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Study of the determination and pharmacokinetics of bufadienolides in dog's plasma after administration of Liu-Shen-Wan by high performance liquid chromatography time-of-flight mass spectrometry

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Abstract

A sensitive and reliable high performance liquid chromatography-tandem time-of-flight mass spectrometry method (HPLC/TOF MS) has been developed to determine three active bufadienolides from Liu-Shen-Wan (LSW) in dog's plasma. Enhanced selectivity and sensitivity in comparison with traditional HPLC/DAD method could be obtained through this method. Bufodienolides could be well separated and distinguished from its nominally isobaric endogenous components by HPLC/TOF MS, with the linear calibration range covering from 0.5 ng/mL to 100 ng/mL and Limit of Detection (LOD) being about 0.15 ng/mL. This method was also proved to be quite stable, with the intra-day precision and the inter-day precision results being lower than 6.39% and 7.44%, respectively. Meanwhile HPLC/TOF MS was successfully used in the pharmacokinetic study of LSW. For resibufogenin, the major pharmacokinetic parameters AUC_{0-t}, C_{max} and $t_{1/2\alpha}$ were 160.72 ± 21.97 ng/mL min, 2.35 ± 0.71 ng/mL and 20.74 ± 5.89 min, respectively, and for bufalin the corresponding parameters were 55.55 ± 7.55 ng/mL min, 0.91 ± 0.15 ng/mL and 25.45 ± 13.28 min, respectively.

Keywords: Liu-Shen-Wan; Pharmacokinetics; Bufadienolides; HPLC/TOF MS

1. Introduction

Liu-Shen-Wan (LSW), a well-known heatclearing and detoxicating formula, is mainly composed of animal and mineral derived medicines including Musk (*Moschus berezovskii* Flerov), bezoar (*Bos taurus domesticus* Gmelin), toad venom (*Bufo bufo gargarizans* Cantor), borneol (*Dryobalanops aromatica* Gaertner. f.), pearl (*Pteria martensii* Dunker) and realgar (*Realgar*). This ancient prescription was confirmed to be effective in treating infectious inflammatory diseases such as diphtheria, scarlet fever, acute tonsillitis, purulent parotitis, encephalitis B, viral pneumonia and throat pain for more than 200 years [1–3].

Bufadienolides (Fig. 1) are the major effective constituents in Toad venom [4,5]. Bufadienolide is a type of steroid with a characteristic α-pyrone ring at C-17, and show significant cardiotonic, blood-pressure-stimulating, anesthetic, and antitumor activities [6,7].

The detection and accurate measurement of bufadienolides in body fluids are in especially urgent need for therapeutic drug monitoring of LSW. Recent literature revealed that several methods have been used to determine bufadienolides in toad venom [8–10], in traditional Chinese medicines formula [11–13] and in human liver [14]. In all of these reports, reversed-phase HPLC/UV was used as the quantitative determination technique. However, HPLC/UV is not sufficiently sensitive to determine bufadienolides in biological matrix for pharmacokinetics study. Recently, high performance liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometry tandem mass spectrometry technique was applied to the study of Chansu (toad venom), but it was just used as a identification method [9,10].

To our knowledge, no method has been reported for the simultaneous quantitative determination of these active constituents by using HPLC/MS for pharmacokinetic investigation. Being a useful tool for analysing compounds in complex matrices,

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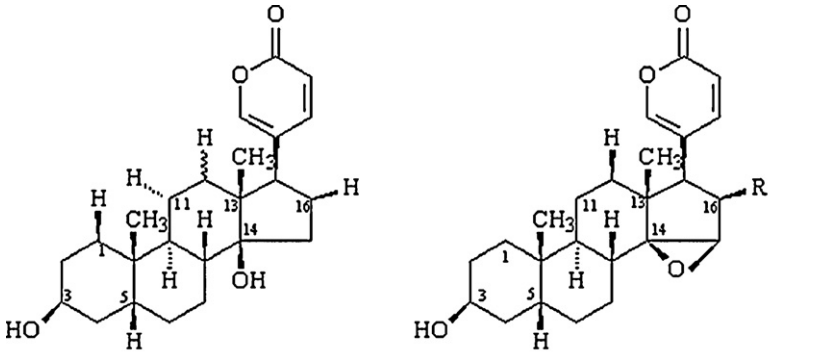

Fig. 1. Structures of three bufadienolides.

high performance liquid chromatography coupling with time-of-flight mass spectrometry (HPLC/TOF MS) has advantages over HPLC/UV, for it has high resoluteion and narrow extracted-ion windows (±0.02 *m/z*), which can then lead to reduced background, lower detection limit, and higher confidence. Further more, TOF MS has highly linear, wide dynamic range, and lock-mass correction. By extracting Nominal Mass Chromatograms from the complex biological matrix, bufodienolides could be detected and distinguished from the nominally isobaric endogenous components. All these advantages make it suitable for quantitative analysis of dog plasma after administration of LSW.

In this paper, a HPLC/TOF MS method in combination with solid phase extraction (SPE), for simultaneous quantitative determination of the three major bufadienolides in dog's plasma after administration of LSW is described.

2. Experimental

2.1. Chemicals and reagents

LSW (3.125 mg/pill, Lot No. 20050110) was supplied by Shanghai Lei Yun Shang Pharmaceutical Company (Shanghai, China). Reference standard Bufalin and resibufogenin were purchased from DELTA Information Centre For Natural Organic Compounds (Xi-an, China, 99% purity), and cinobufagin was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Lot.803-9202 Beijing, China, 99% purity). 5α-dihydrotestosterone, which was used as the internal standard (I.S.), were obtained from Sigma Co. (St. Louis, MO 63178 USA, 98% purity). Solid-phase extraction columns (Oasis® HLB, 1 cc/30 mg 30 μm) were obtained from Waters (Milford, MA, USA). Acetonitrile and methanol were of HPLC grade and purchased from Merck (Darmstadt, Germany) and formic acid of HPLC grade was obtained from Fluka (Buchs, Switzerland). Ultra-pure water was prepared from a Milli-Q system (Millipore, Milford, MA). All other reagents and solvents were of analytical grade and obtained from Beijing Chemical Company (Beijing, China).

2.2. HPLC condition

HPLC/DAD analysis was carried out on an Agilent 1100 series HPLC system (Agilent Series 1100, Palo Alto, CA, USA) comprising a binary pump, thermostated column compartment, a 1200 series injector with temperature controller, and a diode array detector. Separation was performed using a reversed-phase column (250 mm × 4.6 mm i.d., 5.0 μm, Alltima C18) with an Alltech RP18guard column (3.9 mm × 20 mm) at 30 °C. For qualitative investigations a binary gradient mobile phase was used, comprising 0.2% formic acid (solvent A) and acetonitrile (solvent B). Initially the proportions were 70% A and 30% B and then altered in a linear gradient to 56% A and 44% B over the period of 0–8 min at flow rate of 0.9 mL/min, followed by a linear gradient to 30% A and 70% B at the same flow rate in the subsequent 17 min. This gradient elution allowed the efficient separation both of the analytes and interfering components present in the plasma. After the 25 min analysis process, the column was washed with 95% acetonitrile for 5 min and then equilibrated with starting mobile phase (70% A and 30% B) for 10 min prior to the next run. One-third of the eluent was introduced into the TOF-MS system with a split valve. An injection volume of 20 μL was used throughout, and the injector was maintained at 4 °C during the analysis.

2.3. TOF-MS conditions

The HPLC system was coupled to an Agilent 1100 HPLC/MSD TOF (Agilent Corp, Waldbronn, Germany) equipped with an electrospray interface. The electrospray source includes dual nebulizers, one nebulizer for the HPLC eluent and the other for the internal reference solution. The reference nebulizer, along with the HPLC/MSD TOF's automated celebrant delivery system (CDS), provides continuous introduction of reference mass standards into the ion source for automated mass calibration. Accurate mass measurements were obtained with this CDS and thus enhanced accuracy was achieved.

The HPLC conditions for the HPLC/TOF-MS analysis were the same as those used in the HPLC analysis. TOF-MS analysis was performed in positive (ESI+) ion mode under the following operation parameters: capillary voltage 4.0 kV; nitrogen drying gas 9 L/min; nebulizer 40 psi; gas temp. 350 °C; fragmentor voltage 215 V (ESI+); skimmer voltage 60 V; octopole dc1 30 V (ESI+); octopole RF 250 V. Reference masses: 149.0233 and 922.0098 *m/z*. Data files were acquired in continuum (profile) mode, and spectras were stored from *m/z* 50–1000.