Journal of Chromatography B, 1001 (2015) 107–113

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Simultaneous determination of cinnamaldehyde, cinnamic acid, and 2-methoxy cinnamic acid in rat whole blood after oral administration of volatile oil of Cinnamoni Ramulus by UHPLC-MS/MS: An application for a pharmacokinetic study

Bin Ji[a], Yunli Zhao[a], Qili Zhang[a], Pei Wang[a], Jiao Guan[a,b], Rong Rong[a], Zhiguo Yu[a,*]

[a] School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China
[b] School of Pharmacy, Jilin Medical College, Jilin 132013, China

ARTICLE INFO

Article history:
Received 13 June 2015
Received in revised form 24 July 2015
Accepted 26 July 2015
Available online 1 August 2015

Keywords:
Cinnamomi Ramulus
Cinnamaldehyde
Cinnamic acid
2-Methoxy cinnamic acid
UHPLC-MS/MS
Pharmacokinetics

ABSTRACT

A simple and rapid ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method was developed for the simultaneous determination of cinnamaldehyde, cinnamic acid, and 2-methoxy cinnamic acid in rat whole blood. It was the first time to study the pharmacokinetics of 2-methoxy cinnamic acid in rat whole blood. Samples were processed by a one-step protein precipitation with acetonitrile-37% formaldehyde (90:10, v:v). Chromatographic separation was performed on a Thermo Scientific C18 column (2.1 mm × 50 mm, 1.9 μm) at room temperature. The total run time was 4 min. The detection was accomplished by using positive and negative ion electrospray ionization in multiple reaction monitoring mode. The method was linear for all of the analytes over 1000 times concentration range with correlation coefficients greater than 0.99. The lower limits of quantification (LLOQ) were 0.1 ng/mL for cinnamaldehyde, 5.8 ng/mL for cinnamic acid, and 10 ng/mL for 2-methoxy cinnamic acid, respectively. To our knowledge, this was the first time that the LLOQ for cinnamaldehyde in validated methods for biological samples was as low as 0.1 ng/mL. Intra- and inter-day precision and accuracy were within ±9% for all of the analytes during the assay validation. Assay recoveries were higher than 80% and the matrix effects were minimal. The half-life were 8.7 ± 0.7 h for cinnamaldehyde, 1.0 ± 0.5 h for cinnamic acid, and 1.4 ± 0.4 h for 2-methoxy cinnamic acid, respectively. The validated assay was firstly applied to the simultaneous quantification of cinnamaldehyde, cinnamic acid, and 2-methoxy cinnamic acid, especially for 2-methoxy cinnamic acid in rat whole blood after oral administration of 15 mg/kg essential oil of Cinnamoni Ramulus. It was observed that the C_{max} and AUC of 2-methoxy cinnamic acid (0.01% in essential oil of Cinnamoni Ramulus) were greater than those of cinnamaldehyde (83.49% in essential oil of Cinnamoni Ramulus), which implied that 2-methoxy cinnamic acid might be the major bioactive constitutes in essential oil of Cinnamoni Ramulus.

1. Introduction

Cinnamomi Ramulus, the stem of *Cinnamomum cassia* Presl, is one of the most important Chinese medicinal materials as recorded in Chinese Pharmacopoeia. The essential oil of Cinnamoni Ramulus (CRO) had been widely used as medicine, food, and cosmetic additives due to its unique properties, such as anti-inflammation, antiviral, antibacterial, and antioxidant activities [1–4]. CRO contains large quantities of aromatic compounds, such as cinnamaldehyde, cinnamic acid, 2-methoxy cinnamic acid, coumarin, cinnamic alcohol, and 2-methoxy cinnamaldehyde. Among them, cinnamaldehyde, cinnamic acid, and 2-methoxy cinnamic acid were reported as the major bioactive compounds. Cinnamaldehyde, the most plentiful content, had been reported to have anti-stress urinary incontinence, anti-inflammation, anti-thrombic and anti-antitumor activities [5–9]. Cinnamic acid had been considered to possess anti-bactericidal, anti-oxidation, and anti-cancer activities [10–12]. 2-Methoxy cinnamic acid was claimed to have significant antityrosinase and antioxidant effects [13–15].

* Corresponding author. Fax: +86 24 23986295.
E-mail address: zhiguo-yu@163.com (Z. Yu).

http://dx.doi.org/10.1016/j.jchromb.2015.07.049
1570-0232/

Table 1
Optimized mass-spectrometric conditions.

Analytes	Ion transition	CV (eV)	CE (eV)	Acquisition time (min)
Cinnamaldehyde	132.99 → 55.05	15	10	1.6 → 2.8
Cinnamic acid	146.96 → 103.02	32	14	1.3 → 2.3
2-Methoxycin namic acid	176.90 → 133.12	5	10	1.5 → 2.5
Acetaminophen (IS)	151.86 → 109.75	30	16	0.5 → 1.3

During the past two decades, most studies relevant to CRO and its major components were focused on its biological activity, but little attention was paid to its *in vivo* pharmacokinetics. Only one method based on HPLC-UV was established for the simultaneous determination of cinnamaldehyde and cinnamic acid in rat whole blood after oral administration of cinnamaldehyde monomer [16]. Nevertheless, the LLOQ for the HPLC-UV method was 0.1 and 1 μg/mL for cinnamaldehyde and cinnamic acid, respectively, the total analytical time was up to 9 min. Indeed, several literatures had been reported for the determination of cinnamaldehyde or cinnamic acid separately. The most sensitive GC–MS method had a LLOQ of 0.6 ng/mL for cinnamaldehyde with a run time of up to 11 min [17], which was not sensitive enough for a pharmacokinetic study under clinical does.

In the present study, a sensitive and selective UHPLC-MS/MS method was developed for the simultaneous determination of cinnamaldehyde, cinnamic acid, and 2-methoxy cinnamic acid in rat whole blood. The LLOQ for cinnamaldehyde was 0.1 ng/mL. The total run time was 4 min. To our knowledge, this was the first time that the LLOQ for cinnamaldehyde in validated methods for biological samples was as low as 0.1 ng/mL. In addition, this would be the first account to analyze the pharmacokinetics of 2-methoxy cinnamic acid. It was expected that the present work would provide some references for the apprehension of the action mechanism and further clinical efficacy studies of Cinnamoni Ramulus.

2. Experimental

2.1. Chemicals and reagents

Cinnamomi Ramulus was obtained from GuoDa Pharmacy (Shenyang, China). The reference standards of cinnamaldehyde, cinnamic acid, and acetaminophen (IS) were purchased from the National Institutes for Food and Drug Control (Beijing, China). 2-Methoxy cinnamic acid was obtained from Hubei Pharmaceutical Corporation (Wuhan, China). Formaldehyde solution (37–40%) was purchased from Tianjin Damao Chemical reagent factory (Tianjin, China). HPLC-grade acetonitrile and methanol were purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade formic acid was purchased from Tianjin Kermel Chemical Reagent factory (Tianjin, China). Deionized water was purchased from Hangzhou Wahaha Corporation (Hangzhou, China).

2.2. Instrumentation and chromatographic conditions

An Acquity UPLC coupled with a Xevo TQ-S mass spectrometer (Waters Corp., Milford, MA, USA) was employed. Electrospray ionization in multiple reaction monitoring mode was used and positive/negative switching was applied whenever required. Nitrogen was used as the source gas. The following setups of the analyzers were used: the capillary voltage was set to 3.0 kV, the source and desolvation temperature were set at 150 and 350 °C, respectively. The cone and desolvation gas were set at 150 and 700 L/h, respectively. MS collision energy and MS/MS collision energy were maintained at 4 and 25 V, respectively. A summary of the ion transitions, cone voltage (CV), collision energy (CE), and acquisition time are presented in Table 1. Chromatographic separation was performed on a Thermo Hypersil GOLD C_{18} column (2.1 mm × 50 mm, 1.9 μm). Mobile phase A consisted of 0.005% formic acid in water and mobile phase B consisted of acetonitrile. The gradient elution was carried out for 4 min at a flow rate of 0.2 mL/min. The gradient started at 30% B, followed by linear increased to 70% B from 0 to 3 min, then decreased to 30% B from 3 to 4 min. The injection volume was 5 μL for each sample.

2.3. Preparation of CRO

Cinnamomi Ramulus (100 g) was placed in a 2-L round bottomed flask with 1000 mL deionized water and the essential oil

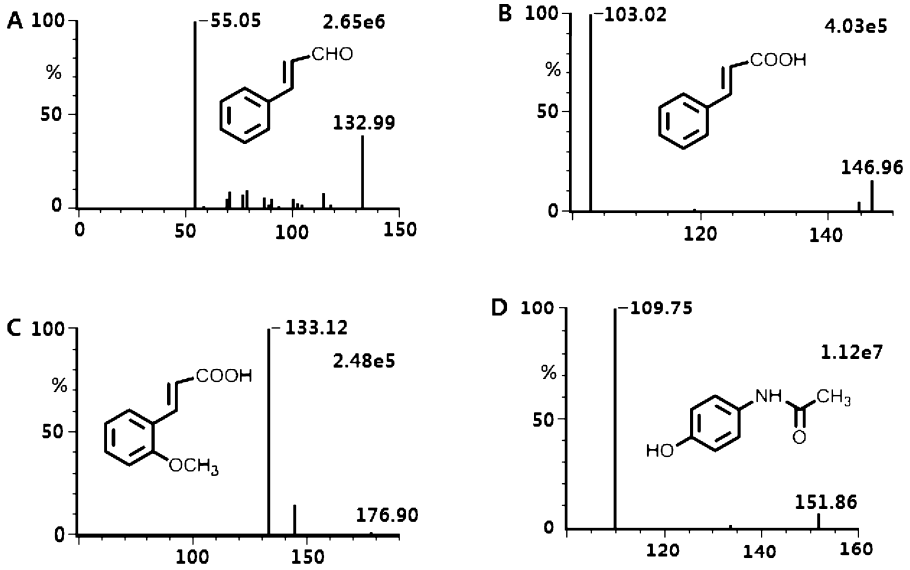

Fig. 1. MS/MS spectra of cinnamaldehyde (A), cinnamic acid (B), 2-methoxy cinnamic acid (C) and acetaminophen (D).