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Simultaneous quantitative determination of sanguinarine, chelerythrine, dihydrosanguinarine and dihydrochelerythrine in chicken by HPLC–MS/MS method and its applications to drug residue and pharmacokinetic study

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ABSTRACT

A specific and reliable HPLC–MS/MS method was developed and validated for simultaneously determination of sanguinarine, chelerythrine and their metabolites (dihydrosanguinarine and dihydrochelerythrine) in chicken tissue for the first time. This is important because these compounds are related to the use of a naturally occurring and novel feed additive with many benefits, but the levels of these compounds must be strictly controlled. The compounds were extracted by acetonitrile and 1% HCl–methanol solution successively and then separated on a C_{18} column. A triple-quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source was used for detection. Quantification was performed using multiple reaction monitoring with positive mode. The method was validated in terms of specificity, linearity, precision, accuracy and stability. The calibration curves were linear over the concentration range of 0.5–100.0 ng/g for sanguinarine, 0.5–100.0 ng/g for chelerythrine, 0.2–100.0 ng/g for dihydrosanguinarine and 0.1–100 ng/g for dihydrochelerythrine, respectively. All of the recovery rates of the four analytes were over 85%. The RSD of intra-day and inter-day precision was less than 5.0%, and the relative error was all within 12.0%. This validated method has been successfully applied to assess the drug residue and metabolite residue characteristics of sanguinarine and chelerythrine in chicken tissue after oral administration of the extracts of *Macleaya cordata (Willd.) R. Br*, and to investigate the pharmacokinetic parameters of sanguinarine and dihydrosanguinarine in chicken plasma.

1. Introduction

Sanguinarine (SG) and chelerythrine (CHE) are types of quaternary benzo[c]phenanthridine alkaloids (QBAs) which existed in the Papaveraceae plant such as *Chelidonium majus*, *Macleaya cordata (Willd.) R. Br.* and *Eomecon chionantha Hance* [1–3]. The two alkaloids of QBAs found in *M. cordata (Willd.) R. Br.* were most well studied. SG and CHE have broad spectrum of biological activities such as anti-microbial [4], anti-fungal [5], anti-inflammatory and pesticidal [6,7]. The extract containing SG and CHE has been used in animal breeding field as veterinary drugs and feed additives [8,9]. In the European Union, the *M. cordata* extract was first exploited as a natural feed additive in animal production [10,11], and is on the European Food Safety Authority list of plants [12]. In China, the extract of *M. cordata* containing SG and CHE was approved as the first traditional Chinese medicine of veterinary-drug product. It has been proved to possess antibacterial, anti-inflammatory, appetite inducing, and growth promoting functions [13].

In the animal body, QBAs can be reduced, leading to the formation of dihydro metabolites. The reduction products are probably the major metabolites of SG and CHE in mammals [14,15]. Alain Deroussent characterized the oxidative metabolites produced by human CYP1A1 and CYP1A2 and rat liver microsomes using liquid chromatography tandem mass spectrometry [16]. Psotová verified dihydrosanguinarine (DHSG) as the first metabolite of SG transformation in rat with a liquid chromatographic mass

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Table 1
HPLC–MS/MS parameters for quantitative analysis of SG, CHE, DHSG and DHCHE.

Analytes	Retention time (min)	Transitions (*m*/*z*)	Collision energy (eV)	Cone-voltage (V)
SG	3.5	332.1→273.9[a]	41	140
		332.1→316.8	29	
CHE	5.3	348.1→332.0[a]	29	150
		348.1→304.1	29	
DHSG	14.9	334.1→319.0[a]	21	135
		334.1→276.2	33	
DHCHE	13.8	350.1→335.0[a]	29	135
		350.1→319.1	45	

[a] Quantitative ion.

spectrometric method [14]. Wu reported the reductive metabolism of the SG bond by rat liver preparations [17]. Vacek investigated the biotransformation patterns of CHE and dihydrochelerythrine (DHCHE) in human hepatocytes [15]. Kosina et al. had identified the benzo[c]phenanthridine metabolites in human hepatocytes by liquid chromatography with electrospray ion-trap and quadrupole time-of-flight mass spectrometry[18].

Many HPLC or HPLC–MS/MS methods have been developed for the determination of SG and CHE in plant and plasma [19–22]. But the simultaneous quantitative determination of SG CHE and the reduced metabolites, such as DHSG and DHCHE in chicken as a target animal with HPLC–MS/MS method has not been reported. The aim of this study was to establish a sensitive and simple HPLC–MS/MS method to determine the concentration of SG, CHE, DHSG and DHCHE in chicken. In this study, a HPLC–MS/MS method was developed for simultaneous quantitative determination of SG, CHE and the metabolites of DHSG and DHCHE. The method was validated and successfully applied to the determination of drug residues and metabolites of SG and CHE in chick following long-term feeding of Sangrovit (*M. cordata* extracts) which containing SG and CHE.

2. Experiment

2.1. Chemicals and reagents

SG (99.5% purity) and CHE (99.5% purity) standard were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). DHSG (99.5% purity) and DHCHE (99.5% purity) were provided by Micolta Bioresource Inc. (Changsha, China). Sangrovit (extracts from *M. cordata (Willd.) R. Br.*, containing SG ≥ 1.5% and CHE ≥ 0.75%) was provided by Micolta Bioresource Inc. (Changsha, China). Formic acid (HPLC-grade), methanol (HPLC-grade) and acetonitrile (HPLC-grade) were purchased from Merk Darmstadt (Germany). Hydrochloric acid (analytical reagent) and *n*-hexane (analytical reagent) were purchased from China National Pharmaceutical Group (Shanghai, China). Ultrapure water was prepared by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

An Agilent 1290 ultra-performance liquid chromatography and an Agilent 6460 triple-quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA) were used for the determination of the analytes. An Agilent poroshell 120 EC C_{18} (2.1 mm × 150 mm, 2.7 μm) chromatographic column was used to separate the analytes. A KQ5200DE ultrasonic cleaner (Kun Shan Ultrasonic Instruments Co. Ltd, China) was used to extract the target compounds from the chick tissue. A pressure blowing concentrator was used for concentration of the sample solution. A vortex mixer and a high-speed homogenate machine were used to treat the chick tissue sample. All data were acquired and processed using Masshunter™ 2.0 software.

2.3. HPLC/MS/MS conditions

A gradient program was conducted for chromatographic separation with the mobile phase consisting of 0.2% formic acid water (A) and acetonitrile (B) as follows: 0–4 min (28%, A), 4–6 min (50%, A), 6–12 min (65%, A), 12–20 min (65%, A), 20–22 min (28%, A). The flow rate was 0.3 mL/min, and the column temperature was 35 °C. Injection volume was 2 μL.

The mass spectrometer was operated in the positive ion electrospray mode. The target compounds were determined by multiple reactions monitoring (MRM). N_2 (purity of 99.9%) was used as drying gas (10 L/min). N_2 (purity of 99.999%) was used as collision gas. The dwell was 200 ms. Ion source temperature was 350 °C. Capillary voltage was 4000 V. The MRM *m*/*z* transitions, collision energy (CE) and cone-voltage shown as Table 1 were used for the qualitative and quantitative measurement of SG, CHE, DHSG and DHCHE.

2.4. Preparation of standard and quality control samples

Stock solution of SG, CHE, DHSG and DHCHE was prepared by dissolving appropriate amount of each standard in methanol for the concentration of 100.0 μg/mL respectively. The stock solution was serially diluted with methanol to provide working standard solution of 100 ng/mL. SG, CHE, DHSG and DHCHE stock solution were blended to make the mixture stock solution. The stock solution was stored at 4 °C and used for preparing standard curve and quality control (QC) samples.

The calibration curve samples with seven non-zero standard levels containing SG, CHE, DHSG and DHCHE in the concentration of 0.1–150.0 ng/mL were prepared. The standard calibration samples of the four alkaloids were prepared by spiking 10 μL of the mixing standard solution of SG, CHE, DHSG and DHCHE into 50 μL of blank chick tissue homogenate to get the nominal concentrations of 0.1, 0.5, 1.0, 5.0, 50, 100, 150 ng/mL. The quality control samples at low, medium and high concentration levels were prepared in the same method as the calibration curves. The nominal concentration of QC samples were 10.0, 50.0, 100.0 ng/mL for SG, CHE, and 5.0, 20.0, 50.0 ng/mL for DHSG, DHCHE respectively.

2.5. Sample preparation

1.0 g of chicken tissue (muscle, liver, etc.) sample was weighed critically and added to a 50 mL of centrifugal tube; 2.0 mL of ultrapure water was added. Then 15 mL of acetonitrile–water (95:5, v/v) solution was added after the tissue was homogenized with high speed homogenizer. The tube was capped, and the contents were vigorously mixed on a vortex mixer for 3 min. The sample was extracted with ultrasonic for 30 min in an ultrasonic cleaner at 30 °C, then centrifuged at 10,000 r/min for 10 min. The supernatant