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Analysis of lignan constituents from *Schisandra chinensis* by liquid chromatography–electrospray mass spectrometry

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Abstract

An electrospray high-performance liquid chromatography–mass spectrometry interface coupled with a photodiode-array detector has been applied successfully to the identification of a number of lignan constituents from an ethanol extract of the fruits of *Schisandra chinensis* Baill. A total of fifteen peaks were identified, six of them unambiguously identified as schisandrin (1), schisantherin A (19), schisantherin B (20), schisanhenol (4), deoxyschisandrin (3) and γ-schisandrin (11), based on their abundant $[M+H]^+$, $[M+Na]^+$ ions, UV spectra and retention times, compared with those data of reference compounds. Another nine peaks were tentatively identified, based on their intense $[M+H]^+$ and $[M+Na]^+$ ions and UV spectra.

Keywords: Mass spectrometry; *Schisandra chinensis* Baill; Lignans

1. Introduction

The development of various liquid chromatography–mass spectrometry (LC–MS) interfaces has solved the technical problems for coupling LC and MS. LC–MS nowadays has become a routine method in many areas of analytical chemistry. A literature survey showed that electrospray (ES) is used more than other interfaces in recent years. However, many of the ES-related papers are in biomacromolecular studies as a sample-introduction device. Only a few of the papers published applied on-line high-performance liquid chromatography (HPLC)–ES-MS [1]. HPLC–thermospray (TS) MS, for phytochemical analysis, has been reviewed [2]. HPLC–ES-MS has not been widely applied for the analysis of natural compounds, which usually are small molecules.

In an ES interface, a solution of analytes and solvents is sprayed through a glass needle into the spray chamber, which is operated at atmospheric pressure. It is actually a process of ion evaporation ionization and can handle virtually any analytes in a gentle manner [3], which is very appropriate for the analysis of natural products.

HPLC–ES-MS coupled with a photodiode-array detector enables us to obtain UV and MS spectra for each peak. Therefore, identification of the peaks from a chromatogram is possible by comparing those data with literature, sometimes even without reference compounds for comparison of their retention times.

In our continuing research on the applicability of HPLC–ES-MS for analysis of different classes of

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natural products, we have analyzed the ethanol extract of schisandra fruit.

The fruit of *Schisandra chinensis* Baill. (Magnoliaceae) is a famous traditional Chinese medicine, used as a tonic and sedative. There are 25 species of the schisandra genus worldwide, with most in China (about eighteen species). However, in the Chinese pharmacopoeia, only two species are recorded for medical use, *Schisandra chinensis* Baill. and *Schisandra spenanthera* Rehd. et Wils. More than 40 lignan compounds were isolated from schisandra plants, some of which showed significant biological activities [4,5]. Table 1 lists 25 lignan compounds that are discussed in this report.

The content and distribution of lignans are extremely varied in different species. The major lignans in *S. chinensis* are schisandrin (1) (2–9%), γ-schisandrin (11) (1–5%), deoxyschisandrin (30) (0.2–1.1%) and schisandrol B (8) (0.7–3%), depending on the plant origin and harvest season (% of dry weight). There is only a minor amount of other lignans (below 0.2% of dry weight). However, the fruits of *S. spenanthera* have very little schisandrin (1) and γ-schisandrin (11), but are rich in deoxyschisandrin (3) and many wuweizi esters [6].

A HPLC chromatogram may provide a fingerprint to distinguish different species, although the total peak assignment of a HPLC chromatogram of schisandra extract can be completed. However, this type of work has not been published up to now. Lee et al. [7] applied countercurrent chromatography to analyze the extract of *Schisandra rubriflora*, another schisandra species. The chromatogram only showed five peaks, and those peaks were identified by a coupled thermospray MS. Zhu et al. [8] have separated six reference lignan compounds by HPLC. They only use this chromatogram to quantify the lignan content of Chinese patent medicines containing *Schisandra chinensis*. We prepared an ethanol extract of the fruits of *S. chinensis* for HPLC–ES-MS analysis in order to identify all the peaks of a chromatogram.

2. Experimental

2.1. Instrumentation

A HP 1090 Series II HPLC (Hewlett-Packard, USA) with a photodiode-array detector set at 225 nm was in-line-coupled with a HP 5989 B quadrupole mass spectrometer (Hewlett-Packard), without stream splitting.

UV spectra were taken in the region of 200–500 nm. Chromatographic conditions were as follows: Column, Prodigy ODS, 5 μm, 150×2.0 mm (Phenomenex, USA); mobile phase, A, H_2O; B, MeOH; Gradient elution was with 60–100% B from 0–15 min, 100% B from 15–20 min and 100–60% B from 20–25 min; the flow-rate was 0.2 ml/min and the temperature was 45°C.

Mass range measured, 350–580 amu; quadrupole temperature, 150°C; EM volts, 2010. The spectra were acquired in the positive mode.

The electrospray interface used was a HP 59987 A (Hewlett-Packard); end plate voltage, VEnd-3500; capillary exit voltage, CapEx 110; drying N_2 temperature, 350°C; flow-rate, 40 ml/min; nebulizing N_2, 80 p.s.i.

2.2. Reference compounds and chemicals

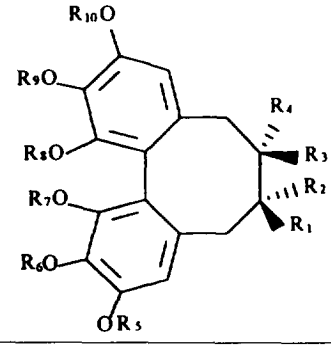
Deoxyschisandrin (3), γ-schisandrin (11), schisantherin A (19) and B (20) were given to us by Dr. Jiasen Liu and schisanhenol (4) was provided by Dr. Dan-yun Zhu, from the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. Schisandrin (1) was isolated in our laboratory. Methanol and water were of HPLC grade (VWR, USA). A 1.5-mg amount of compounds (1) and (20) and 2 mg of compounds (3), (11), (19) and (4) were dissolved in 10 ml of methanol, to form the solution of mixed reference compounds. A 2-μl volume of the solution was injected into the HPLC column.

2.3. Plant material and sample preparation

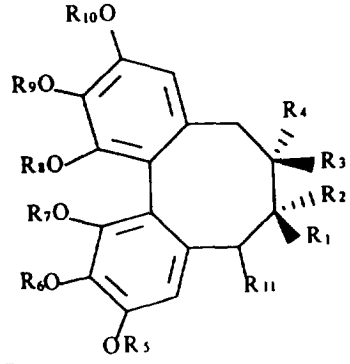
The fruits of *Schisandra chinensis* Baill. were purchased from Tai Sang Trading (USA).

A 2-g amount of dried fruits was extracted with H_2O at 90°C for 2 h and filtered. The residue was refluxed with 20 ml of ethanol for 2 h. The ethanol solution was filtered and concentrated to dryness by rotovaporation. The 50 mg ethanol extract was dissolved in 10 ml of methanol and filtered through a 0.45-μm nylon Acrodisk 13 filter. A 2-μl volume of the sample solution was injected into the HPLC column.

Table 1
Lignan constituents found in *Schisandra chinensis*



Number	Compound name	MF	M_r	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
1	Schisandrin	$C_{24}H_{32}O_7$	432	OH	Me	H	Me	Me	Me	Me	Me	Me	Me
2	Isoschisandrin	$C_{24}H_{32}O_7$	432	H	Me	OH	Me	Me	Me	Me	Me	Me	Me
3	Deoxyschisandrin	$C_{24}H_{32}O_6$	416	H	Me	H	Me	Me	Me	Me	Me	Me	Me
4	Schisanhenol	$C_{23}H_{30}O_6$	402	H	Me	H	Me	Me	Me	Me	H	Me	Me
5	Gomisin N	$C_{23}H_{28}O_6$	400	H	Me	H	Me	-CH_2-		Me	Me	Me	Me
6	Angeloygomisin H	$C_{28}H_{36}O_{10}$	500	OH	Me	H	Me	Me	Me	Me	Angeloyl	Me	Me
7	Tigloylgomisn H	$C_{28}H_{36}O_8$	500	OH	Me	H	Me	Me	Me	Me	Tigloyl	Me	Me
8	Schisandrol B	$C_{23}H_{28}O_7$	416	OH	Me	H	Me	Me	Me	Me	Me	-CH_2-	
9	Gomisin M1	$C_{22}H_{26}O_6$	386	H	Me	H	Me	-CH_2-		Me	H	Me	Me
10	Gomisin M2	$C_{22}H_{26}O_6$	386	H	Me	H	Me	-CH_2-		H	Me	Me	Me
11	γ-Schisandrin	$C_{23}H_{28}O_6$	400	H	Me	H	Me	-CH_2-		Me	Me	Me	Me
12	Schisandrin C	$C_{22}H_{24}O_6$	384	H	Me	H	Me	-CH_2-		Me	Me	-CH_2-	
13	Gomisin J	$C_{22}H_{28}O_6$	388	H	Me	H	Me	H	Me	Me	Me	Me	H
14	Gomisin L1	$C_{22}H_{26}O_6$	386	H	Me	H	Me	-CH_2-		Me	H	Me	Me
15	Gomisin L2	$C_{22}H_{26}O_6$	386	H	Me	H	Me	-CH_2-		Me	Me	Me	H



	Compound name	MF	M_r	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}
16	Gomisin R	$C_{22}H_{24}O_7$	400	Me	H	Me	H	-CH_2-		Me	Me	-CH_2-		β-OH
17	Gomisin O	$C_{23}H_{28}O_7$	416	Me	H	Me	H	Me	Me	Me	Me	-CH_2-		β-OH
18	Epi-gomisin O	$C_{23}H_{28}O_7$	416	Me	H	Me	H	Me	Me	Me	Me	-CH_2-		α-OH
19	Schisantherin A	$C_{30}H_{32}O_9$	536	OH	Me	Me	H	Me	Me	Me	Me	-CH_2-		β-O-Benzoyl
20	Schisantherin B	$C_{28}H_{34}O_9$	514	OH	Me	Me	H	Me	Me	Me	Me	-CH_2-		β-O-Angeloyl
21	Schisantherin C	$C_{28}H_{34}O_9$	514	OH	Me	Me	H	Me	Me	Me	Me	-CH_2-		β-O-Tigloyl
22	Gomisin F	$C_{28}H_{34}O_9$	514	OH	Me	Me	H	-CH_2-		Me	Me	Me	Me	β-O-Angeloyl
23	Gomisin G	$C_{30}H_{32}O_9$	536	OH	Me	Me	H	-CH_2-		Me	Me	Me	Me	β-O-Benzoyl
24	Angeloylgomisin P	$C_{28}H_{34}O_9$	514	Me	OH	Me	H	Me	Me	Me	Me	-CH_2-		β-O-Angeloyl
25	Tigloylgomisin P	$C_{28}H_{34}O_9$	514	Me	OH	Me	H	Me	Me	Me	Me	-CH_2-		β-O-Tigloyl

3. Results

3.1. HPLC–MS of lignan reference compounds

Six reference compounds, schisandrin (1), schisantherin A (19), schisantherin B (20), schisanhenol (4), deoxyschisandrin (3) and γ-schisandrin (11), were chromatographed in order to determine their retention times. UV spectra and mass spectra were obtained for comparison with those obtained from compounds represented by the extract chromatogram of schisandra fruit. Their HPLC and MS chromatograms are shown in Fig. 1.

3.2. HPLC–MS of an extract of schisandra fruits

HPLC and MS chromatograms of an ethanol extract of schisandra fruit are shown in Fig. 2. The value of t_R, $[M+H]^+$, $[M+Na]^+$, UV λ_{max} and the identification of individual peaks are listed in Table 2.

The similarity of the UV spectra of peaks 1–17 indicated that they possessed the same skeletal structure, dibenzocyclo-octadien system.

Peaks 1, 7, 8, 9, 12 and 15 were unambiguously identified as schisandrin (1), schisantherin A (19), schisantherin B (20), schisanhenol (4), deoxyschisandrin (3) and γ-schisandrin (11), based on their retention times, UV spectra, $[M+H]^+$ and $[M+Na]^+$ ions compared with those data of reference compounds.

Because a positional isomer and stereoisomer of some lignans would have the same molecular mass, it is possible that they are co-eluted. Therefore, another nine peaks were only tentatively identified, based on their intense $[M+H]^+$ and $[M+Na]^+$ ions. For example, isoschisandrin (2) has the same molecular mass, 432, as schisandrin (1). Its content in *S. chinensis* is very low (0.001%) [9]. Peak 1 probably also contains isoschisandrin (2).

Peak 2 has an intense protonated molecule $[M+H]^+$ at *m/z* 389 and an adduct ion $[M+Na]^+$ at *m/z* 411. It could be gomisin J (13). Its reported content in schisandra is 0.02% [10].

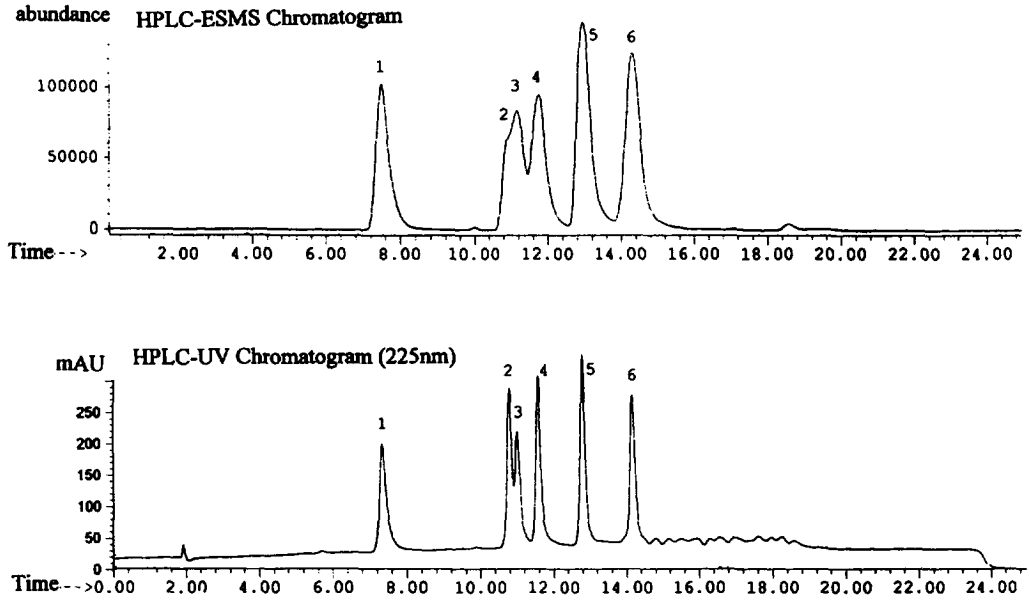


Fig. 1. Simultaneous HPLC–UV and HPLC–ES-MS chromatograms of lignan reference compounds without post-column stream splitting. Chromatographic conditions were as described in Section 2. The following compounds are indicated: 1=schisandrin (1); 2=schisantherin A (19); 3=schisantherin B (20); 4=schisanthenol (4); 5=deoxyschisandrin (3) and 6=γ-schisandrin (11).

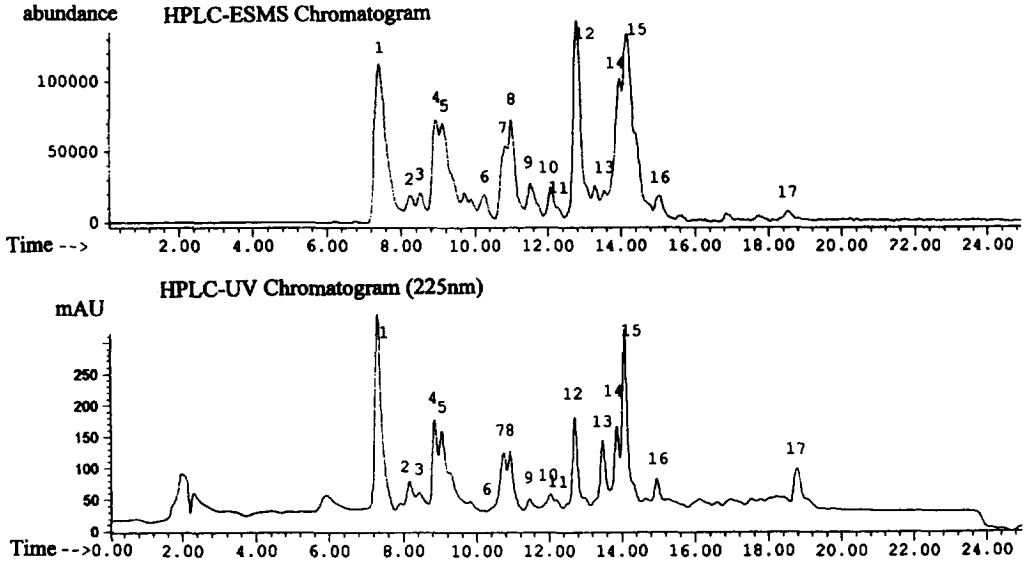


Fig. 2. Simultaneous HPLC–UV and HPLC–ES-MS chromatograms of an ethanol extract from *Schisandra chinensis* fruit, without post-column stream splitting. Chromatographic conditions were as described in Section 2. Peak assignments are shown in Table 2.

Table 2
Peak assignments for analysis of an ethanol extract from *Schisandra chinensis* fruit

Peak number	Retention time (min)	$[M+H]^+$ (*m/z*)	$[M+Na]^+$ (*m/z*)	UV λ_{max} (nm)	Identification
1	7.3	433	455	217, 251sh, 280sh	Schisandrin, isoschisandrin
2	8.1	389	411	220, 258, 280sh	Gomisin J
3	8.4	501	523	220, 256, 288sh	Angeloylgomisin H or tigloylgomisin H
4	8.9	417	439	220, 255, 284sh	Schisandrol B (gomisin A), Gomison O or epi-gomisin O
5	9.2	501	523	220, 255, 284sh	Angeloylgomisin H or tigloylgomisin H
6	10.2	537	559	229, 260	gomisin G
7	10.8	537	559	221, 254sh, 285sh	Schisantherin A
8	11.1	no	537	221, 260sh, 295sh	Schisantherin B
9	11.5	403	425	218, 252sh, 285sh	Schisanhenol
10	12.0	515	537	222, 255, 280sh	Gomisin F, angeloylgomisin P or tigloylgomisin P
11	12.2	387	409	230, 259sh	Gomisin M1, M2, L1 or L2[a]
12	12.9	417	439	218, 248sh, 280sh	Deoxyschisandrin
13	13.5	485	507	235, 280	nd
14	13.9	401	423	222, 275, 310sh	Gomisin R or N[a]
15	14.2	401	423	218, 254sh, 280sh	γ-Schisandrin
16	15.0	385	407	220, 258, 284sh	Schisandrin C
17	18.9	[b]	[b]	205, 268	nd

nd, Unable to identify.
[a] UV spectra data does not match literature data well.
[b] No dominant molecular ion.

Peaks 3 and 5 both showed a weak protonated molecule $[M+H]^+$ at m/z 501 and an intense adduct ion $[M+Na]^+$ at m/z 523. They are possibly angeloylgomisin H (6) or tigloylgomisin H (7). The reported content of them in schisandra is 0.03 and 0.01%, respectively [11].

Peak 6 has an intense protonated molecule $[M+H]^+$ at m/z 537 and an adduct ion $[M+Na]^+$ at m/z 559. It was tentatively identified as gomisin G (23). Its reported content in schisandra is 0.016% [12].

Peak 4 shows an intense protonated molecule $[M+H]^+$ at m/z 417 and an adduct ion $[M+Na]^+$ at m/z 439, the same as deoxyschisandrin (3), so it was tentatively identified as schisandrol B (8), gomisin O or (17) epi-gomisin O (18). The reported content of them in schisandra is 0.18, 0.002 and 0.001%, respectively [13]. The results of Zhu et al. [8] showed that the retention time of schisandrol B was between that of schisandrin and schisantherin A, which matches our results.

Peak 10 has an intense protonated molecule $[M+H]^+$ at m/z 515 and an adduct ion $[M+Na]^+$ at m/z 537. It is possibly gomisin F (22), angeloylgomisin P (24) or tigloylgomisin P (25). The reported content of these compounds in schisandra is 0.004, 0.01 and 0.001%, respectively [14].

Peak 11 shows an intense protonated molecule $[M+H]^+$ at m/z 387 and an adduct ion $[M+Na]^+$ at m/z 409. It is possibly gomisin M_1 (9), M_2 (10), L_1 (14) or L_2 (15). The reported content of each compound in schisandra is less than 0.002% [15]. Peak 13 has an intense protonated molecule $[M+H]^+$ at m/z 485 and an adduct ion $[M+Na]^+$ at m/z 507. We are unable to identify it, because it does not match the molecular mass of any reported lignans. Peak 14 has an intense protonated molecule $[M+H]^+$ at m/z 401 and an adduct ion $[M+Na]^+$ at m/z 423. Its possible identification is as gomisin R (16) or gomisin N (5). These reportedly make up 0.001 and 0.3% of the content in schisandra, respectively [16,17].

Peak 16 shows an intense protonated molecule $[M+H]^+$ at m/z 385 and an adduct ion $[M+Na]^+$ at m/z 407. It could be schisandrin C (12). Its reported content in schisandra is 0.07% [18]. Peak 17 shows four intense ions at m/z 393, 413, 455 and 507. This is a co-eluted peak containing several compounds which we were unable to identify.

4. Discussion

All lignan compounds in this HPLC–ES-MS study showed abundant molecular ions. The previous HPLC–ES-MS experiments in our laboratory also showed that many natural products with neutral molecules such as lignans, coumarins, lactones, saponins and some flavonoids can be easily protonated to form molecular ions in a neutral or weak acid environment at positive mode. After we added 0.25% acetic acid to water (elution solvent A), the HPLC separation of lignan compounds was not affected but the MS signal enhancement was increased by 50%. In addition, the presence of adduct ions $[M+Na]^+$ was reduced.

The mechanism of ES ionizations of analytes seems complicated. Besides ion evaporation of preformed ions from the microdroplet, gas-phase ion-molecule reactions also could play an important role [19]. We believe that HPLC–ES-MS studies of different classes of natural products will provide us with more information for understanding the mechanism of ES ionizations.

Under our experimental conditions, the single-ion detection limit for deoxyschisandrin (3) was 1 ng. We are concerned more with a qualitative rather than a quantitative study at the present time. However, further quantitative studies need to be done. In Figs. 1 and 2, the ES-MS peaks were broader than the UV signal. We think that this was due to the diffusion of post-column eluent. We use a 90-cm long, 0.010 in. I.D. PEEK tubing to connect the exit port of the UV detector with the mass spectrometer. This is the shortest distance that can be reached in this HP instrument. The device needs to be improved.

Our experiments show that HPLC–ES-MS is a powerful tool for direct, on-line qualitative identification previously known compounds in plant extracts. The mass range we measured in our analyses was 350–580 amu, because we knew that the molecular masses of most of the lignan compounds from schisandra are found in this range. The UV and MS detectors are complementary. In Fig. 2, the separation of peaks 2 and 3 in HPLC–ES-MS was better than in HPLC–UV, but peak 17 in HPLC–UV is stronger than in HPLC–MS. This is caused by different sensitivities of compounds to UV and MS detectors. This method can also provide much in-

formation to guide the isolation of previously unknown compounds. The isolation of unidentified peaks is in progress.

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