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Characterization of lignans in *Schisandra chinensis* oil with a single analysis process by UPLC-Q/TOF-MS

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ABSTRACT

Schisandra chinensis is a medicinal and edible plant that contains various bioactive compounds. Among these, lignans are the major functional compounds. Nevertheless, detailed information about lignans in *Schisandra chinensis* oil remains scarce. A powerful UPLC-Q/TOF-MS method was established for the rapid identification of the lignan constituents of *Schisandra chinensis* oils. The results showed that 21 lignans have been unambiguously identified, and four lignans have been tentatively identified in the *Schisandra chinensis* oils. In addition, semi-quantitative analysis indicated that the total lignan content in the *Schisandra chinensis* oils was distributed from 67.73 ± 0.06 to 87.61 ± 1.83 mg/g. Schisandrin and schisandrin B were the most abundant lignans in the *Schisandra chinensis* oils, their content ranging from 15.85 ± 0.09 to 20.57 ± 0.38 mg/g. Additionally, this study provided a systematic characterization of lignans in *Schisandra chinensis* oil and indicated that the oil might be used as lignan-related functional foods.

1. Introduction

Schisandra chinensis (*S. chinensis*), or Wuweizi (Fructus Schisandrae, Chinese Magnoliavine Fruit), is a traditional Chinese medicine that is wide spread in northeast Asia (Japan, China, and Korea) (Szopa et al., 2017). Recently, it has been listed as a medicinal and edible plant (Mocan et al., 2016b). The Chinese Pharmacopoeia records indicated that it can cure night sweating, enuresis and chronic cough, and it presents anti-diabetes and anti-hepatitis properties (Panossian and Wikman, 2008; Sowndhararajan et al., 2018). Most of the bioactivity and nutritional value is related to the lignans of *S. chinensis*. The most popular research has been about isolated, identified and evaluated compounds from *S. chinensis* fruits, including lignans, volatile oil, phenolic components, polysaccharide, and proanthocyanins (Mocan et al., 2016a; Szopa et al., 2017).

However, few studies have been established on *S. chinensis* oils. According to our pre-experiments, the fruits of *S. chinensis* fruits also contain oils in an amount of approximately 10% (dry weight). This amount indicates that *S. chinensis* might be a potential functional oil resource. To date, most of research reports have been about the essential oil of *S. chinensis,* including its extraction and bioactivity evaluation (antioxidant and antibacterial) (Ma et al., 2012). The protective effect of *S. chinensis* oil on pancreatic b-cells in diabetic rats has been explored (An et al., 2015). However, there are few reports on the chemical constituents of *S. chinensis* oil. Therefore, it is of great significance to explore the chemical compositions of *S. chinensis* oil.

Among all the bioactive substances existed in *S. chinensis* fruits, lignans are the most widely studied compounds. Data from several studies suggested that the content and species of lignans in *S. chinensis* fruits are very abundant and bioactivities. The bioactivity of *S. chinensis* is mainly related to its lignans (Wan et al., 2017; Sowndhararajan et al., 2018). Schisandrin, schisandrin B, schisandrol B and deoxyschizandrin are the most abundant and functional dibenzocyclooctadiene lignans in *S. chinensis* (Hu et al., 2014a; Sowndhararajan et al., 2018). A number of studies indicated that these compounds exhibit anti-inflammatory, hepato-protective, anticancer, anti-oxidative effects, anti-oxidative and anti-cardiovascular disease traits, as well as improve cognitive function (Szopa et al., 2017; Sowndhararajan et al., 2018). However, little is known about the lignans that compose *S. chinensis* oil. Therefore, the characteristics of the lignans in *S. chinensis* oil have attracted our attention, and this study might be meaningful to the further development, practical application, and functional evaluation of the *S. chinensis* oils.

Over the past two decades, many liquid chromatography methods have been developed for the determination of lignans in *S. chinensis*.

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These are used to determine the structural characteristics (Huang et al., 2007), molecular species (Deng et al., 2008), purification and quantification (Lu and Chen, 2009; Mocan et al., 2016a), and the pharmacokinetics of lignans (Liu et al., 2017a). Among all the analysis technologies, MS^E has shown that it has great power in the analysis of complex pharmaceutical and biomedical samples (high sensitivity, simultaneous determination, low detection limits and without sample pretreatment) (Su et al., 2016; Liu et al., 2017a; Rybin et al., 2017). All the useful information can be obtained by a single analytical process. Additionally, this method has also been widely applied in the analysis of complex pharmaceutical and biomedical samples (Frenich et al., 2014; Mocan et al., 2016a; Donazzolo et al., 2017). Accordingly, in view of the efficiency and feasibility of analysis, UPLC equipped with an MS^E detection device of MS^E has been selected in this study.

The aim of this study was to comprehensively identify and quantify the nature of lignans in *S. chinensis* oil. UPLC-Q/TOF-MS technology along with MS^E data acquisition were developed for the determination of lignans. The results of this study were expected to be helpful for the comprehensive recognition, further pharmacological research, and potential application of *S. chinensis* oil.

2. Materials and methods

2.1. Materials and reagents

S. chinensis fruits were purchased from Lan xinbao Co., Ltd. (Jilin, China). They were identified by the food and drug administration of Yanbian (Jilin, China). More specifically, the *S. chinensis* fruits obtained in August 2017 belong to the city of Baishan (Jilin, China) and the *S. chinensis* fruits obtained in June 2018 belong to the city of Tonghua (Jilin, China). All the *S. chinensis* fruits were dried, ground, and stored at 4 °C until used. The standard schisandrin B (> 98%) for semi-quantification was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China).

Formic acid, *n*-hexane, and chloroform were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Methanol and acetonitrile were of HPLC grade and obtained from Fisher Scientific Co., Ltd. (Shanghai, China). Water was purified with a Milli-Q water purification system (Millipore Co., Ltd. Milford, USA). High-purity nitrogen (99.999%) was purchased from Shanghai Likang Gas Co., Ltd. (Shanghai, China).

2.2. Preparation of S. chinensis oil

2.2.1. S. chinensis oil prepared by ultrasound-assisted n-hexane extraction

S. chinensis powders were extracted by n-hexane (m/v = 1:7) with the assistance of ultrasonic water bath (Xinzhi biotechnology Co., Ltd, KQ3200E, Ningbo, China). It was operated at an ultrasonic output power of 240 W and a frequency of 40 kHz. The sonication time and temperature were 30 min and 20 °C, respectively. Then, the mixtures were left for 3–4 hours, and the supernatant liquid was collected and concentrated at 45 °C using a rotary evaporator (Eyela, N-1100, Shanghai, China) to obtain the oils. All the samples were extracted by this method, and the obtained oils were named Oil A and Oil B.

2.2.2. S. chinensis oil prepared by supercritical CO_2 fluid extraction

The extraction experiments were carried out in a semi-pilot supercritical CO_2 fluid extraction (SFE) equipment (Hua'an supercritical fluid extraction equipment Co., Ltd, HA-120-50-01, Nantong, China). In the SFE process, approximately 350 g of *S. chinensis* powder was placed in the extractor. The temperatures of the extraction kettle, separation kettle I and separation kettle II were 37, 60 and 30 °C, respectively. The pressures of the extraction kettle and separation kettle were approximately 20–25 MPa. The flow rate of CO_2 was approximately 50–55 L/h. Only the sample obtained in June 2018 was extracted by SFE technology. The corresponding oil was named Oil C.

2.3. Pretreatment of oil samples and standard solutions

An 0.01 g aliquot of the extracted oils was diluted with 10 mL methanol/chloroform (v/v = 3:1), vortexed and dissolved thoroughly. Then, the dissolved solution was transferred to sample vials for UPLC-Q/TOF-MS analysis. All the oil samples were analyzed in triplicate.

The standard material of schisandrin B was accurately weighed and dissolved in methanol/chloroform (v/v = 3:1) to obtain a 1 mg/mL mother solution. Then, the mother solution was diluted to 20 μ g/mL with methanol. Finally, a four-fold step dilution was conducted from the mother solution five times. The corresponding concentrations of standard solution were 1.95×10^{-2}, 7.81×10^{-2}, 0.31, 1.25, 5 and 20 μg/mL.

2.4. UPLC-Q/TOF-MS analysis

S. chinensis oil samples were analyzed via high resolution mass spectrometry analysis, carried out on a Waters ACQUITY UPLC I-Class system (Waters, Shanghai, China) with a BEH C18 column (100 mm × 2.1 mm, 1.7 μm). The mobile phase consisted of solvent A (0.1% formic acid water) and B (0.1% formic acid/methanol/acetonitrile), with a total flow rate of 0.4 mL/min. The column temperature was 50 °C, and the injection volume was 1 μL. The elution test was performed as follows: 0 - 0.5 min, 10% B linear; 0.5–2 min, 50% B; 2–7 min, 80% B linear; 7–15 min, 100% B; 15–17 min, 10% B.

Mass spectrometry was performed using a Waters Vion-IMS-Q-ToF mass spectrometer (Waters, Shanghai, China) operating in both positive and negative ion electrospray ionization. The detection used the MS^E function in profile mode with a 6 V collision energy in function one (parent ions experiment: MS^E-L) and a collision energy ramp of 20–40 V in function two (fragment ions experiment: MS^E-H) (frequency of low to high collision switch was 30 Hz). The scan range was from 50 to 1000 m/z. The scan time for each function was set to 0.2 s. Nitrogen (99.999%) was used as the desolvation gas (1000 L/h) and cone gas (50 L/h). Ion monitoring conditions were defined as capillary voltage of 2.0 kV, source temperature of 120 °C, and desolvation temperature of 500 °C. Data were acquired and processed using UNIFI 1.8.1 (Nonlinear Dynamics, Newcastle, UK) and QI analysis software (Nonlinear Dynamics, Newcastle, UK).

2.5. Identification of lignans in the S. chinensis oils

The modified process of lignan identification in the *S. chinensis* oils was applied in this research (Dong et al., 2016; Huang et al., 2007; Liu et al., 2017a). To obtain better information about lignans, the detection parameters (electrospray voltage, capillary voltage and capillary temperature) were optimized manually. The mass spectra data of the lignans in the *S. chinensis* oils were acquired in the positive ion mode, and the main adduct ion was $[M+Na]^+$. The forms of $[M+H]^+$, $[M+NH_4]^+$ and $[M\text{-}H_2O+H]^+$ were also observed during the electrospray process. In addition, the absolute value of mass detection error for each component was less than 3 ppm. The lignans were identified by comparing their information of parent ions (MS^E-L) and fragment ions (MS^E-H) to the substance library.

2.6. Semi-quantification of lignans in the S. chinensis oil

The external reference method was used for the semi-quantitative analysis of the lignans in the *S. chinensis* oils. All the oil samples were analyzed in triplicate.

3. Results and discussion

3.1. Compositions of lignans in the S. chinensis oils

Lignans are a subclass of phenolic compounds that are widely

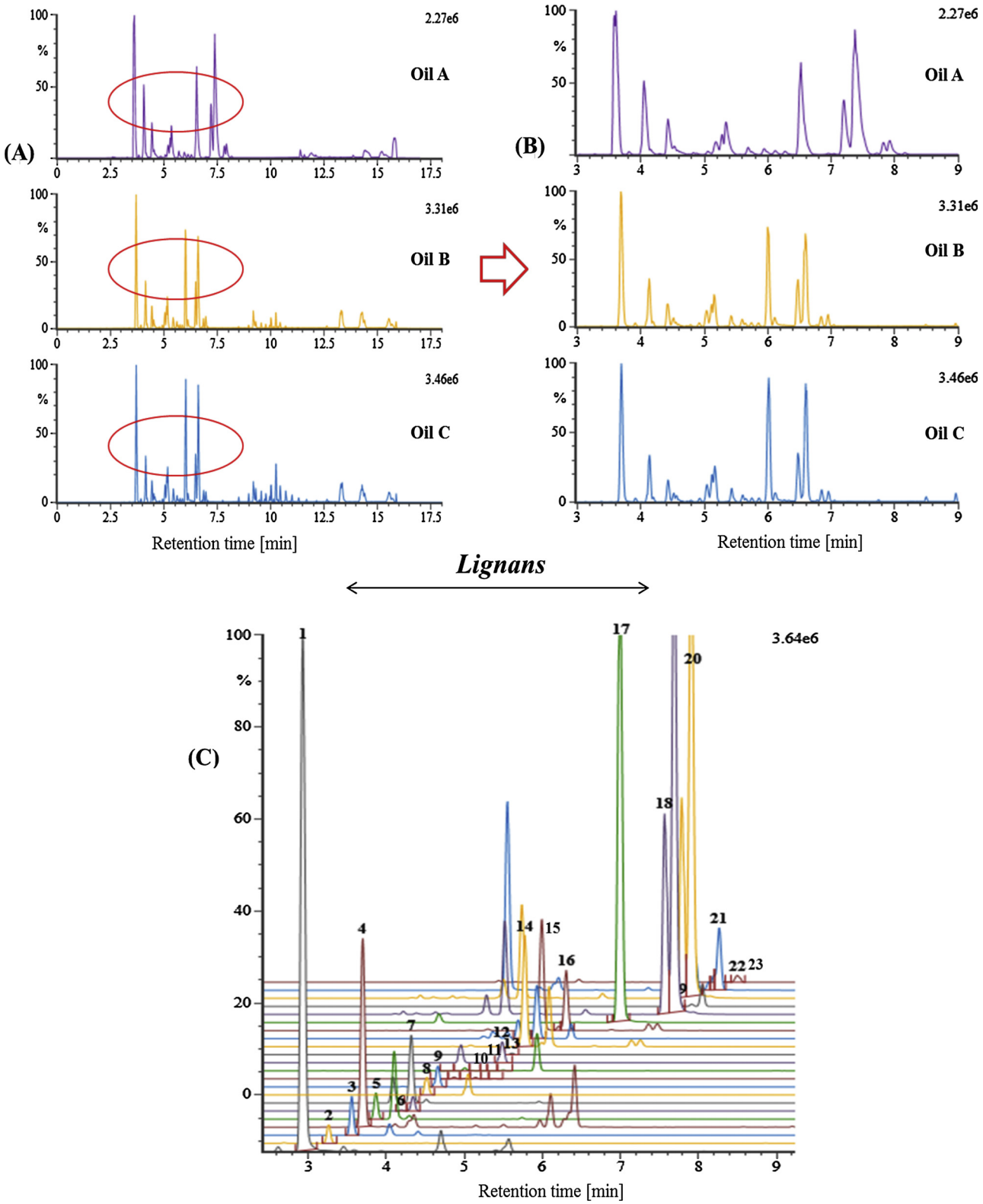


Fig. 1. (A) The BPI chromatogram of *S. chinensis* oil A, B and C obtained by an MS^E data collection technique (UPLC-Q/TOF-MS) in positive mode. (B) Details of the separation of lignans achieved between 3 and 9 min. (C) Extracted ion chromatograms (EICs) obtained when the optimum parameters for the separation and detection were used; the numbered compounds correspond to Table 1.

distributed in the fruits of *S. chinensis*. The method of UPLC-Q/TOF-MS was applied to the characterization of lignans in the *S. chinensis* oils in a single chromatographic run. The base peak intensity chromatogram of lignans in the three *S. chinensis* oils obtained by UPLC-Q/TOF-MS are presented in Fig. 1(A). All the lignan compounds were identified in the region marked with a red circle. The magnification of the marked region is shown in Fig. 1(B). The typical extracted ion chromatograms of each identified compounds are exhibited in Fig. 1(C). There are approximately 23 ion peaks, and twenty-one of them were unambiguously identified as different lignans. Four deduced lignans matched with the

Table 1
Lignans identified in the three *S. chinensis* oils extracted by ultrasound-assisted n-hexane extraction and supercritical CO_2 fluid technology.

Peak [a]	Component name	Identification				Quantification [mg/g]		
		Formula	Neutral mass [*m/z*]	MS^E-L, $[M + A]^+$	MS^E-H, [*m/z*] (Abundance, %)	Oil A	Oil B	Oil C
1	Schisandrin	$C_{24}H_{32}O_7$	432.2148	+Na, -H_2O+H	[369](100), [384](91), [331](57), [346](44), [354](33), [415](27)	18.62 ± 0.53	20.20 ± 0.26	20.57 ± 0.38
2	Gomisin D	$C_{28}H_{34}O_{10}$	530.2152	+Na, +NH_4	[341](100), [401](61), [352](37), [383](34), [326](22),[270](5)	0.42 ± 0.01	0.72 ± 0.01	0.74 ± 0.01
3	Gomisin J	$C_{22}H_{28}O_6$	388.1886	+Na, +H	[330](100), [315](75), [326](69), [339](33), [300](25), [181](1)	0.62 ± 0.00	1.21 ± 0.03	1.25 ± 0.04
4	Schisandrol B	$C_{23}H_{28}O_7$	416.1835	+Na, +NH_4	[330](100), [353](84), [369](61), [368](57), [339](33), [399](31)	6.02 ± 0.32	6.96 ± 0.13	6.73 ± 0.08
5	Angeloylgomisin H	$C_{28}H_{36}O_8$	500.2410	+Na, +NH_4	[317](100), [401](74), [370](66), [386](56), [301](35), [483](29)	0.98 ± 0.02	1.04 ± 0.03	0.99 ± 0.03
6	Angeloylgomisin Q	$C_{29}H_{38}O_9$	530.2516	+Na, +NH_4	[342](100), [415](55), [371](50), [312](32), [327](31), [356](28)	0.27 ± 0.02	< 0.01	0.02 ± 0.00
7	Tigloylgomisin H	$C_{28}H_{36}O_8$	500.2410	+Na, +NH_4	[370](100), [317](99), [355](81), [483](54), [401](51), [384](39)	1.76 ± 0.02	2.83 ± 0.05	2.72 ± 0.05
8	Gomisin G	$C_{30}H_{32}O_9$	536.2046	+Na, +NH_4	[340](100), [342](65), [415](51), [371](47), [325](37), [285](5)	0.49 ± 0.01	0.64 ± 0.01	0.66 ± 0.02
9	Pregomisin	$C_{22}H_{30}O_6$	390.2042	+Na, +H	[341](100), [356](51), [340](43), [343](37), [326](28), [167](19)	0.50 ± 0.02	0.61 ± 0.03	0.67 ± 0.02
10	Gomisin E	$C_{28}H_{34}O_9$	514.2203	+Na, +NH_4	[340](100), [415](51), [371](46), [312](29), [327](28), [356](26)	0.08 ± 0.00	0.03 ± 0.00	0.04 ± 0.01
11	Angeloylgomisin P	$C_{28}H_{34}O_9$	514.2203	+Na, +NH_4	[342](100), [415](55), [371](50), [312](32), [327](31), [356](28)	0.56 ± 0.01	0.08 ± 0.00	0.08 ± 0.01
12	Schisantherin A	$C_{30}H_{32}O_9$	536.2046	+Na, +NH_4	[341](100), [415](99), [340](43), [356](41), [373](29), [383](24)	0.46 ± 0.02	0.73 ± 0.00	0.91 ± 0.05
13	Schisantherin D	$C_{29}H_{28}O_9$	520.1733	+Na	[340](100), [342](65), [371](47), [325](37), [327](20), [312](10)	0.04 ± 0.00	0.02 ± 0.00	0.03 ± 0.01
14	Schisanhenol	$C_{23}H_{30}O_6$	402.2042	+Na, +NH_4	[331](100), [388](81), [333](43), [233](41), [372](39), [235](39)	2.18 ± 0.09	3.51 ± 0.06	4.26 ± 0.11
15	Schisantherin B [C]	$C_{28}H_{34}O_9$	514.2203	+Na, +NH_4	[366](100), [385](58), [437](52), [351](50), [328](20), [353](20)	1.49 ± 0.02	2.28 ± 0.03	2.45 ± 0.07
16	Gomisin K3	$C_{23}H_{30}O_6$	402.2042	+Na, +NH_4	[371](100), [355](93), [341](84), [388](36), [301](20), [181](8)	0.96 ± 0.01	1.73 ± 0.02	2.12 ± 0.04
17	Deoxyschisandrin	$C_{24}H_{32}O_6$	416.2199	+Na, +NH_4	[402](100), [370](65), [355](64), [347](63), [386](42), [332](32)	8.78 ± 0.08	11.65 ± 0.33	14.57 ± 0.24
18	Gomisin H	$C_{23}H_{30}O_7$	418.1992	+Na, +H	[370](100), [401](94), [386](66), [354](47), [331](47), [316](43)	5.61 ± 0.02	6.26 ± 0.23	7.05 ± 0.57
19	Angeloylgomisin O	$C_{28}H_{34}O_8$	498.2254	+Na	[368](100), [353](72), [399](69), [343](34), [357](33), [337](17)	0.09 ± 0.00	0.23 ± 0.00	0.25 ± 0.01
20	Schisandrin B	$C_{23}H_{28}O_6$	400.1886	+Na, +H	[370](100), [386](82), [329](61), [331](53), [368](25), [301](20)	15.80 ± 0.06	17.61 ± 0.77	18.13 ± 0.60
21	Gomisin O [Epi-]	$C_{23}H_{28}O_7$	416.1835	+Na	[330](100), [368](99), [353](63), [354](37), [339](37), [313](36)	1.58 ± 0.16	1.87 ± 0.01	2.18 ± 0.06
22	Gomisin R	$C_{22}H_{24}O_7$	400.1522	+Na	[314](100), [353](63), [368(42), [383](26), [313](7), [300](7)	0.32 ± 0.01	0.25 ± 0.00	0.26 ± 0.01
23	Angeloylgomisin R	$C_{28}H_{34}O_8$	482.1941	+Na	[368](100), [330](76), [315](54), [353](50), [399](37), [339](20)	0.06 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
		Total content of lignans				67.73 ± 0.06	80.49 ± 0.99	87.61 ± 1.83

Oil-A: Extracted from *S. chinensis* (Baishan, Jilin, China) by UAE; Oil-B: Extracted from *S. chinensis* (Tonghua, Jilin, China) by UAE; Oil-C: Extracted from *S. chinensis* (Tonghua, Jilin, China) by SFE.

[a] Peak numbering refer to peaks in Fig. 1 **(C)**. A: $[M + Na]^+$, $[M+H]^+$, $[M + NH_4]^+$, $[M\text{-}H_2O+H]^+$. Abbreviations: MS^E-L: low collision energy; MS^E-H: higher collision energy.

other two peaks based on the analysis of MS information. All the 25 corresponding lignans are listed in Table 1. For all the identified lignans in the three *S. chinensis* oils (Table 1), there are some questions that need to be emphasized. First, all the lignans identified in the three *S. chinensis* oils have been detected in the extracts of *S. chinensis*. Second, their elution principles were slightly inconsistent with previous reports (Huang et al., 2007; Deng et al., 2008; Zhu et al., 2015; Kim et al., 2015). In this study, schisandrin was eluted as the first substance, and schisantherin B and schisantherin C (Peak 15), as well as gomisin O and epigomisin O (Peak 21), were assigned to the same ion peak. Additionally, they were tentatively assigned two compounds to one peak. In addition, the elution sequence of schisantherin A–D, was A, D, B and C. Pregomisin was eluted between schisandrin and schisantherin A. While in previous research, the elution sequence of schisantherin A–D, was A, B, C and D. The pregomisin was eluted later than schisandrin B (Huang et al., 2007), and another report indicated that it was eluted far before schisandrin B, it kept behind the elution of schisandrol B (Mocan et al., 2016a). This kind of inconsistent phenomenon has occurred in previous research (Liu et al., 2017a; Dong et al., 2016). The reason might be that the same compounds respond variously to different equipment or the tested materials were different. Therefore, the differences in the elution sequence of the lignans between *S. chinensis* oil and *S. chinensis* are acceptable.

In addition, it should be noted that the elution region of lignans in Oil A was broader than those of Oil B and Oil C. Possible reasons were different column efficiency (polluted or outdated) or systematic error (detection environments or equipment conditions) during various detection periods. Under the above-mentioned conditions, the increases or decreased of compound elution times are reasonable.

3.2. Fragmentation analysis of lignans in S. chinensis oils by UPLC-Q/TOF-MS

To elucidate the whole identification process of lignans in *S. chinensis* oil by UPLC-Q/TOF-MS, the standard compound schisandrin B was taken as an example. The schisandrin B was chromatographed to determine its retention time, information of parent ions (MS^E-L) and fragment ions (MS^E-H).

The MS^E-L and MS^E-H spectra information of schisandrin B (peak 20) in standard solution and *S. chinensis* oil A, B and C are shown in Fig. 2. The following evident information can be obtained from Fig. 2. First, under the same detection pathway, they have the same retention time. Second, the MS^E-L and MS^E-H analysis of theoretical adduct ions and MS fragments indicated that they are the same substance. Third, an analysis of the comparison of MS theoretical values indicated that the same adducts ions and MS fragments produced by various samples had different intensities. Therefore, it is normal that the component identified in the Oil A, Oil B and Oil C was the same as standard schisandrin B.

Specifically, according to the MS^E-L spectra information, it is obviously that the main adduct ion of schisandrin B (Peak 20) formed during the analysis process was $[M+Na]^+$ at *m/z* approximately 423.1776 and $[M+H]^+$ at *m/z* 401.1958. Schisandrin B was identified in both the standard solution and oil samples, in which the two adduct ion forms were generated. Normally, they had the same chemical formula of $C_{23}H_{28}O_6$ (*m/z* = 400.1886). In the comparative analysis of the information captured in MS^E-L and MS^E-H, we can draw the conclusion that they were essentially the same. According to our analysis, MS^E-H produced the feature fragments ions at *m/z* 370 (100), 386 (82), 331 (53), 301 (20), *etc.*, which were observed in the MS^E-H spectra. The information specific to the MS^E-H spectra is listed in Table 2. The peaks were at *m/z* 370 and 386 owing to the loss of OCH_2 at C-5 and CH_2 at C-4, respectively. The peak of *m/z* 329 was formed by the loss of the C_5H_{11} at the eight-membered dibenzene ring. Another product ion at *m/z* 331, was due to the loss of C_5H_9 at the eight-membered dibenzene ring. The loss of CH_4O at the position of C-5, formatted the compounds of $C_{22}H_{24}O_5$, *m/z* 368. The peak of *m/z* 339 was justified by the combined losses of CH_2 at C-5 and the C_2H_8O at the eight-membered dibenzene ring. These results were consistent with previous research (Huang et al., 2007). It is reported that the C—C bonds of carbocyclic eight-membered rings existing in natural dibenzocyclooctadiene lignans are weaker than those in the aromatic ring. In addition, the fragments of M-100, M-172 and M-143 were the typical mass fragments of schisandrin B (Huang et al., 2007). Therefore, the schisandrin B compound has been totally identified, and these principles could be applied in the identification of other lignans.

The most abundant compound schisandrin (peak 1) and the tentatively identified compound of schisantherin B (C) (peak 15) in the Oil A, Oil B and Oil C have been analyzed in detail. Their MS^E spectra are presented in Fig. 3(A) and (B). Similarly, their specific MS^E-H spectra information are shown in Table 2. From Fig. 3(A) and Table 2, it is easy to draw the conclusion that all the identified schisandrin (Peak 1) in the three oils was occupied as the main adduct ion of $[M\text{-}H_2O+H]^+$ at *m/z* 415.2122 and the adduct ion of $[M+Na]^+$ at *m/z* 455.2046. According to the spectral information of MS^E-H, the peak of *m/z* 369 was produced by the loss of CH_3 at C-5, OCH_3 at C-3′ and OH at C-8′. The peak of *m/z* 384 was formed by the loss of OCH_3 at C-4′ and OH at C-8′. The product ions at *m/z* 331 and 346 were due to the loss of $C_6H_{13}O$ and $C_5H_{10}O$ at the eight-membered dibenzene ring. The fragment of *m/z* 354 was formed with the loss of CH_3 at C-3′and C-4′, OCH_3 at C-5′ and OH at C-8′. The peak of *m/z* 415 was produced by the loss of OH at C-8′. Conspicuously, the compound identified as schisandrin in all oil samples had almost the same MS^E-L and MS^E-H spectra information. Thus, the peak is considered to be schisandrin. For the compounds of schisantherin B (C) (Peak 15), it belonged to the same ion peak. As seen in Fig. 3 (B) and Table 2, the MS^E spectra of schisantherin B (C) was identical under the detection process. This result indicated that at each identification stage, the feature ions belonging to them were the same. More accurate identification requires reference substances. On the basis of the MS^E-L information, the compound in question (peak 15 in Fig. 1(C)) was tentatively identified as the both possible compounds. All of this evidence indicated that the identification pattern of lignans in *S. chinensis* oil by UPLC-Q/TOF-MS was creditable.

3.3. Concentration of the identified lignans in the S. chinensis oils

Quantitative analyses were performed by the external standard method to make intuitional information about lignans in the *S. chinensis* oils, the. The regression equations were $y = 7.27 \times 10\hat{}5x + 8686.78$ ($R^2 = 0.9999$) and $y = 6.97 \times 10\hat{}5x + 13{,}617$ ($R^2 = 0.9999$), respectively. All calibration curves of the standard compounds of lignans presented good linearity and excellent correlation coefficients. The recovery of the analytical method was 93.24% and 95.12%, respectively. The CV% of components ranged from 0 to 8.09%. The limit of detection (LOD) was determined based on the signal to noise ratio of three. The limit of quantification (LOQ) was calculated as the lowest injection concentration of the detected components. The LOD was and the LOQ was $0.4 \times 10\hat{}{-6}$ and $1.95 \times 10\hat{}{-2}$ μg/mL, respectively. The results of the lignan content in the three *S. chinensis* oils are shown in Table 1. As shown in Table 1, it is clear to see that schisandrin, schisandrin B, deoxyschisandrin, schisandrol B and gomisin H were the most abundant lignans in the three *S. chinensis* oils. Their content in the *S. chinensis* oils was higher than 5 mg/g, and the rest of the lignans were lower than 5 mg/g. The content of those five kinds of lignans accounted for more than 75% (approximately 54–67 mg/g) of the total identified lignans in the three *S. chinensis* oils. The distribution and chemical structure of those five kinds of lignans in the three oil samples is exhibited in Fig. 4. Generally, their content in Oil C is higher than those of Oil A and Oil B, whether in single or total content. The total content of lignans in *S. chinensis* Oil A, B and C were 67.73 ± 0.06, 80.94 ± 0.99, and 87.61 ± 1.83 mg/g, respectively. This phenomenon might be caused by the differences in *S. chinensis* material and oil extraction methods. It

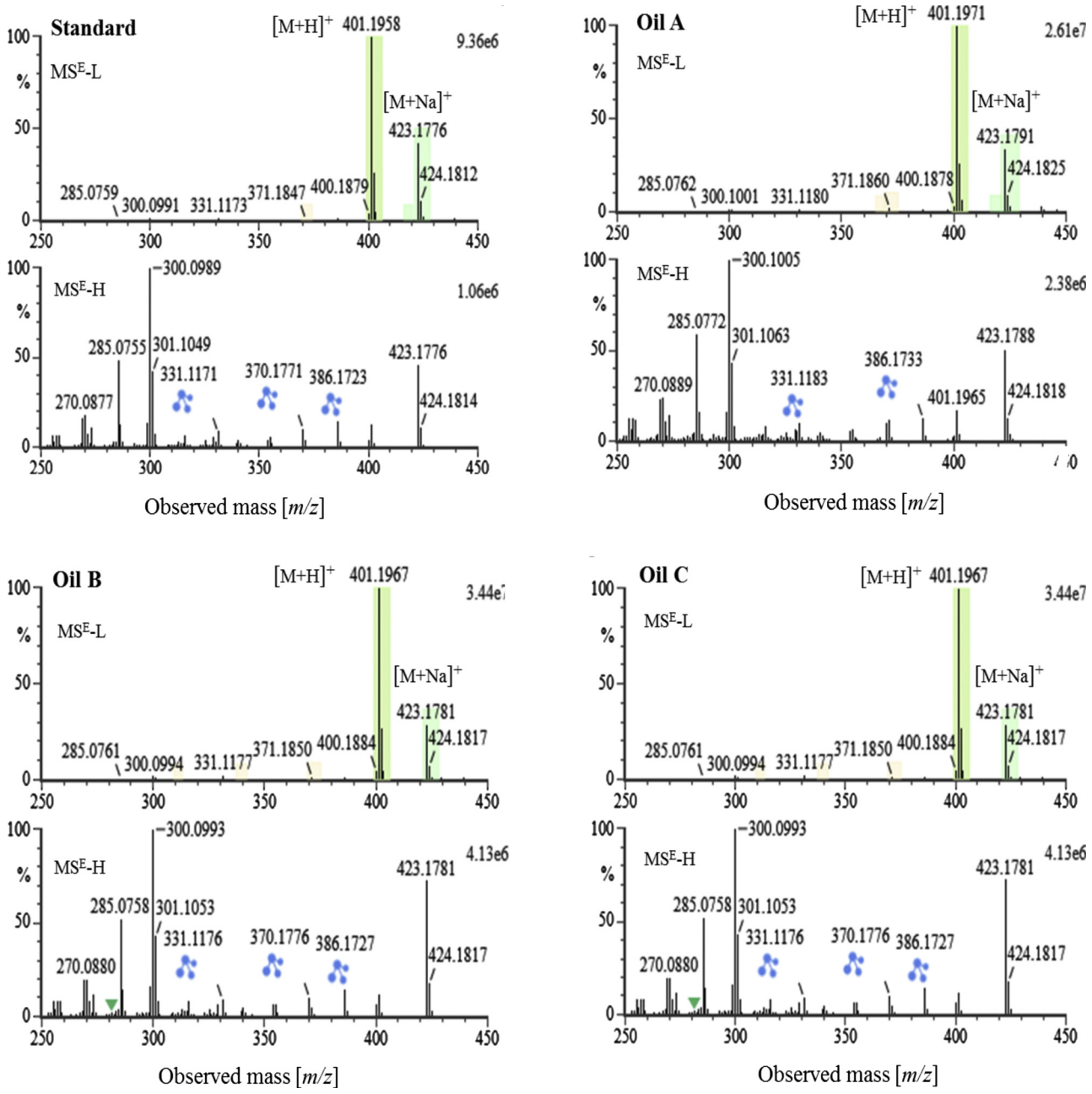
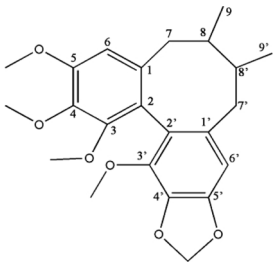
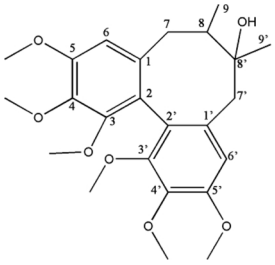
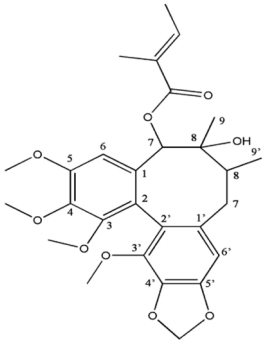


Fig. 2. Typical MS^E spectra of schisandrin B identified in standard solution and *S. chinensis* Oil A, B and C.

is well known that the SFE technology is an effective and suitable method for extracting oils. It has special thermodynamic properties, has high heat transferring and is environmentally friendly, and all components of the plants remain in the extract. For Oil A and Oil B, difference in content might be due to their maturity, variety, climate or growing environment. Meanwhile, the content trends of schisandrol B in oils is slightly different from other compounds. The reason might be ascribed to the operation or detection error. That is, the lignan constitution of various *S. chinensis* oil is the same, but their content is affected by the *S. chinensis* species and the oil extraction method. SFE technology is an alternative method for the *S. chinensis* oil extraction.

Compared to the previous reports, the content of most lignans in the *S. chinensis* oils were higher than that extracted from *S. chinensis* fruits. The most abundant compounds were schisandrin (approximately 18–20 mg/g) and schisandrin B (approximately 15–18 mg/g) in the *S. chinensis* oils. They have been widely investigated in bioactivity and content. Many studies indicated the anti-inflammatory activity, antitumor characteristics, hepato-protective property, anti-Alzheimer's effect, potent anti-oxidative activity and antidepressant-like effect of schisandrin B (Szopa et al., 2017; Wang et al., 2017; Liu et al., 2017b; Lai et al., 2017). Schisandrin has been indicated to protect against neurotoxicity and enhance cognitive performance (Wan et al., 2017; Sowndhararajan et al., 2018). The content of schisandrin and schisandrin B in methanol extract *S. chinensis* fruits were approximately 8 mg/g and 1 mg/g, respectively (Deng et al., 2008). Similarly, the amounts of schisandrin and schisandrin B from *S. chinensis* fruits by methanol extraction were the highest, with values of 5.68 and 7.94 mg/g, respectively (Huang et al., 2007). Additionally, the concentration of schisandrin and schisandrin B from *S. chinensis* fruits(F), leaves(L) and stems(S) by methanol were approximately 4.24 and 2.27 (F), 1.29 and 1.01 (L), 0.96 and 0.32 (S) mg/g, respectively (Mocan et al., 2016a). In addition, Dong et al investigated the patterns of seven lignans in 12 *Schisandra* species using ultrasonic-assisted temperature switch ionic liquid micro-extraction. The contents of schisandrin and schisandrin B

Table 2
The MS^E-H data of the standard schisandrin B and typical lignans compounds schisandrin and schisantherin B [C] in the *S. chinensis* oils.

Name/Structure	Peak number in Fig. 1 (C)	Proposed formula	Observed mass [m/z]	Mass error[ppm]	Proposed neutral loss
Schisandrin B 	20	$C_{23}H_{28}O_6$	400.1886	1.0	–
		$C_{22}H_{26}O_5$	370.1779	0.4	CH_2O
		$C_{22}H_{26}O_6$	386.1724	0.5	CH_2
		$C_{18}H_{17}O_6$	329.1020	0.2	C_5H_{11}
		$C_{18}H_{19}O_6$	331.1179	0.74	C_5H_9
		$C_{22}H_{24}O_5$	368.1618	−0.7	CH_4O
		$C_{20}H_{19}O_5$	301.0707	−0.9	C_3H_9O
Schisandrin 	1	$C_{24}H_{32}O_7$	432.2148	1.7	–
		$C_{22}H_{25}O_5$	369.1697	−1.5	$C_2H_7O_2$
		$C_{23}H_{28}O_5$	384.1931	0.5	CH_4O_2
		$C_{18}H_{19}O_6$	331.1176	−0.3	$C_6H_{13}O$
		$C_{19}H_{22}O_6$	346.1412	1.0	$C_5H_{10}O$
		$C_{21}H_{22}O_5$	354.1462	−2.8	$C_3H_{10}O_2$
		$C_{24}H_{31}O_6$	415.2115	−0.3	OH
Schisantherin B [C] 	15	$C_{28}H_{34}O_9$	514.2203	0.8	–
		$C_{22}H_{22}O_5$	366.1462	−0.6	$C_6H_{12}O_4$
		$C_{22}H_{25}O_6$	385.1646	−0.2	$C_6H_9O_3$
		$C_{25}H_{25}O_7$	437.1595	−0.1	$C_3H_9O_2$
		$C_{21}H_{19}O_5$	351.1227	−3.0	$C_7H_{15}O_4$
		$C_{19}H_{20}O_5$	328.1305	−0.1	$C_9H_{14}O_4$
		$C_{21}H_{21}O_5$	353.1384	−2.4	$C_7H_{13}O_4$

were only 0.72 and 0.22 mg/g, respectively (Dong et al., 2016). Additionally, all the reported contents were much lower than the content shown in our research. Otherwise, researchers compared the pressure liquid extraction, reflux and sonication extraction methods for nine lignans in *S. chinensis* fruits. The content of schisandrin ranged from 5.72 to 6.37 mg/g, while schisandrin B ranged from 0.64 to 0.81 mg/g (Lee and Kim, 2010). The varible contents of schisandrin and schisandrin B in the different research reports might be due to the various raw materials, different pretreatments, extraction methods or detection conditions.

Except for schisandrin and schisandrin B, deoxyschisandrin was distributed from 8.78 ± 0.08 to 14.57 ± 0.24 mg/g in the three *S. chinensis* oils. It is acknowledged that deoxyschisandrin is one of the main pharmacologically active ingredients of *S. chinensis*, which shows anti-hepatotoxic effects (Hu et al., 2014b). In addition, it is widely reported that schisandrol B (or gomisin A) exerts a potent vaso-relaxation activity via endothelium-dependent and independent mechanisms, maintaining blood pressure and NO levels by reversing abnormal phenotypes (Szopa et al., 2017; Liu et al., 2017a; Kim et al., 2017). The concentration of schisandrol B in the three *S. chinensis* oils ranged from 6.02 ± 0.32 to 6.96 ± 0.13 mg/g. A recent study showed that gomisin H is capable of inhibiting the proliferation of breast cancer cells MCF7 and tongue cancer cells CAL27 (Hou et al., 2016). Gomisin H content in the *S. chinensis* oil was approximately 6.26 ± 0.23 mg/g. Similarly, this content is different from previous reports, and the reasons could be the same as those for schisandrin and schisandrin B.

The last item to mention is that most of the identified lignans in *S. chinensis* oil are dibenzocyclooctadiene lignans except for pregomisin. The result was in accordance with recent research, which only determined dibenzocyclooctadiene lignans in *S. chinensis* wine by UPLC-QTOF-MSE and UPLC-QTOF-MRM (Liu et al., 2017a). A possible reason for this phenomenon might be assigned to the abundance, stability, or lipid solubility of dibenzocyclooctadiene lignans in *S. chinensis* fruits. Undoubtedly, it is widely reported that dibenzocyclooctadiene lignans play an important role in the bioactivity of *S. chinensis* fruits (Lu and Chen, 2009; Hu et al., 2014b; Szopa et al., 2017).

Compared to the previous studies, the bioactive lignans in *S. chinensis* oil were more diverse and abundant. Especially for the components of schisandrin, schisandrin B, deoxyschisandrin, schisandrol B and gomisin H. They have been widely acknowledged that owns various biological activities, including anti-inflammatory activity, antitumor characteristics, hepato-protective property and potent anti-oxidative activity, et al. (Hu et al., 2014b; Szopa et al., 2017; Sowndhararajan et al., 2018) As for their content in *S. chinensis* oil is far more than in the *S. chinensis* fruits and roots. The comparative analysis indicated that the content in *S. chinensis* oil is about two to eight times higher than on *S. chinensis* fruits and roots (Deng et al., 2008; Mocan et al., 2016a; Dong et al., 2016). All the above-mentioned information demonstrates that S. chinensis oil might be a great source of lignans and has great potential in the development of lignan-related health food.

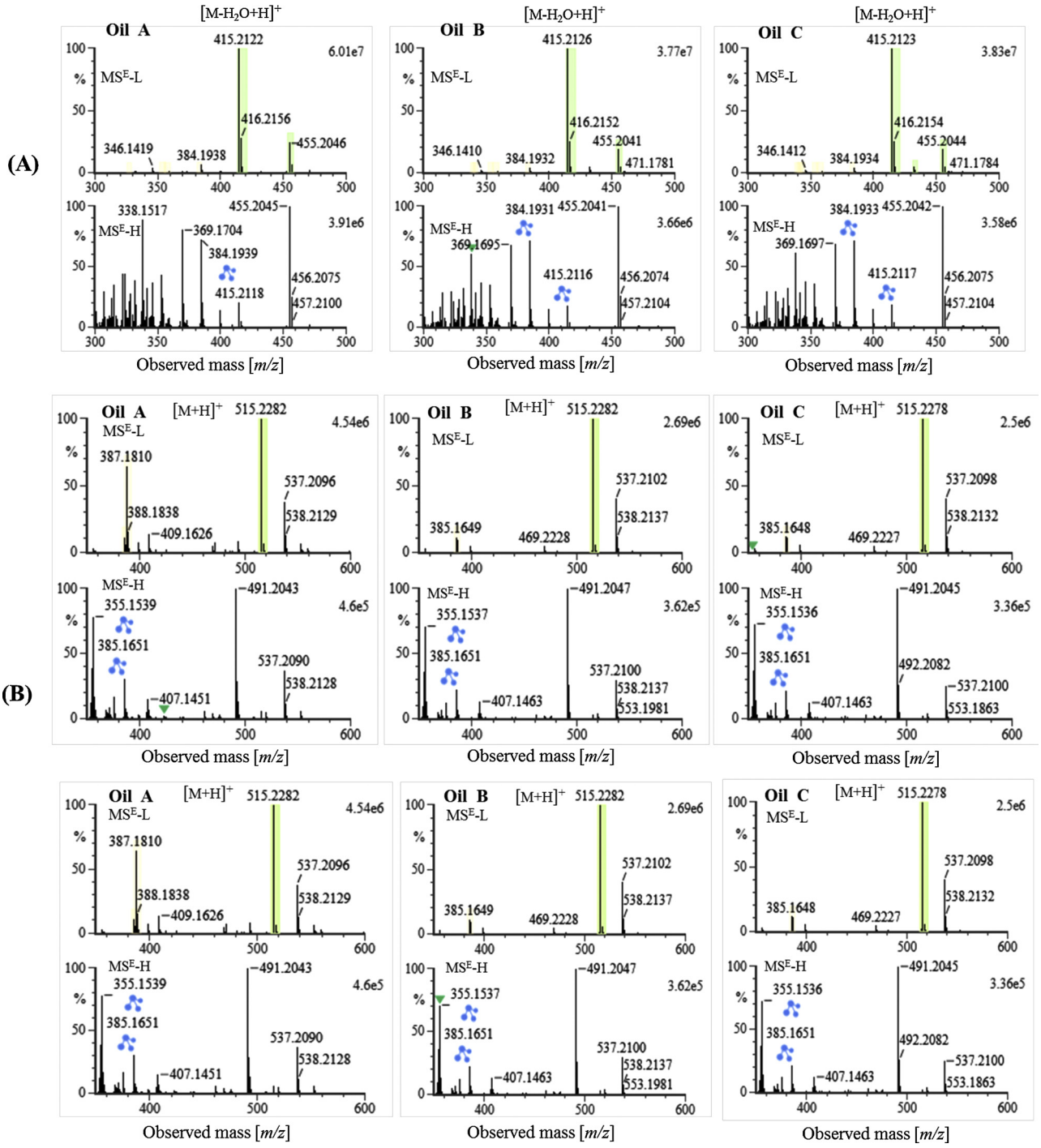


Fig. 3. The MS^E spectra of the most abundant compound [schisandrin] and tentatively identified compound [schisantherin B (C)] in *S. chinensis* Oil A, B and C.

4. Conclusion

In this study, a rapid and efficient method was performed to comprehensively analyze the lignans in three *S. chinensis* oils by using UPLC-Q/TOF-MS. There are approximately 25 lignans identified by their MS^E-L and MS^E-H spectral information. Quantitative analysis indicated that the *S. chinensis* oils had a high content of total lignans, accounting for 67.73–87.61 mg/g. Schisandrin, schisandrin B, deoxyschisandrin, schisandrol B and gomisin H were the most abundant lignans in the *S. chinensis* oils. In the future, the composition of lignans in the *S. chinensis* oils may be of great value for its dietary, nutritional and product application.

Conflict of interest

The authors declare that there is no conflict of interests regarding this paper publication.

Acknowledgments

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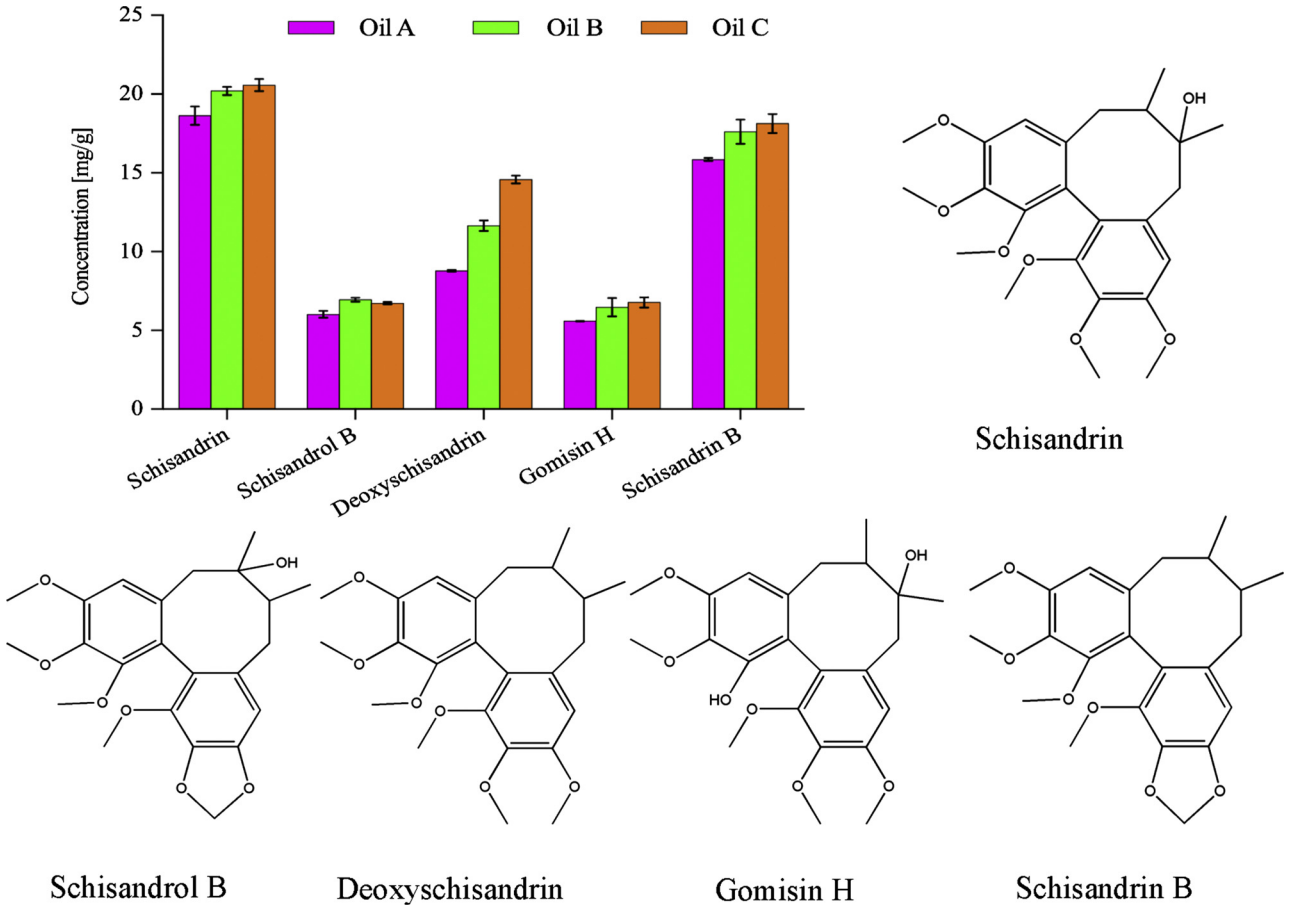

Fig. 4. Distribution and chemical structure of the five major lignans in the three *S. chinensis*. oils.

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