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# Application of a liquid chromatography–tandem mass spectrometry method to the pharmacokinetics, tissue distribution and excretion studies of sweroside in rats

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ABSTRACT

A sensitive, reliable and accurate high-performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS) was developed and validated for the quantification of sweroside in rat plasma, tissue and excretion. A single-step protein precipitation by methanol was used to prepare samples. Sweroside and swertiamarin (internal standard, IS) were separated by using a $C_{18}$ column and a mobile phase consisted of methanol and water containing 0.1% formic acid running at a flow rate of 0.8 ml/min for 6 min. Detection and quantification were performed using a mass spectrometer by the multiple-reaction monitoring (MRM) in positive electrospray ionization mode. The optimized mass transition ion pairs ($m/z$) for quantitation were $[M+H]^+ 359.1 \rightarrow 197.2$ for sweroside and $[M+Na]^+ 397.4 \rightarrow 165.3$ for swertiamarin (IS), respectively. The inter-day precision (RSD %) was less than 11.20% and intra-day precision (RSD %) was less than 10.90%, while the inter-day accuracy (RE %) was ranged from −9.69 to 9.17% and intra-day accuracy (RE %) was ranged from −10.56 to 13.47%. The mean elimination half-life ($t_{1/2}$) of sweroside for 5, 10 and 15 mg/kg dose were 78.8, 67.6 and 77.2 min, respectively. And sweroside follows linear plasma pharmacokinetics across the investigated dosage range in rats (5–15 mg/kg). The absolute bioavailability ($F$ %) of sweroside was 11.90% on average. The results of tissue distribution showed the higher sweroside concentrations were found in kidney, liver, spleen and lung, and the small amount of drug was distributed into the brain tissue. The high distribution in liver confirms the reports that sweroside has hepatoprotective activity and promoted liver regeneration, and there was no long-term accumulation of sweroside in rat tissues. Total recoveries of sweroside within 48 h were 0.67% in bile, 1.55% in urine and 0.46% in feces, which might be resulted from liver first-pass effect. The above results suggested that sweroside was mainly excreted as the metabolites.



## 1. Introduction

Iridoid and secoiridoid glycosides exist widely in plants of many families [1,2] and showed a wide variety of biological activities such as hypotensive, sedative, antipyretic and antitussive activities [3–6]. Swertia herbs belonging to Gentianaceae have been used to treat digestive dysfunction, acute and chronic dysentery, and hepatitis in China. Numerous pharmacological effects have been attributed to these plant species e.g. stomachic, cholagogue, hepatoprotective and antipyretic effects [7,8]. Sweroside ($C_{16}H_{22}O_9$, molecular weight 358.344), an iridoid glycoside, belongs to a large group of natural monoterpenoids with a glucose moiety attached to C-1 in the pyrane ring. It widely exists in plants of Swertia herbs and shows a wide variety of pharmacological and biological activities. Modern pharmacology shows that sweroside performs many pharmacological and biological activities including hepatoprotective effect, antiproliferation, vasorelaxation, antihepatitis, anti-inflammatory, antiaggregatory, and antiallergic effects [9–14].

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Additional, sweroside has displayed bright prospects in prevention and therapy of osteoporosis [15].

It is well-known that a good understanding of pharmacokinetic properties in proper models is crucial to any drug candidate [16]. Moreover, proper knowledge on the distribution and excretion is vital to investigate the major target sites and interpret the *in vivo* disposition of a drug candidate [17]. Considering the growing beneficial role of sweroside in human health, the detailed *in vivo* pharmacokinetic, disposition and excretion study of sweroside by proper administration route, such as oral or intravenous administration, are necessarily required.

Over the years, a variety of high-performance liquid chromatography (HPLC) methods have been reported for the quantitative determination of sweroside in pharmaceutical formulations [18], However, these methods often have disadvantages, such as poor sensitivity or low specificity, which is not suitable for the analysis of sweroside in biological samples. Some assays with HPLC–UV [19,20] and HPLC–MS [21] methods have been described for the determination of sweroside in plasma samples. These HPLC–UV methods, however, have generally shown low sensitivity, providing LOQ with ~0.05 μg/mL. Nowadays, HPLC–MS/MS has gained importance for the quantitative estimation of drugs in various biological matrices, due to its high sensitivity, selectivity and reproducibility. Suryawanshi et al. [21] quantified the sweroside in rat plasma using a liquid chromatography–tandem mass spectrometry (LC–MS/MS) method, but this study mainly focus on the quantitation of mangiferin and four secoiridoid glycosides. So far, no HPLC–MS method has been published for the quantitation of sweroside in plasma, tissue, bile, urine and feces after a single oral or intravenous administration. Therefore, a high-performance liquid chromatography–tandem mass spectrometry method with electrospray ionization (HPLC–ESI-MS/MS) method has been developed and successfully applied to the quantification of sweroside in rat biological samples such as plasma, tissue, bile, urine and feces samples. The present study provides a full investigation of the pharmacokinetic profiles, tissue distribution and excretion patterns of sweroside in healthy Sprague–Dawley rats following a single oral (at three dosages) or intravenous administration of sweroside, respectively. To the best of our knowledge, it is the first time to report the pharmacokinetic, tissue distribution and excretion profiles of sweroside. The chemical structures of sweroside and the internal standard (IS) swertiamarin are shown in Fig. 1.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Sweroside (no. PN1125SA13) and swertimarin (internal standard, IS, no. 20120129) were obtained from Shanghai Tauto Biotech Co., Ltd, China. The purities of the above ingredients were more than 98% according to LC analysis. Methanol and formic acid (HPLC-grade) were purchased from DIKMA Company (USA). The chemical structures of sweroside and the IS swertimarin are shown in Fig. 1. Deionized water was freshly generated by Heal Force-PWVF Reagent Water System (Shanghai CanRex Analyses Instrument Corporation Limited, China). Heparin (Liquemine, 125,000 IU, The First Biochemical Pharmaceutical Co. Ltd., Shanghai, China) was used as an anticoagulant in all plasma samples.

### 2.2. Instrumentation and analytical conditions

#### 2.2.1. Instruments

The LC–MS/MS system consisted of an Agilent 1200 liquid chromatography system (USA) equipped with a quaternary solvent delivery system, an autosampler and a column compartment and a 3200 QTRAP™ system (Applied Biosystems, Foster City, CA, USA) with a hybrid triple quadrupole linear ion trap mass spectrometer equipped with Turbo V sources and TurboIonspray interface.

#### 2.2.2. Liquid chromatography

The chromatographic separation was performed on a Diamonsil $C_{18}$ column (150 mm × 4.6 mm, 5 μm; DIKMA Company, USA). The column temperature was kept at 30 °C and the injection volume was 20 μL. The mobile phase consisted of A (methanol) and B (0.1% formic acid, v/v) using gradient elution as follow: 0–4.5 min, linear change from A–B (45:55, v/v) to A–B (75:25, v/v); 4.5–4.6 min, linear change from A–B (75:25, v/v) to A–B (95:5, v/v); 4.6–6.0 min, isocratic elution A–B (95:5, v/v); and then quickly returned to initial A–B (45:55, v/v). This was followed by the equilibration period of 6 min prior to the injection of each sample. The liquid flow-rate was set at 0.8 mL/min. In order to reduce the contamination flow into the ion source, the eluent was discharged to waste for the first 2 min.

#### 2.2.3. Mass spectrometry

The electrospray ionization source was operated in the positive ion mode using the following conditions: the ion spray voltage was set to 5500 V, the turbo spray temperature was 650 °C, nebulizer gas (gas 1) and heater gas (gas 2) was set at 60 and 65 arbitrary units, respectively. The curtain gas was kept at 25 arbitrary units and the interface heater was on. Collision cell exit potential (CXP) and entrance potential (EP) was set at 5.0 V and 10.0 V, respectively. Nitrogen was used in all cases. The multiple-reaction monitoring (MRM) mode was employed for the determination. Quantification was performed using MRM of the transitions of $m/z$ 359.1 to $m/z$ 197.2 for sweroside and $m/z$ 397.4 to $m/z$ 165.3 for swertimarin (IS), respectively. Fig. 1 provides data on the precursor ion, the product ion, the corresponding declustering potential (DP) and collision energy (CE). The dwell time was set to 200 ms in the positive mode. The Biosystems/MDS Sciex Analyst software (version 1.5.2) was used for the data acquisition and processing.

### 2.3. Animals

All the studies on animals were in accordance with the Guidelines for Care and Use of Laboratory Animals. SPF Male Sprague–Dawley rats, weighing 250 ± 20 g, were supplied by Experimental Animal Research Center, Hebei Medical University, China. Rats were acclimated in the laboratory for 1 week prior to the experiments, housed in separate cages at a temperature of 23 ± 2 °C with a 12 h light/dark cycle and a relative humidity of 50%, free access to standard diet and water. All the rats were fasted for 12 h before the experiments with free access to water. This project all animal experiments were carried out according to guide lines for experimental animal management committee of Hebei Medical University, China.

### 2.4. Sample preparation and extraction

In the present study, a conventional step protein precipitation method was applied to extract sweroside and IS from biological samples (plasma, tissue homogenates, urine, feces or bile). A rat plasma sample (100 μL) was placed in a 1.5 ml Eppendorf tube and then precipitate agent with 400 μL methanol containing 20 μL internal standard solution was added. The mixtures were extracted by vortexing for 5 min, and then centrifuged 12,000 rmp for 10 min. 400 μL of the supernatant was transferred to a new 1.5 ml Eppendorf tube and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The residuals were dissolved in 50 μL of methanol