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Comprehensive two-dimensional liquid chromatography coupled with quadrupole time-of-flight mass spectrometry for chemical constituents analysis of tripterygium glycosides tablets

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ABSTRACT

Comprehensive two-dimensional liquid chromatography platform (LC × LC) coupled with quadrupole time-of-flight (QTOF) mass spectrometry (MS) is developed to separate, identify and relatively determine the chemical constituents of two types of tripterygium glycosides tablets (TGT). The types and relative contents of the constituents discovered in two kinds of TGT tablets were subsequently compared. C8 and C18 column were used for the separation of the first and second dimensional chromatography (^{1}D and ^{2}D) respectively, and an integrated shift and full gradient mode was used in ^{2}D. Using this LC × LC–QTOF-MS platform, 92 and 132 constituents were detected in TGT preparations from Hubei and Hunan manufacturers respectively (HB-TGT and HN-TGT), most of which belonged to the diterpenoid, triterpenoid and alkaloid families. 50 and 90 compounds were unique in HB-TGT and HN-TGT, respectively, and their relative contents proportion were 52.0% and 54.2% of HB-TGT and HN-TGT, respectively. Furthermore, two TGT tablets could both lead to obvious change in biochemical parameters, oxidative stress related parameters and histopathological status to different degree. In all, the LC × LC–QTOF-MS platform offer a powerful and efficient method for characterizing, identifying and semi-quantifying chemical components in TGT preparations.

1. Introduction

Comprehensive two-dimensional liquid chromatography (LC × LC) is widely used to analyze complex samples [1–10]. LC × LC can utilize two series of chromatography systems connected by an "interface valve" to separate complex samples continuously, and offers high peak capacity and resolution power compared to conventional one-dimension LC [11]. Especially, RPLC × RPLC mode, using two reversed-phase separation process, was increasingly used nowadays due to its high efficiency, fast equilibration, and great compatibility with mass detector [12]. This technique has been successfully used in the analysis of the chemical components of herbal medicines [1–3], drug metabolite *in vivo* [13,14], biopharmaceutical analysis [15], and quality control of agriculture products [16–25].

The HPLC-TOF-MS system is an accurate and sensitive method that is suitable for identifying chemical substances in complex matrices. So far, it has been widely used to analyze the constituents of herbal medicines [26,27]. The ability of the mass technique to separate co-elution components according to their specific mass is well known; however, related studies have demonstrated that radical cations from the co-elution constituents can interact with each other in ion sources, which inevitably and significantly interrupts the results of ion detection [28]. Thus, a powerful separation is still irreplaceable to analyze complex samples.

Tripterygium wilfordii Hook.f (TWHF), derived from genus tripterygium, shows great therapeutic efficacy for many diseases including rheumatoid arthritis, asthma, chronic renal syndrome, cupus erythematous [29–32]. However, TWHF has also been shown to have severe toxic effects on the digestive, reproductive system and circulatory systems [33,34]. Tripterygium glycosides tablets (TGT) containing ethyl acetate extracts of TWHF were orally administered as a common clinical preparation for TWHF. Unfortunately,

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the substantive basis of its therapeutic and toxic effect is not yet completely understood; therefore, it is necessary to comprehensively research the chemical constituents of TGT. To date, more than 170 chemical constituents have been discovered in TWHF, which mainly comprise members of the diterpenoid, triterpenoid and alkaloid families [35–48]. Some of them have been found to show multiple bioactivity and toxicity effects [49,50]. Theoretically, Chinese herbal medicine exert typical synergistic effects of multiple components on multiple target sites [51], and it is therefore necessary to comprehensively characterize the components of these herbal medicine; however, up to date, quality control of TWHF and related preparations have been focused on the contents of several components, including triptolide, celastrol, and wilforlide A [52,53]. Previous studies of TWHF and its related preparations have all made use of conventional one-dimensional HPLC analysis, but its peak capacity and resolution power could not be sufficient when analyzing TWHF samples. The neighboring peaks of TGT preparations commonly overlapped and baseline disturbances were observed. So far, only approximately 20 constituents in TWHF have been analyzed successfully. Hence, a powerful analysis method with high peak capacity and resolution power is required to analyze TWHF and its preparation samples.

The aim of this study is to systematically analyze numerous constituents in TGT preparations using an integrated strategy of advanced LC × LC and QTOF-MS [54]. Highly accurate mass data and information on TWHF composition were used to comprehensively characterize the chemical compounds. Moreover, the types and contents of chemical components in two TGT preparations from different manufacturers were compared, and the differences above maybe caused the differential toxicity of two kinds of TGT preparations in some extent.

2. Method

2.1. Experiment and reagent

2.1.1. Experiment

The Agilent 1290 infinity LC × LC system (Waldbronn, Germany) was equipped with a binary system, online degasser, diode-array UV absorbance detectors, thermostat column compartments, an autosampler, and an interface valve to connect two-dimensional systems with double 50-μL stainless steel sample loops. A 6530 quadrupole time of flight mass spectrometer (QTOF-MS) detector with an ESI ion source was used. A Sartorius CP 224S balance (Göttingen, Germany) and Mettler Toledo XP 205 balance (Mettler Toledo, Greifensee, Switzerland) were also used.

2.1.2. Reagent

Triptolide (No. MUST-12121901), triptonide (No. MUST-12120302), triptophenolide (No. MUST-12111205), wilforlide A (No. MUST-12121001), and celastrol (No. MUST-12021802) were all purchased from Chengdu MUST Bio-Technology Co., Ltd (HPLC > 98.0%). HB-TGT (No. 20131201) and HN-TGT (No. 20120602) were obtained from Hubei Huangshi Feiyun Pharmaceutical Co., Ltd and Hunan Xieli Pharmaceutical Co., Ltd (10 mg/tablet in terms of weight of TWHF extracts). Methanol, acetonitrile and HPLC-grade formic acid were obtained from Dikma Co. Ltd. (USA). HPLC-grade water was prepared using a Milli-Q Nano pure water purification system (Billerica, MA).

2.2. Chromatographic conditions

For the first dimensional separation (^{1}D), an Agilent Polaris 3 C8-ether (3 μm, 3.0 mm × 100 mm i.d.; Agilent Technologies, Little Falls, DE) was utilized at 30 °C. In the section of ^{1}D separation selection, Agilent Zorbax Bonus-RP C18 (3.5 μm, 2.1 mm × 100 mm i.d.) and Agilent narrowBore Aq C18 (3.5 μm, 3.0 mm × 100 mm i.d.) columns were also used. The injection volume was 10 μL. The flow rate was set at 80 μL/min. Water and methanol were set as mobile phases A and B, respectively. The initial solvent was 40% mobile B and the gradient increased to 100% mobile B after 65 min and was subsequently maintained at 100% mobile B for 10 min. The entire analysis time was 75 min.

The column of ^{2}D separation was an Agilent Poroshell 120 SB-C18 (2.7 μm, 3.0 mm × 50 mm i.d.; Agilent Technologies, Little Falls, DE) and the column temperature was set at 45 °C. The analysis was integrated using the shift and full gradient modes at different time segments, as described in detail in Table 1. Water with 0.1% formic acid and acetontrile with 0.08% formic acid were used as solvents A and B, respectively. The flow rate was 2 mL/min.

In this LC × LC system, a 2-position 8-port valve was employed to connect two-dimensional chromatography and was operated using the process shown in Supplementary Fig. 1. Two equivalent loops were responsible for collecting fractions from ^{1}D and transferring fractions into ^{2}D in an alternative mode with a modulation cycle of 33 s throughout the overall chromatographic process.

2.3. Mass condition

The mass spectrometric parameters were set as follows: positive ion mode (ESI+); nebulizer gas pressure of 35 psi; sheath gas temperature of 325 °C; drying gas flow rate of 8 L/min; capillary voltage of 3500 V; fragmentor voltage of 200 V, skimmer of 65 V; scan mode set to 100–1000 *m*/*z*; collision energy of 40 eV; acquisition rate of 2 spectra/s; and a split ratio of 1:4. A reference solution was used for mass axis calibration prior to the analysis, width of precursor ion was set as narrow (1.3 Hz).

2.4. Preparation of the TWHF standard solution

Approximately 5 mg of standard substances including triptolide, triptonide, triptophenolide, wilforlide A, and celastrol were precisely weighed and dissolved in methanol to obtain a standard solution with a concentration of 1 mg/mL, respectively. The standard solution with a concentration of 10 μg/mL was prepared by diluting the above five types of standard solution (1 mg/mL).

Table 1
Chromatographic parameters of the LC × LC system.

^{1}D column	Agilent Polaris 3 C8-ether (3 μm, 3.0 mm × 100 mm i.d)				
^{1}D Gradient condition (B%)	0 min, 40%; 0–65 min, 40–100%; 65–75 min, 100%				
^{2}D column	Agilent Poroshell 120 SB-C18 (3.0 mm × 50 mm, 2.7 μm)				
	Time (min)	Shift gradient (0 min)	Shift gradient (40 min)	Full gradient (40.1 min)	Full gradient (75 min)
	0	30	55	55	55
^{2}D Gradient condition (B%)	0.25	50	70	100	100
	0.45	70	85	100	100
	0.50	70	85	100	100