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Optimization of solid-phase extraction and liquid chromatography-tandem mass spectrometry for simultaneous determination of capilliposide B and its active metabolite in rat urine and feces: Overcoming nonspecific binding

Zhongzhe Cheng[a], Xing Zhou[b], Wenyi Li[b], Bingying Hu[b], Yang Zhang[c], Yong Xu[d], Lin Zhang[e,*], Hongliang Jiang[b,*]

[a] School of Pharmacy, Weifang Medical University, 7166 Baotong West Street, Weifang, Shandong 261053, China
[b] Tongji School of Pharmacy, Huazhong University of Science and Technology, 13 Hangkong Road, Wuhan, Hubei 430030, China
[c] Department of Pharmacy, Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Road, Wuhan, Hubei 430030, China
[d] Medical Research Center, Humanwell Healthcare (Group) Co., Ltd, 666 Gao xin Road, Wuhan, Hubei 430075, China
[e] Department of Biomedical Engineering, Zhejiang University, Hangzhou 310027, Zhejiang, China

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ABSTRACT

Capilliposide B, a novel oleanane triterpenoid saponin isolated from *Lysimachia capillipes* Hemsl, showed significant anti-tumor activities in recent studies. To characterize the excretion of Capilliposide B, a reliable liquid chromatography-tandem mass spectrometry (LC–MS/MS) method was developed and validated for simultaneous determination of Capilliposide B and its active metabolite, Capilliposide A in rat urine and feces. Sample preparation using a solid-phase extraction procedure was optimized by acidification of samples at various degrees, providing extensive sample clean-up with a high extraction recovery. In addition, rat urinary samples were pretreated with CHAPS, an anti-adsorptive agent, for overcoming nonspecific analytes adsorption during sample storage and process. The method validation was conducted over the curve range of 10.0–5000 ng/ml for both analytes. The intra- and inter-day precision and accuracy of the QC samples showed ≤11.0% RSD and −10.4–12.8% relative error. The method was successfully applied to an excretion study of Capilliposide B following intravenous administration.

1. Introduction

Lysimachia capillipes Hemsl (Primulaceae), a folk medicinal plant which grows in southeastern China, is used as a remedy for the treatment of colds and arthritis and as a spice in China. Capilliposide B (LC-B) is a novel oleanane triterpenoid saponin isolated from *Lysimachia capillipes* Hemsl, and exhibits significant cytotoxicities against human A-2780 cell lines in vitro [1]. In addition, after oral administration of total saponins of *L. capillipes* Hemsl extract with LC-B as the major bioactive component, the proliferation of gastric cancer BGC-823 cells, prostate cancer PC3 cells, ovarian cancer SK-OV-3 cells, lung cancer A549, H1299 and H460 cells in nude mice can be effectively inhibited in a dose-dependent manner without overt toxicity [2,3]. The pharmacokinetic behavior of LC-B in rat plasma was investigated in our previous study [4]. During this study, Capilliposide A (LC-A), an esterolytic product of LC-B, was characterized in rat plasma by LC-QTRAP-MS/MS. LC-A was obtained and its chemical structure was identified by ^{1}H NMR and ^{13}C NMR in a subsequent study [5]. Furthermore, the cytotoxicity of LC-A was observed in HepG2 cell lines [5]. The evaluation of urinary and fecal excretion plays an important role in understanding a compound's route of metabolism. To date, however, there is little information about the disposition of LC-B and its major metabolite, LC-A, in vivo. Therefore, the excretion study of LC-B and LC-A becomes necessary for further development of LC-B as a therapeutic agent.

The initial published analytical method for quantification of LC-B in the raw materials of *L. capillipes* Hemsl employed HPLC separation and evaporative light scattering detection (ELSD) [6]. Later, the bioanalysis of LC-B in rat plasma by LC–MS/MS using a simple protein precipitation approach was well established [4]. However,

* Corresponding authors.
E-mail addresses: zhanglin@zju.edu.cn (L. Zhang), jianghongliang@hust.edu.cn (H. Jiang).

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the urine and feces samples are very different from plasma samples since various elements including water, electrolytes, endogenous molecules even or food residue are present, which can cause severe mass spectrometric contamination or matrix effect if these endogenous and exogenous components are not removed prior to LC injection. Solid-phase extraction (SPE) can be suitable for sample desalting and clean-up of complex matrix samples in LC–MS/MS analysis [7]. In addition, urine assays are usually more challenging than plasma assays owing to potential nonspecific binding issues of analytes and individual variability of urine pH, etc [8]. In this study, great efforts in minimizing the nonspecific binding of analytes to urine collection containers by adding an appropriate anti-adsorptive agent and in acidifying urine samples at its optimal concentration were enforced.

The main purpose of the present study was to develop and validate a sensitive, specific, and reliable bioanalytical method for simultaneous determination of LC-B and its metabolite, LC-A in urinary and fecal samples. The present study emphasized on resolution of the analyte nonspecific binding issue and optimization of the sample pretreatment and preparation procedures. The proposed method was successfully applied to evaluate urinary and fecal excretion of LC-B following intravenous administration.

2. Experimental

2.1. Chemical, reagents and materials

LC-B was in-house isolated with a purity of > 98% as measured using a HPLC-ELSD method [1,4]. LC-A was obtained by hydrolysis of Capilliposide C (LC-C), an analogue of LC-B [5]. The chemical structure of LC-A was identified by ^{1}H NMR and ^{13}C NMR (Avance-400 NMR spectrometer, Bruker, Switzerland), respectively. The purity of LC-A was above 98% determined by a HPLC-ELSD approach [5]. The internal standard (IS) dioscin, with a purity of >98%, was purchased from National Pharmaceutical Engineering Center for Solid Preparation in Chinese Herbal Medicine (Nanchang, China). Their chemical structures were presented in Fig. 1 HPLC grade methanol was obtained from Fisher Scientific (NJ, USA). 3-[3-cholamidopropyl)-dimethylammonio]- 1-propane sulfonate (CHAPS) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic acid (≥96%) was obtained from Sinopharm Chemistry Reagent Co., Ltd (Shanghai, China). A Milli-Q water purification system (Millipore, Milford, MA, USA) was used in the laboratory to produce deionized water.

2.2. Chromatographic and mass spectrometric conditions

The LC–MS/MS method used is similar to that reported in our previous studies with some modifications [4]. The HPLC system consisted of a solvent delivery system LC-20ADXR, an autosampler SIL-20AC, a column oven CTO-20AC, a degasser DGU-20A3, and a controller CBM-20A from Shimadzu (Kyoto, Japan). An analytical Aquasil C18 column (50 × 2.1 mm, 5 μm) from Thermo Electron (Bellefont, PA, USA) was employed. The mobile phase was composed of 0.1% formic acid in water as mobile phase A (MA) and methanol as mobile phase B (MB) using a gradient elution of 20% of MB (0–0.2 min), from 20 to 60% of MB (0.2–0.3 min), from 60 to 95% of MB (0.3–2.6 min), 95% of MB (2.6–3.1 min), from 95 to 20% of MB (3.1–3.2 min), and 20% of MB (3.2–4.0 min). Separation was carried out at a flow rate of 0.8 ml/min with a column oven temperature at 40 °C. The temperature of the autosampler was kept at 4 °C.

An 4500 QTRAP® mass spectrometer (AB SCIEX, Concord, Canada) equipped with a Turboionspray® source (TIS) was operated in the negative ion mode with multiple reaction monitoring (MRM) for LC-QTRAP-MS/MS analysis. The MS parameters were optimized as follows: TIS temperature, 600 °C; ionspray voltage, −4500 V; curtain gas, nitrogen, 30; nebulizing gas, 50; TIS gas, 50; declustering potential, −216 V for LC-B, −243 V for LC-A and −215 V for IS; entrance potential, −10 V; collision energy (CE), −67 eV for LC-B, −66 eV for LC-A and −44 eV for IS; collision cell exit potential, −15 V. The following MRM transitions were used: *m/z* 1175.6 → 1043.7 for LC-B, 1077.7 → 927.3for LC-A, and *m/z* 867.5 → 721.6 for IS, respectively.

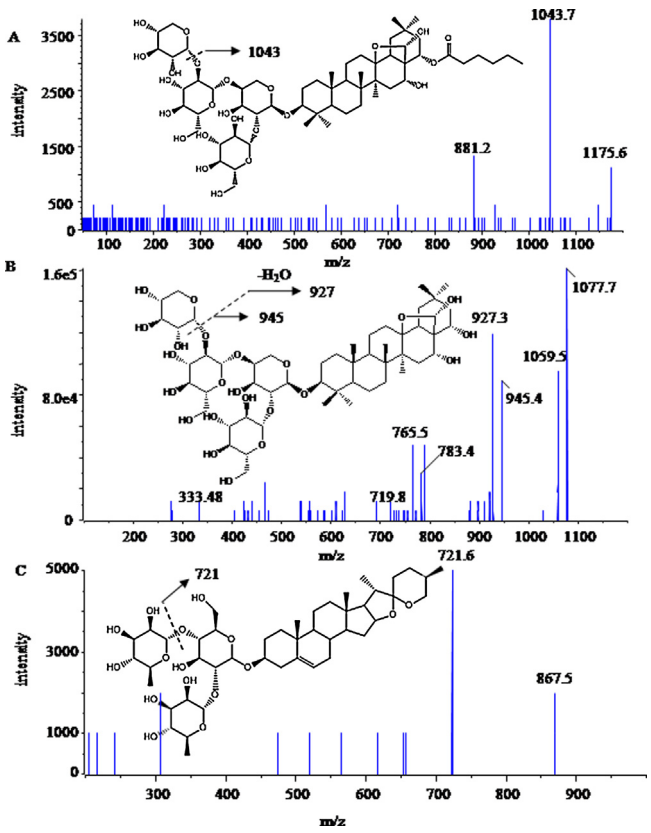

Fig. 1. Chemical structures and product ion mass spectra of (A) capilliposideB, (B) capilliposideA, and (C) IS.

2.3. Preparation of stock and working solutions, calibration standards and QC samples

Stock solutions of LC-B, LC-A and IS were prepared in methanol: water (50:50, v/v) at 1.25, 1.25 and 0.500 mg/ml, respectively. Working solutions of analytes were prepared by serial dilution of the stock solutions with methanol: water (50:50, v/v). Blank urine was acidified with a certain volume of formic acid to yield 1% formic acid in sample. CHAPS, an anti-adsorptive agent, was applied to overcome nonspecific adsorption of analytes in urine assays. 300 mM CHAPS in water was added to acidified blank urine at a ratio of 1/100. Blank feces were homogenized by acidic water containing 1% formic acid at a ratio of 1/20 (g/ml). Pretreated blank urine and feces homogenate were stored at −80 °C. Calibration standards containing LC-B and LC-A were both prepared at 10.0, 20.0, 50.0, 250, 500, 2500, 4000 and 5000 ng/ml, respectively. Quality control (QC) samples were prepared at 10.0, 30.0, 300 and 3750 ng/ml for lower limit of quantification (LLOQ), low QC (LQC), medium QC (MQC), and high QC (HQC), respectively. QC samples were stored at −80 °C, for stability evaluation. All calibration standards and QC samples were prepared by spiking an appropriate amount of working solutions into blank urine or feces homogenates to reach the