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Simultaneous determination of 11 characteristic components in three species of *Curcuma* rhizomes using pressurized liquid extraction and high-performance liquid chromatography

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Abstract

A high-performance liquid chromatography coupled with diode array detection (HPLC-DAD) and pressurized liquid extraction was developed for simultaneous quantitative determination of 11 characteristic compounds, including curcumenone, curcumenol, neocurdione, curdione, isocurcumenol, furanodienone, curcumol, germacrone, curzerene, furanodiene and β-elemene, in rhizomes of three species of *Curcuma*. The analysis was performed on an ODS C_{18} column. The mobile phase consisted of (A) water and (B) acetonitrile using a gradient elution. The peaks were monitored at both 214 nm and 256 nm. All calibration curves showed good linearity ($r^2 > 0.9996$) within test ranges. This method showed good repeatability for the quantification of these eleven components in three species *Curcuma* rhizomes with intra- and inter-day variations of less than 1.57% and 1.98%, respectively. The validated method was successfully applied to quantify 11 investigated components in eighteen samples of three species of *Curcuma*, which is helpful to control their quality.
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Keywords: HPLC-DAD; Sesquiterpenoids; Pressurized liquid extraction; *Curcuma*; Quality control

1. Introduction

Ezhu, one of commonly used traditional Chinese medicines, is derived from rhizomes of three species of *Curcuma*, including *Curcuma phaeocaulis*, *Curcuma kwangsiensis* and *Curcuma wenyujin*, according to the record of China Pharmacopoeia [1]. At present, the essential oil of *Ezhu* is considered possessing anti-tumour [2,3] and antiviral activities [4,5]. Sesquiterpenoids including β-elemene, curcumol, germacrone, curdione and neocurdione are thought to be the biological active ingredients in the essential oil [6–8]. Therefore, determination of the sesquiterpenoids is very important for pharmacological study and quality control of *Ezhu*. Several methods including thin-layer chromatography (TLC) [9–13], gas chromatography-flame ionization detection (GC-FID) [14–19], gas chromatography–mass spectrometry (GC–MS) [20,21], high-performance liquid chromatography (HPLC) [8,22–28] have been reported to quantify sesquiterpenoids in *Ezhu* and its products. However, only a limited number of sesquiterpenoids, such as curcumol [8–11,13–18,20–22,24,25,28], germacrone [8,12,20–28], curdione [8,11,20,21,24,28], isocurcumenol [8] and β-elemene [19] were determined because of the absence of chemical standards. To date, the accurate contents of most sesquiterpenoids in *Ezhu* have not been determined though relative quantitation of 11 sesquiterpenoids was achieved using germacrone as a reference [29]. In addition, though GC–MS is a powerful tool for analysis of volatile oil, essential oil of *Ezhu* contains heat-sensitive components such as germacrone [30] and furanodiene [31,32], which may degrade and result in wrong results during GC analysis. Our studies showed that the degradation of furanodiene and furanodienone was still existent under optimum GC conditions (see Fig. 1), which suggested that development of an alternative method for quantitative determination of the two sesquiterpenoids is necessary.

In present study, pressurized liquid extraction (PLE) and high-performance liquid chromatography (HPLC) were developed for quantitative determination of 11 characteristic

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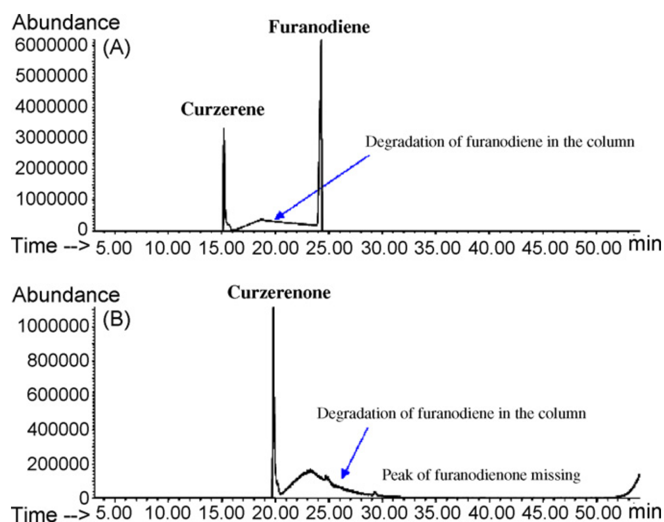

Fig. 1. Degradation of (A) furanodiene and (B) furanodienone during GC–MS analysis under optimum conditions. GC–MS conditions: column, a capillary (30 m × 0.25 mm I.D.) coated with 0.25 μm film 5% phenyl methyl siloxane; carrier gas, high purity helium; flow-rate, 1.0 ml/min; inlet mode and temperature, pulsed splitless at 190 °C; The column temperature was set at 60 °C and held for 2 min for injection, then programmed at 5 °C/min to 145 °C and held for 25 min at the temperature of 145 °C, then at 5 °C/min to 200 °C, and finally, at 20 °C/min to 280 °C, and held for 3 min at the temperature of 280 °C. The mass spectrometers were operated in electron-impact (EI) mode, the scan range was 40–550 amu, the ionization energy was 70 eV and the scan rate was 0.34 s per scan. The quadrupole, ionization source temperature were 150 °C and 280 °C, respectively.

components, i.e. curcumenone, curcumenol, neocurdione, curdione, isocurcumenol, furanodienone, curcumol, germacrone, curzerene, furanodiene and β-elemene, in *Ezhu*. The validated method was successfully applied to quantify the investigated components in eighteen samples of three species of *Curcuma* used as *Ezhu*.

2. Experimental

2.1. Chemicals, reagents and materials

Acetonitrile for HPLC was purchased from Merck (Darmstadt, Germany). Water for HPLC was prepared using a Millipore Milli-Q Plus system (Millipore, Bedford, MA, USA). Curcumenone, curcumenol, neocurdione, curdione, isocurcumenol, furanodienone, curcumol, germacrone, curzerene, furanodiene and β-elemene, with more than 98% of purity, were separated and purified by ourselves. The structures were confirmed by comparing their UV, EI-MS and NMR data with literatures [33–44]. The structures were shown in Fig. 2.

Six batches (CW1–CW6) of *C. wenyujin* rhizomes were obtained from Yueqing, Zhejiang Province; *C. phaeocaulis rhizomes* (CP1–CP6) were separately collected from Tingjiang, Jiangyuan, Sanjiang, Zhoudu, Wangdan and Shuangliu, Sichuan Province; *C. kwangsiensis* (CK1–CK6) rhizomes were collected from Nanning, Guixian, Wuming and Yunshan, Guangxi Province, as well as Wenshan and Malipo, Yunnan Province, respectively. All the plant materials were collected in November 2003. The samples were sealed and stored at −20 °C before analysis. The voucher specimens of *Curcuma* rhizomes were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macau SAR, China.

2.2. Pressurized liquid extraction (PLE)

Pressurized liquid extractions were performed on a Dionex ASE 200 system (Dionex, Sunnyvale, CA, USA) as described before with minor modification [29]. In brief, raw materials of *Ezhu* were dried at 40 °C for 6 h and were grounded into powder of 0.2–0.3 mm. Powder of *Ezhu* (0.5 g) was mixed with diatomaceous earth (0.5 g) and placed into 11-ml stainless steel extraction cell, respectively. The sample was extracted under the optimized conditions: solvent, methanol; temperature, 100 °C; particle size, 0.2–0.3 mm; static extraction time, 5 min; pressure, 1000 psi.; static cycle, 1 and 40% of the flush volume. Then, extract was transferred to a 25 ml volumetric flask which was made up to its volume with extraction solvent and filtered through a 0.45 μm Econofilter (Agilent Technologies, Palo Alto, CA, USA) prior to injection into the HPLC system.

2.3. HPLC analysis

All separations were performed on an Agilent Series 1100 (Agilent Technologies, USA) liquid chromatograph, equipped with a vacuum degasser, a quaternary pump, an autosampler, and a diode array detection (DAD) system. A Zorbax ODS column (250 mm × 4.6 mm I.D., 5 μm) with a Zorbax ODS C18 guard column (20 mm × 3.9 mm I.D., 5 μm) was used. Solvents that constituted the mobile phase were A (water) and B (acetonitrile). The elution conditions applied were: 0–15 min, linear gradient 30–47% B; 15–30 min, isocratic 47% B; 30–40 min, linear gradient 47–60% B; 40–50 min, linear gradient 60–90% B; 50–60 min, linear gradient 90–100% B; and finally, washing the column with 100% B for 10 min before reconditioning the column for 15 min with 30% B. The flow-rate was 1 ml/min and the injection volume was 10 μl. The column operated at 25 °C. The analytes were monitored with DAD at 214 nm and 256 nm.

3. Result and discussion

3.1. Optimization of HPLC conditions

All compounds except curcumenone and curcumenol have poor UV absorptivities based on UV spectra of the investigated compounds. Therefore, 214 nm had to be chosen for detection of the analytes though curcumenone and curcumenol were detected at 256 nm considering the sensitivity. The baseline was good and complete separation was achieved using gradient elution of water and acetonitrile. Under the optimum chromatographic conditions, the peaks corresponding to different analytes were well-separated in 60 min (see Fig. 3). The peaks of these sesquiterpenoids were identified by two means: (i) by comparing the retention times of the peaks with those of the reference compounds eluted under the same conditions and (ii) by spiking the sample with stock standard solutions of analytes.

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