Supplementary material

Rapid Analysis for Differential Chemical Compositions of *Poria cocos* by Using Thin-Layer Chromatography Spray Ionization-Mass Spectrometry

Ying Yang [1], Linnan Li [1, *], Na Li [1], Fan Li [1], Wenxiang Fan[1], Yitian He [1], Zhengtao Wang [1, 2, *], Li Yang [1, 2, *]

1. The MOE Key Laboratory of Standardization of Chinese Medicines, The SATCM Key Laboratory of New Resources and Quality Evaluation of Chinese Medicines, The Shanghai Key Laboratory for Compound Chinese Medicines, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

2. Shanghai R&D Center for Standardization of Chinese Medicines, Shanghai 201203, China

Corresponding author:

Prof. Linnan Li, E-mail: linnanli@shutcm.edu.cn;

Prof. Li Yang, E-mail: yl7@shutcm.edu.cn;

Prof. Zhengtao Wang, E-mail: ztwang@shutcm.edu.cn

Experimental

Comparison of TLCSI-MS and HPLC-MS methods

The *Poria cocos*-22 was selected and the samples were firstly analyzed using thin-layer chromatography. The spot areas on the TLC plates were then analyzed using TLCSI-MS and HPLC-MS, respectively. As shown in Fig. S14, the results of TLCSI-MS and HPLC-MS were generally consistent. In addition, to exclude background interference, the blank TLC plate was tested before the experiments (Fig. S15).

Stability experiments of TLCSI-MS method

The same test solution (*Poria cocos*-22) was precisely aspirated at 0 h, 2 h, 4 h, 6 h, 12 h, 24 h and 36 h for determination. Poria acid and dehydrococodylic acid were used as an indicator component, and the results of pachymic acid content with RSD (n=3) of 1.80%, depachymic acid content with RSD (n=3) of 1.87% were obtained. This indicated that the stability of the method is good (Table S3).

UPLC-Q-TOF-MS analysis

Chromatographic separation was performed on a Waters ACQUITY UPLC™ system (Waters, Milford, MA, USA), equipped with a binary solvent delivery system and an auto-sampler. An ACQUITY UPLC® BEHC18 chromatographic column (2.1 mm×100 mm, 1.7 μm, Waters) was used for separation at 30°C. The mobile phase consisted of (A) acetonitrile and (B) 0.1% formic acid aqueous solution. A gradient elution program was set as follows: 0-15 min 10%-95% A, 15-18 min 10% A. The flow

rate was set at 0.3 $mL \cdot min^{-1}$ and the injection volume of the sample was 5 μL. The temperature of the auto-sampler was maintained at 10°C.

UPLC™-QTOF Synapt G2 HDMS (Waters, Milford, MA, USA) equipped with an electrospray ionization source and Micromass ZQ 2000 single quadrupole detector was detected. The mass spectrometer was employed at negative ion mode and operated over a range of m/z 100 to 1500, and nitrogen was used as nebulization and desolvation gas. The desolvation gas flow rate was 600 $L \cdot h^{-1}$ at the temperature of 350°C. The source temperature was held at 120°C. The capillary voltage and cone voltage were set at 2.8 kV and 55 V, respectively. The cone gas flow was set at 50 $L \cdot h^{-1}$ and detection mode was MSE mode.

Supplementary figures

Supplementary tables

Table S1 Information of 20 batches of *Poria cocos*.

Table S2 Information table of *Poria cocos* preparations.

Table S3 Results of stability test measurements.

Table S4 Chemical characterization of triterpene compounds in PC, RP, WP and PRP by TLCSI-MS/MS.

Table S5 Recovery of triterpene acids in different *Poria cocos* preparations by TLCSI-MS in negative-ion mode.

Table S6 Comparison of TLCSI-MS and HPLC-MS method for analysis of two triterpene acids in different batches of Poria cocos samples.

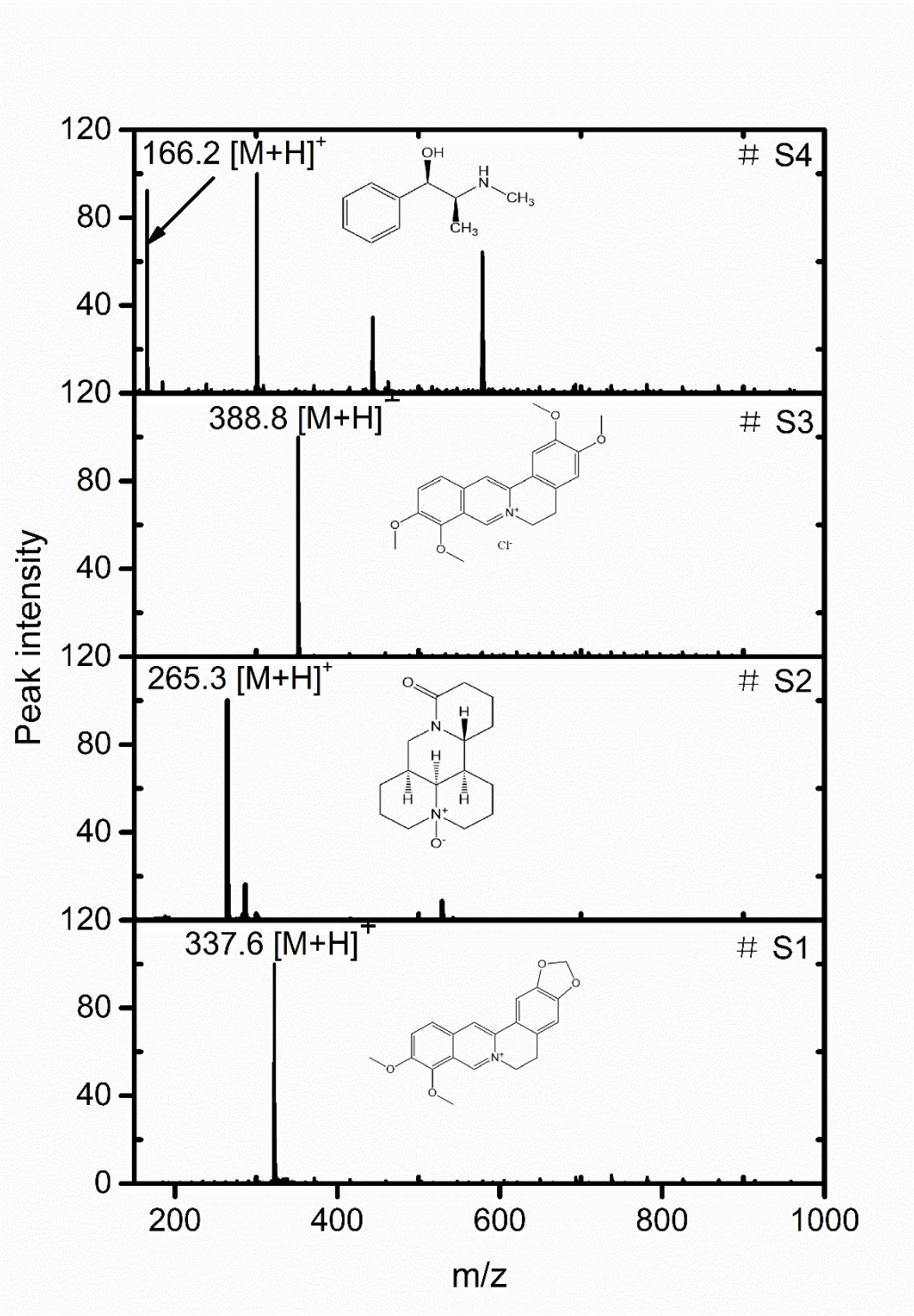

Fig. S1 TLCSI-MS spectra of alkaloids: berberine (S1), oxymatrine (S2), palmatine chloride (S3), and ephedrine (S4).

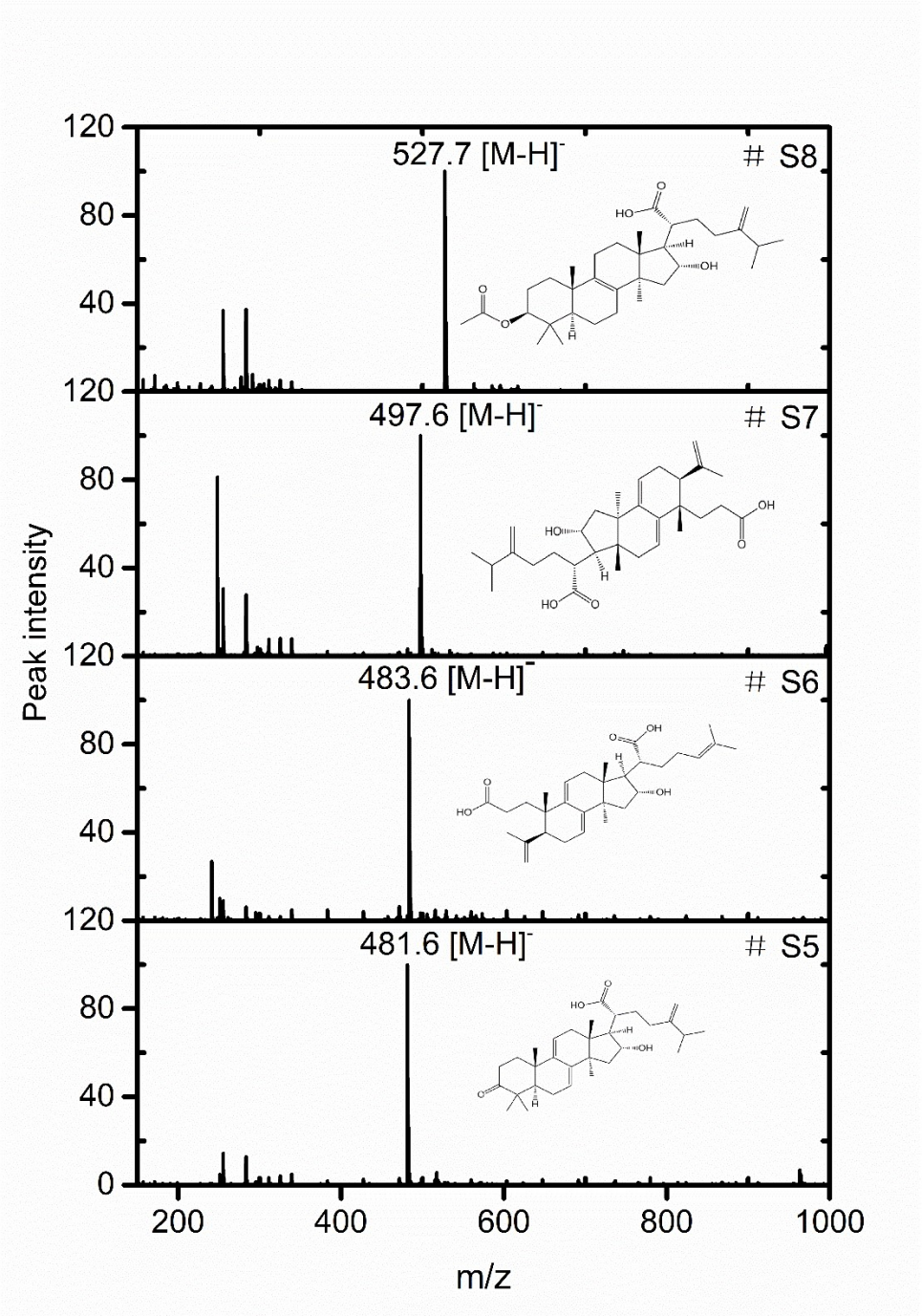

Fig. S2 TLCSI-MS spectra of triterpene acids: polyporenic acid C (S5), poricoic acid B (S6), poricoic acid A (S7), and pachymic acid (S4).

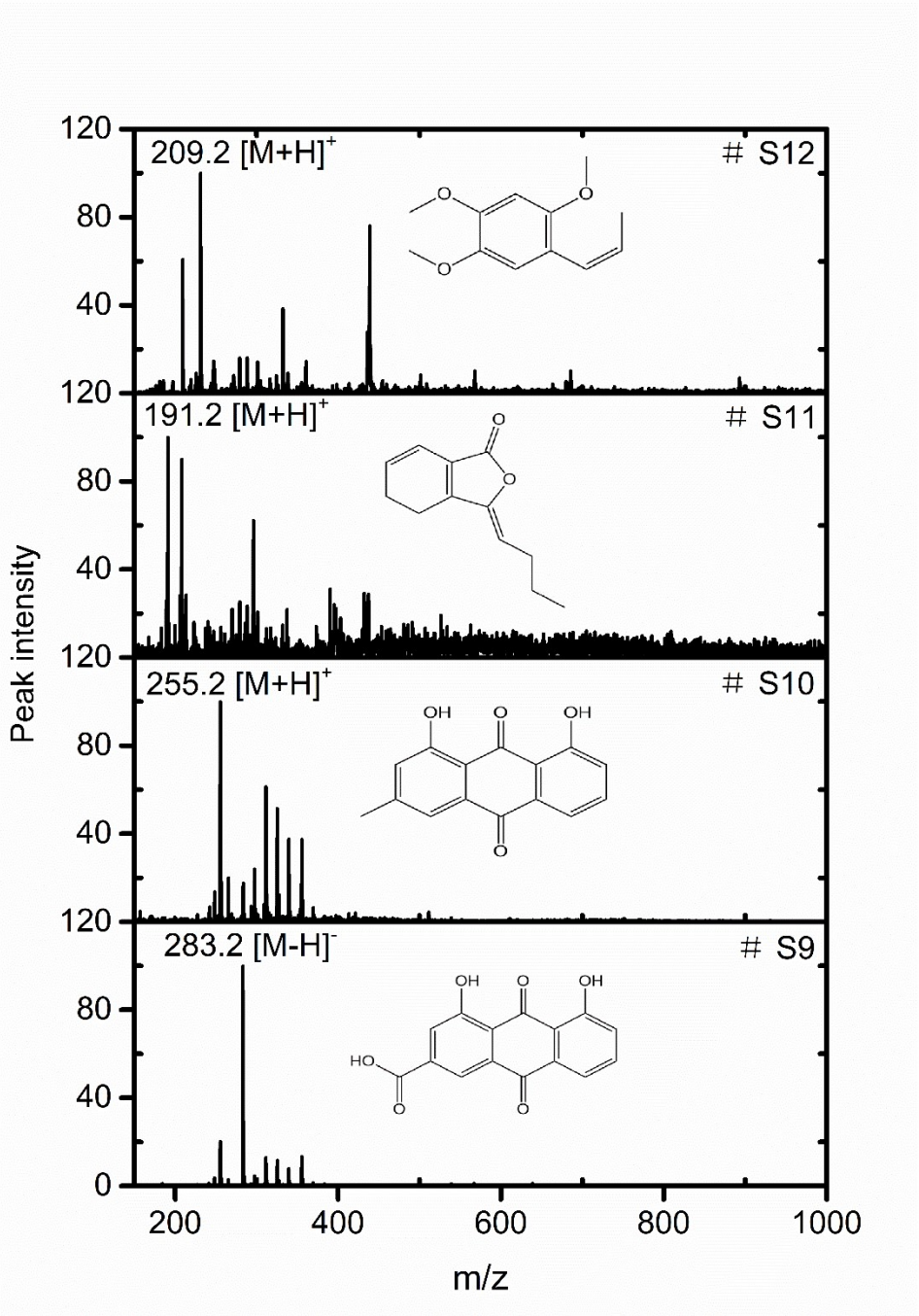

Fig. S3 TLCSI-MS spectra of anthraquinones and volatile oils: rhein (S9), chrysophanol (S10), ligustilide (S11), and β-asarone (S12).

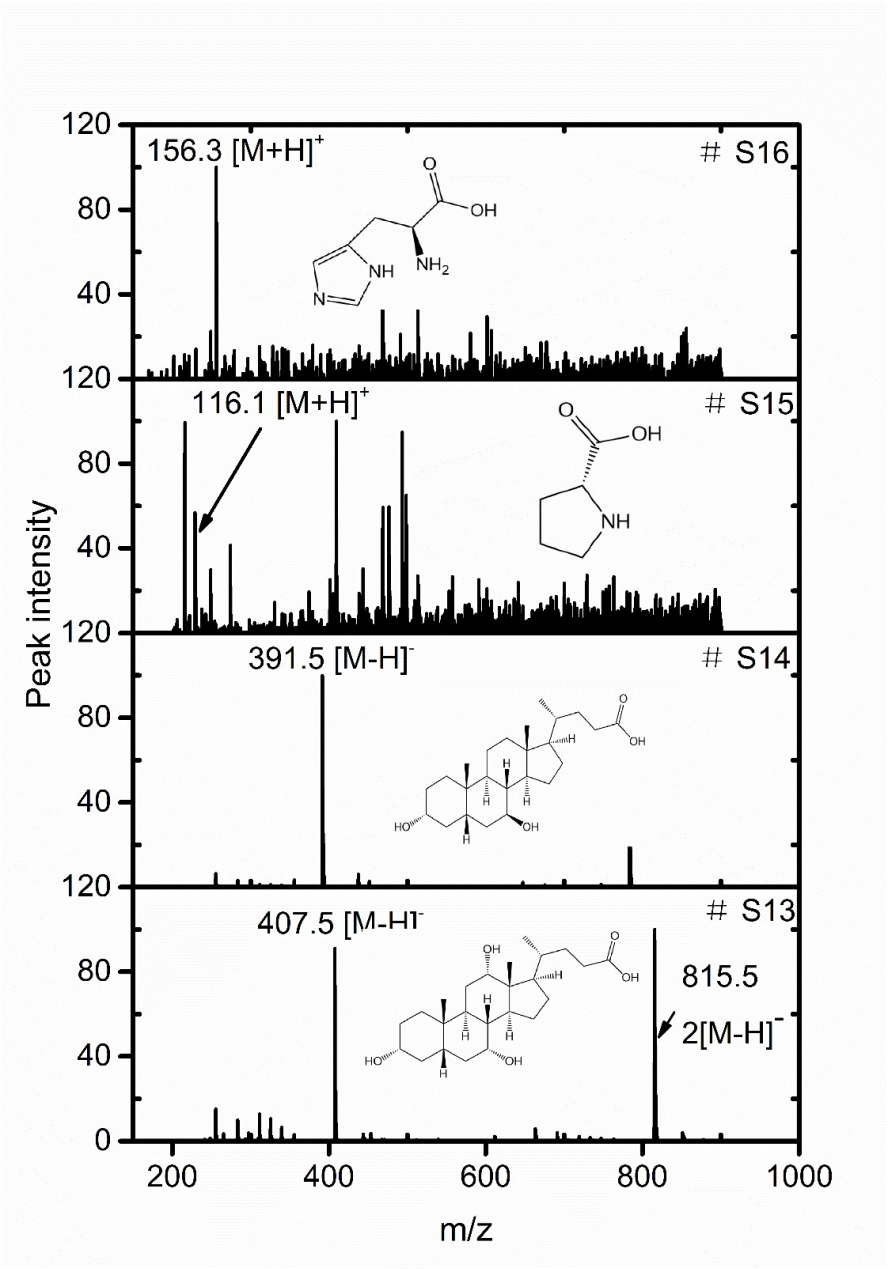

Fig. S4 TLCSI-MS spectra of amino acids and bile acids: cholic acid (S13), ursodeoxycholic acid (S14), proline (S15), and histidine (S16).

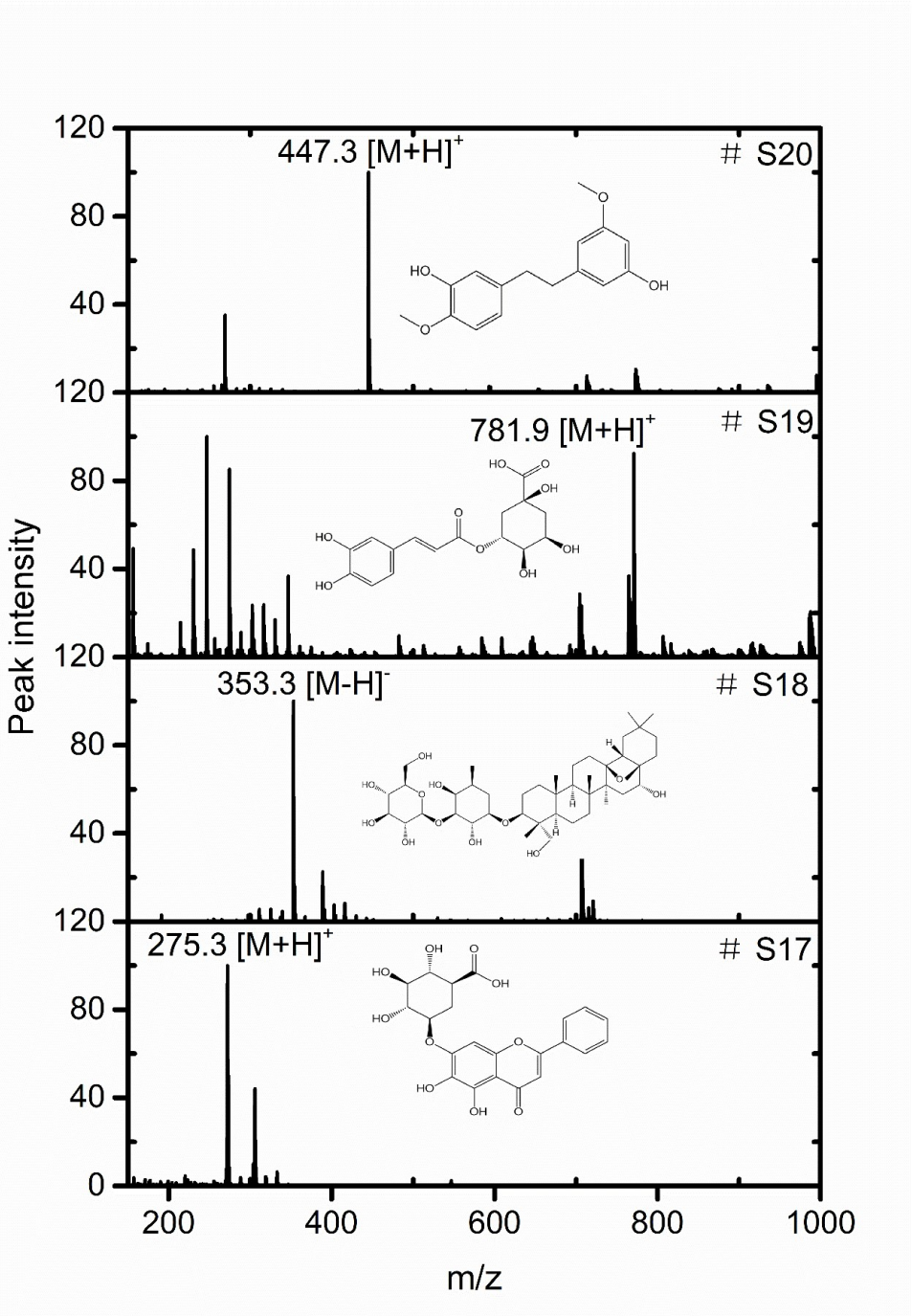

Fig. S5 TLCSI-MS spectra of phenolic acids, phenylpropanoids, triterpenoid saponins, and flavonoids: gigantol (S17), chlorogenic acid (S18), saikosaponin D (S19), and baicalin (S20).

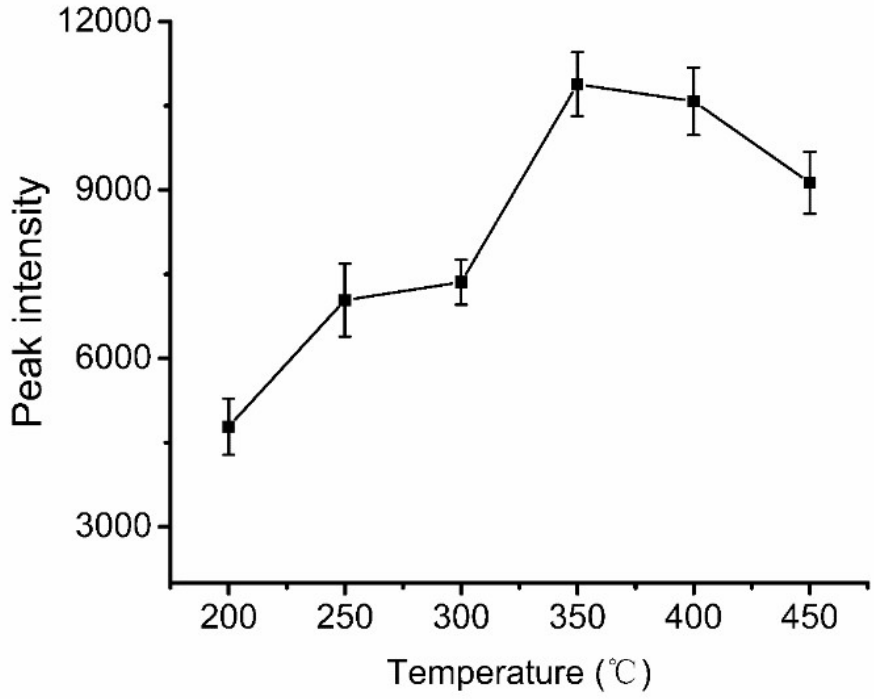

Fig. S6 The effect of gas heater temperature on the ionization efficiency of pachymic acid.

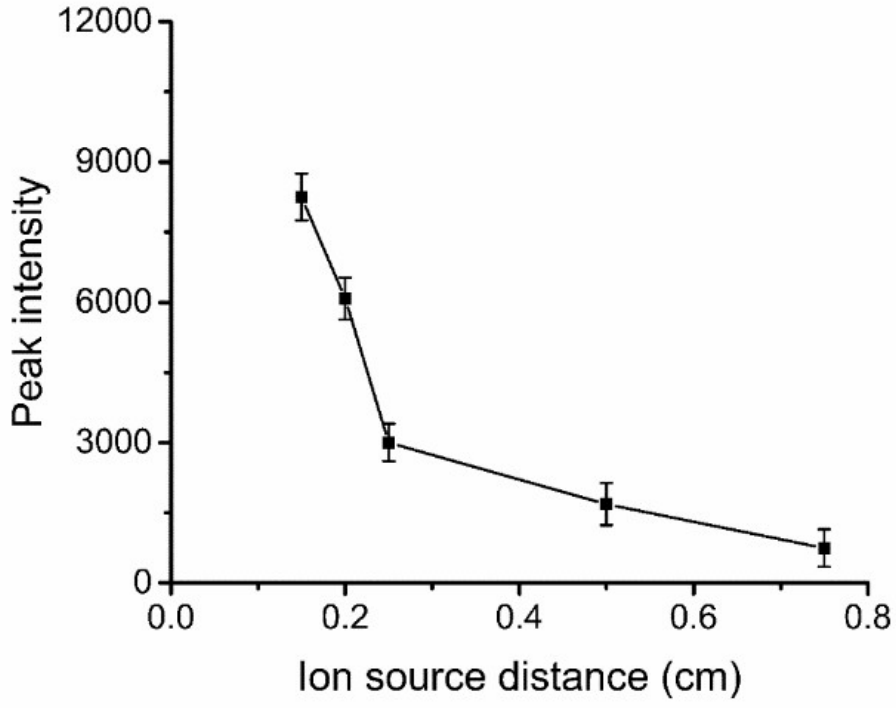

Fig. S7 The effect of ion source distance on the ionization efficiency of pachymic acid.

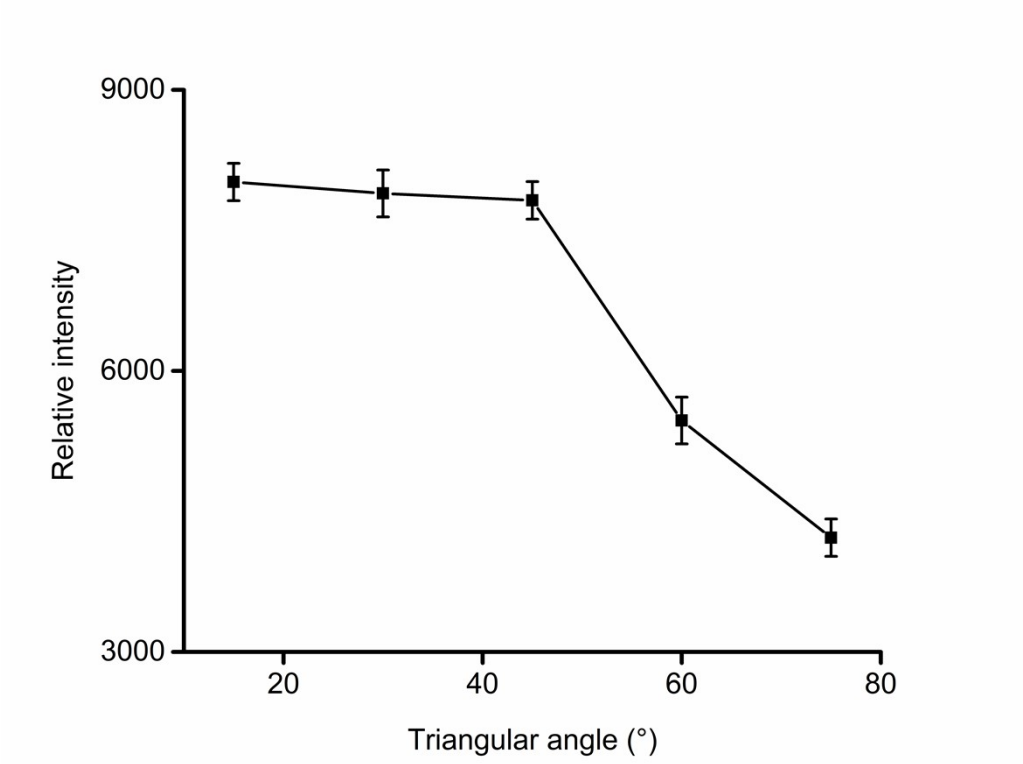

Fig. S8 The effect of triangular angle on the ionization efficiency of pachymic acid.

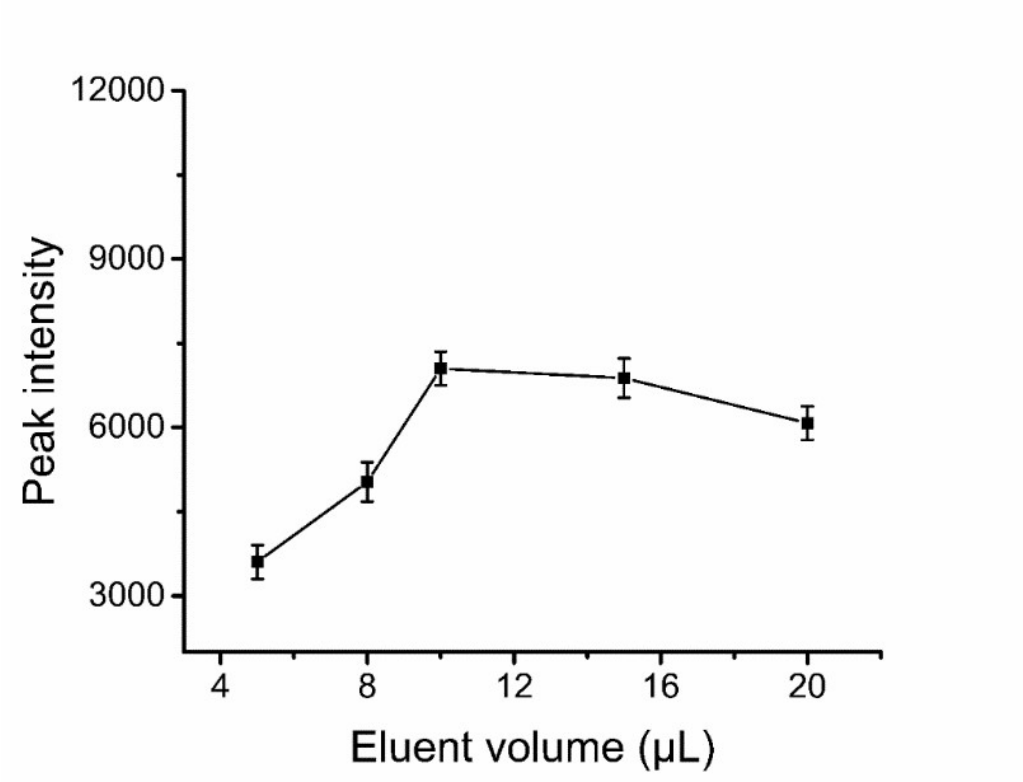

Fig. S9 The effect of eluent volume on the ionization efficiency of pachymic acid.

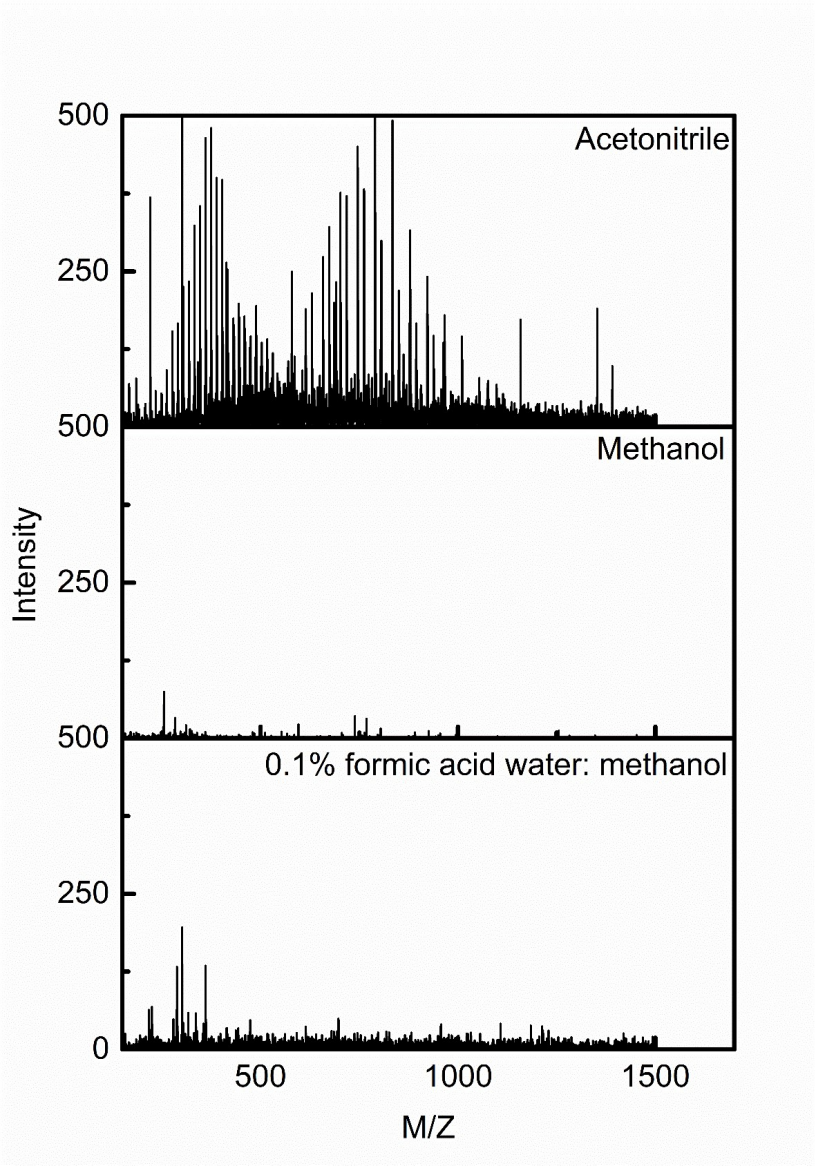

Fig. S10 The interference effect of different eluent solvents.

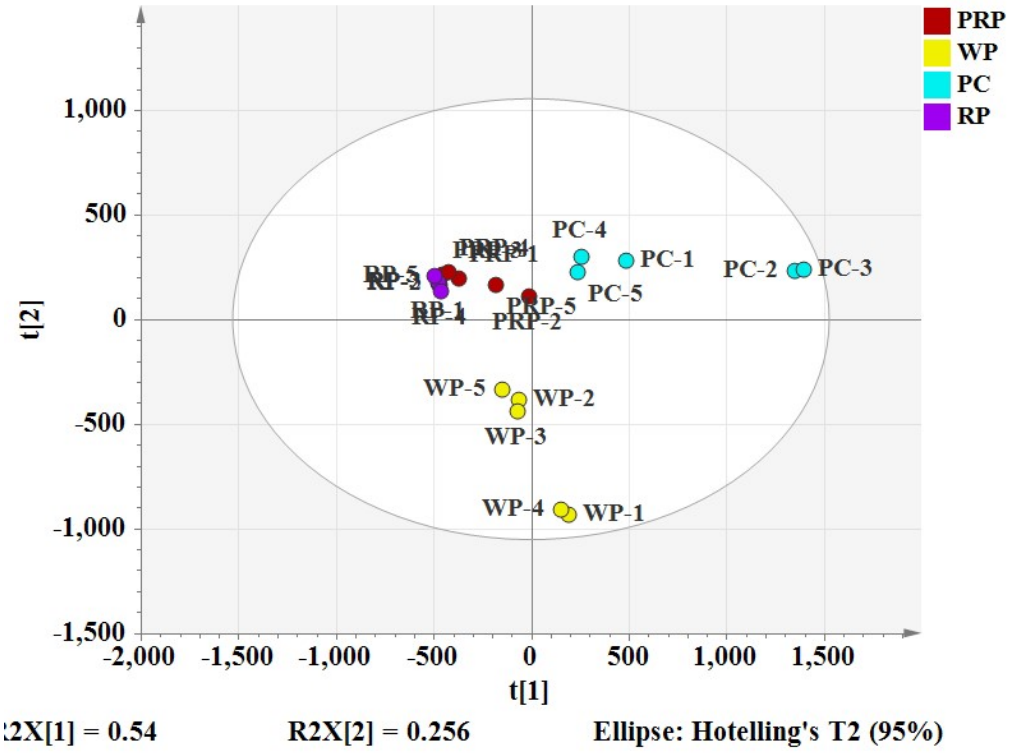

Fig. S11 PCA analysis of *Poria cocos*: White Poria (WP), Poriae Cutis (PC), Poria cum Radix Pini (PRP), and Rubra Poria (RP).

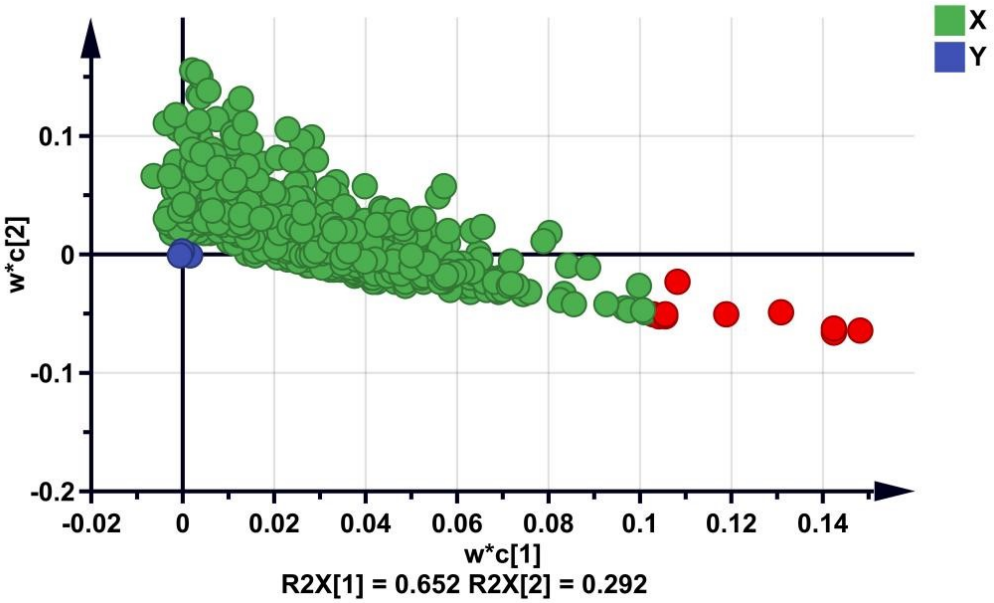

Fig. S12 PLS-DA/Loading Plot based on the holistic chemical profiling of four parts from 20 *Poria cocos* samples.

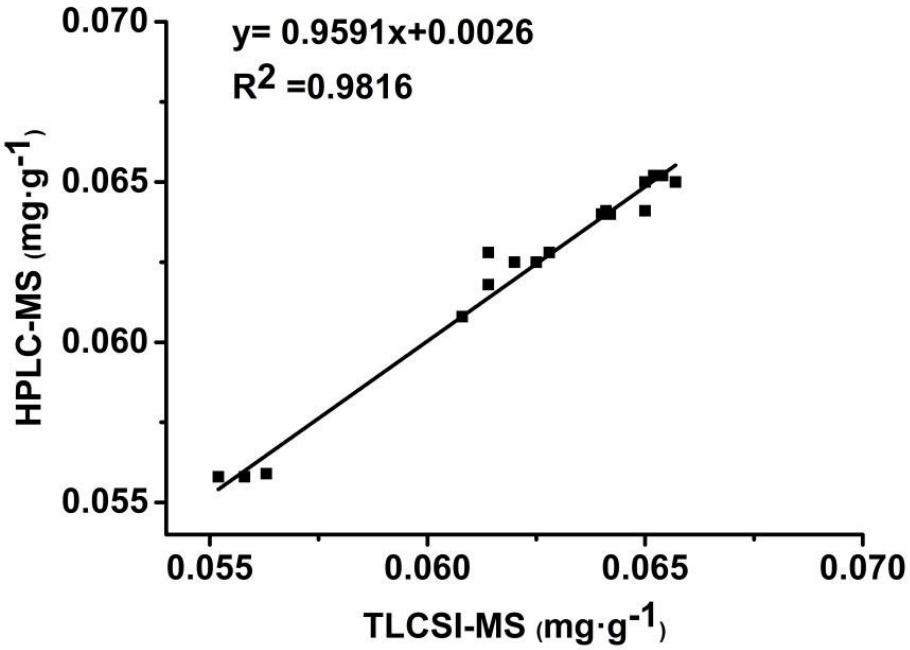

Fig. S13 Correlation analysis diagram of TLCSI-MS and HPLC-MS methods.

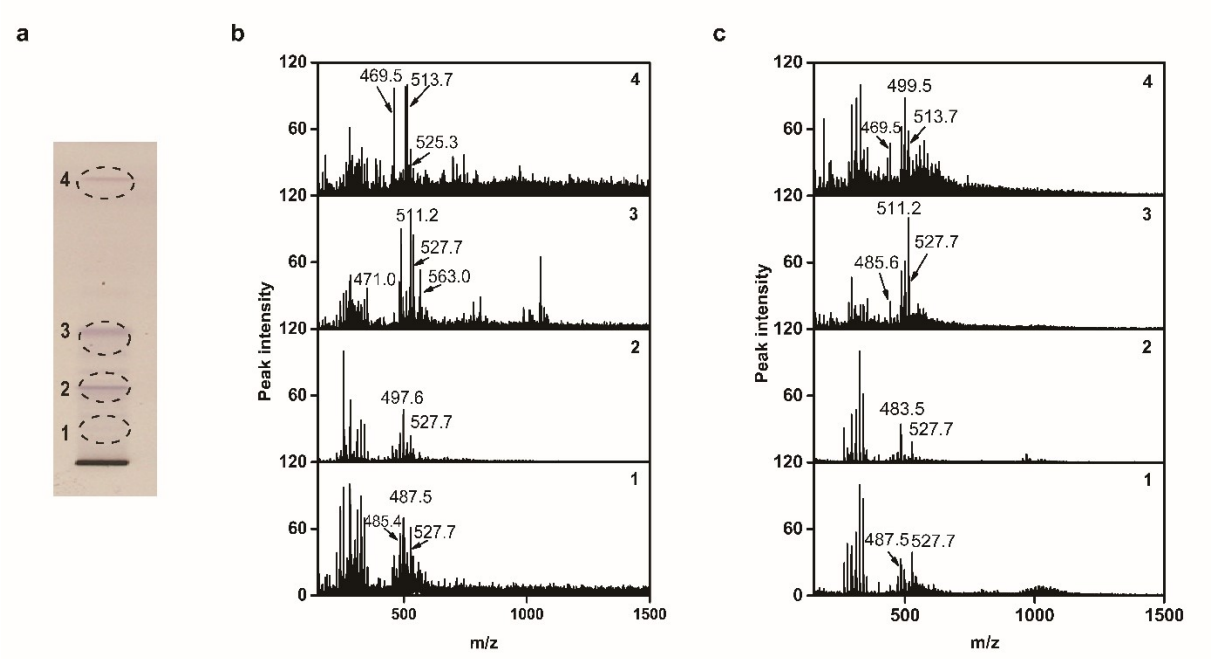

Fig. S14 TLC profile (a), TLCSI-MS (b) and HPLC-MS spectra (c) of *Poria cocos*.

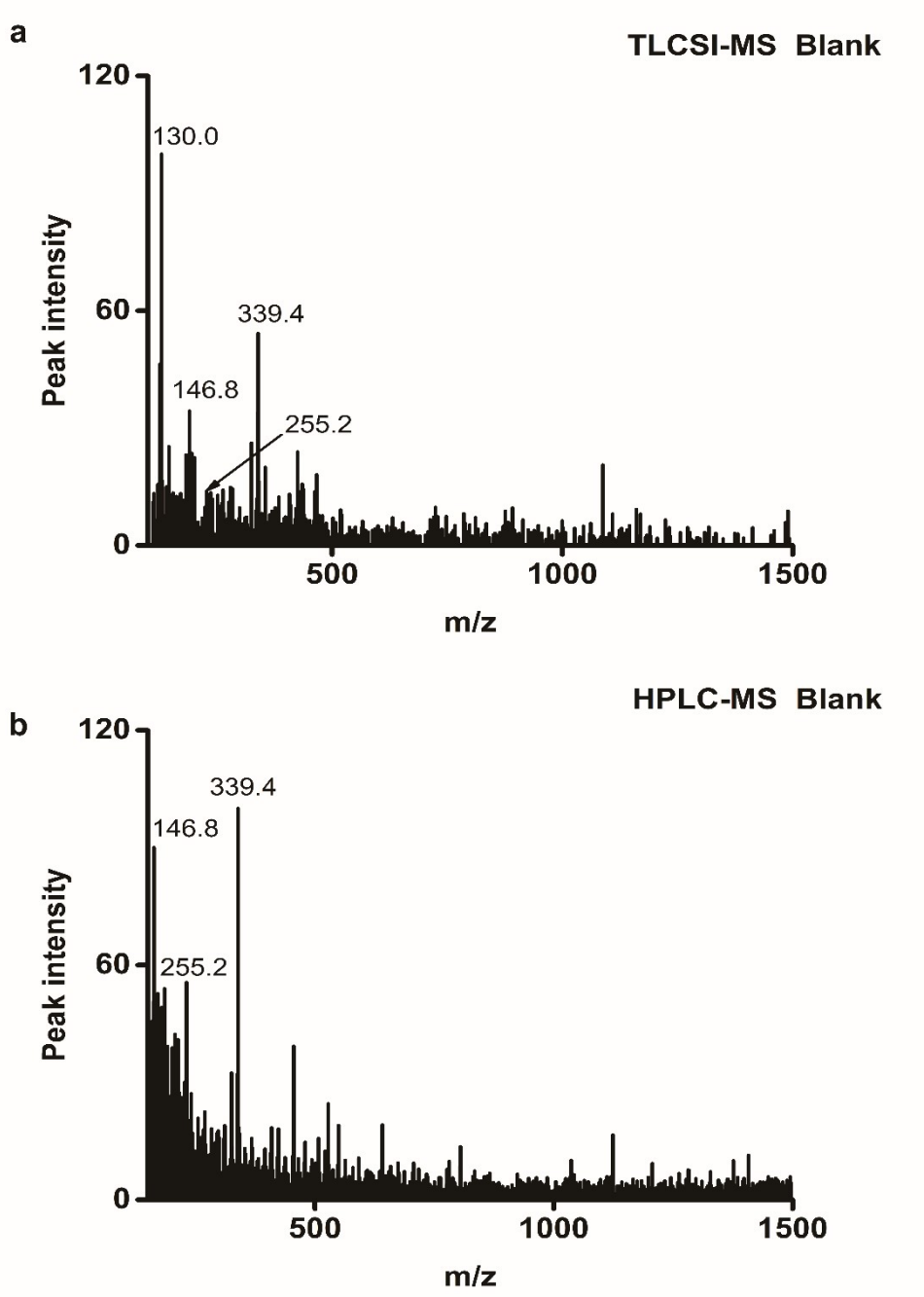

Fig. S15 Mass spectra of blank backgrounds for TLCSI-MS (a) and HPLC-MS (b).

Table S1 Information of 20 batches of *Poria cocos*.

No.	Sample	Locality	Producer
1	Poria cum Radix Pini	Yingshan, Hubei	Huanggang Jingui Chinese Medicine Industry Development Co., Ltd.
2	Poria cum Radix Pini	Yingshan, Hubei	
3	Poria cum Radix Pini	Yingshan, Hubei	
4	Poria cum Radix Pini	Yingshan, Hubei	
5	Poria cum Radix Pini	Jinzhai, Anhu	
6	Poriae Cutis	Yingshan, Hubei	
7	Poriae Cutis	Yingshan, Hubei	
8	Poriae Cutis	Yingshan, Hubei	
9	Poriae Cutis	Yingshan, Hubei	Treasure source Chinese herbal medicine flower tea shop
10	Poriae Cutis	Yingshan, Hubei	Shangyuantang Pharmacy Flagship Store
11	Rubra Poria	Anqing, Anhui	Anhui Dechang Pharmaceutical Co., Ltd.
12	Rubra Poria	Anqing, Anhui	
13	Rubra Poria	Anqing, Anhui	
14	Rubra Poria	Anqing, Anhui	
15	Rubra Poria	Anqing, Anhui	
16	White Poria	Laibang, Yuexi	Yuexi County Linyuan Agricultural and Sideline Products Cooperative
17	White Poria	Jingdong, Yunnan	
18	White Poria	Jingdong, Yunnan	Anhui Shanlihuo Agriculture Co., Ltd.
19	White Poria	Jingdong, Yunnan	Yuexi County Linyuan Agricultural and Sideline Products Cooperative
20	White Poria	Luotian, Hubei	

Table S2 Information table of *Poria cocos* preparations.

No.	Sample	Compounds	Producer
1	LDC-1	Poria, Rehmanniae Radix Praeparata, Moutan Cortex, Kaempferiae Rhizome, Corni Fructus, Alismatis Rhizome	Amendment to Pharmaceutical Group Co., Ltd
2	LDC-2	Poria, Rehmanniae Radix Praeparata, Moutan Cortex, Kaempferiae Rhizome, Corni Fructus, Alismatis Rhizome	Amendment to Pharmaceutical Group Co., Ltd
3	LDG	Poria, Rehmanniae Radix Praeparata, Moutan Cortex, Kaempferiae Rhizome, Corni Fructus, Alismatis Rhizome	Baohetang (Jiaozuo) Pharmaceutical Co., Ltd
4	GFC	Poria, Moutan Cortex, Cinnamomi Ramulus, Paeoniae Radix Alba	Jiangsu Kang Yuan Pharmaceutical Co., Ltd.

Table S3 Results of stability test measurements.

Time (h)	Depachymic acid ($mg \cdot g^{-1}$)	Pachymic acid ($mg \cdot g^{-1}$)
0	0.25	0.42
2	0.25	0.42
4	0.25	0.43
6	0.24	0.41
8	0.25	0.42
12	0.24	0.41
24	0.25	0.43
36	0.25	0.42
RSD (%)	1.87	1.80

Table S4 Chemical characterization of triterpene compounds in PC, RP, WP and PRP by TLCSI-MS/MS.

Identified compounds	[M-H]- (m/z)	MS/MS
3β,5α,9α-Trihydroxyergosta-7,22-dien-6-one	443.5	327.2, 369.3, 407.2, 281.1
16α-Hydroxy-lanosta-7,9(11),24-trien-21-oic acid	453.3	239.0, 324.3, 371.2, 435.5
Dehydrotrametenolic acid	453.6	324.3, 337.2, 435.5, 438.7
3-Hydrocortisonic acid	453.6	324.3, 337.2, 435.5, 438.7
Dehydrotrametenolic acid	455.7	437.3, 295.2
3-Oxo-16-hydroxylanosta-7,9(11),24-trien-21-oic acid	467.7	325.2, 399.3
Dehydroeburiconic acid	467.7	311.2, 449.3
16α-Hydroxytrametenolic acid	471.3	339.0, 397.3, 415.4, 427.4
Pachymenin C	479.3	311.2, 405.2
Polyporenic acid C	481.3	335.0, 388.4, 403.4, 419.5, 435.5
Poricoic acid B	483.6	313.3, 337.2, 405.3, 439.4, 471.3
Dehydrotumulosic acid	483.3	337.4, 367.4, 411.4, 465.3
Poriacosone A	485.3	441.4, 467.5,470.0
Tumulosic acid	485.3	339.3, 387.4, 441.4
Poriacosone B	485.3	339.3, 423.4, 325.2
16α-Acetoxy-lanosta-7,9(11),24-dien-21-oic acid	495.3	435.3, 293.1
Poricoic acid A	497.6	323.0, 382.4, 424.3, 480.3
6α-Hydroxy-polyporenic acid C	497.7	341.0, 381.3, 423.4
16α, 25-Dihydroxyeburiciconic acid	499.5	311.3, 401.3,

16α, 29-dihydroxyeburiconic acid	499.5	369.1, 383.3, 481.5
3-O-acetyldehydroeburicoic acid	509.3	449.3, 353.2, 293.2
Pachymenin AM	511.7	325.1, 351.3, 369.3, 413.4, 467.4, 481.3
Pachymenin D	513.3	379.3, 421.4, 437.4, 495.2
3-O-acetyl-16α-hydroxytrametenolic acid	513.3	379.3, 425.4, 441.4, 495.4
5,8α-Dioxy-3β,16-dihydroxyl-lanost-7(11),24-dien-21-oic acid	515.3	383.4, 405.3, 421.4
Poricoic acid D	515.3	383.4, 439.4
Poricoic acid M	517.6	423.4, 441.5, 409.4, 355.3
Dehydropachymic acid	525.3	463.3
Pachymic acid	527.7	399.3, 411.4, 429.4, 467.5
25-Methoxyporicoic acid A	527.5	399.3, 467.5, 509.5
3β-Acetyloxy-16α-hydroxy-24-oxolanost-8-en-21-oic acid	529.3	397.5, 413.3, 469.3, 511.5
6α-Hydroxydehydropachymic acid	541.3	403.3, 421.5, 437.4, 497.5

Table S5 Recovery of triterpene acids in different *Poria cocos* preparations by TLCSI-MS in negative-ion mode.

Samples	Compounds	Background concentration ($ng \cdot mL^{-1}$)	Spiked concentration ($ng \cdot mL^{-1}$)	Concentration ($ng \cdot mL^{-1}$)	Recovery (%)	RSD (%) (n=3)
LDC-1	pachymic acid	30.5	0	-	-	-
			25	30.1	85.8	2.3
			50	81.1	81.0	0.5
			75	101.4	85.3	2.5
	poricoic acid B	24.8	0	-	-	-
			10	25.7	92.4	5.3
			20	48.9	93.4	2.7
			30	90.7	99.1	3.0
	polyporenic acid C	11.6	0	-	-	-
			10	18.5	88.3	1.8
			20	30.5	95.3	1.4
			30	50.2	102.4	5.0
LDC-2	pachymic acid	28.4	0	-	-	-
			25	75.8	82.3	2.0
			50	88.5	97.8	3.3
			75	118.3	102.5	2.5
	poricoic acid B	22.9	0	-	-	-
			25	48.9	85.3	1.9
			50	90.7	92.2	2.4
			75	101.2	102.3	2.4
	polyporenic acid C	10.2	0	-	-	-
			10	25.0	85.2	2.8
			20	45.6	87.5	2.6
			30	57.5	95.5	3.0
LDG	pachymic acid	27.0	0	-	-	-
			10	28.7	85.0	1.3
			20	56.2	92.8	2.0
			30	110.6	103.4	0.6
	poricoic acid B	7.5	0	-	-	-

			5	7.5	80.5	1.2
			10	20.5	83	3.4
			15	38.9	85	5.8
			0	-	-	-
	polyporenic acid C	11.6	10	10.7	83.7	5.2
			20	23.6	90.2	2.0
			30	45.3	105.8	1.6
GFC	pachymic acid	125	0	-	-	-
			50	114.3	80	2.0
			100	239.5	95.5	1.9
			150	348.3	101.2	3.6
	poricoic acid B	25	0	-	-	-
			25	48.5	82.5	3.7
			50	94.2	85.4	0.8
			75	159.3	99.3	0.8
	polyporenic acid C	150	0	-	-	-
			50	140.3	88.6	2.8
			100	289.5	98.8	2.6
			150	350.4	102.5	3.0

Note: LDC-1/LDC-2 represents different manufacturers.

Table S6 Comparison of TLCSI-MS and HPLC-MS method for analysis of two triterpene acids in different batches of *Poria cocos* samples.

Triterpene acids ($mg \cdot g^{-1}$)	*Poria cocos*-12		*Poria cocos*-20		*Poria cocos*-22	
	TLCSI-MS	HPLC-MS	TLCSI-MS	HPLC-MS	TLCSI-MS	HPLC-MS
Pachymic acid	0.51	0.49	0.48	0.45	0.41	0.40
Depachymic acid	0.19	0.19	0.19	0.20	0.32	0.21