there have been several methods involved in the literature to determine carbinoxamine maleate as drug substance as well as in pharmaceutical formulations in combination with other antihistamine, which included spectrophotometry [9–11], micro-emulsion

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electrokinetic chromatography [12], capillary electrophoresis [13–15], and liquid chromatography [16–18]. These conventional quantitative methods with classical ultraviolet detection have possessed insufficient sensitivity, longer chromatographic run time and a labor intensive work-up procedure, which nowadays are less favored in industries to support clinical researches. Among the separation analysis technology currently available, liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) has been springed up as the prominent analytical way for assay of small molecule drugs in biological matrix. Gurupadayya *et al.* [19] developed a convenient method using liquid chromatography-electrospray ionization mass spectrometry for quantitative determination of carbinoxamine in human plasma, which was the only relevant literature published so far using LC–MS/MS method. This method possessed the lower limit of quantification (LLOQ) of 0.5 ng/mL, while that of our method was 0.1 ng/mL which could quantitatively detect the analyte at 36 h after administration with the clinical dosage. In addition, the method of our report had a lower injection volume and flow rate, which could reduce pollutions of the instrument and help the analyte to be ionized adequately in mass spectrometry. Moreover, as a biological sample analysis method, the referenced method lacked of verification of the stability and the matrix effect which was crucial for the reliability of the data.

In the current research, a reliable and well-rounded UHPLC ESI-MS/MS method was established and validated for quantification of carbinoxamine in beagle plasma for the first time. It was successfully applied to reveal the pharmacokinetic profiles of carbinoxamine after intragastric administration of Carbinoxamine Maleate Oral Solution. What's more, it was anticipated that this study would be available for improvinng clinical therapeutic efficacy with minimal incidence of side effects and overcoming limitations of the development of new preparations in clinical practice.

2. Experimental

2.1. Chemicals and materials

Carbinoxamine maleate (purity of 99.9%, Batch No.1096000) was acquired from Sigma (St. Louis, MO, USA). Carbinoxamine Maleate Oral Solution was supplied by Beijing Nuokangda Pharmaceutical Technology Company Ltd. (Beijing, China). Diazepam (internal standard, IS) (purity of 99.9%, Batch No.171225-201304) (Fig. 1) was procured from the National Institutes for Food and Drug Control (Shengyang, China). Sodium hydroxide was obtained from Tianjin regent chemicals Company Ltd. (Tianjin, China). Methanol and acetonitrile were offered by Fisher Company Inc. (USA) (HPLC). Ethyl acetate was rendered by Shandong Yuwang Industrial Company Ltd. (Yucheng, China) (HPLC). Formic acid was obtained from Kemiou Chemical Reagent Company Ltd. (Tianjin, China) (HPLC). Deionized water was bought from Hangzhou Wa-ha-ha Group Company Ltd. (Hangzhou, China). By the way, all other reagents were of HPLC grade and used without further purification.

2.2. Animals

Six male beagles (12 ± 4 kg) were obtained from Zhen He Scientific Research Animal Domestrate Service Center (Fuzhou, China). All of the experimental processes were fulfilled in strict conformity with the Guide to the Nursing and Use of Experimental Animals in China, and authorized by the Animal Ethics Commission of Shenyang Pharmaceutical University (Shenyang, China). In the controlled environment, animals were bred freely and availably to normal laboratory eatables no less than one month prior to the trial.

2.3. LC–MS/MS conditions

An XR LC – 20 ADCE Prominence ™ HPLC system (Shimadzu, Japan) comprised of a binary pump, an online solvent degasser, an automatic sampler and a thermostatically controlled column compartment were chosen for this method. Chromatographic resolution was successfully implemented on a Hypersil GOLD C18 column (100 mm × 2.1 mm, 3.0 μm) by utilizing a mobile phase comprising of methanol (B) and water containing 0.1% formic acid (A). The gradient elution procedure followed by a 2 min re-equilibration step to the initial conditions was conducted as shown below: 40% B → 60% B at 0.01–0.50 min; 60% B → 90% B at 0.51–2.00 min; 90% B at 2.01–3.00 min. The column oven temperature was maintained at 30 °C and a constant rate of flow of eluent was operated at 0.4 mL/min.

The HPLC system was coupled to a QTrap™ 4000 MS/MS system fitted out with a turbo ion spray source (AB Sciex, USA). Tandem mass spectrometer was manipulated under positive electrospray ionization mode with multiple reactions monitoring mode (MRM) transition acquisition. The protonated transitions of the parent to daughter ion were primarily monitored at *m/z* 291.2 → 202.1 for carbinoxamine and *m/z* 285.0 → 193.2 for diazepam (IS). The ion spray voltage (IS) and turbo spray temperature were maintained at 5500 V and 500 °C. The curtain gas (CUR), nebulizing gas (GS1) and turboionspray gas (GS2) (gas: nitrogen) were optimized at 20, 50 and 40 psi, respectively. Data collection and processing were completed with the Analyst 1.6 software supplied by Applied Biosystems MDS Sciex. The quantitative parameters were listed in Table 1.

2.4. Preparation of stock and working solutions

The stock standard solutions of the analyte and IS were prepared separately by dissolving the precisely weighted reference substances with absolute methanol to obtain an ultimate concentration of 0.1 mg/

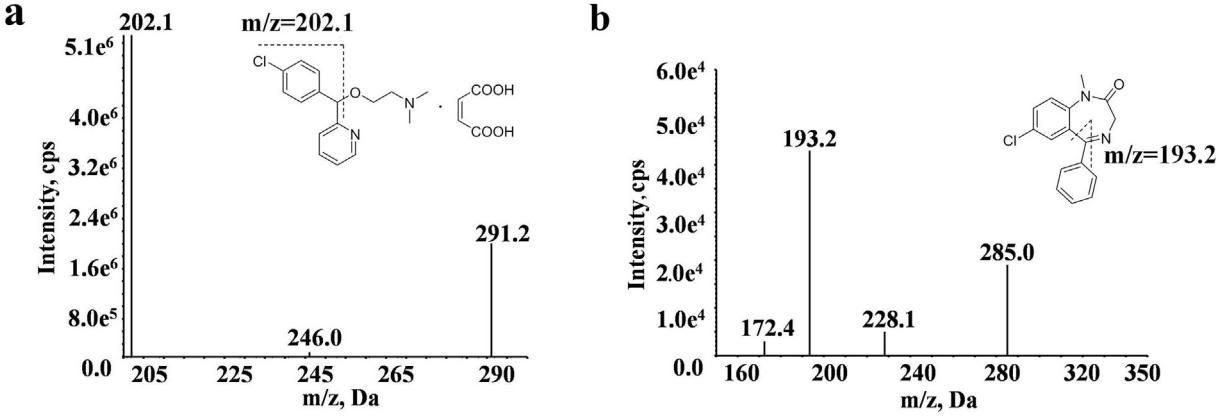

Fig. 1. Chemical structures with fragmentation and produce ion spectrum of carbinoxamine (a) and diazepam (b, IS).