Journal of Chromatography B, 947–948 (2014) 57–61

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination of xanthatin by ultra high performance liquid chromatography coupled with triple quadrupole mass spectrometry: Application to pharmacokinetic study of xanthatin in rat plasma

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ARTICLE INFO

Article history:
Received 28 May 2013
Accepted 5 December 2013
Available online 18 December 2013

Keywords:
Xanthatin
Pharmacokinetics
Ultra high performance liquid chromatography tandem mass spectrometry (UHPLC–ESI-MS/MS)

ABSTRACT

A sensitive, specific and rapid ultra high performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) method has been established to study pharmacokinetic properties of xanthatin. Xanthatin, a compound which belongs to sesquiterpene lactone group, was determined in rat plasma with psoralen as internal standard. Chromatographic separation was performed on an Agilent Zorbax Eclipse plus C_{18} column (50 mm × 2.1 mm, 3.5 μm) with gradient elution system at a flow rate of 0.3 mL/min. The mobile phase was composed of methanol and 0.1% formic acid water solution. Analysis was performed under a triple-quadruple tandem mass-spectrometer with an electrospray ionization (ESI) source via the multiple reaction monitoring (MRM) mode to determine xanthatin at $[M+H]^+$ m/z 247.3 → m/z 205.2 and that of IS at $[M+H]^+$ m/z 187.1 → m/z 143.0 within 5 min. The assay method exhibited good separation of xanthatin from the interference of endogenous substances. The lower limit of quantification (LLOQ) was 1 ng/mL, with a good linearity within the concentration range of 1–5000 ng/mL (r = 0.9990). Intra-day and inter-day precision RSD was less than 9.27%; intra-day and inter-day accuracy was 88.48% and 102.25% respectively. The extraction recoveries of xanthatin range from 82.12% to 89.55%, and the extraction RSD was less than 9.01%. The established LC–ESI-MS/MS method is rapid and sensitive, which has been successfully applied to quantify xanthatin in rat plasma for the first time.

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1. Introduction

Xanthium strumarium L. (Asteraceae), a traditional medical plant in Asia and Africa, was widely used for the treatment of rhinitis, rheumatism, eczema, cancer, ulcer and malaria [1–3]. Xanthatin, with an α-methylene-γ-butrolactone structure, was one of the major active components isolated from the leaves of *X. strumarium* L. [4]. In recent years, various biological activities of this compound have been reported, such as anti-inflammation [5], antileishmanial, antifungal [6], antioxidant [7], trypanocidal and cytotoxic properties [8].

Particularly, xanthatin can significantly inhibit the growth of various animal and human tumors [9–11]. The molecular mechanisms of its anticancer properties are attracting more and more attention. Previous studies have shown that xanthatin exhibited anti-proliferative effects by inducing the expression of the GADD45γ gene in MDA-MB-231 cells [12], thus induce cell cycle arrest and apoptosis via disrupting NF-κB pathway in A549 Cells [13]. Xanthatin also induces human gastric carcinoma MKN-45 cells apoptosis by activating p53 pathway [14]. In addition, our previous study demonstrated that xanthatin could significantly induce apoptosis of murine melanoma B16-F10 by activating Wnt/β-catenin signaling in vitro. It is obviously that xanthatin could inhibit B16-F10 tumor growth in the murine melanoma model by intraperitoneal injection at designated concentration of 4.8 mg/200 g [15]. On the other hand, scientists are focusing on

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http://dx.doi.org/10.1016/j.jchromb.2013.12.006

developing an efficient method to synthesize xanthatin and investigating its structure-activity relationship [16]. All of these results suggest that xanthatin maybe a promising drug entity, which can be developed for treating human cancers.

In the process of developing a new drug, relative pharmacokinetic parameters are necessary to be explored. To the best of the authors' knowledge, the pharmacokinetic characters of xanthatin have not been reported yet. Therefore, it is necessary to develop a method to determine the concentration of xanthin in plasma and investigate its pharmacokinetic properties. The results will contribute to the following pharmacological research of xanthatin. The purpose of this paper was to establish a sensitive, rapid and specific UHPLC–MS/MS method for the determination of xanthatin in rat plasma.

2. Materials and methods

2.1. Reagents and materials

Methanol (Calepure, Canada, HPLC grade). The water used in this research was purified with an EPED water purification system from Nanjing EPED system (Nanjing, China), and the other reagents were HPLC grade. The air-dried aerial parts of *X. strumarium* L. were collected from the suburbs of Xuzhou, Jiangsu province of China. The herb was authenticated by Professor Jianwei Chen of Nanjing University of Chinese Medicine. Xanthatin (Fig. 1a, purity ≥98%) was isolated from the air-dried aerial parts of *X. strumarium* L. in the authors' laboratory. Its chemical structure was confirmed by using ^{1}H NMR, DEPT, ^{1}H, ^{1}H-COSY, NOESY, HSQC, and HMBC. Psoralen (Fig. 1b, purity ≥99%) was obtained from Shanghai U-sea Biotech Co. Ltd. (Shanghai, China). Other reagents and solvents were of the highest quality available.

2.2. Equipments

All separations were performed on a DGU-20A 5R series UHPLC system equipped with a LC-30AD binary pump (Shimadzu Corporation UFLC XR, Kyoto, Japan). Mass spectrometry was conducted by using a 5500 triple quad tandem mass spectrometer equipped with electrospray ionization (ESI) source (AB SCIEX, Foster City, CA, USA).

2.3. Chromatographic and mass-spectroscopic conditions

The analytical column was Agilent Eclipse plus C_{18} column (50 mm × 2.1 mm, 3.5 μm). The mobile phase was composed of methanol (B) and 0.1% formic acid water solution (A) with gradient elution system (0–1.5 min, 5–50% B; 1.5–3.5 min, 50–70% B; 3.5–4 min, 70–85% B; 4–4.5 min, 85–50% B; 4.5–5 min, 50–5% B) at a flow rate of 0.3 mL/min. Injection volume was 5 μL and the column temperature was 40 °C.

Mass analysis was performed in the positive ion electrospray ionization mode with an electrospray ionization (ESI) source. The ion spray voltage was set at 5.5 kV. The optimized parameters were recorded as following data: Ion source temperature, 600 °C; curtain gas (CUR), 241.33 kPa; ion source gas 1 (GAS1), 344.75 kPa; ion source gas 2 (GAS2), 413.70 kPa; ion spray voltage (IS), 5500 V; declustering potential (DP), 69 V; collision energy (CE), 14 V; entrance potential (EP), 10 V and collision exit potential (CXP), 14 V. Multiple reaction monitoring (MRM) mode was applied for the quantitation at $[M+H]^+$ m/z 247.3 → m/z 205.2 for xanthatin and at $[M+H]^+$ m/z 187.1 → m/z 143.0 for psoralen (IS). The chemical structure and the mass spectrum of xanthatin and psoralen were shown in Fig. 1.

2.4. Preparation of stock solutions and quality control samples

Stock solution was prepared by dissolving xanthatin and psoralen in methanol at concentration of 200 μg/mL each. The internal standard (IS) working solution (10 ng/mL) was obtained by dilute the stock solution with methanol. Calibration samples were prepared by spiking 90 μL blank plasma with 10 μL xanthatin working solutions to produce final concentrations of 1, 10, 50, 100, 500, 1000 and 5000 ng/mL. The quality control (QC) samples were prepared at concentrations of 2, 50, 500 and 3000 ng/mL respectively. All solutions were kept in 4 °C refrigerator and brought to room temperature before use.

2.5. Sample preparation

To each sample, 10 μL of IS solution (10 ng/mL) was added into 100 μL of rat plasma. The mixture was extracted with 1 mL of ethyl acetate by vortexing for 3 min. The aqueous and organic layer was separated by centrifugation at 4000 r/min for 5 min and the organic layer was transferred to another centrifuge tube and evaporated to dry under a gentle stream of nitrogen gas at 40 °**C**. The residue was reconstituted with 100 μL of mobile phase (0.1% formic acid-methanol at ratio of 5: 95), then centrifuged at 4000 r/min for 5 min. For analyzing, 5 μL of the supernatant was injected into the LC–MS/MS system.

2.6. Method validation

2.6.1. Specificity

Specificity of the method was evaluated by comparing chromatograms of blank plasma samples, samples spiked with IS plus xanthatin and treated plasma samples.

2.6.2. Linearity and quantification

Various concentrations of xanthatin calibration standard solution (1, 10, 50, 100, 500, 1000, 5000 ng/mL) with IS (10 ng/mL) were prepared as plasma sample preparation method and assayed by UHPLC–MS/MS system. Peak area ratios of xanthatin/IS plotted against the corresponding concentrations were calculated to construct calibration curve. The calibration curve was established via $1/x^2$ weighted linear least-squares regression model. The lower limit of quantification (LLOQ) was determined as the lowest concentration on the QC samples.

2.6.3. Precision and accuracy

Accuracy and precision of the method were determined by analyzing six replicates of the QC and LLOQ samples. The intra-day precision and accuracy of the method were assessed by determining the QC samples six times within a single day, while the inter-day precision and accuracy were estimated by determining the QC samples over three consecutive days.

2.6.4. Recovery and matrix effects

The extraction recovery was determined by comparing the concentration of extracted QC samples with the same concentration of xanthatin reference standard solutions. Matrix effects of the method were calculated by comparing the peak areas of reference standard in extracts of blank plasma with that of neat standard solution. Recovery and matrix effects experiments at four QC concentrations for plasma were examined (n = 5).

2.6.5. Stability

The stability of xanthatin in plasma was investigated by analyzing of four QC samples (n = 5). The short-term stability was investigated by analyzing plasma samples kept at room temperature for 4 h. Freezing-thawing stability was determined after

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