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Ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometric procedure for qualitative and quantitative analyses of nortriterpenoids and lignans in the genus *Schisandra*

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ABSTRACT

An ultra performance liquid chromatography (UPLC) coupled with quadrupole time-of-flight mass spectrometry (QTOF-MS) procedure is designed for the first simultaneous analysis of nortriterpenoids and lignans in *Schisandra* samples. The method consists of three individual mass spectrometric experiments, including the full scan MS, MS/MS experiment and in-source collision induced dissociation (CID) MS/MS, which enable the identification of diagnostic fragmentation pathways of nortriterpenoids and lignans. As such, a total of 6 nortriterpenoids and 10 lignans were unequivocally identified, and one nortriterpenoid and 20 lignans were tentatively identified from different *Schisandra* samples within 12.5 min. In addition, 6 nortriterpenoids and 10 lignans were quantified in 48 samples of *S. chinensis* and *S. sphenanthera* using an extract ion chromatogram (XIC) of the full scan MS experiment. Dataset obtained from UPLC–MS was processed with principal component analysis (PCA) and orthogonal partial least squared discriminant analysis (OPLS-DA) to compare the difference between the two *Schisandra* species.

1. Introduction

The dicotyledon family Schisandraceae includes two genera *Schisandra* and *Kadsura*. Together there are about 50 species worldwide with the majority of their distribution found in Southeast Asia and North America. *Schisandra* is one of the medicinally important genuses with 30 species spread across the world, of which 19 are found in China [1]. Many species of the genus *Schisandra* have long been used as folk medicines in China. Among them, the most commonly used medicinal plants are *Schisandra chinensis* (Turcz.) Baill and *Schisandra sphenanthera* Rehd et Wils, which were also known as "Bei-Wuweizi" and "Nan-Wuweizi", respectively. Since the majority of *S. chinensis* is distributed in northeastern China, Russia, Korea and Japan [1,2], and *S. sphenanthera* is distributed in the southern provinces of China [1,2]. Reports have shown that the ripe fruits of the two plants exhibit similar actions, and have been primarily used as sedative, tonic agents to replenish and promote the production of body fluid, as well as tonify the kidney to relieve mental strain and for the treatment of hepatitis [2]. Although the fruits of these two plants have been accepted as two different crude drugs by the Chinese Pharmacopoeia since 2000 [2], both plants are found to be a rich source of lignans [3–7] and terpenoids [8–11] in particular the dibenzocyclooctadiene lignans exhibiting various biological activities.

Schisandra nortriterpenoids are a structurally intriguing group of a highly oxygenated, polycyclic, fused heterocyclic natural triterpenoids isolated from *Schisandra*. Typically the schisanartane skeleton triterpenoids featured octacyclic backbone and a 7/8/5 fused carbocycle with more than 12 chiral-carbon centers. This unusual assembly ring and the highly oxygenated nortriterpenoids have attracted interests of many chemists. To date, over 60 highly oxygenated nortriterpenoids have been identified from different *Schisandra* species by H.D. Sun's group [12], but these compounds have not yet been studied by electrospray ionization (ESI) MS/MS. Many analytical methods, including high performance liquid chromatography (HPLC) [13] and high performance liquid chromatography–mass spectrometry (HPLC–MS) [7,14–18], have focused on the analysis of lignans of *S. chinensis* and *S. sphenanthera*. To the best of our knowledge, there have been no previous reports on the simultaneous determination of nortriterpenoids and

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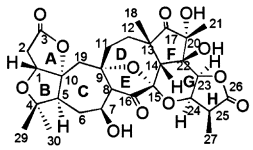
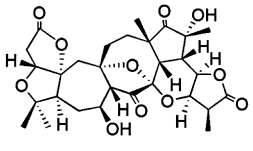
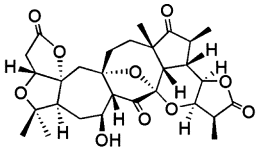


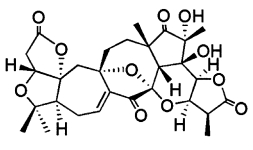
Micarndilactone A (**1**)



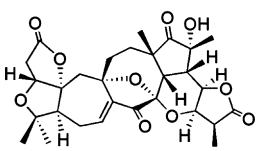
Micarndilactone D (**2**)



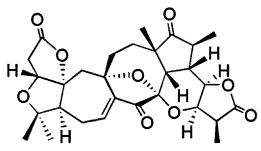
Henridilactone D (**3**)



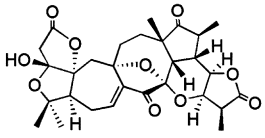
Henridilactone B (**4**)



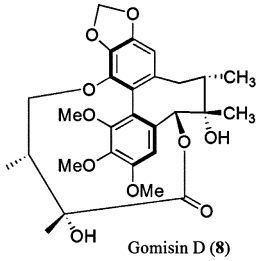
Henridilactone A (**5**)



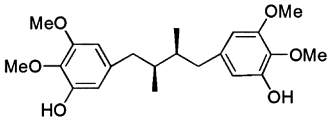
Lancifodilactone D (**6**)



Schindilactone A (**17**)



Gomisin D (**8**)



Pre-gomisin (**37**)

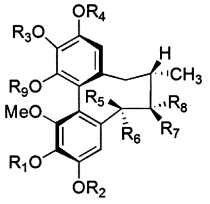
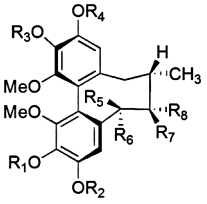
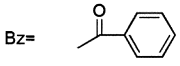
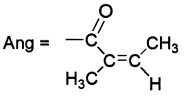
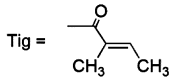
Compounds	Structure		R1	R2	R3	R4	R5	R6	R7	R8	R9
Schisandrin		**7**	Me	Me	OH	Me	-	-	-	-	Me
Gomisin A		**9**	Me	Me		$-CH_2-$	-	-	OH	Me	Me
Deoxyschisandrin		**13**	Me	Me	Me	Me	-	-	H	Me	Me
Aangeloylgomisin H		**18**	Me	Me	Me	Me	-	-	OH	Me	Ang
Tigloylgomisin H		**19**	Me	Me	Me	Me	-	-	OH	Me	Tig
Schisandrol B		**20**	Me	Me		$-CH_2-$	-	-	OH	Me	Me
Benzoylgomisin H		**21**	Me	Me	Me	Me	-	-	OH	Me	Bz
Gomisin K3		**23**	Me	Me	Me	Me	-	-	H	Me	H
Gomisin K1		**28**	Me	Me	Me	OH	-	-	H	Me	Me
Gomisin M2		**31**	$-CH_2-$		Me	Me	-	-	H	Me	Me
γ -Schisandrin		**32**	Me	Me	Me	Me	-	-	OH	Me	Me
Gomisin G		**10**	$-CH_2-$		Me	Me	OBz	H	Me	OH	
Gomisin F		**11**	$-CH_2-$		Me	Me	OAng	H	Me	OH	
Schisantherin A		**12**	Me	Me		$-CH_2-$	OBz	H	Me	OH	
Schisandrin B		**14**	Me	Me		$-CH_2-$	H	H	H	Me	
Schisandrin C		**15**	$-CH_2-$			$-CH_2-$	H	H	H	Me	
Angeloylgomisin O		**16**	Me	Me		$-CH_2-$	OAng	H	H	Me	
Angeloylgomisin Q or Tigloylgomisin Q		**22**	Me	Me	Me	Me	Me	OAng	Me	OH	
Schisantherin B		**24**	Me	Me		$-CH_2-$	OAng	H	Me	OH	
Schisantherin C		**25**	Me	Me		$-CH_2-$	OTig	H	Me	OH	
Wuweilignan D		**26**	Me	Me	Me	Me	OBz	H	-	-	
Schisantherin D		**27**	$-CH_2-$			$-CH_2-$	OBz	H	Me	OH	
Wuweilignan L		**29**	$-CH_2-$		Me	Me	OTig	H	Me	OH	
Gomisin O		**30**	Me	Me	Me	Me	OH	H	H	Me	
Schisandrene		**33**	$-CH_2-$			$-CH_2-$	OBz	H	$=CH_2$		
Benzoylgomisin O		**34**	Me	Me		$-CH_2-$	OAng	H	H	Me	
Tigloylgomisin O		**35**	Me	Me		$-CH_2-$	OTig	H	H	Me	
Gomisin R		**36**	$-CH_2-$			$-CH_2-$	-	-	-	-	

Fig. 1. Structures assigned in the extracts of *S. chinensis* and *S. sphenanthera* samples.

lignans in *Schisandra* species. Therefore, it is important to develop a sensitive and selective method to accurately detect the presence and contents of the nortriterpenoids and lignans in *S. chinensis* and *S. sphenanthera*.

Traditionally, the analyses of traditional Chinese medicines (TCMs) require time-consuming chromatography to achieve optimal resolution of the complex samples. UPLC has been proven to be a valuable separation tool for TCMs with higher speed, improved sensitivity, selectivity and specificity while maintaining separation capacity together with QTOF-MS/MS, which allows the generation of abundant structural mass information with greater accuracy and precision, UPLC coupled to QTOF-MS/MS have great potential as a rapid qualitative and quantitative analysis method for TCMs [19–21].

In the present study, we developed an UPLC–QTOF-MS/MS method for the simultaneous qualitative and quantitative analyses of lignans and nortriterpenoids in 48 samples of *S. chinensis* and *S. sphenanthera* collected from various sources and the evaluation of the chemical consistency between the *Schisandra* samples.

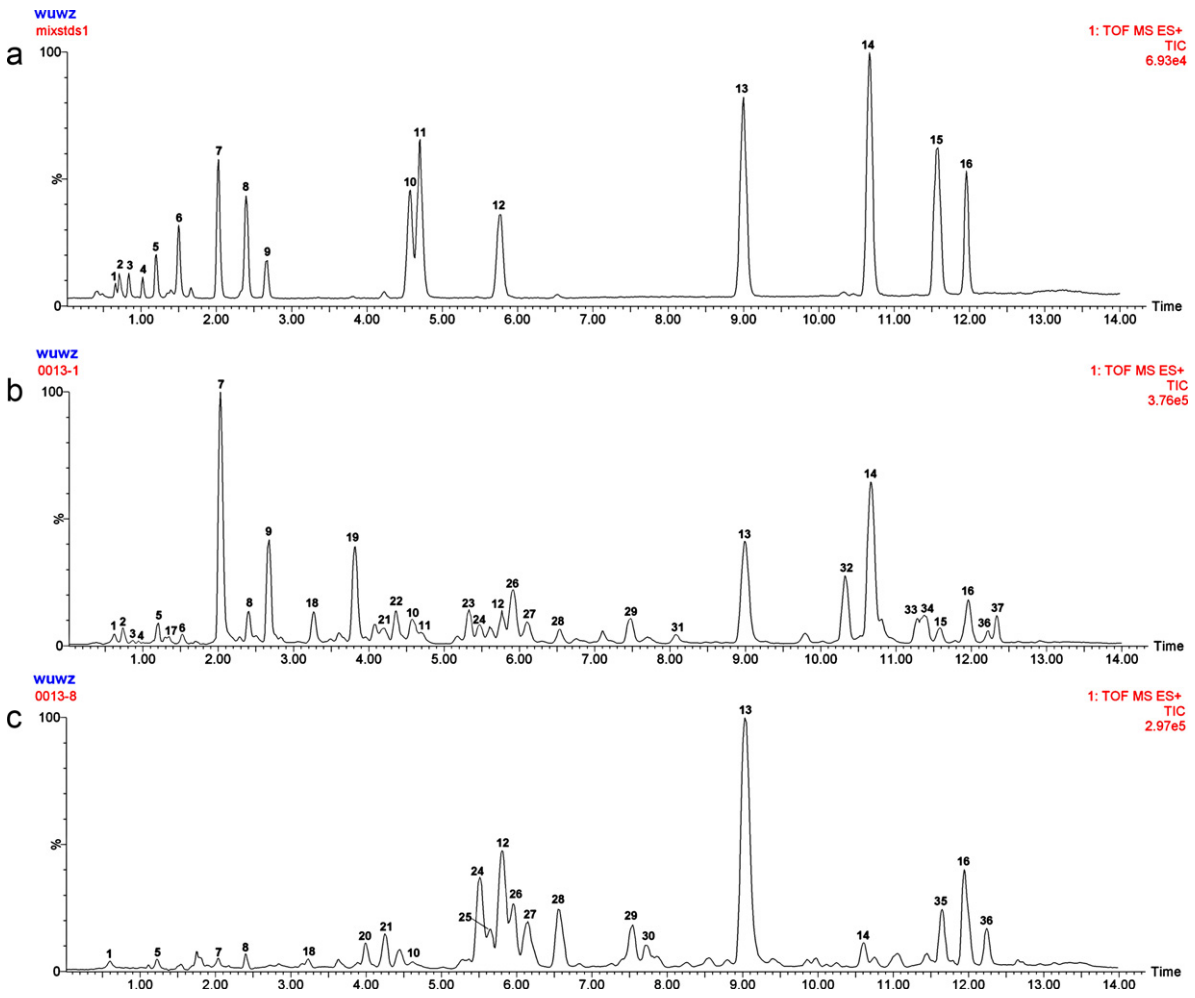


Fig. 2. Representative total ion current (TIC) of standard solution and extracts of *S. chinensis* and *S. sphenanthera* for identification: (A) TIC of ESI-MS of 16 standard solution; (B–C) TIC of ESI-MS from *S. chinensis* and *S. sphenanthera*.

2. Experimental

2.1. Chemicals and materials

Acetonitrile (ACN, HPLC–MS grade) was purchased from Fishier Scientific UK (Loughborough, UK) and formic acid (spectroscopy grade) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Pure water was prepared from a Milli-Q SP Regent Water system (Millipore, Bedford, MA, USA). Leucine–enkephalin was obtained from Sigma–Aldrich.

The 28 samples of *S. chinensis* (CMED-0013-01–06, 14–21, 23–35, 38) and 20 samples of *S. sphenanthera* (CMED-0013-07–13, 22, 36–37, 39–48) were collected from different provinces of China. The plant materials were authenticated by Prof. Chen Dao-Feng, Fudan University of China. All voucher specimens (CMED-0013-01–048) were deposited in Chengdu Institute of Biology, Chinese Academy of Sciences.

2.2. Sample preparation

All air dried plant samples were ground into fine powder by a pulverizer separately. A sample of 0.2 g fine powder was placed in a 50 mL capped conical flask. 10 mL 80% methanol–H_2O was added, and was extracted under ultrasonic bath (50 Hz) for 30 min. Then the extract was filtered and the residue was extracted again with same amount of fresh solvent for two times. The three extracts were combined, evaporated under vacuum at 50 °C, and diluted to 10 mL with 80% methanol–H_2O in a volumetric flask before being filtered through a 0.22 μm PTFE syringe filter (Whatman, MN, Nalgene, Advantec). An aliquot of each filtrate (2 μL) was injected into the UPLC instrument for analysis.

As shown in Fig. 1, six *Schisandra* nortriterpenoids and ten lignans reference standards, namely micrandilactone A (1) [22], micrandilactone D (2) [23], henridilactone D (3) [24], henridilactone B (4) [24], henridilactone A (5) [24], lancifodilactone D (6) [25], schisandrin (7) [26], gomisin D (8) [27], gomisin A (9) [27], gomisin G (10) [26,27], gomisin F (11) [27], schisantherin A (12) [28], deoxyschisandrin (13) [29], schisandrin B (14) [28], schisandrin C (15) [28] and angeloylgomisin O (16) [30] were isolated from different *Schisandra* species and were identified by IR, UV, NMR spectroscopic analyses, and the purity of these authentic compounds was verified to be over 95% by UPLC–DAD-MS detection. The extracts were dissolved in acetonitrile to give a working concentration range of 0.1–3 μg/mL.

2.3. Chromatography

UPLC was performed using a Waters Acquity UPLC system (Waters, Milford, MA, USA), equipped with a binary solvent delivery system, an autosampler, and a photodiode array detection (DAD) system. UPLC separation was achieved on a Waters ACQUITY

Table 1
The retention time (R_t) and MS characteristics of the main detected peaks in *Schisandra* samples.

Peaks No.	R_t	Calculated	Observed	Error (ppm)	Elemental composition	Identification
1	0.72	577.2285	577.2281	−0.7	$C_{29}H_{37}O_{12}$	Micrandilactone A
		517.2074	517.2070	−0.8	$C_{27}H_{33}O_{10}$	
		499.1968	499.1962	−1.2	$C_{27}H_{31}O_{9}$	
		481.1862	481.1852	−2.1	$C_{27}H_{29}O_{8}$	
		471.2019	471.2024	1.1	$C_{26}H_{31}O_{8}$	
		453.1913	453.1923	2.3	$C_{26}H_{29}O_{7}$	
		435.1808	435.1827	4.3	$C_{26}H_{27}O_{6}$	
		425.1600	425.1617	4.0	$C_{24}H_{25}O_{7}$	
2	0.80	561.2336	561.2326	−1.8	$C_{29}H_{37}O_{11}$	Micrandilactone D
		501.2125	501.2121	−0.8	$C_{27}H_{33}O_{9}$	
		483.2019	483.2006	−2.7	$C_{27}H_{31}O_{8}$	
		437.1964	437.1968	0.9	$C_{26}H_{29}O_{6}$	
		409.1651	409.1654	0.8	$C_{24}H_{25}O_{6}$	
3	0.90	545.2387	545.2400	2.4	$C_{29}H_{37}O_{10}$	Henridilactone D
		485.2175	485.2169	−1.2	$C_{27}H_{33}O_{8}$	
		467.2070	467.2074	0.9	$C_{27}H_{31}O_{7}$	
		439.2121	439.2114	−1.6	$C_{26}H_{31}O_{6}$	
		421.2015	421.2025	2.4	$C_{26}H_{29}O_{5}$	
		411.1808	411.1814	1.5	$C_{24}H_{27}O_{6}$	
4	1.09	559.2179	559.2187	1.4	$C_{29}H_{35}O_{11}$	Henridilactone B
		499.1968	499.1964	−0.8	$C_{27}H_{31}O_{9}$	
		481.1862	481.1859	−0.6	$C_{27}H_{29}O_{8}$	
		463.1757	463.1739	−3.9	$C_{27}H_{27}O_{7}$	
		425.1600	425.1607	1.6	$C_{24}H_{25}O_{7}$	
		373.1287	373.1279	−2.1	$C_{20}H_{21}O_{7}$	
5	1.26	543.2230	543.2243	2.4	$C_{29}H_{35}O_{10}$	Henridilactone A
		525.2125	525.2131	1.2	$C_{29}H_{33}O_{9}$	
		507.2019	507.2025	1.3	$C_{29}H_{31}O_{8}$	
		447.1808	447.1802	−1.3	$C_{27}H_{27}O_{6}$	
		409.1651	409.1658	1.7	$C_{24}H_{25}O_{6}$	
		391.1909	391.1916	1.8	$C_{25}H_{27}O_{4}$	
		373.1440	373.1448	2.1	$C_{24}H_{21}O_{4}$	
		341.1389	341.1385	−1.2	$C_{20}H_{21}O_{5}$	
		265.1076	265.1074	−0.8	$C_{14}H_{17}O_{5}$	
6	1.57	527.2281	527.2289	1.5	$C_{29}H_{35}O_{9}$	Lancifodilactone D
		467.2070	467.2068	−0.4	$C_{27}H_{31}O_{7}$	
		449.1964	449.1958	−1.3	$C_{27}H_{29}O_{6}$	
		393.1702	393.1713	2.8	$C_{24}H_{25}O_{5}$	
		359.1495	359.1487	−2.2	$C_{20}H_{23}O_{6}$	
7	2.03	433.2226	433.2227	0.2	$C_{24}H_{33}O_{7}$	Schisandrin
		415.2121	415.2122	0.2	$C_{24}H_{31}O_{6}$	
		384.1937	384.1945	2.1	$C_{23}H_{28}O_{5}$	
		369.1702	369.1712	2.7	$C_{22}H_{25}O_{5}$	
8	2.45	531.2230	531.2239	1.7	$C_{28}H_{35}O_{10}$	Gomisin D
		401.1599	401.1608	2.2	$C_{22}H_{25}O_{7}$	
		341.1389	341.1382	2.1	$C_{20}H_{21}O_{5}$	
9	2.71	417.1913	417.1919	1.4	$C_{23}H_{29}O_{7}$	Gomisin A
		399.1808	399.1816	2.0	$C_{23}H_{27}O_{6}$	
		368.1624	368.1631	1.9	$C_{22}H_{24}O_{5}$	
10	4.61	537.2125	537.2128	0.6	$C_{30}H_{33}O_{9}$	Gomisin G
		415.1757	415.1758	0.2	$C_{23}H_{27}O_{7}$	
		371.1858	371.1852	−1.6	$C_{22}H_{27}O_{5}$	
11	4.74	515.2281	515.2289	1.5	$C_{28}H_{35}O_{9}$	Gomisin F
		415.1757	415.1751	−1.4	$C_{23}H_{27}O_{7}$	
		341.1753	341.1759	1.8	$C_{21}H_{25}O_{4}$	
12	5.81	537.2125	537.2128	0.6	$C_{30}H_{33}O_{9}$	Schisantherin A
		415.1757	415.1755	−0.7	$C_{23}H_{27}O_{7}$	
		371.1858	371.1848	2.7	$C_{22}H_{27}O_{5}$	
		340.1675	340.1679	2.6	$C_{21}H_{24}O_{4}$	
13	9.04	417.2277	417.2272	−1.2	$C_{24}H_{33}O_{6}$	Deoxyschisandrin
		347.1495	347.1499	1.2	$C_{19}H_{23}O_{6}$	
		316.1311	316.1320	2.8	$C_{18}H_{20}O_{5}$	
14	10.72	401.1964	401.1966	0.5	$C_{23}H_{29}O_{6}$	Schisandrin B
		370.1780	370.1768	−3.2	$C_{22}H_{26}O_{5}$	
		331.1182	331.1189	2.1	$C_{18}H_{19}O_{6}$	
		300.1725	300.1719	−2.0	$C_{19}H_{24}O_{3}$	
15	11.53	385.1651	385.1648	−0.8	$C_{22}H_{25}O_{6}$	Schisandrin C
		315.1596	315.1592	−1.3	$C_{19}H_{23}O_{4}$	
16	12.02	499.2332	499.2330	−0.4	$C_{28}H_{35}O_{8}$	Angeloylgomisin O
		399.1808	399.1811	0.8	$C_{23}H_{27}O_{6}$	
		330.1103	330.1098	−1.5	$C_{18}H_{18}O_{6}$	
17	1.33	543.2230	543.2236	1.10	$C_{29}H_{35}O_{10}$	Schindilactone A
		525.2125	525.2128	0.6	$C_{29}H_{33}O_{9}$	
		507.2019	507.2012	−1.4	$C_{29}H_{31}O_{8}$	
		483.2019	483.2011	−1.7	$C_{27}H_{31}O_{8}$	
		447.1808	447.1821	2.9	$C_{27}H_{27}O_{6}$	

Table 1 (*Continued*)

Peaks No.	R_t	Calculated	Observed	Error (ppm)	Elemental composition	Identification
		409.1651	409.1658	1.7	$C_{24}H_{25}O_6$	
		391.1909	391.1901	−2.0	$C_{25}H_{27}O_4$	
		373.1440	373.1445	1.3	$C_{24}H_{21}O_4$	
		365.1753	365.1758	1.4	$C_{23}H_{25}O_4$	
		341.1389	341.1382	−2.1	$C_{20}H_{21}O_5$	
		265.1076	265.1073	1.1	$C_{14}H_{17}O_5$	
18	3.27	501.2488	501.2487	−0.2	$C_{28}H_{37}O_8$	Angeloylgomisin H
		483.2383	483.2402	3.9	$C_{28}H_{35}O_7$	
		401.1964	401.1976	3.0	$C_{23}H_{29}O_6$	
		370.1780	370.1770	−2.7	$C_{22}H_{26}O_5$	
19	3.82	501.2488	501.2484	−0.8	$C_{28}H_{37}O_8$	Tigloylgomisin H
		483.2383	483.2389	1.2	$C_{28}H_{35}O_7$	
		401.1964	401.1965	0.2	$C_{23}H_{29}O_6$	
		370.1780	370.1786	1.6	$C_{22}H_{26}O_5$	
20	3.99	417.1913	417.1912	−0.2	$C_{23}H_{29}O_7$	Schisandrol B
		399.1808	399.1805	−0.8	$C_{23}H_{27}O_6$	
		368.1624	368.1635	3.0	$C_{22}H_{24}O_5$	
		353.1753	353.1758	1.4	$C_{22}H_{25}O_4$	
21	4.25	523.2326	523.2329	0.6	$C_{30}H_{35}O_8$	Benzoylgomision H
		505.2221	505.2216	−1.0	$C_{30}H_{33}O_7$	
		401.1959	401.1965	1.5	$C_{23}H_{29}O_6$	
22	4.36	531.2594	531.2589	−0.9	$C_{29}H_{39}O_9$	Tigloylgomisin Q
		431.2070	431.2063	−1.6	$C_{24}H_{31}O_7$	
		372.1573	372.1577	1.1	$C_{21}H_{24}O_6$	
		356.1624	356.1629	1.4	$C_{21}H_{24}O_5$	
23	5.33	403.2121	403.2125	1.0	$C_{23}H_{31}O_6$	Schisanhenol
		341.1753	341.1748	−1.5	$C_{21}H_{25}O_4$	
		302.1518	302.1511	−2.3	$C_{18}H_{22}O_4$	
24	5.51	515.2281	515.2277	−0.8	$C_{28}H_{35}O_9$	Schisantherin B
		415.1757	415.1751	−1.4	$C_{23}H_{27}O_7$	
		340.1675	340.1670	−1.5	$C_{21}H_{24}O_4$	
25	5.67	515.2281	515.2286	1.0	$C_{28}H_{35}O_9$	Schisantherin C
		415.1757	415.1758	0.2	$C_{23}H_{27}O_7$	
		340.1675	340.1668	−2.2	$C_{21}H_{24}O_4$	
26	5.81	537.2125	537.2122	−0.6	$C_{30}H_{33}O_9$	Wuweilignan D
		415.1757	415.1752	−1.2	$C_{23}H_{27}O_7$	
		371.1495	371.1502	1.9	$C_{21}H_{23}O_6$	
27	6.15	521.2175	521.2173	−0.4	$C_{30}H_{33}O_8$	Schisantherin D
		415.1757	415.1754	−0.7	$C_{23}H_{27}O_7$	
		340.1675	340.1682	2.1	$C_{21}H_{24}O_4$	
28	6.55	403.2121	403.2125	1.0	$C_{23}H_{31}O_6$	Gomisin K1
		340.1675	340.1678	0.9	$C_{21}H_{24}O_4$	
		325.1440	325.1438	−0.6	$C_{20}H_{21}O_4$	
29	7.45	515.2281	515.2285	0.8	$C_{28}H_{35}O_9$	Wuweilignan L
		415.1757	415.1751	−1.4	$C_{23}H_{27}O_7$	
		371.1495	371.1489	−1.6	$C_{21}H_{23}O_6$	
30	7.73	417.1913	417.1910	−0.7	$C_{23}H_{29}O_7$	Gomisin O
		399.1808	399.1803	−1.3	$C_{23}H_{27}O_6$	
		369.1702	369.1703	0.3	$C_{22}H_{257}O_5$	
31	8.07	387.1808	387.1802	−1.5	$C_{22}H_{26}O_6$	Gomisin M2
		355.1545	355.1550	1.4	$C_{21}H_{23}O_5$	
		339.1596	339.1590	−1.9	$C_{21}H_{23}O_4$	
32	10.32	401.1964	401.1970	1.5	$C_{23}H_{29}O_6$	γ-Schsandrin
		331.1182	331.1175	−2.1	$C_{18}H_{19}O_6$	
		300.0998	300.0992	−2.0	$C_{17}H_{16}O_5$	
33	11.25	503.1706	503.1711	1.0	$C_{29}H_{27}O_8$	Schisandrene
		467.1651	461.1659	1.7	$C_{28}H_{25}O_6$	
		399.1444	399.1436	−2.0	$C_{22}H_{23}O_7$	
34	11.40	521.2175	521.2166	−1.5	$C_{30}H_{33}O_8$	Benzoylgomisin O
		399.1808	399.1800	−2.0	$C_{23}H_{27}O_6$	
		368.1624	368.1620	−1.1	$C_{22}H_{24}O_5$	
35	11.65	499.2332	499.2330	−0.4	$C_{28}H_{35}O_8$	Tigloylgomisin O
		399.1808	399.1802	−1.5	$C_{23}H_{27}O_6$	
		353.1753	353.1758	1.4	$C_{22}H_{25}O_4$	
36	12.22	401.1600	401.1598	−0.8	$C_{22}H_{25}O_7$	Gomisin R
		383.1495	383.1490	−1.3	$C_{22}H_{23}O_6$	
		353.1389	353.1394	1.4	$C_{21}H_{21}O_5$	
37	12.57	391.2121	391.2125	1.0	$C_{22}H_{31}O_6$	Pregomisin
		361.2015	361.2020	1.4	$C_{21}H_{29}O_5$	
		331.1909	331.1902	−2.2	$C_{20}H_{27}O_4$	

BEH C_{18} column (2.1 × 100 mm I.D., 1.7 μm, Waters, Milford, MA, USA). The mobile phase consisted of (A) 0.1% formic acid in water and (B) ACN containing 0.1% formic acid. The UPLC eluting conditions were optimized as follows: linear gradient from 40% to 45% B (0–5 min), linear gradient from 45% to 70% B (5–12 min), isocratic at 70% B (12–13 min), and linear gradient from 70% to 40% B (13–14 min). The flow rate was 0.6 mL/min. The column and autosampler were maintained at 35 °C and 10 °C, respectively.

Table 2
Regression equation, correlation coefficients, linearity ranges and limit of detection (LOD) and quantitation (LOQ) for the 16 marker compounds.

No.	Compounds	Regression equation	SD of the slope	SD of the intercept	Linear range (μg/mL)	R^2	LOD (ng/mL)	LOQ (ng/mL)
1	Micrandilactone A	$Y = 509.9x - 0.31$	3.18	0.02	0.09–85	0.9995	11	33
2	Micrandilactone D	$Y = 3111x + 0.674$	9.07	0.06	0.1–35	0.9997	0.42	1.3
3	Henridilactone D	$Y = 3916.6x + 3.7$	8.50	0.21	0.2–20	0.9982	8.7	12
4	Henridilactone B	$Y = 218.7x + 0.13$	2.28	0.01	0.1–28	0.9998	0.033	0.10
5	Henridilactone A	$Y = 1726.2x + 48$	11.0	4.10	0.6–65	0.9981	1.07	3.2
6	Lancifodilactone D	$Y = 2557.3x + 0.63$	15.9	0.05	0.5–120	0.9976	3.6	13
7	Schisandrin	$Y = 1120x - 252.3$	7.65	12.9	0.45–450	0.9985	0.26	0.78
8	Gomisin D	$Y = 3616.3x + 453.7$	10.9	18.0	0.25–250	0.9998	0.50	1.5
9	Gomisin A	$Y = 1212.8x + 429.4$	9.25	14.2	0.5–560	0.9985	0.28	0.84
10	Gomisin G	$Y = 556.6x + 107.1$	3.04	8.05	0.62–620	0.9995	6.0	18
11	Gomisin F	$Y = 3208.1x + 122.2$	7.87	7.17	0.6–300	0.9974	0.55	1.6
12	Schisantherin A	$Y = 418.2x + 104.9$	3.49	9.12	1.1–550	0.9985	10	30
13	Deoxyschisandrin	$Y = 9108.4x + 432.1$	19.6	12.0	1.3–675	0.9991	0.80	1.4
14	Schisandrin B	$Y = 1849x + 294.8$	10.3	10.1	2.2–220	0.9989	0.77	2.2
15	Schisandrin C	$Y = 1360.4x + 73.9$	12.3	5.56	1–500	0.9993	3.0	9.0
16	Angeloylgomisin O	$Y = 4944.6x + 71.3$	21.8	7.27	3–300	0.9988	0.83	2.5

SD: standard deviation.

Each wash of the autosampler syringe cycle consisted of 200 μL strong solvent (80% acetonitrile) and 400 μL weak solvent (40% acetonitrile). The on-line UV spectra were recorded in the range of 190–400 nm.

2.4. Mass spectrometry

Mass spectrometry was performed using a Waters QTOF Premier (Micromass MS Technologies, Manchester, UK) equipped with an electrospray ionization (ESI) source in W mode, which gives a resolution of 10,000 (FWHM) and mass accuracy error less than 5 ppm. The molecular masses of the precursor ion and the product ions were accurately determined with reference compound leucine–enkephalin (m/z 556.2771) in the LockSpray mode at a concentration of 50 pg/μL at an infusion flow rate of 10 μL/min. A dwell time of 0.2 s was employed with an inter-acquisition delay of 0.01 s.

Three different MS scanning experiments were used. (1) MS full scan in positive mode was conducted. The nebulization gas was set to 600 l/h at 300 °C, the cone gas was set to 50 l/h, and the source temperature was set to 105 °C. The capillary voltage and cone voltage were set to 3000 V and 45 V, respectively. (2) MS/MS experiments were carried out by ramping collision energies from 25 and 50 V. (3) The in source collision-induced dissociation (CID) MS/MS experiments were optimized to allow fragmentation of the precursor ions to occur through cone voltage fragmentation by adjusting the cone voltage to 50 V before the passage of ions of interest into the collision cell. This additional fragmentation step allowed further fragmentation in the collision cell with collision energies from 25 V to 50 V to produce abundant product ions.

2.5. Validation procedure

2.5.1. Calibration curves

Stock solutions containing 16 reference compounds were prepared and diluted to appropriate concentrations for the construction of calibration curves. Each concentration of the mixed standard solution was injected in triplicates, and then the calibration curves were constructed by plotting the peak area versus the concentration of each analyte.

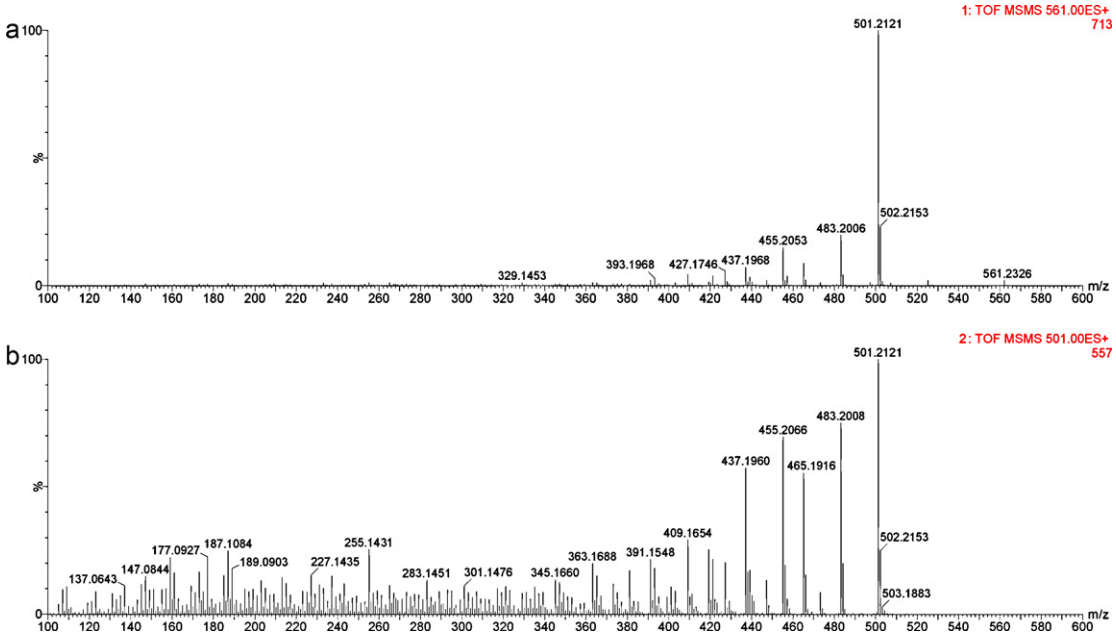
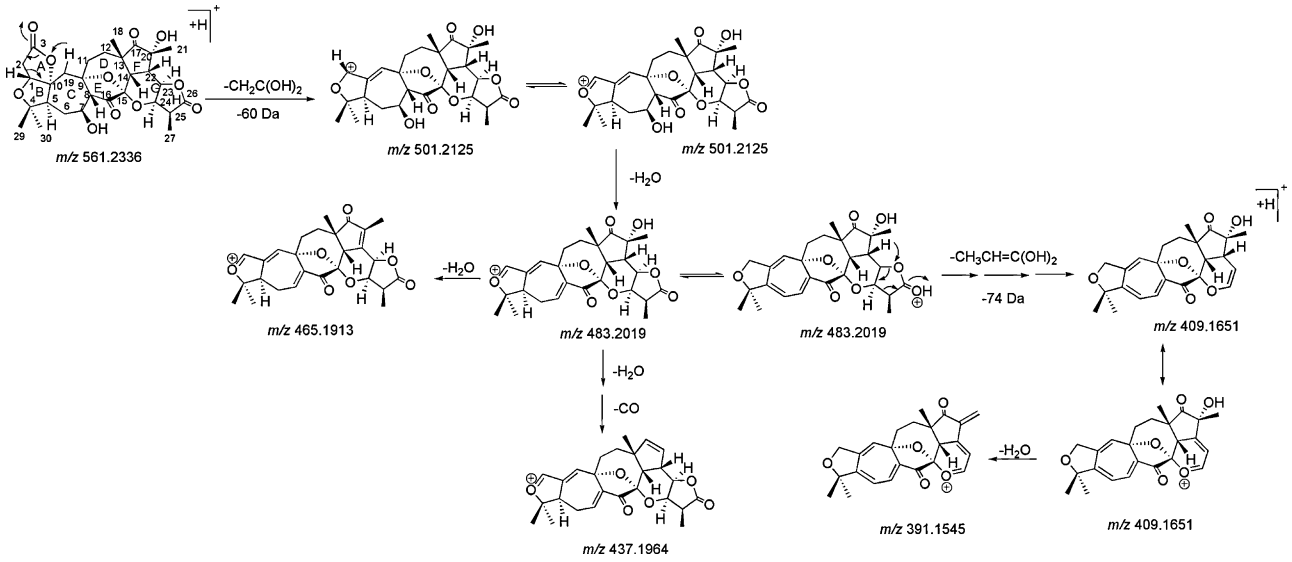


Fig. 3. (A) MS/MS of $[M+H]^+$ at m/z 561 of **2**, (A) MS^3 of selected ion at m/z 501 from m/z 561 of **1**.



Scheme 1. Partial fragmentation pathways of micrandilactone D (**2**).

2.5.2. LOD and LOQ

The stock solutions containing 16 reference compounds were diluted to a series of appropriate concentrations, and an aliquot of the diluted solutions were injected into UPLC–QTOF-MS for analysis. The limits of detection (LOD) and quantification (LOQ) under the present chromatographic conditions were determined at a signal-to-noise ratio (S/N) of about 3 and 10, respectively.

2.5.3. Precision, accuracy and repeatability

Intra- and inter-day variations were chosen to determine the precision of the developed method. For intra-day variability test, the mixed standard solutions were analyzed for six replicates within one day, while for inter-day variability test; the solutions were examined in duplicates for three consecutive days. Variations were expressed by relative standard deviation (RSD).

The recovery was used to evaluate the accuracy of the method. A known amount of standards were added into a certain amount (0.20 g) of sample 0013-01. The mixture was extracted and analyzed using the method mentioned above. Six replicates were used for the test. To confirm the repeatability, six replicates of the same samples (0013-01) were extracted and analyzed as mentioned above. The RSD value was calculated as a measurement of method repeatability.

2.6. Chemometric data analysis

The UPLC–QTOF-MS data of 48 samples of *S. chinensis* and *S. sphenanthera* were analyzed to identify potential discriminate variables. The peak finding, peak alignment, and peak filtering of ESI(+) raw data were carried out by the Markerlynx Applications Manager (version XS, Waters, Manchester, UK). The parameters used were retention time (R_t) range 0.3–13 min, mass range 70–800 Da, mass tolerance 0.01 Da. Isotopic peaks were excluded for analysis, noise elimination level was set at 10.00, minimum intensity was set to 10% of base peak intensity, maximum masses per R_t was set at 6 and R_t tolerance was set at 0.01 min. The R_t and m/z data pair for each peak were determined by the software.

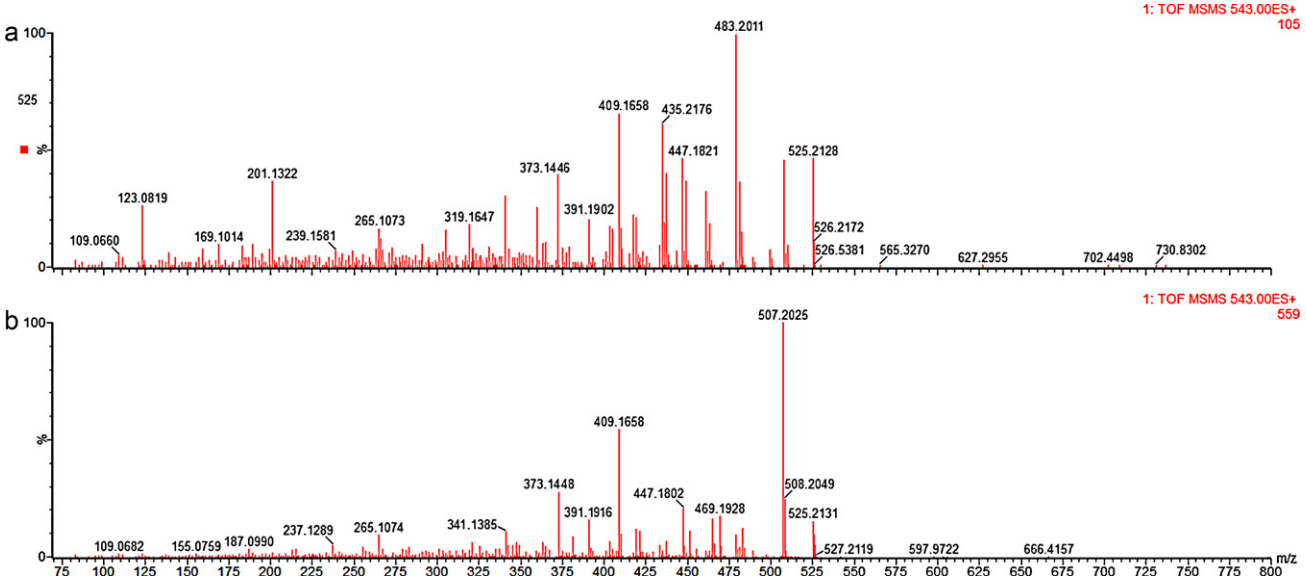


Fig. 4. (A) MS/MS of $[M+H]^+$ at m/z 543 of **17**, (B) MS/MS of $[M+H]^+$ at m/z 543 of **5**.

Table 3
Precision and recovery for the 16 markers.

No.	Compounds	Precision		Recovery	
		Intra-day RSD% (n = 6)	Inter-day RSD% (n = 6)	%	RSD% (n = 6)
1	Micrandilactone A	2.27	4.30	97.4	4.79
2	Micrandilactone D	2.72	5.34	103.5	5.28
3	Henridilactone D	3.70	6.12	86.7	6.03
4	Henridilactone B	1.05	4.66	87.9	7.31
5	Henridilactone A	3.14	7.08	92.1	6.77
6	Lancifodilactone D	1.08	3.48	95.0	4.12
7	Schisandrin	2.53	4.17	93.2	9.39
8	Gomisin D	2.84	5.91	90.8	5.26
9	Gomisin A	3.21	6.75	95.3	5.87
10	Gomisin G	3.37	5.43	96.7	7.63
11	Gomisin F	2.82	4.65	89.8	4.45
12	Schisantherin A	3.25	6.12	91.7	5.06
13	Deoxyschisandrin	4.35	8.17	105.6	6.13
14	Schisandrin B	2.33	5.60	94.4	5.72
15	Schisandrin C	3.72	3.04	92.5	4.29
16	Angeloylgomisin O	2.03	3.90	89.8	5.04

3. Results and discussion

3.1. Optimization of chromatographic separation

In order to develop optimal elution conditions for the simultaneous separation of nortriterpenoids and lignans, chromatographic parameters were optimized with speed and resolution in mind. A mixed solution of 6 nortriterpenoids and 10 lignans standards and the crude extract of *S. chinensis* (0013-01) were used for the screening and optimization of UPLC conditions. The chromatographic behavior was explored using C_8 and C_{18} columns with varying concentrations of formic acid. The results obtained showed as mobile phase acidic that the separations on the C_{18} column with two mobile phase systems, consisting of water (containing 0.1% formic acid, Mobile Phase A) and ACN (containing 0.1% formic acid, Mobile Phase B), were better than other conditions tested (C_8 column with H_2O and ACN, H_2O and MeOH). 0.1% formic acid was added to both aqueous solution and ACN to improve chromatographic behavior and to reduce the peak tailing as well as facilitating ionization. With the optimal gradient elution as described in Section 2.3, all of the 16 main standards were well separated within 12.5 min. The representative UPLC–QTOF chromatograms of standard solution and the extracts of *S. chinensis* and *S. sphenanthera* are presented in Fig. 2.

3.2. Qualitative analysis of nortriterpenoids and lignans

3.2.1. Mass spectrometry analysis of reference nortriterpenoids

The reference standards of six nortriterpenoids, namely micrandilactone A (**1**), micrandilactone D (**2**), henridilactone D (**3**), henridilactone B (**4**), henridilactone A (**5**) and lancifodilactone D (**6**), are all of the schisanartane skeleton type (Fig. 1) and were analyzed by UPLC–QTOF-MS. All reference nortriterpenoids produced abundant $[M+H]^+$ ions as the base peak in positive ESI-MS spectra. The $[M+H]^+$ ions were selected as the precursor ions for fragmentation to produce MS/MS spectra. Conditions for the in-source CID MS/MS experiments were optimized and developed through raising cone voltage and ramping collision voltage to induce more fragment ions. Micrandilactone D (**2**) is a good representative of them. In its MS/MS spectrum (as shown in Fig. 3), by using the protonated ion $[M+H]^+$ at m/z 561.2326 as the precursor ion, a predominant ion $[M+H\text{-}60]^+$ at m/z 501.2121 was detected, which was formed through the H-19 transfer to the lactone A ring via a four-membered transition state by the elimination of the neutral loss of $CH_2C(OH)_2$ (that possibly rearranges to CH_3COOH, acetic acid). The in-source CID MS/MS spectrum of the precursor ion at m/z 501.2121 displayed abundant fragment ions at m/z 483.2006, 465.1915, 455.2075, 437.1968, 409.1654, 391.1548 and 363.1592, corresponding to the losses of H_2O, $2H_2O$, CO_2, $H_2O + CO$, $2H_2O + CO$, 74 Da, 74 Da + H_2O and 74 Da + CO, respectively. Notably, the diagnostic product ion at m/z 409.1668 formed from the precursor m/z 501.2110 was interpreted as a hydrogen (H-22) transfer to the lactone H ring via a four-member transition state to eliminate the neutral fragment $CH_3CH{=}C(OH)_2$ (that possibly rearranges to C_2H_5COOH, propionic acid). The fragmentation mechanism was proposed in Scheme 1.

3.2.2. Mass spectrometry analysis of components in Schisandra samples

UPLC–DAD-QTOF-MS/MS was employed to analyze the crude extracts of different *Schisandra* samples. More than 40 peaks were detected from the crude extracts of the *Schisandra* samples (as shown in Table 1 and Fig. 2). Trace amounts of nortriterpenoids were detected which showed higher polarity than the lignans, with peaks eluting before R_t of 2.5 min corresponding to the nortriterpenoids, while the peaks eluting after R_t of 2.5 min corresponding to lignans. This class of nortriterpenoids had similar maximal UV absorption wavelengths of around 220 nm and 250 nm. By contrast, the dibenzocyclooctadiene lignans exhibited a maximal UV absorption wavelength at about 220 nm. By comparing the retention time, UV and ESI-MS/MS spectra data with those of authentic compounds, 16 peaks, including six *Schisandra* nortriterpenoids and ten dibenzocyclooctadiene lignans, were unequivocally identified, namely micrandilactone A (**1**), micrandilactone D (**2**), henridilactone D (**3**), henridilactone B (**4**), henridilactone A (**5**), lancifodilactone D (**6**), schisandrin (**7**), gomisin D (**8**), gomisin A (**9**), gomisin G (**10**), gomisin F (**11**), schisantherin A (**12**), deoxyschisandrin (**13**), schisandrin B (**14**), schisandrin C (**15**), and angeloylgomisin O (**16**). Twenty other peaks (peaks 17–37), including one nortriterpenoid and 19 dibenzocyclooctadiene lignans were tentatively identified by comparing their HR-MS and MS/MS data with those published.

The trace peak 17 showed an accurate mass of $[M+H]^+$ ion at m/z 543.2236, corresponding to the molecular formula $C_{29}H_{35}O_{10}$, is an isomer of compound **5**. This was verified by studying the fragmentation pattern of compound **17** and compared it with compound **5** (Fig. 4). For instance, the MS/MS spectrum of **17** contained major fragment ions at m/z 525.2128 $[M+H-H_2O]^+$, 507.2012 $[M+H-2H_2O]^+$, 483.2011 $[M+H-C_2H_4O_2]^+$, 447.1821 $[M+H-2H_2O-CH_2C(OH)_2]^+$, 409.1658 $[M+H-CH_2C(OH)_2-CH_3CHC(OH)_2]^+$, 391.1901 $[M+H-2H_2O-CH_2C(OH)_2-2CO]^+$, 373.1445 $[M+H-2H_2O-CH_2C(OH)_2-CH_3CHC(OH)_2]^+$, 365.1758 $[M+H-CH_2C(OH)_2-CH_3CHC(OH)_2-CO_2]^+$, 341.1382 $[M+H-2H_2O-C_{10}H_{15}O_3]^+$, 265.1073

Table 4
The contents of 16 standards in the tested samples (μg/g).

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0013-01	0.113	0.043	0.043	0.033	0.80	0.049	3495.6	503.1	837.0	348	203	1465	2754.0	3552.4	2.58	228.4
0013-02	–	0.014	0	0	0.34	0.024	3647.9	505.2	824.8	354	172	1828	2292.4	3011.5	3.12	237.5
0013-03	0.123	0.064	0.032	0	0.78	0.046	4307.0	518.7	1453.0	537	207	20.8	2902.8	3612.5	1.52	252.4
0013-04	0.162	0.115	0.127	0.034	0.12	0.23	3345.2	463.0	1360.1	543	221	1767	3068.0	3869.1	4.78	180.3
0013-05	0.092	0.077	0.043	0.008	0.80	0.041	4650.7	497.6	714.3	354	240	1815	3303.2	4208.7	3.28	211.8
0013-06	0.113	0.013	0.070	0	0.58	0.069	4092.1	535.8	510.3	400	217	1990	3059.6	3782.8	4.16	231.5
0013-07	0.07	0	0	0	0.05	0	68.2	16.7	0	28	1	2812	9826.0	39.8	0	213.4
0013-08	0	0	0	0	0.047	0.007	60.5	1.9	0	0	0	2009	9536.2	0	0	15.5
0013-09	0.009	0	0	0	0.023	0	65.0	27.8	0	37	0	2826	8818.2	28.8	0	12.9
0013-10	0	0	0	0	0.055	0	37.9	16.4	0	0	0	116	1758.0	0	0.3	130.7
0013-11	–	0	0	0	0	0	37.8	0	0	0	1.8	23	103.6	51.0	0	7.8
0013-12	0.069	0.022	0	0.007	0.023	0.005	37.9	0	0	0	1	41	85.5	35.3	0	9.9
0013-13	0	0	0	0.031	1.32	0.009	44.6	352.4	0.8	189	47	183	10.3	842.6	0.41	37.7
0013-14	0	0	0	0	0.10	0.011	43.8	4.4	0	25	6	1036	2764.1	117.5	0	134.4
0013-15	0.01	0	0	0	0	0	38.5	29.4	0	3	0	248	521.8	0	0	42.3
0013-16	0	0	0	0	0	0.003	43.1	27.0	0	10	0	528	1014.8	19.8	0	51.7
0013-17	0.021	0.043	0.015	0	0.30	0	1352.4	112.3	383.5	228	89	1983	3941.8	1568.5	1.5	222.6
0013-18	0.012	0	0	0	0.070	0.011	76.5	36.0	8.3	158	21	3589	6699.6	387.9	0	662.1
0013-19	0.007	0	0	0	0.12	0	113.0	66.6	3.2	413	33	5294	11333.1	589.0	0	424.1
0013-20	0	0	0	0	0	0	168.2	52.1	0	176	6	4361	9426.4	125.1	0	778.2
0013-21	0.01	0	0	0.002	0.015	0.001	70.0	5.9	0	56	0	3010	6420.4	0	0	557.9
0013-22	0	0.249	0.047	0.32	0.72	0.058	158.8	2.6	56.6	75	9	3287	1033.1	186.2	0.4	1423.5
0013-23	0	0	0	0	0.087	0	78.1	21.6	0	0	3	2072	4891.6	70.0	0	18.8
0013-24	0.013	0	0	0.012	0.14	0.001	66.8	2.5	0	71	2	2922	5524.5	53.3	0	31.1
0013-25	0.057	0	0.022	0	0.36	0.059	1982.6	159.8	378.2	302	119	1430	1426.1	2082.1	1.4	208.1
0013-26	0	0	0	0	0.012	0	79.5	104.7	0	45	8	2148	5868.9	154.8	0	1093.0
0013-27	0.022	0.022	0.008	0.023	0.59	0.016	1593.7	137.6	464.5	290	2	1757	3352.9	53.9	2.4	11.1
0013-28	0.139	0.085	0	0	0.88	0.065	3759.2	237.0	585.4	1208	178	2589	3269.5	3109.1	2.5	1221.7
0013-29	0.023	0.063	0.016	0	0.91	0.018	1464.2	146.2	354.6	255	94	893	1112.3	1657.0	3.6	141.4
0013-30	0	0	0	0	0	0	72.8	0	5.0	187	8	1584	4493.6	164.2	0	161.7
0013-31	0.019	0.079	0	0	1.6	0.046	2232.6	220.1	598.5	9	133	1793	1565.2	2336.4	4.8	234.9
0013-32	0.013	0	0	0	0.34	0.003	63.1	0	0	285	13	2358	5714.5	254.6	0	177.2
0013-33	0.008	0.020	0	0	0.47	0.006	1789.2	114.5	350.4	451	97	5034	10808.6	1708.1	1.0	737.2
0013-34	0.013	0.017	0	0	0.15	0.001	917.1	66.6	238.8	164	63	1892	4040.7	1122.6	0.7	213.0
0013-35	0.019	0.012	0	0	0	0.003	2262.7	200.7	565.2	627	143	1833	10669.5	2510.4	1.1	655.6
0013-36	2.75	3.74	1.14	2.9	0.38	0.19	1334.8	9.3	51.2	52	154	1855.2	2116.7		0.54	374.6
0013-37	0	0.032	0.151	3.7	1.2	0.16	170.6	3.7	0	9	14	607	270.1		42.5	16.4
0013-38	0.79	0.642	1.83	0.085	0.077	0.13	722.5	88.0	237.1	1743	360	131.6	1249.4		7.2	35.4
0013-39	0	0	0.016	0	0	0	8108.1	650.4	4325.7	4531	313	249.2	1653.4		0.9	106.0
0013-40	0	0	0.0136	0	0	0.033	28.6	2.6	9.5	48	8.9	22.74	1144.0		3.8	128.2
0013-41	0	0	0	0.23	0.013	0	519.6	30.8	196	810	371	2391.1	6483.9		1.6	269.6
0013-42	0.162	0.064	0	0.36	0	0	358	10.6	170.4	1079	399	2128	6851.1		1.7	771.4
0013-43	0.202	0.061	0.011	0.31	0.014	0.075	91.5	0	14.4	96	25	39.05	936.6		0	103.6
0013-44	0	0	0	0.27	0	0	965.2	6.7	108.8	665	332	1814.7	6498.1		12.5	117.6
0013-45	0.084	0.154	0	0.30	0	0.076	421.8	0	64.4	607	617	1699.4	6737.9		22.9	67.8
0013-46	0	0	0	0.084	0	0	16.5	0	0	0	0	20	341.6		0	0
0013-47	0	0.022	0	0.82	0.038	0	974.6	8.4	169.5	1723	667	17.15	7782.8		9.3	68.8
0013-48	0	0	0	0.22	0.018	0	861.1	9.8	217.0	1232	347	419.7	7490.7		6.6	80.2

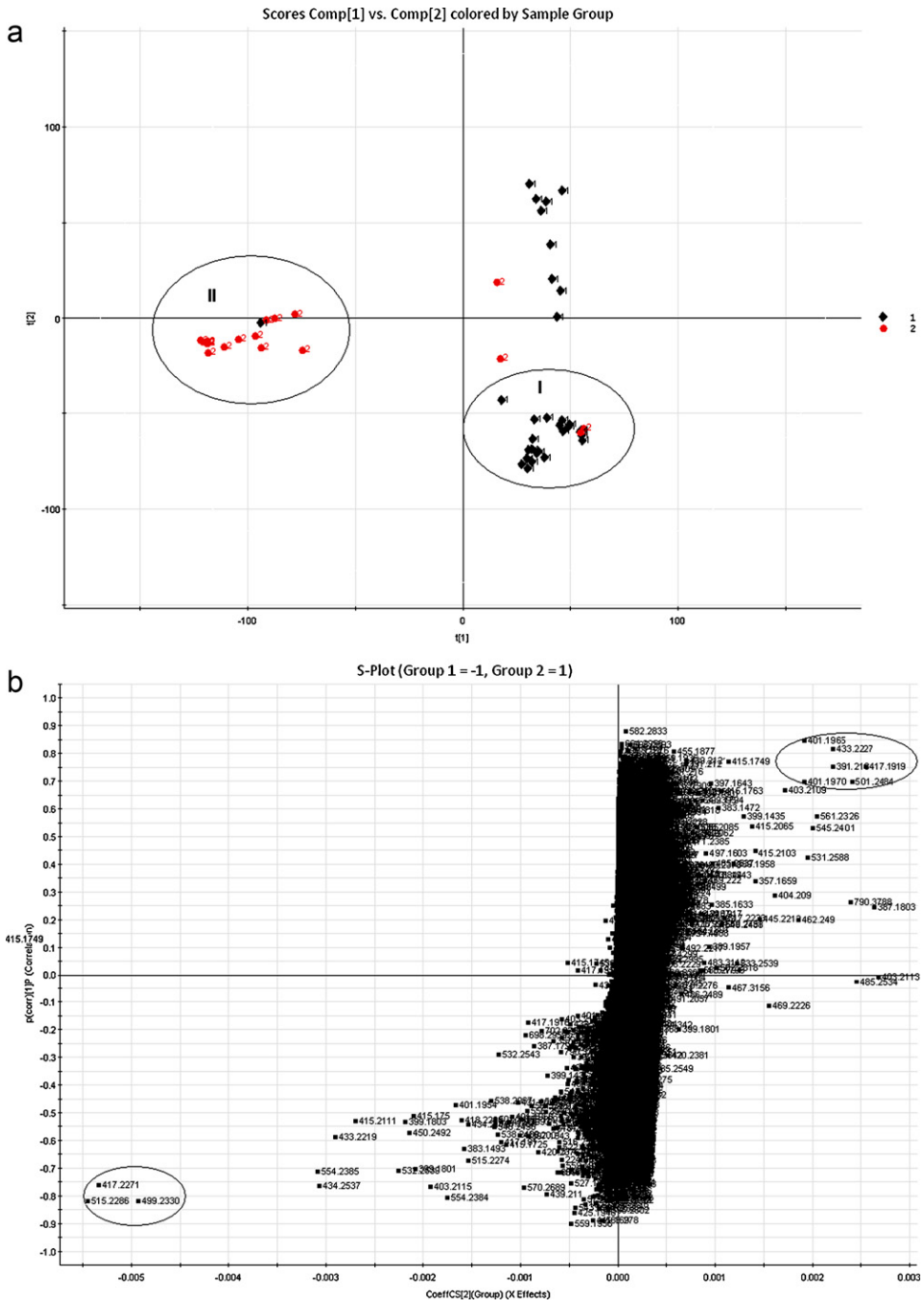


Fig. 5. (A) Score plot. (B) OPLS-DA/S-plot of 48 *Schisandra* samples.

$[M+H-2H_2O-C_{16}H_{19}O_3]^+$. The MS/MS spectrum showed the characteristic fragment ions at *m/z* 483.2011, and 373.1445, which was formed through the losses of $CH_2C(OH)_2$ (60 Da) and $CH_3CH{=}C(OH)_2$ (74 Da). The fragment ion at *m/z* 365.1758 formed from the precursor *m/z* 409.1658 via the loss of CO_2 indicated the presence of the hydroxyl unit connected to C-1 in the structure of **17**. According to the above findings as well as other supporting evidence from literature, compound **17** was tentatively assigned to be schindilactone A [31].

Comprehensive studies have been reported for lignans from various *Schisandra* species [13–18]. These lignans have the same framework structure but different peripheral substituents at C-2, C-3, C-6, C-7, C-12, C-13 and C-14. The elimination of CH_3, CH_2O and CH_3O from the OCH_3 groups and the loss of H_2O and/or organic acid from the eight-member dibenzene ring are diagnostic fragmentation pathways. A formula database of known lignans in *S. chinensis* and *S. sphenanthera* has been established in our laboratory, which was used to tentatively identify 19 lignans (peaks 18–37) by comparing the exact masses and formulae of their MS and MS/MS data acquired by UPLC–QTOF-MS with those of literatures. Those include angeloylgomisin H (**18**) [14,15], tigloylgomisin H (**19**) [14,15], schisandrol B (**20**) [14,15], benzoylgomision H (**21**) [15], tigloylgomisin Q (**22**) [14,15], schisanhernol (**23**) [15,17], schisantherin B (**24**) [14,15], schisantherin C (**25**) [14,15], wuweilignan D (**26**) [32], schisantherin D (**27**) [14,16], gomisin K1 (**28**) [18], wuweilignan L (**29**) [32], gomisin O (**30**) [14], gomision M2 (**31**)

Table 5
The marker compounds which attributed to differentiate *S. chinensis* and *S. sphenanthera* samples by PCA.

Peak No.	*S. chinensis*	*S. sphenanthera*	Compounds	Reference
2	+		Micrandilactone D	[23]
7	+		Schisandrin	[7]
9	+		Gomisin A	[7,26]
13	+	++	Deoxyschisandrin	[7]
14	+		Schisandrin B	[7]
19	+		Tigloylgomisin H	[7,14,15]
25		+	Schisantherin C	[7]
32	+		γ-Schsandrin	[7,14,17]
35		+	Tigloylgomisin O	[7]
37	+		Pregomisin	[7,14]

[17], γ-schsandrin (**32**) [14,17] schisandrene (**33**) [33], benzoylgomisin O (**34**) [18], tigloylgomisin O (**35**) [18], gomisin R (**36**) [14] and pregomisin (**37**) [14].

3.3. Quantification of nortriterpenoids and lignans

3.3.1. Validation of method

The linearity, regression, and linear ranges of 16 analytes were determined using the developed UPLC–QTOF-MS method (Table 2). The correlation coefficient values ($r > 0.998$) indicated appropriate correlations between the investigated compound concentrations and their peak area within the test ranges. The LOD and LOQ were less than 11 and 33 ng/mL (Table 2), which were determined at a signal-to-noise ratio (S/N) of about 3 and 10, respectively. The overall intra- and inter-day variations (RSD) of the 16 analytes were less than 4.35% and 8.17%, respectively (Table 3). The developed method had good accuracy and repeatability, with the recoveries being in the range of 86.7–105.6% (RSD < 9.39%) (Table 3).

3.3.2. Quantification results

The high precision and accuracy of the optimized UPLC–QTOF-MS method was then applied to the simultaneous quantification of main components of 6 nortriterpenoids and 10 lignans in 48 samples of *S. chinensis* and *S. sphenanthera*, by using XICs with a 0.01 Da window under full-scan MS conditions.

The results obtained as shown in Table 4 showed that lignans were found abundantly in *S. chinensis* and *S. Sphenanthera*. The content of schisandrin (**7**), schisantherin A (**12**), deoxyschisandrin (**13**) and schisandrin B (**14**) was high in most *Schisandra* samples, and the content of gomisin D (**8**), gomisin A (**9**), gomisin G (**10**), gomisin F (**11**) and angeloylgomisin O (**16**) was moderate in *Schisandra* samples. On the other hand, schisandrin C (**15**) content was low in most *Schisandra* samples. In contrast, the content of the nortriterpenoids was very low in all *Schisandra* samples, with the stem samples (0013-13, 22, 36-38) containing relatively higher contents of nortriterpenoids. In addition, there were large variations in the content of the investigated lignans and nortriterpenoids in the *Schisandra* samples among different species and different geographic resources, which may be responsible for the different therapeutic efficacies of these herbal plants.

3.4. Principal component analysis (PCA)

To identify the differences between *S. chinensis* and *S. sphenanthera*, unsupervised principal component analysis (PCA) and supervised orthogonal partial least squared discriminant analysis (OPLS-DA) were performed. After Pareto scaling with mean-centering, a two-component PCA score plot of UPLC–QTOF-MS data was utilized to depict general variations of components among the *Schisandrin* species. As shown in the score plot (Fig. 4A), the determined samples could be separated into two clusters, the *S. chinensis* cluster and *S. sphenanthera* cluster, indicating that there is global chemical difference between the two species. However, within the same species the clustering was not very tight and some individual samples were sparsely distributed. This implies that the combined usage of commercial *S. chinensis* and *S. sphenanthera* is a common practice.

Since a total of 30,725 variables were initially included, and in order to find the potential chemical markers for the discrimination between *S. chinensis* and *S. sphenanthera*, statistical analysis was performed to generate a S-plot (Fig. 5B). In the S-plot, each point represents an ion t_R-m/z pair; the *X* axis represents variable contribution, so the farther the distance the ion t_R-m/z pair points is from zero, the more the ion contributes to the difference between the two groups; the *Y* axis represents variable confidence, so the farther the distance the ion t_R-m/z pair points is from zero, the higher the level of confidence to the difference between the two groups. So, the t_R-m/z pair points at the two ends of "**S**" represent characteristic markers with the most confidence to each group.

Eight peaks at top right corner of "**S**" are the peaks of *S. chinensis* samples that contribute most to the difference between the two *Schisandrin* species. It was found that peaks **7** (t_R 2.03 min, m/z 433.2227), **9** (t_R 2.71 min, m/z 417.1919), **14** (t_R 10.72 min, m/z 401.1966), **19** (t_R 3.82 min, m/z 501.2484), **32** (t_R 10.32 min, m/z 401.1970), **37** (t_R 12.57 min, m/z 391.2125) have higher intensity in most *S. chinensis* samples, but lower intensity in most *S. sphenanthera* samples. Moreover, norterpenoids **2** (t_R 0.80 min, m/z 561.2326) was detectable in most *S. chinensis* samples but were undetectable in *S. sphenanthera*. Similarly, peaks **13** (t_R 9.04 min, m/z 417.2272), **25** (t_R 5.67 min, m/z 515.2286) and **35** (t_R 11.65 min, m/z 499.2330) at the bottom left corner of "**S**" are the ions of *S. sphenanthera*, which were detected in higher intensity in most *S. sphenanthera* samples, and so would be the most suitable chemical markers for the identification of *S. sphenanthera*. Detailed comparison of the results obtained by PCA with previous works in the literature was listed in Table 5.

4. Conclusion

An UPLC–QTOF-MS/MS method was developed for the first simultaneous qualitative and quantitative analyses of the nortriterpenoids and lignans in *Schisandra* samples in the evaluation of the biodiversities of *S. chinensis* and *S. sphenanthera* with high sample throughput. The fragmentation pathways of the nortriterpenoids were proposed to rationalize the observed MS/MS behavior; the elimination of $CH_2C(OH)_2$ (or CH_3COOH) and CH_3CH–$C(OH)_2$ (or C_2H_5COOH) from the lactone A and H ring is the dominant fragmentation pattern. Meanwhile, this newly established method was validated to be sensitive, precise and accurate, and has been successfully applied to the quantitative determination of 6 nortriterpenoids and 10 lignans in 48 *Schisandra* samples. The results showed that there were low content of nortriterpenoids, but high content of lignans in both *S. chinensis* and *S. sphenanthera*

samples. PCA was utilized for screening and identification of marker components for the differential analysis of *S. chinensis* and *S. sphenanthera*. Obviously, the *S. chinensis* were separated from *S. sphenanthera samples*, and the marker compounds, which attribute to the signification of the two species, were identified by OPLS-DA.

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References

[1] Y.-H. Liu, X.-R. Luo, R.-F. Wu, B.-N. Zhang, in: Y.-H. Liu (Ed.), Flora of China, vol. 30(1), Science Press, 1996, pp. 243–244.
[2] Chinese Pharmacopoeia Commission, Pharmacopoeia of the People's Republic of China, vol. 1, People's Medical Publishing House, Beijing, 2010, p. 61, 227.
[3] Y.-H. Kuo, L.-Y. Kuo, C.-F. Chen, Four new C19 homoligans, scharisanrins A, B, and D and cytotoxic schiarianrin C, from Schizandra arisanensis, J. Org. Chem. 62 (1997) 3242–3245.
[4] D.-F. Chen, Y.-H. Wang, J.-P. Gao, Q.-Z. Sun, J.-Y. Hu, in: H.R. Zhou, J.A. Duan (Eds.), Plant Chemotaxonomy, Shanghai Scientific and Technical Publisher, Shanghai, China, 2005, p. 451.
[5] Y.-G. Chen, P. Wang, Z.-W. Lin, H.-D. Sun, G.-W. Qin, Y.-Y. Xie, Dibenzocyclooctadiene lignans from Kadsura angustifolia, Phytochemistry 48 (1998) 1059–1062.
[6] J.-B. Chang, J. Reiner, J.-X. Xie, Progress on the chemistry of dibenzocyclooctadiene lignans, Chem. Rev. 105 (2005) 4581–4609.
[7] Y. Lu, D.-F. hen, C. hen, Analysis of *Schisandra chinensis* and *Schisandra sphenanthera*, J. Chromatogr. A 1216 (2009) 1980–1990.
[8] L.-N. Li, H. Xue, Triterpenoids from roots and stems of Kadsura coccinea, Planta Med. 52 (1986) 492–493.
[9] K. Kangouri, T. Miyoshi, A. Kawashima, A. Ikeda, T. Mizutani, S. Omura, L.-N. Li, H. Xue, Planta Med. 55 (1989) 297–299.
[10] H.-D. Sun, S.-X. Qiu, L.-Z. Lin, Z.-Y. Wang, Z.-W. Lin, T. Pengsuparp, J.-M. Pezzuto, H.-H.-S. Fong, C.-A. Cordell, N.-R. Farnsworth, Nigranoic acid a triterpenoid from Schisandra sphaerandra that inhibits HIV-1 reverse transcriptase, J. Nat. Prod. 59 (1996) 525–527.
[11] D.-F. Chen, S.-X. Zhang, H.-K. Wang, S.-Y. Zhang, Q.-Z. Sun, M. Cosentino, K.-H. Lee, Novel anti-HIV lancilactone C and related triterpenes from Kadsura lancilimba, J. Nat. Prod. 62 (1999) 94–97.
[12] W.-L. Xiao, R.-T. Li, S.-X. Huang, J.-X. Pu, H.-D. Sun, Triterpenoids from the Schisandraceae family, Nat. Prod. Rep. 25 (2008) 871–891.
[13] C.-W. Halstead, S. Lee, C.-S. Khoo, J.-R. Hennell, A. Bensoussan, Validation of a method for the simultaneous determination of four schisandra lignans in the raw herb and commercial dried aqueous extracts of *Schisandra chinensis* (Wu Wei Zi) by RP-LC with DAD, J. Pharm. Biomed. Anal. 45 (2007) 30–37.
[14] X.-G. He, L.-Z. Lian, L.-Z. Lin, Analysis of lignan constituents from *Schisandra chinensis* by liquid chromatography–electrospray mass spectrometry, J. Chromatogr. A 757 (1997) 81–87.
[15] X. Huang, F.-R. Song, Z.-Q. Liu, S.-Y. Liu, Studies on lignan constituents from Schisandra chinensis (Turcz.) Bail. fruits using high-performance liquid chromatography/electrospray ionization multiple-stage tandem mass spectrometry, J. Mass Spectrom. 42 (2007) 1148–1161.
[16] X. Huang, F.-R. Song, Z.-Q. Liu, S.-Y. Liu, Structural characterization and identification of dibenzocyclooctadiene lignans in *Fructus Schisandrae* using electrospray ionization ion trap multiple-stage tandem mass spectrometry and electrospray ionization Fourier transform ion cyclotron resonance multiple-stage tandem mass spectrometry, Anal. Chim. Acta 615 (2008) 124–135.
[17] Z.-Y. Lou, H. Zhang, C.-G. Gong, Z.-Y. Zhu, L. Zhao, Y.-J. Xu, B. Wang, G.-Q. Zhang, Analysis of lignans in *Schisandra chinensis* and rat plasma by high-performance liquid-chromatography diode-array detection time-of-flight mass spectrometry and quadrupole ion trap mass spectrometry, Rapid Commun. Mass Spectrom. 23 (2009) 831–842.
[18] P.-Y. Shi, Q. He, Y.-F. Zhang, H.-B. Qu, Y.-Y. Chen, Quantitative determination of lanostane triterpenes in Fomes officinalis and their fragmentation study by HPLC-ESI, Phytochem. Anal. 20 (2010) 197–206.
[19] Y. Zhou, Q.-B. Han, J.-Z. Song, C.-F. Qiao, H.-X. Xu, Characterization of polyprenylated xanthones in *Garcinia xipshuanbannaensis* using liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry, J. Chromatogr. A 1206 (2008) 131–139.
[20] Y. Zhou, X. Liu, J. Yang, Q.-B. Han, J.-Z. Song, S.-L. Li, C.-F. Qiao, L.-S. Ding, H.-X. Xu, Analysis of caged xanthones from the resin of *Garcinia hanburyi* using ultra-performance liquid chromatography/electrospray ionization quadrupole time-of-flight tandem mass spectrometry, Anal. Chim. Acta 629 (2008) 104–118.
[21] Y. Zhou, G. Xu, F.F.-K. Choi, L.-S. Ding, Q.-B. Han, J.-Z. Song, C.-F. Qiao, Q.-S. Zhao, H.-X. Xu, Qualitative and quantitative analysis of diterpenoids in *Salvia* species by liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry, J. Chromatogr. A 1216 (2009) 4847–4858.
[22] R.-T. Li, Q.-S. Zhao, S.-H. Li, Q.-B. Han, H.-D. Sun, Y. Lu, L.-L. Zhang, Q.-T. Zheng, A. Micrandilactone, A novel triterpene from *Schisandra micrantha*, Org. Lett. 5 (2003) 1023–1026.
[23] R.-T. Li, W.-L. Xiao, Y.-H. Shen, Q.-S. Zhao, H.-D. Sun, Structure characterization and possible biogenesis of three new families of nortriterpenoids: schisanartane, schiartane, and 18-norschiartane, Chem. Eur. J. 11 (2005) 2989–2996.
[24] R.-T. Li, Y.-H. Shen, W. Xiang, H.-D. Sun, Four novel nortriterpenoids isolated from *Schisandra henryi* var. *yunnanensis*, Eur. J. Org. Chem. 80 (2004) 7–811.
[25] R.-T. Li, W. Xiang, S.-H. Li, Z.-W. Lin, H.-D. Sun, Lancifodilactones B–E new nortriterpenes from *Schisandra lancifolia*, J. Nat. Prod. 67 (2004) 94–97.
[26] Y. Ikeya, H. Taguchi, I. Yosioka, The constituents of *Schizandra chinensis* BAILL. I. Isolation and structure determination of five new lignans, gomisin A B, C, F, and G, and the absolute structure of schizandrin, Chem. Pharm. Bull. 27 (1979) 1383–1394.
[27] H. Taguchi, Y. Ikeya, The structures of two new lignans, gomisin F and G, and absolute structures of gomisin A, B, and C, Chem. Pharm. Bull. 25 (1977) 364–366.
[28] J.-S. Liu, S.-D. Fang, M.-F. Huang, Y.-L. Gao, J.-S. Hsu, Chemistry of lignans from plants of Schisandraceae, Huaxue Xuebao 34 (1976) 229–240.
[29] C.-S. Liu, M.-F. Huang, Y.-L. Kao, The structures of schisanhenol and deoxyschizandrin, Huaxue Xuebao 36 (1978) 193–197.
[30] Y. Ikeya, N. Ookawa, H. Taguchi, I. Yosioka, The structures of three new lignans, angeloylgomisin O, and angeloyl- and benzoylisogomisin O, Chem. Pharm. Bull. 30 (1982) 3202–3206.
[31] S.-X. Huang, R.-T. Li, J.-P. Liu, Y. Lu, Y. Chang, C. Lei, W.-L. Xiao, L.-B. Yang, Q.-T. Zheng, H.-D. Sun, Isolation and characterization of biogenetically related highly oxygenated nortriterpenoids from *Schisandra chinensis*, Org. Lett. 9 (2007) 2079–2082.
[32] S.-X. Huang, Investigation on the chemical constituents and bioactivity of five medicinal plants, PhD thesis of Kun-Ming Institute of Botany, Chinese Academy of Sciences, 2007.
[33] Y.-W. Choi, S. Takamatsu, S.-I. Khan, P.-V. Srinivas, D. Ferreira, J.-P. Zhao, I.-A. Khan, Schisandrene a dibenzocyclooctadiene lignan from *Schisandra chinensis*: structure–antioxidant activity relationships of dibenzocyclooctadiene lignans, J. Nat. Prod. 69 (2006) 356–359.