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A rapid and sensitive UHPLC–MS/MS method for quantification of 2-(2-hydroxypropanamido) benzoic acid in rat plasma: Application to a pharmacokinetic study

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ABSTRACT

A rapid, sensitive and high throughput UHPLC–MS/MS method was established and validated to assay the concentration of 2-(2-hydroxypropanamido) benzoic acid (HPABA), a promising anti-inflammatory drug, in rat plasma. Plasma samples were processed by liquid-liquid extraction with ethyl acetate and separated on a Shim-pack XR-ODS C_{18} column (75 mm × 3.0 mm, 2.2 μm) at an isocratic flow rate of 0.4 mL/min using acetonitrile–0.1% formic acid in water (50:50, *v*/*v*) as mobile phase, and total run time was 2 min. MS/MS detection was accomplished in multiple reaction monitoring (MRM) mode with positive electrospray ionization. The calibration curve was linear over the concentration range of 0.01–50 μg/mL with lower limit of quantification of 0.01 μg/mL. The intra- and inter-day precisions were below 8.5% in terms of relative standard deviation (RSD), and the accuracy was within ±4.0% in terms of relative error (RE). Extraction recovery, matrix effect and stability were satisfactory in rat plasma. The developed method was successfully applied to a pharmacokinetic study of HPABA following intragastric administration of 25, 50, 100 mg/kg and an intravenous injection at a dose of 12.5 mg/kg to Sprague-Dawley rats. Results indicated that HPABA had linear pharmacokinetic properties within the tested intragastric dosage range and the absolute bioavailability was above 59.1%.

1. Introduction

Marine organisms have long been recognized as a rich source for novel compounds with great potential as drugs, nutritional supplements, cosmetics, agrichemicals and enzymes, and each of these marine bioproducts has a significant potential market value [1–3]. Marine natural products, especially produced from primary or secondary metabolism of microorganisms, are being developed with growing intensive interest to discovery novel bioactive substances. In recent years, a lot of structurally and pharmacologically important substances have been isolated with a wide range of activities such as anticancer [4–6], anti-angiogenesis [7], antioxidant [8,9], antimicrobial [10–12] and anti-inflammatory [13–16].

2-(2-Hydroxypropanamido) benzoic acid (HPABA, Fig. 1A) was isolated from the fermentation broth of a marine fungus *Penicillium chrysogenum* which was acquired from North China sea. Initial investigations demonstrate that HPABA possesses remarkable response against acetic acid induced abdominal constriction and xylene induced ear edema in mice at a dose of 100 mg/kg, but it exhibits no ulcerogenic effect like aspirin [17]. In addition, results of carrageenan induced hind paw edema in rats show that the anti-inflammatory mechanism of HPABA is synthetically, including nitric oxide (NO) and malondialdehyde (MDA) suppression and increase in the activities of superoxide dismutase (SOD) (data are not listed). To research further on HPABA, we developed an efficient route for the synthesis and purification of it and established a RP-HPLC method to separate it and its related substances [18].

During the development of a new drug, preliminary pharmacokinetic study regarding its absorption, distribution, metabolism and excretion (ADME) need to be carried out because they are responsible for 60% failures of all drugs in the clinical phases [19]. Computational approaches are being used nowadays to predict ADME properties of the compounds at early stages of drug development process, which may help to remove the compounds with

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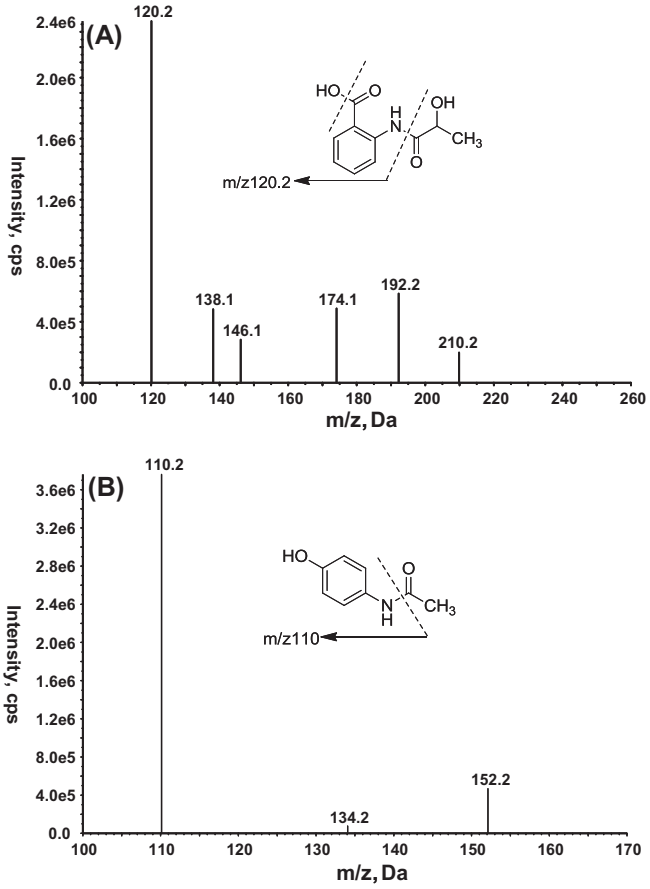

Fig. 1. Full-scan product ion spectra of $[M+H]^+$ ions of HPABA (A) and IS (B) in positive ionization mode.

poor ADME properties and lead to significant savings in research and development costs. In our previous study, ADME prediction of HPABA was carried out by Discovery Studio (Version 3.0, Accelrys® software corporation, San Diego, CA, USA) [20–22]. It was predicted that HPABA may possess good intestinal absorption and high affinity to plasma proteins; it could not cross the blood–brain barrier and will not inhibit the CYP2D6 enzyme during metabolism. Hence HPABA is likely to be a promising anti-inflammatory drug with good oral bioavailability and little side effect.

The computational results can be used to assist drug discovery and development, but pharmacokinetic characteristics including elimination half time ($t_{1/2}$), plasma clearance (CL), oral bioavailability and pharmacokinetic linearity of promising drugs still need to be experimentally determined. As far as we know, there is no research to reveal the pharmacokinetic profile of HPABA. In order to accomplish preclinical pharmacokinetic investigation of HPABA, it is necessary to develop appropriate analytical method for determination of it in biological matrices. Liquid chromatography with tandem mass spectrometry (LC–MS/MS) capable of high selectivity and sensitivity as well as high sample throughput has become the dominant tool to assay compounds in complex biological matrices [23–27]. This paper reports on a simple and sensitive UHPLC–MS/MS method with an electrospray ionization (ESI) source in multiple reaction monitoring (MRM) mode to determine the concentration of HPABA in rat plasma. After full validation, the method was successfully applied to a pharmacokinetic study of HPABA after intragastric administration of 25, 50, 100 mg/kg and an intravenous injection of 12.5 mg/kg. Pharmacokinetic characteristics of HPABA such as pharmacokinetic linearity and absolute bioavailability were revealed, which could facilitate the further research and development of HPABA.

Table 1
Optimized MRM parameters, collision energy (CE), declustering potential (DP), entrance potential (EP) and cell exit potential (CXP) for HPABA and IS.

Analyte	Precursor ion (m/z)	Product ion (m/z)	CE (eV)	DP (V)	EP (V)	CXP (V)
HPABA	210.2	120.2	25	26	8	3
IS	152.2	110.2	21	47	4	3

2. Experimental

2.1. Materials, reagents and animals

HPABA (purity > 99%) was synthesized in School of Pharmacy, Shenyang Pharmaceutical University (Shenyang, China). Paracetamol (IS, purity > 99%, Fig. 1B) was purchased from National Institute for Food and Drug Control (Beijing, China). Acetonitrile of HPLC grade was purchased from Fisher Scientific (Fair Lawn, NJ, USA). All the other reagents were of analytical grade. Deionized water was purified using a Milli-Q system (Millipore, Milford, MA, USA) and used throughout the study.

Pathogen free Sprague-Dawley rats (220–250 g) were obtained form Experimental Animal Center of Shenyang Pharmaceutical University and bred with unlimited access to food and water in an air-conditioned animal center at temperature of 22 ± 2 °C and relative humidity of 50 ± 10% with a natural light–dark cycle for a week and then fasted with only access to water for 12 h prior to the experiment. Animal experiment was carried out according to the Guideline for Animal Experimentation of Shenyang Pharmaceutical University, and the protocol was approved by the Animal Ethics Committee of the institution.

2.2. Instrument and analytical conditions

The UHPLC-ESI-MS/MS system consists of a Prominence™ UHPLC system equipped with a binary pump, a degasser, an autosampler, a thermostatted column compartment (Shimadzu, Kyoto, Japan) and a QTRAP 3200™ MS/MS system (AB Sciex, Foster, CA, USA). Chromatographic separation was performed on a Shimpack XR-ODS C_{18} column (75 mm × 3.0 mm, 2.2 μm; Shimadzu, Kyoto, Japan), which was protected by a high pressure column prefilter (2 μm). The mobile phase was composed of acetonitrile–0.1% formic acid in water (50:50, v/v) at a flow rate of 0.4 mL/min. The temperature of column and autosampler were maintained at 35 °C and 4 °C, respectively. The injection volume was 2 μL.

Mass spectrometric detection was operated in positive ionization mode using a TurboIonSpray source in multiple reaction monitoring (MRM) mode. The ionspray voltage and source temperature were maintained at 5500 V and 500 °C, respectively. High-pure nitrogen was used as nebulizing gas (50 psi), auxiliary gas (50 psi) and curtain gas (20 psi). The optimized MRM parameters for HPABA and IS are listed in Table 1. Analyst 1.5.1 software (AB Sciex, Foster, CA, USA) was used for the control of equipment, data acquisition and analysis.

2.3. Preparation of standard and quality control samples

Individual stock solutions of HPABA and IS were prepared in methanol at 1.0 mg/mL and 400 μg/mL, respectively. The stock solution of HPABA was then serially diluted with methanol to obtain the working solutions. The IS working solution of 2 μg/mL was also prepared by diluting of the stock solution with methanol. All the