Journal of Chromatography A, 1358 (2014) 110–116

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Identification and analysis of gastrodin and its five metabolites using ultra fast liquid chromatography electrospray ionization tandem mass spectrometry to investigate influence of multiple-dose and food

Yuanwei Jia[a,b], Jie Shen[b], Xin Li[a], Haitang Xie[b], Junsong Wang[c], Jun Luo[a], Kelvin D.G. Wang[a], Qingwang Liu[a], Lingyi Kong[a,*]

[a] Department of Natural Medicinal Chemistry, China Pharmaceutical University, 24# Tongjia Xiang, Nanjing 210009, Jiangsu, China
[b] Anhui Provincial Center for Drug Clinical Evaluation, Yijishan Hospital, Wannan Medical College, Wuhu 241001, Anhui, China
[c] Center for Molecular Metabolism, School of Environmental and Biological Engineering, Nanjing University of Science and Technology, Nanjing 210094, China

ARTICLE INFO

Article history:
Received 4 March 2014
Received in revised form 21 June 2014
Accepted 23 June 2014
Available online 2 July 2014

Keywords:
Gastrodin
Metabolites
UFLC-ESI–MS/MS
Multiple-dose
Food
Pharmacokinetics

ABSTRACT

A reliable and highly sensitive ultra performance liquid chromatography electrospray ionization tandem mass spectrometry (UFLC-ESI–MS/MS) analytical method was developed for identification and quantification of gastrodin (GAS) and its metabolites in rat plasma. Five metabolites were identified: *p*-formylphenyl-β-D-glucopyranoside (M1), *p*-hydroxybenzonic acid (M2), *p*-hydroxybenzyl alcohol (M3), *p*-formaldehydephenyl-β-D-glucopyranoside (M4), *p*-hydroxybenzaldehyde (M5). The molecular structures of metabolites were proposed based on the characters of their precursor ions, product ions and chromatographic retention time. Four of them were reported firstly in rat plasma. This method involved the addition of bergeninum as the internal standard (IS), UFLC separation, and quantification by MS/MS system using negative electrospray ionization in the multiple reaction monitoring (MRM) mode. The lower limit of quantification of gastrodin and five metabolites were all 1 ng/mL. The method was linear in the concentration range of 0.001–10 μg/mL. The intra- and inter-day precisions (R.S.D %) were within 15.0% for all analytes. No interference was noted due to endogenous substances. All analytes were stable in rat plasma stored at room temperature and 4 °C for at least 4 h, −20 °C combined with three freeze–thaw cycles for at least 1 month. By this method, the influence of multiple-dose and food on the pharmacokinetics behaviors of GAS and its metabolites were studied for the first time. We hope pharmacokinetic data of present study may inspire rational clinical usage of GAS.

1. Introduction

Gastrodia elata (GE) Blume (Chinese name "Tianma") is one of the most important traditional plants, which has been used as both herbal medicine and food in East Asia (such as China, Japan, and Taiwan) since ancient times. GAS, the main bioactive ingredient of GE [1–5], has excellent effects in clinical treatment of central nervous system (CNS) disorders such as vertigo, headache, insomnia, neuralgia, neurasthenia and epilepsy [6–8]. Long-term oral administration and multiple-doses are the common methods when GAS used clinically. However, there are few researches on the metabolism and pharmacokinetic (PK) behaviors of GAS designed according to its accustomed application in clinical practice. Furthermore, one of the problems which any long-term dosage cannot evade is food influence to the PK parameters of pharmacologic active substances of that drug.

Up to now, as for how GAS played its pharmacological effects, some interesting results were reported [9–11]. *P*-Hydroxybenzyl alcohol was considered the main pharmacological effect matter after GAS was taken, which can pass through blood brain barrier. However, previous studies and our obtained data all found the concentrations of *p*-hydroxybenzyl alcohol in plasma and tissues were very low and declined rapidly [12,13]. Taking GAS molecular structure into consideration, we assume that *p*-hydroxybenzyl alcohol is not the only metabolite, which can contribute to pharmacological action of GAS.

The aim of this study was to identify novel metabolites other than *p*-hydroxybenzyl alcohol, the one we already knew, as well as to investigate influence of multiple-dose and food on in vivo disposal of GAS and its metabolites when given through

* Corresponding author. Tel.: +86 025 83271405; fax: +86 025 83271405.
E-mail addresses: cpu_lykong@126.com, lykong@cpu.edu.cn (L. Kong).

http://dx.doi.org/10.1016/j.chroma.2014.06.080
0021-9673/

gastrointestinal tract using simultaneous quantification UFLC-ESI–MS/MS method verified in this research.

2. Experimental

2.1. Chemicals and reagents

GAS (286 g/mol), *p*-hydroxybenzonic acid (M2, 138 g/mol), *p*-hydroxybenzyl alcohol (M3, 123 g/mol) and *p*-hydroxybenzaldehyde (M5, 122 g/mol) were kindly provided by Kun Ming Baker Norton Co., Ltd. Bergeninum (Batch No. 1532-200202) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purity of all chemicals was above 99.9% and their molecular structures were shown in Fig. 1. HPLC grade acetonitrile was obtained from Fisher Scientific (Toronto, Canada). Deionized water was prepared using a Milli-Q purification system (Millipore, Bedford, MA, USA). High-purity nitrogen (99.999%) was purchased from Gas Supplier Center of Nanjing University (Nanjing, China). All solvents and other reagents used were of analytical grade.

The stock solutions (400 μg/mL) of four compounds were prepared in deionized water. These stocks were further diluted to sub-stock solutions with deionized water. Working solutions (5 μL) were diluted with drug-free plasma (45 μL) and mixed by vortex mixer (Scientific Industries, Inc., USA) for 1 min to span calibration standard ranges of 0.001–10 μg/mL. The standard 2.5 μg/mL stock solution of the internal standard (IS) was prepared using acetonitrile. Quality control (QC) samples (0.001, 1, 10 μg/mL) were

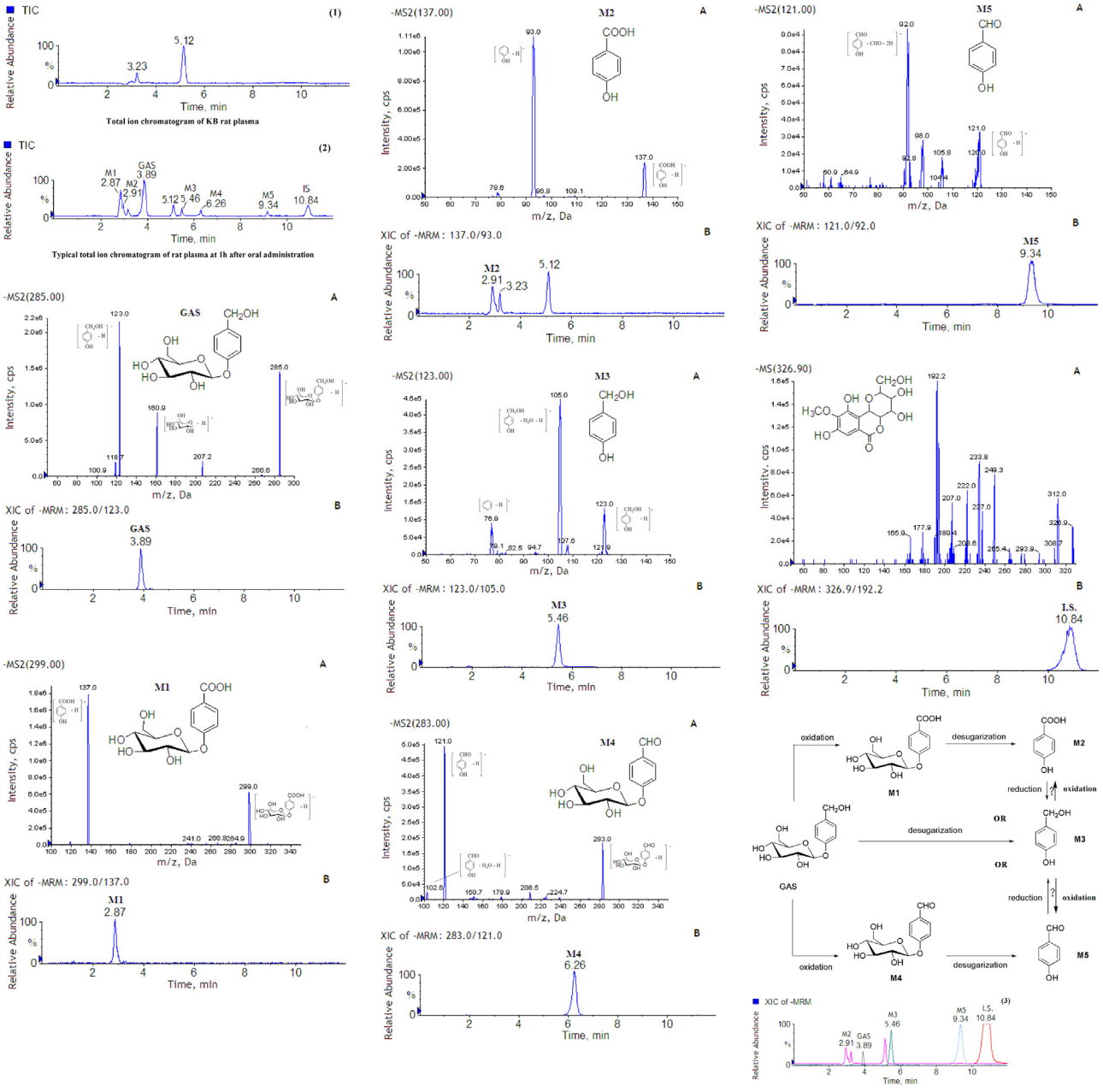

Fig. 1. Total ion chromatogram, extracted ion chromatograms and MS^2 product ions spectra in rat plasma after GAS intragastric administration 100 mg/kg: (1) total ion chromatogram of KB rat plasma; (2) typical total ion chromatogram of rat plasma at 1 h after oral administration; (3–9) MS2 product ions spectra and extracted ion chromatograms of GAS, M1–M5 and IS from rat plasma sample; (10) proposed metabolite pathway of GAS in rats. (11) LLOQ chromatograms of GAS and M2, M3, M5, IS (1 ng/mL, zoom in).