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Quantitative determination and pharmacokinetic study of solamargine in rat plasma by liquid chromatography–mass spectrometry

Xiao Zheng[a], Lianming Xu[b], Yan Liang[a], Wei Xiao[b,*], Lin Xie[a], Yan Zhang[b], Li Zhao[b], Liang Cao[b], Jun Chen[b], Guangji Wang[a,**]

[a] Key Lab of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, No. 24 Tongjiaxiang Street, Nanjing 210009, China
[b] Jiangsu Kanion Pharmaceutical Co., Ltd., 58 Haichang South Road, Xinpu District, Lianyungang 222001, China

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ABSTRACT

A sensitive and simple liquid chromatography–mass spectrometry (LC–MS) method has been developed and validated for the quantification of solamargine, a steroidal glycoalkaloid, in rat plasma. Vincristine was selected as the internal standard. Sample preparation involved simple liquid–liquid extraction by ethyl acetate with high efficiency. The chromatographical separation was performed on a Shimadzu C_{18} column (150 mm × 2.0 mm, 5 μm) with a gradient elution of acetonitrile and 0.02% (v/v) formic acid. The elutes were detected under positive electrospray ionization (ESI) and the target analytes quantified by selected ion monitoring (SIM) mode. The method was sensitive with the lowest limit of quantitation (LLOQ) at 0.5 ng/mL in 50 μL of rat plasma. Good linearity (r^2 = 0.9996) was obtained covering the concentration of 0.5–2000.0 ng/mL. The intra- and inter-day assay precision ranged from 2.87 to 3.60% and 0.52 to 6.81%, respectively. In addition, the stability, extraction recovery and matrix effect involved in the method were also validated. The practical utility of the aforementioned method was successfully confirmed in the pharmacokinetic evaluation of solamargine in Sprague-Dawley rats after intravenous administration.

1. Introduction

Steroidal glycoalkaloids represent a class of naturally occurring compounds possessing a variety of biological activities. Solamargine (Fig. 1), a steroid alkaloid glycoside existing in at least 100 *Solanum* species [1], was originally found to be toxic to herbivores and microbial pathogens [2]. Following pharmacological studies revealed that solamargine could exert inhibitory effects on tumor cells including human hepatoma cells [3], colon cancer cells [4] and lung cancer cells [5]. Importantly, combined anti-tumor therapy with solamargine could increase the susceptibility of lung cancer cells [6] and breast cancer cells [7] to chemotherapeutics and the synergistic action highlighted the unique anti-tumor efficacy of solamargine. Detailed study of the underlying molecular mechanisms has received widespread attention and yielded important discoveries [7–12]. In fact, Coramsine, a chemotherapeutic and immunomodulating agent with a 1:1 mixture of solasonine and solamargine, was demonstrated to exhibit strong anti-neoplastic activity in specific cancer cell lines, animals and humans, and its pre-clinical studies have been successfully completed in 2008 [http://en.wikipedia.org/wiki/Coramsine]. Taken together, these evidences strongly indicate that solamargine could become a promising candidate in the development pipeline of anti-tumor drugs.

For the further development of solamargine and better elucidation of its pharmacological efficacy, reliable analytical methodologies of solamargine in biological samples are warranted for characterizing its disposition and pharmacokinetic behaviors, thus providing essential information for drug-like assessment in the early stage of drug development. Currently, however, in contrast to the extensive pharmacological studies available, the quantitative method of solamargine is largely lacking. Due to the absence of a typical UV chromophore, detection of solamargine by a previously reported high-pressure liquid chromatography with UV detection (HPLC-UV) was compromised by poor sensitivity and repeatability [1]. A recently reported quantitative method for solamargine in plant, based on post chromatographic derivatization after separation through high-performance thin-layer chromatography (HPTLC), was feasible for the quality assessment of medicinal plants [13]. However, in light of the requirement for sensitive and accurate determination of solamargine in biosamples, this method could be compromised by its poor sensitivity. Liquid

* Corresponding author. Tel.: +86 25 86587928; fax: +86 25 86587926.
** Corresponding author. Tel.: +86 25 83271128; fax: +86 25 85306750.
E-mail addresses: xiaowkanion@hotmail.com (W. Xiao), guangjiwang@hotmail.com (G. Wang).

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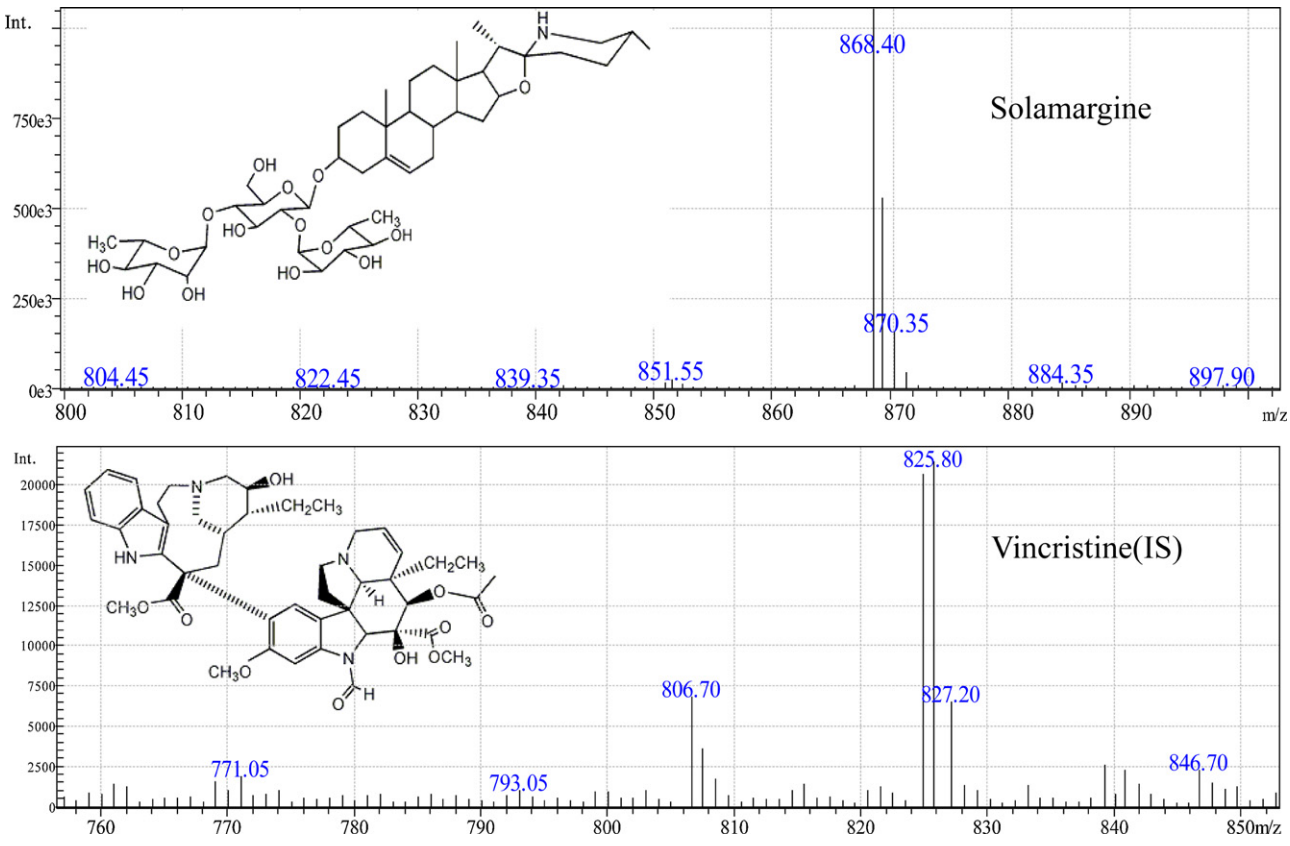

Fig. 1. Chemical structures and mass scan spectra for solamargine and vincristine (IS).

chromatography–mass spectrometry (LC–MS) proves a feasible alternative attributed to its good separation and detection capacity. Moreover, LC–MS based techniques have been extensively applied in the bioanalysis and pharmacokinetic studies of numerous drugs [14–16]. Notably, a LC–MS based method has been developed for the quantitative study of solamargine in the extract from a herbal medicine [17]. Nevertheless, it is conceivable that the sensitivity was far from the requirement of quantitative assay by biological samples. According to our knowledge, no LC–MS procedure for quantitative measurement of solamargine in biological samples has been reported in the literature at present. Herein, based on LC–MS, we aimed to develop a validated quantitative method for solamargine in rat plasma after a single-step liquid–liquid extraction (LLE) with ethyl acetate, and provided the first report on comprehensive pharmacokinetic parameters of solamargine in rats following intravenous administration. In the context of the extensive mechanistic research underway, this information holds promise for better elucidation and manipulation of the pharmacodynamic effects of solamargine in future studies.

2. Materials and methods

2.1. Chemicals and materials

Solamargine (purity > 95%) was supplied by Jiangsu Kanion Pharmaceutical Co., Ltd. (Jiangsu, China) and vincristine (internal standard) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC grade acetonitrile and methanol were purchased from Merck (Merck Company, Germany). Other chemicals were all of analytical grade. Ultrapure water was generated from the Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Instrumentation and chromatographic conditions

Liquid chromatography was performed on a Shimadzu LC-10AD HPLC system (Shimadzu, Kyoto, Japan) consisting of a LC-10AD pump, a DGU-14AM degasser, a SIL-HTc autosampler and a CTO-10 Avp column oven. The HPLC system was coupled to a Shimadzu LCMS-2010A quadrupole mass spectrometer by an electrospray ionization (ESI) interface. Data acquisition and processing were performed by Shimadzu LCMS Solution software (Version 2.04).

Chromatographic separation was carried out on a Shim-Pack VP-ODS C_{18} column (150 mm × 2.0 mm, 5 μm) with a gradient elution of the mobile phase system consisting of acetonitrile (A) and 0.02% formic acid (B). The elution progressed following the time program: 0.02–4.5 min, B% 15–70; 4.5–5.0 min, B% 70–15; 5.0–8.0 min, B% 15–15. The temperature of the column and auto-sampler was kept constant at 40 °C and 10 °C, respectively.

The tuning parameters for the ESI were set as follows: curved desolvation line (CDL) voltage 25.0 kV, probe voltage 4.5 kV, Q array voltage 60 V, RF 150 V, CDL temperature 250 °C, block temperature 200 °C. Spray gas and drying gas flow rate were set at 1.5 and 2.5 L/min, respectively. The $[M+H]^+$ ions of solamargine (m/z 868.4) and vincristine (m/z 825.8) were selected as ions for selected ion monitoring (SIM) as best sensitivity was found under the positive scanning mode (Fig. 1).

2.3. Preparation of stock and standard solutions

The stock solutions of solamargine and vincristine (IS) were separately prepared in methanol at concentrations of 1.0 mg/mL and 500.0 μg/mL. Immediately before use, serial dilution of the stock solution with methanol provided solamargine working solutions covering the concentration from