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Quantification of ligustilides in the roots of *Angelica sinensis* and related umbelliferous medicinal plants by high-performance liquid chromatography and liquid chromatography–mass spectrometry

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Abstract

A reversed phase high-performance liquid chromatographic method was developed for quantifying *E*-ligustilide (**1**) and *Z*-ligustilide (**3**) in the roots of *Angelica sinensis* (Oliv.) Diels with confirmation using UV, atmospheric pressure chemical ionisation (APCI) MS and APCI-MS–MS techniques. Based on the UV spectra of compounds **1**, *E*-butylidenephthalide (**2**), **3** and *Z*-butylidenephthalide (**4**), the absorption at 350 nm was chosen as measuring wavelength in which baseline separation of compounds **1** and **3** could be obtained but avoided the interference of compounds **2** and **4**. The identity of compounds **1** and **3** in samples were unambiguously determined by the respective quasi-molecular ions ($[M+H]^+$) in APCI-MS. According to the stability data, acetonitrile was chosen for the preparation of standard solutions in order to minimize the isomerization of compound **3**. Compounds **1** and **3** were qualitatively and quantitatively analyzed in seven samples of the roots of *Angelica sinensis* (Oliv.) Diels, *Angelica acutiloba* Kitagawa, *Angelica acutiloba* Kitagawa var. *sugiyamae* Hikino and the rhizome of *Ligusticum chuanxiong* Hort. Analysis of an extract from a sample root of *Angelica gigas* Nakai using LC–MS for the first time could not detect the presence of ligustilide in this herb. The overall analytical procedure is rapid and reproducible which is considered suitable for quantitative analysis of large number of samples.

Keywords: *Angelica* spp.; *Ligusticum chuanxiong*; Plant materials; Pharmaceutical analysis; Ligustilide

1. Introduction

Radix Angelicae Sinensis (Chinese Danggui, CDG) is the processed root of *Angelica sinensis* (Oliv.) Diels, which is widely used as one of the traditional Chinese medicinal materials in prescriptions and composite formulae to enrich blood, activate blood circulation, regulate menstruation, relieve pain and relax bowels, etc. [1]. Moreover, CDG has been used as a health food, one of the ingredients included in cosmetic and some drugs for animal, etc.

According to the traditional experience, the distinct fragrance had been used as an indicator for CDG quality assessment and grading. The fragrance is related to its chemical constituent ligustilide, one of the major chemical components in its volatile oils, which is also an important marker used for assessing CDG quality [2–4]. Pharmacological and clinical studies indicate that ligustilide is a bioactive compound to inhibit platelet aggregation, relax uterus, tracheal muscle and smooth muscle, prevent gynecological diseases, treat menstrual disorders, urgent premature birth and hypertension, etc [5–9]. Ligustilide is also distributed in Japanese Danggui (JDG, Touki), a legally used herbal medicinal material in Japan. JDG is the roots of *Angelica acutiloba* Kitagawa and *Angelica acutiloba* Kitagawa var. *sugiyamae* Hikino. Furthermore, another widely used medicinal plant *Ligusticum chuanxiong* Hort. (Rhizoma Chuanxiong, Chinese Chuanxiong, CCX), was reported containing more than 1.0% of

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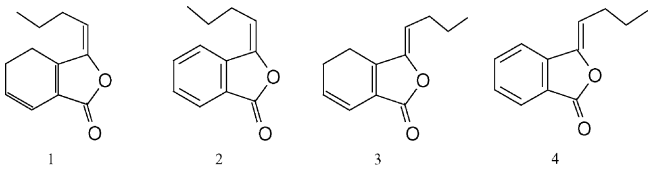

Fig. 1. Chemical structures of *E*-ligustilide (1), *E*-butylidenephthalide (2), *Z*-ligustilide (3) and *E*-butylidenephthalide (4).

ligustilide [10–14]. Hence, ligustilide is usually recognized as one of characteristic markers for assessing the quality and identification of CDG, JDG and CCX.

Ligustilides, including *E*-ligustilide (**1**) and *Z*-ligustilide (**3**) (Fig. 1), are the volatile compounds that have been commonly analyzed by GC–flame ionisation detection (FID) or GC–MS in herbs materials and/or products of their volatile oils [3,4,11,15,16]. However, ligustilide(s) are thermally labile and may easily be isomerized in high temperature. Although there are several publications about ligustilide(s) identified in fingerprints, quantified in herbs or their essential oils using HPLC–UV, HPLC–diode array detection (DAD) or LC–MS, the determination of compounds **1** and **3** were interfered by *E*-butylidenephthalide (**2**), *Z*-butylidenephthalide (**4**) and other possible impurities in the published chromatographic conditions [12,13,17–23]. Moreover, the particular unstable property of ligustilide renders quantitation difficult.

This study mainly focuses on developing a rapid, accurate quantitative method for the determination of ligustilide to cope with a large number of samples in standardization and quality control of herbal medicinal materials. Compounds **1**, **2**, **3** and **4** were firstly identified by LC–MS and LC–MS–MS spectra in the CDG samples. Based on the UV absorption characteristic, a new measuring wavelength was chosen for ideal separation of compounds **1** and **3** with no interference peak observed in the HPLC chromatograms. A new RP-HPLC method was developed to quantify compound **3** in the CDG samples based on a series of method validation studies. Besides, compound **1** was also quantified in samples using the linear regression relationship as in compound **3** owing to their similarity in UV absorption characteristics. Three CDG samples, two JDG samples and two CCX samples were first qualitatively analyzed for compounds **1** and **3** using the LC–MS technique, and further quantified by HPLC. Furthermore, a sample root of *Angelica gigas* Nakai (Korea Danggui, KDG) was also analysed for the first time by LC–MS to detect the presence of compounds **1** and **3**. These findings provide alternative observations of the existence of these compounds in this herb as reported previously [20,24–26].

2. Experimental

2.1. Instrumentation

An Agilent/HP 1100 series HPLC–DAD system consisting of a vacuum degasser, binary pump, autosampler, thermostated column compartment and DAD (Agilent, Palo Alto, CA, USA) was used for quantitative analyzing and UV spectra acquisition. An Applied Biosystems/PE-Sciex API 365 LC–MS–MS system with atmospheric pressure ionisation source (Applied Biosystems, Foster City, CA, USA) was used for mass spectrometric determination. Branson 5210E-MTH ultrasonic cleaner (Branso Ultrasonics, CT, USA) was used for sample extraction. For chromatographic analyses, an Alltima C_{18} column (5 μm, 150 mm × 4.6 mm) with a compatible guard column (C_{18}, 5 μm, 7.5 mm × 4.6 mm) was used. The mobile phase consisted of water–acetonitrile (40:60). The flow rate was 1.0 mL/min and column temperature was maintained at ambient conditions. The atmospheric pressure chemical ionisation (APCI) mass spectra were acquired in the positive ion mode.

2.2. Solvents and chemicals

HPLC grade acetonitrile (Labscan, Bangkok, Thailand) was used for preparation of standard solutions and mobile phase. Analytical grade methanol (Merck, Darmstand, Germany) was used for sample preparation. Deionized water was generated from a Milli-Q water system (Millipore, Bedford, MA, USA).

Z-Ligustilide was extracted, separated and purified from fresh roots of *Angelica sinensis* (Oliv.) Diels in our laboratory. Purified *Z*-ligustilide was identified by electron impact ionisation (EI) MS, ^{1}H NMR and ^{13}C NMR spectrometric techniques. The purity was determined by HPLC–UV with two wavelengths and was shown to be greater than 98%. The detailed procedures for separation and spectrometric identification will be reported in another paper.

2.3. Plant materials

Samples CDG-1 to CDG-3 were whole roots of *Angelica sinensis* (Oliv.) Diels. CDG-1 was collected from the CDG cultivating base of Good Agricultural Practice which is being developed jointly by Hong Kong Baptist University, Agriculture Center of Dingxin district (Gausu, China) and Gansu Sheng Tai Traditional Chinese Medicine Development (Gansu, China) in Minxian, Gansu, China when CDG was being harvested on 22 October 2002. CDG-2 was collected from Pingwu, Sichuan, China on 8 November 2002 by Professor Li Liang (Institute of Mianyang Chinese Traditional Medicine, Sichuan, China). CDG-3 was collected from Heqing, Yunnan, China, which was authenticated by Dr. Zhao Zhongzhen (School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China). Sample JDG-1 and JDG-2 were coarse granule of commercial products of JDG. JDG-1 was the roots of *Angelica acutiloba* Kitagawa, produced by Uchida Wakanyaku (Tokyo, Japan; lot YA352620). JDG-2 was the roots of *Angelica acutiloba* Kitagawa var. *sugiyamae* Hikino, produced by Tochimoto (Osaka, Japan; lot no. 060103). Sample CCX-1 and CCX-2 were the rhizome of *Ligusticum chuanxiong* Hort., which were authenticated by Professor Zhang Hao (West China

School of Pharmacy, Sichuan University, Chengdu, China). CCX-1 was collected from Pengzhou, Sichuan, China on 5 September 2003. CCX-2 was bought at Chengdu Chinese Medicine Material Market, Sichuan, China on 4 September 2003. Sample KDG was the roots of *Angelica gigas* Nakai harvested in Antu, Jilin, China in October 2003 and was authenticated by Professor Yan Zhongkai (Academy of Jilin Traditional Chinese Medicine, Jilin, China).

Representative samples were cut into smaller pieces and mixed thoroughly. A representative part of sample pieces or whole was further ground into powder, passed through a 20 mesh (0.9 mm) sieve. The ground powders were stored in amber glass bottles at about 4 °C before use.

2.4. Preparation of standard solutions for linearity and calibration test

Accurately weighed 26.6 mg of *Z*-ligustilide standard were introduced into a 25 mL volumetric flask and made up to the volume with acetonitrile as stock standard solution (1060 mg/L). Aliquots of 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mL stock standard solutions were transferred into 10 mL volumetric flasks and made up to the volume with acetonitrile as working calibration solutions. The concentrations of *Z*-ligustilide in calibration solutions were equivalent to 10.6, 53.2, 106.4, 159.6, 212.8, 266.0, 319.2, 372.4 and 425.6 mg/L, respectively. An aliquot of 10 μL solution for each calibration was injected for HPLC analysis.

2.5. Sample preparation

An accurately weighed 0.5 g of sample powder was introduced into a 60 mL amber collection vial and 25 mL of methanol was added. The amber collection vial was covered and sonicated for 100 min. Methanol was used to restore the volume after sonication. The extract was filtered with a 0.2 μm membrane filter. An aliquot of 10 μL solution was in-

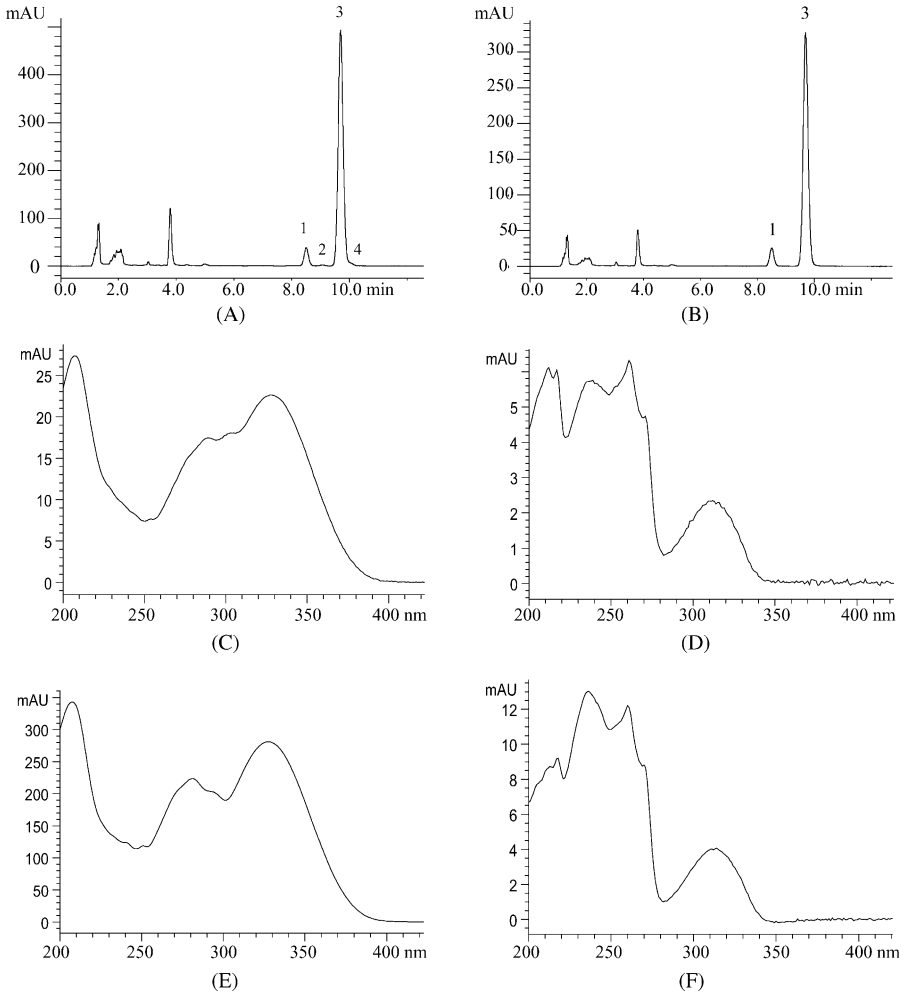

Fig. 2. Chromatogram of methanolic extract of the root of *Angelica sinensis* (Oliv.) Diels measured at 328 nm (A) and 350 nm (B), and their on-line detected UV-spectra of *E*-ligustilide (peak 1, C), *E*-butylidenephthalide (peak 2, D), *Z*-ligustilide (peak 3, E) and *Z*-butylidenephthalide (peak 4, F). (Analytical column: Alltima C_{18}, 5 μm, 150 mm × 4.6 mm; guard column: C_{18}, 5 μm, 7.5 mm × 4.6 mm; sample injection volume: 10 μL; mobile phase: water–acetonitrile (40:60); flow rate: 1.0 mL/min; temperature: ambient.)

jected for HPLC analysis. Sample duplicates were prepared as shown above for analysis.

3. Results and discussion

3.1. Selection of detection wavelength

Compounds **1**–**4** are phthalide-type compounds, which are commonly found in herbs. These compounds were very difficult to be fully resolved by chromatographic conditions as reported in literatures for herbal samples [12,13,17–23]. The resolutions of these compounds were tested and compared with normal and reversed phase conditions using a variety of analytical columns such as Prevail Cyano (3 μm, 150 mm × 2.1 mm), Alltima C_{18} (5 μm, 250 mm × 4.6 mm) or Alltima C_{18} (5 μm, 150 mm × 4.6 mm). Various different mobile phases including hexane–ethanol, hexane–dichloromethane, benzene–dichloromethane, hexane–benzene or acetonitrile–water were tried with detection wavelength at 328 nm, the maximum absorption wavelength of *Z*-ligustilide. The preferred chromatographic condition was found to be using Alltima C_{18} (5 μm, 150 mm × 4.6 mm) column with acetonitrile–water (60:40) as the mobile phase. However, it still encountered difficulty of resolving compounds **3** and **4** to baseline (Fig. 2A). Although the resolution between peaks 3 and 4 was achieved by modifying the composition of mobile phase to produce better resolution, severe peak broadening and reduction in peak height were observed with a prolonged retention time and a poor reproducible peak area. In order to achieve a rapid, sensitive and reproducible method, the condition for resolving compounds **1** and **3** described above was adopted with a compromised peak resolution. Thus, Alltima C_{18} (5 μm, 150 mm × 4.6 mm) was chosen as the analytical column with acetonitrile–water (60:40) as mobile phase.

Previous studies reported that the optimum UV absorption wavelengths for ligustilide determination in herb samples using HPLC–UV were suggested to be 210, 240, 270 and 284 nm [10,17–23]. By examining the UV spectra of compounds **1**–**4**, strong UV absorption for compounds **1** and **3** was found at 350 nm. However, similar UV absorption was not located for compounds **2** and **4** (Fig. 2). Therefore, the chromatographic interference from compounds **2** and **4** could be effectively minimized by using the detection wavelength at 350 nm.

The limit of detection (LOD) of *Z*-ligustilide in sample was determined based on visual evaluation with signal-to-noise ratio of about 3:1. The LOD was estimated to be 0.27 mg/L in test solution, which was equivalent to 13.5 μg/g in solid sample. Moreover, the quantitation limit of compound **3** was determined based on signal-to-noise ratio of about 10:1 for five replicated analyses of spiked matrix blank. The quantitation limit was found to be 1.54 mg/L in sample solutions, equivalent to 77.0 μg/g in solid sample. These results were considered satisfactory and acceptable for subsequent quantitative analysis.

3.2. Identification of Z-ligustilide and E-ligustilide in analytes

Apart from comparing the retention time (t_R), compounds **1** and **3** were further identified by HPLC–APCI-MS analysis in order to provide further information on their identities. Protonated ion of ligustilide at 191 *m/z* was observed (Fig. 3B). Consistent LC–MS spectra and LC–MS–MS spectra were obtained for standard and sample solutions, which demonstrate that peaks 1 and 3 were in fact identified as compounds **1** and **3**, respectively (Fig. 3C). In addition, similar UV spectra of peaks 1 and 3 obtained by DAD agreed with those reported in literatures [10,17,21]. In this regard, the seven herbal samples, CDG-1 to 3, JDG-1 to 2 and CCX-1 to 2, were demonstrated containing both compounds **1** and **3**.

Angelica gigas Nakai., Korea Danggui (KDG), also a medicinal plant, is found in Jilin of P. R. China and north of Korea. In early literatures, ligustilide was not reported in the roots of KDG [25,26]. Takano et al. (1990) reported that ligustilide and butylidenephthalide were not detected in this

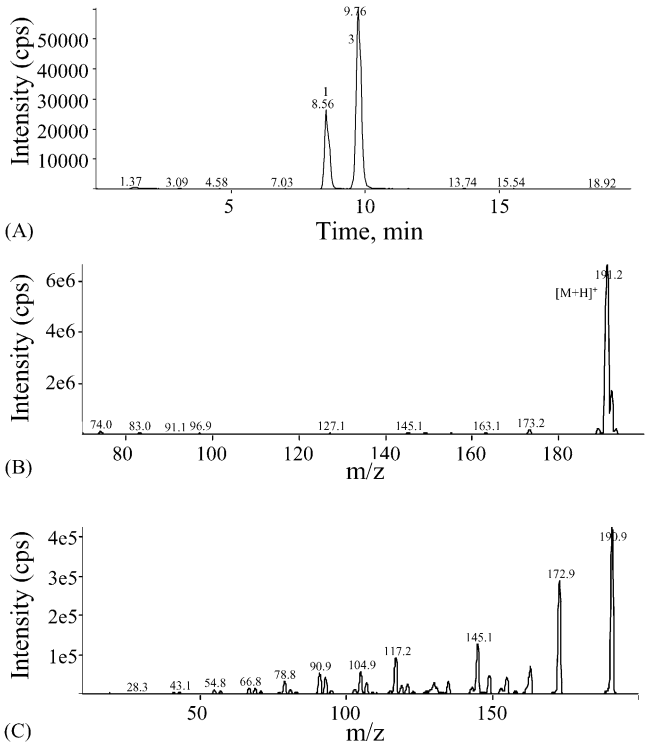

Fig. 3. Total ion chromatogram of methanolic extract of the roots of *Angelica sinenisis* (Oliv.) Diels (A), on-line detected mass spectra of *Z*-ligustilide and *E*-ligustilide (B) and daughter ion mass spectrum of $[M+H]^+$ (C). 1. *E*-ligustilide; 3. *Z*-ligustilide. (Analytical column: Alltima C_{18}, 5 μm, 150 mm × 4.6 mm; guard column: C_{18}, 5 μm, 7.5 mm × 4.6 mm; mobile phase: water–acetonitrile (40:60); flow rate: 1.0 mL/min; temperature: ambient; UV detection: 350 nm; mass spectrometric detection: atmospheric pressure chemical ionisation in positive ion mode.)

herb [24]. However, a recent publication reported that the contents of *Z*-ligustilide were estimated to be 245 ± 2.94 and 285 ± 3.42 mg/100 g in two samples from Chuncheon and Sokcho, South Korea, respectively [20]. In our study, a KDG sample was collected from Jilin, China, in October 2003 and was analyzed by the presently developed chromatographic method. A large chromatographic peak (peak 5) was observed at the same t_R of peak 3. However, its UV spectrum was found inconsistent with that of compound **3**. In our subsequent confirmatory analyses by using HPLC–APCI-MS, protonated ion of ligustilide at 191 *m*/*z* could not be found. It is worth also noting that *E*-ligustilide (peak 1) did not appear in the HPLC chromatogram, in which it should simultaneously co-exist with *Z*-ligustilide (peak 3) (Fig. 4). Therefore, it can be concluded that ligustilide was not present in the root of *Angelica gigas* Nakai. Moreover, additional *Z*-ligustilide peak could not be observed in the HPLC chromatogram of KDG sample after the concentrate was spiked with *Z*-ligustilide. This observation indicates that *Z*-ligustilide (peak 3) and the unknown compound (peak 5) in the samples could not be resolved under the present chromatographic conditions.

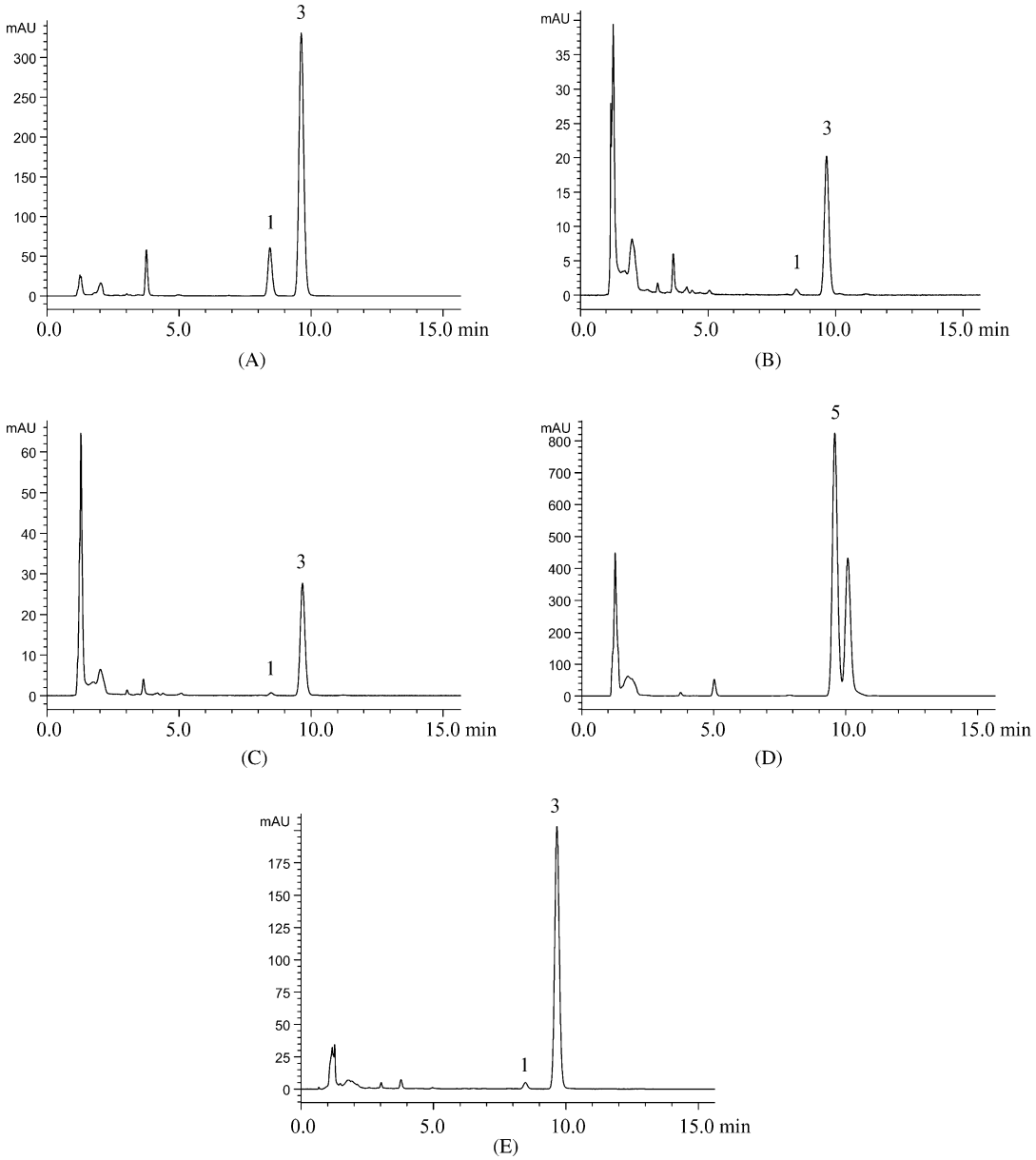

Fig. 4. Chromatogram of methanolic extract of the roots of *Angelica sinenisis* (Oliv.) Diels (A), *A. acutiloba* Kitagawa (B), *A. acutiloba* Kitagawa var. *sugiyamae* Hikino (C), *A. gigas* Naka (D) and the rhizome of *Ligusticum chuanxiong* Hort. (E). 1 = *E*-ligustilide; 3 = *Z*-ligustilide; 5 = unknown compound with same t_R as *Z*-ligustilide. (Analytical column: Alltima C_{18}, 5 μm, 150 mm × 4.6 mm; guard column: C_{18}, 5 μm, 7.5 mm × 4.6 mm; injection sample volume: 10 μL; mobile phase: water–acetonitrile (40:60); flow rate: 1.0 mL/min; temperature: ambient; detection: 350 nm.)

Table 1
A summary of contents for *Z*-ligustilide, *E*-ligustilide and total ligustilides in seven samples of *Angelica sinensis* and related umbelliferous herbs

Sample	Source	Ligustilide content (mg/g)[a]		
		Z-Ligustilide	*E*-Ligustilide[b]	Total
CDG-1	Minxian, Gansu, P. R. China	12.2 ± 0.20	1.96 ± 0.03	14.2
CDG-2	Pingwu, Sichuan, P. R. China	14.2 ± 0.34	1.72 ± 0.05	15.9
CDG-3	Heqing, Yunnan, P. R. China	9.36 ± 0.26	0.916 ± 0.03	10.3
JDG-1	Japan	0.729 ± 0.03	0.0280 ± 0.001[c]	0.757
JDG-2	Japan	0.987 ± 0.03	0.0259 ± 0.001[c]	1.01
CCX-1	Pengzhou, Sichuan, P. R. China	16.1 ± 0.22	0.343 ± 0.01	16.4
CCX-2	Chengdu, P. R. China	15.2 ± 0.16	0.323 ± 0.01	15.5

CDG-1 to CDG-3 were the whole roots of *Angelica sinensis* (Oliv.) Diels. JDG-1 was the coarse granule in a commercially available sample of the root of *Angelica acutiloba* Kitagawa produced by Uchida Wakanyaku, Tokyo, Japan (lot YA352620). JDG-2 was the coarse granule in a commercially available sample of the root of *Angelica acutiloba* Kitagawa var. *sugiyamae* Hikino produced by Tochimoto, Osaka, Japan (lot no. 060103). CCX-1 to CCX-2 were the rhizome of *Ligusticum chuanxiong* Hort.

[a] The value is mean ± S.D. (n = 4). The value is expressed in 3 significant figures.

[b] The amount is expressed in terms of *Z*-ligustilide.

[c] 2.0 g of sample powder was used to compensate the low content relatively of *Z*-ligustilide.

3.3. Quantitative analysis

For quantitation of *Z*-ligustilide, its stability remained one of the major technical concerns. *Z*-ligustilide is readily isomerized in air and/or some solvent systems [27,28]. Its stability in storage was evaluated by dissolving Z-ligustilide in acetonitrile and methanol, respectively, and compared also with a methanolic extract of herb sample in ambient conditions. The amounts of *Z*-ligustilide in these solutions were determined, respectively after storage 0, 1, 2, 4, 7 and 15 days. By comparing the chromatographic peak areas, the level of *Z*-ligustilide were found to be 99.7 and 88.8%, respectively in acetonitrile and methanol on day 15, which indicated that the compound was relatively stable in acetonitrile. On the other hand, the choice of extraction solvent for *Z*-ligustilide in herbs was further compared. Sample was first extracted with methanol and acetonitrile, respectively, then the amount of *Z*-ligustilide was determined. Results showed that the contents of *Z*-ligustilide contents were comparable in both extracts of methanol and acetonitrile. However, persistent turbidity was observed in sample extracted with acetonitrile whilst the methanolic counterpart remained clear throughout. The stability of *Z*-ligustilide in methanolic extract was also tested by the above described method. A 99.8% of peak area was determined even if it was stored in ambient conditions over the period, which showed that *Z*-ligustilide was relatively stable in methanol. Therefore, acetonitrile was recommended as the solvent for preparation *Z*-ligustilide standard solution whilst methanol was used for extraction of *Z*-ligustilide in herb samples.

Z-Ligustilide was quantified in samples using external standard method with reference marker. The method validation data indicated that the procedure was satisfactory. The linearity was determined in the concentration range of 10.6–425.6 mg/L with nine different concentration standard solutions. The linear regression of concentration versus peak area was expressed as $y = 16.472x - 17.535$ in which y is the concentration of *Z*-ligustilide in working calibration solution whilst x is the peak area of *Z*-ligustilide, and their correlation coefficient was found to be 0.9998. This regression equation was used for quantifying *Z*-ligustilide in all sample solutions.

Method reproducibility was evaluated by six injections of standard solutions and six replicates analysis of sample solutions, respectively. Precision of replicated injections was determined and the relative standard deviation (R.S.D.) of *Z*-ligustilide content was reported as 0.78% (n = 6). The R.S.D. of the content of *Z*-ligustilide in samples replicated was estimated to be 0.55% (n = 6).

The recovery of *Z*-ligustilide was determined by spiking sample with different concentration levels, namely: 50, 100 and 150% of *Z*-liugstilide in the samples. The recoveries were estimated as 98.3 ± 1.66% (mean ± R.S.D., n = 3), 96.8 ± 0.93% (n = 3) and 101.7 ± 1.24% (n = 3), respectively. The average recovery was 98.9 ± 2.52% (n = 9).

It is worthnoting that *E*-ligustilide and *Z*-ligustilide are stero-isomeric in nature, they have the same conjugated system in their structures, and therefore their UV absorption spectra highly resemble each other (Figs. 1, 2C and E). Attempt to isolate the *E*-ligustilide for assay was found difficult and met with little success. Therefore, the amount of *E*-ligustilide was expressed in terms of *Z*-ligustilide in the present works. The calibration curve and linear regression equation of *Z*-ligustilide was applied to evaluate the amount of *E*-ligustilide in samples. The results indicated that *E*-ligustilide was present as a small proportion whilst *Z*-ligustilide remained as the predominant component in the samples of CDG, JDG and CCX. A total amount of ligustilide was estimated from the sum of *Z*-ligustlide and *E*-ligustilide in seven samples (Table 1).

4. Conclusions

Z-Ligustilide commonly co-exists in herbs with other phthalide-typed compounds which makes it difficult to separate from its isomers using previously reported HPLC methods. By choosing an appropriate UV absorption wavelength

at 350 nm, satisfactory chromatographic separation of *Z*-ligustilide with its adjacent peaks could be observed under RP-HPLC conditions for four tested herbal species of roots of *Angelica sinensis* (Oliv.) Diels and related medicinal plants. Acetonitrile was used in the preparation of *Z*-ligustilide standard solutions, a stable and repeatable standard solution was obtained. Method validation data indicate that the present method is suitable for the determination of *Z*-ligustilide in the roots of *Angelica sinensis* (Oliv.) Diels, *Angelica acutiloba* Kitagawa, *Angelica acutiloba* Kitagawa var. *sugiyamae* Hikino and the rhizome of *Ligusticum chuanxiong* Hort. The present HPLC procedure makes it possible for analyzing a large amount of samples with its simplicity in analytical procedures and good reproducibility. However, the same HPLC condition is unable to separate *Z*-ligustilide from an interfering peak in the extract of the root of *Angelica gigas* Nakai. The employment of HPLC–APCI-MS is crucial for confirmatory studies in order to eliminate any false positive identification.

Characteristic fragrance has been used as an experience criterion of assessing the quality of Radix Angelicae Sinensis due to the presence of volatile oils. The top quality is usually recognized as the most perfumed as a result of the large amount of the volatile oils. The present results further indicate that Radix Angelicae Sinensis and Rhizoma Chuanxiong contain large amount of ligustilide. Although ligustilide is a bioactive compound, it is not the unique characteristic compound as chemical identity for Radix Angelicae Sinensis. Both of the two herb species are the crude sources of ligustilide. The two analyzed Japanese Dangui samples, the processed products of roots, were found containing lower ligustilide contents. It agreed with previous literature reports that the content of ligustilide in Japanese Danggui was always lower than that in Chinese Danggui [20,29].

The analytical results further demonstrate that ligustilide is not found in the root of *Angelica gigas* Nakai, although the plants of *Angelica sinensis* (Oliv.) Diels and *Angelica gigas* Nakai belong to same genus. In contrast, the plants of *Angelica sinensis* (Oliv.) Diels and *Ligusticum chuanxiong* Hort. belong to different genus, both contain similar content of ligustilide.

Acknowledgements

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