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Rapid screening and identification of sesquiterpene lactones in *Kudiezi* injection based on high-performance liquid chromatography coupled with linear ion trap-orbitrap mass spectrometry

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[ABSTRACT] Sesquiterpene lactones are considered as the major active compounds in *Kudiezi* injection in virtue of their special structures and activities. Herein, an analytical method was developed for rapid screening and identification of sesquiterpene lactones in *Kudiezi* injection using high-performance liquid chromatography coupled with linear ion trap-orbitrap mass spectrometry (HPLC-LTQ-Orbitrap) in negative ion mode. First, two sesquiterpene lactone reference standards were analyzed to obtain their characteristic ESI-MS/MS fragmentation patterns. Second, based on extracted ion chromatography (EIC) data-mining method and characteristic fragmentation pathways analysis, sesquiterpene lactones in *Kudiezi* injection were rapidly screened and identified. Finally, an important parameter Clog *P* was adopted to discriminate the isomers of sesquiterpene lactones. As a result, 50 sesquiterpene lactones were characterized, including 9 sesquiterpene lactone aglycones, 39 sesquiterpene lactone glycosides, and 2 amino acid-sesquiterpene lactone conjugates. Among them, 13 compounds were tentatively identified as new compounds. The results demonstrated that the established method would be a rapid, effective analytical tool for screening and identification of sesquiterpene lactones in the complex system of natural medicines.

[KEY WORDS] HPLC-LTQ-Orbitrap; Sesquiterpene lactones; Characteristic fragmentation pathways; *Kudiezi* injection

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Introduction

Ixeris sonchifolia (Bge.) Hance, belonging to the compositae family, is a kind of bitter perennial herb that is distributed and cultivated widely in northeastern China. It has been traditionally used as folk medicine for invigorating blood circulation, dissipating blood stasis to relieve pain, and normalizing menstruation [1]. As a preparation extracted and refined from the whole herb of *I. sonchifolia*, *Kudiezi* injection has been widely used to treat coronary heart disease, effort angina, and acute cerebral infarction [2-3]. The chemical constituents of *Kudiezi* injection are quite complicated, including sesquiterpene lactones, flavonoids, triterpenes and amino acids, which have been reported to possess anti-inflammatory, anti-atherosclerotic, antioxidant and anticancer activities [4–7]. Until now, few reports are available on systematic analysis of sesquiterpene lactones in *Kudiezi* injection.

Among many different LC-MS platforms, high-resolution mass spectrometry (HRMS) has exhibited excellent performances for components identification because of its high efficiency, sensitivity, and selectivity [8-9]. The hybrid linear ion trap-orbitrap mass spectrometer (LTQ-Orbitrap MS) combined high trapping capacity and MS^n scanning function of linear ion trap along with accurate mass measurements within 5 ppm and a resolving power of up to 100 000 over a wider dynamic range than many other mass spectrometers [10–12]. Meanwhile, the combined application of tandem mass spec-

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trometry for identifying the complicated constituents in traditional Chinese medicines (TCMs) would generate a large quantity of information data, such as elemental compositions and fragmentation patterns of multiple-stage. In addition, off-line data processing also plays an important role in the identification of components, such as extracted ion chromatography (EIC) data-mining method with the remarkable merits of purifying total ion chromatography (TIC) spectra and facilitating the identification of unknown chromatographic peaks [13-14]. These advantages have made LTQ-Orbitrap MS one of the most powerful approaches for the rapid identification of multiple constituents in TCMs.

In the present study, an HPLC-LTQ-Orbitrap data-acquisition approach combined with EIC data-mining technique was established for comprehensive identification of sesquiterpene lactones and their derivatives in *Kudiezi* injection. In addition, an important parameter Clog *P* was used to discriminate the isomers of sesquiterpene lactones.

Materials and Methods

Chemicals

The reference standards of Ixerin Z and 11, 13*α*-dihydroixerin Z (purity greater than 98%) were previously extracted, isolated and identified from *I. sonchifolia* in our laboratory [15]. Their structures (Fig. 1) were fully elucidated by ^{1}H, ^{13}C NMR and ESI-MS data [16-17]. The commercial products of *Kudiezi* injection produced by Tonghua Huaxia Pharmaceutical Co., Ltd. (Jilin, China) were purchased by prescription from Dongfang Hospital in Beijing, China. HPLC grade formic acid, acetonitrile, and methanol were purchased from Merck (Darmstadt, Germany). Ultra-pure water was produced by a Milli-Q purification system (Millipore, Bedford, MA, USA) in the experiment.

Sample preparation

The standard solutions of Ixerin Z and 11, 13*α*-dihydroixerin Z were prepared in methanol at concentrations of 100 μg·mL^{-1}. All the standard solutions were stored at 4 ºC until use. *Kudiezi* injection was filtered through a 0.22 μm membrane, and then an aliquot of 10 μL of the subsequent filtrate was injected into LC-MS system for analysis.

Instrumentation and conditions

HPLC analysis was carried out on an Accela HPLC system equipped with a binary pump and an autosampler (Thermo Scientific, Bremen, Germany). The compounds were separated on a Thermo Hypersil BDS C_{18} column (250 mm × 4.6 mm i.d., 5 μm) at room temperature. The mobile phase consisted of 0.1% (*V*/*V*) formic acid in water (A) and acetonitrile (B) with the elution gradient set as follows: 0−18 min, 2%−8% B; 18−36 min, 8%−12% B; 36−55 min, 12%−18% B; 55−70 min, 18%−25% B; 70−80 min, 25%−30% B; 80−85 min, 30%−40% B. The flow rate was set at 1.0 mL·min^{-1}.

LTQ-Orbitrap XL mass spectrometer (Thermo Scientific, Bremen, Germany) was connected to the LC system *via* an electrospray ionization (ESI) interface. HPLC effluent was introduced into the ESI source in a post-column splitting ratio of 1 : 4. Full scan data acquisition was performed from *m/z* 100 to 600 in negative ion mode. The important ESI parameters were set as follows: sheath gas (nitrogen) flow rate of 30 arb.; auxiliary gas (nitrogen) flow rate of 10 arb.; capillary temperature of 350 ºC; electrospray voltage of 3.0 kV; capillary voltage of −35 V; and tube lens voltage of −110 V. The resolution of Orbitrap analyzer was set at 30 000 with data-dependent ESI-MS^2 analysis triggered by the three most abundant ions from one-stage mass spectrometry scanning, followed by ESI-MS^3 analysis of the most abundant product ions. Collision-induced dissociation (CID) was performed in LTQ with an activation *q* of 0.25 and activation time of 30 ms. The isolation width was 2 amu, and the normalized collision energy was set to 35%. Data were acquired and analyzed using Xcalibur data 2.1 software (Thermo Scientific, Bremen, Germany).

Results and Discussion

Overview of analysis approaches of experimental results

Owing to the low content of sesquiterpene lactones in *Kudiezi* injection, a sensitive and reliable HPLC-LTQ-Orbitrap approach was established for achieving their accurate mass and molecular formula. Meanwhile, considering the lack of reference standards, the characteristic fragmentation pathways of sesquiterpene lactones were concluded and then used for rapid identification of the other sesquiterpene lactones in *Kudiezi* injection. Furthermore, sesquiterpene lactone isomers were differentiated by Clog *P* parameter. As a result, a total of 50 sesquiterpene lactones in *Kudiezi* injection were screened and divided into sesquiterpene lactone aglycones, sesquiterpene lactone glycosides, and conjugates of amino acid-sesquiterpene lactones.

The fragmentation patterns of reference standards

Ixerin Z exhibited its $[M - H]^-$ ion at *m/z* 421.149 3 ($C_{21}H_{25}O_9$) with mass errors within 5 ppm. It produced the base peak ion at *m/z* 259 by neutral loss of a dehydrated glucose. The ion at *m/z* 259 generated prominent ions at *m/z* 215 and *m/z* 241 through losing CO_2 and H_2O, respectively. Moreover, the product ion at *m/z* 241 further generated the predominant ion at *m/z* 197 by loss of one molecular of CO_2. The ion at *m/z* 215 was also followed by loss of H_2O or CO, yielding two minor ions at *m/z* 197 ($[M - H - 162 - CO_2 - H_2O]^-$) and *m/z* 187 ($[M - H - 16 - CO_2 - CO]^-$) (Fig. 2).

11, 13*α*-Dihydroixerin Z firstly produced its base peak ion at *m/z* 261 *via* a glycosidic bond cleavage, and then generated two minor ions at *m/z* 217 ($[M - H - 162 - CO_2]^-$) and *m/z* 199 ($[M - H - 162 - H_2O]^-$). Meanwhile, the ion at *m/z* 187 was also observed in its MS^3 spectrum, corresponding to $[M - H - 162 - C_3H_6O_2]^-$ due to the fragmentation of the *α*-methyl-*γ*-lactone in 11, 13*α*-dihydroixerin Z with saturated $C_{11\text{-}13}$ bond (Fig. 3).

Therefore, the characteristic fragment ions of reference standards were deduced, such as $[M - H - 44]^-$ generated by