Supplementary Information

Experimental

DPPH radical photometric assay

Antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picryl-hydrazylhydrate) radical photometric assay.[1, 2] The original methanol extract, which had been prepared as described in the section 2.4, was diluted by 2048-fold in methanol or 90% PG-1. For comparison, 90% PG-1 was also subjected to the DPPH assay. For the assay, 100 μL of 300 μM DPPH solution in ethanol was added to 100 μL of control solution (methanol and water for the methanol extract and PG-1 extract, respectively) or sample solution and allowed to react at room temperature for 30 min. Then, the absorbance was measured at 518 nm using a Multiskan^{TM} GO microplate spectrophotometer (Thermo Fisher Scientific, MA, USA). The antioxidant activity was expressed as the radical scavenging activity (RSA, %) using the following formula:

$$RSA\% = [(Abs_{control}-Abs_{sample})/Abs_{control}] \times 100$$

, in which $Abs_{control}$ and Abs_{sample} are the absorbance of control solution and sample solution, respectively.

Recovery of extracted flavonoids from DES

Water was used as the anti-solvent. Rutin was dissolved in 90% PG-1 at 5200 ppm, which is close to the rutin concentration of the *Flos sophorae* extract prepared by the optimized method as described in the section 3.7. Water was added to the rutin solution at 20:1 (v/v), vigorously mixed, and incubated on ice for 2 hr. After rutin precipitate was filtered through 0.2 μm filter, the filtrate was hydrolyzed and analyzed by LC-UV.

Recovery of rutin based on solid phase extraction (SPE) was performed using reversed phase Strata^{TM}-X 33 μm Polymeric sorbent cartridges (500 mg, 6 mL) from Phenomenex (Torrance, CA, USA). The rutin solution above was loaded onto the cartridge preconditioned with water (3 x 1 mL). Polar DES components were removed with water (3 x 1 mL) and the retained rutin was eluted in methanol (3 x 1 mL). The pooled eluate was filtered, hydrolyzed, and analyzed by LC-UV.

Supplementary Table S1. Experimental orders and levels of coded and uncoded variables used for the CCD method.

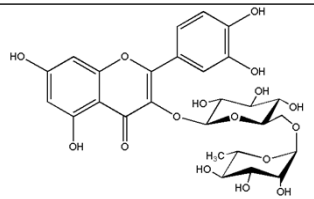
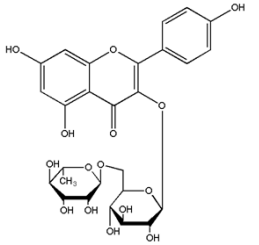
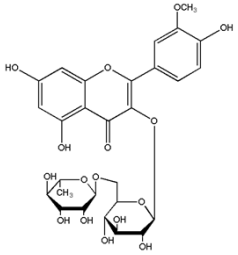
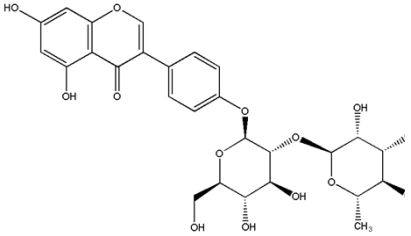
Std	Run	Variable		
		A [1)]	B [2)]	C [3)]
1	4	-1 (89.8)	-1 (1.00)	-1 (15)
2	15	+1 (59.8)	-1 (1.00)	-1 (15)
3	19	-1 (89.8)	+1 (2.00)	-1 (15)
4	10	+1 (59.8)	+1 (2.00)	-1 (15)
5	12	-1 (89.8)	-1 (1.00)	+1 (45)
6	1	+1 (59.8)	-1 (1.00)	+1 (45)
7	17	-1 (89.8)	+1 (2.00)	+1 (45)
8	2	+1 (59.8)	+1 (2.00)	+1 (45)
9	18	-1.682 (100)	0 (1.50)	0 (30)
10	8	+1.682 (49.6)	0 (1.50)	0 (30)
11	5	0 (74.8)	-1.682 (0.66)	0 (30)
12	13	0 (74.8)	+1.682 (2.34)	0 (30)
13	16	0 (74.8)	0 (1.50)	-1.682 (4.8)
14	14	0 (74.8)	0 (1.50)	+1.682 (55.2)
15	3	0 (74.8)	0 (1.50)	0 (30)
16	11	0 (74.8)	0 (1.50)	0 (30)
17	9	0 (74.8)	0 (1.50)	0 (30)
18	7	0 (74.8)	0 (1.50)	0 (30)
19	20	0 (74.8)	0 (1.50)	0 (30)
20	6	0 (74.8)	0 (1.50)	0 (30)

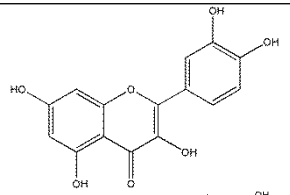
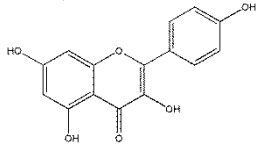
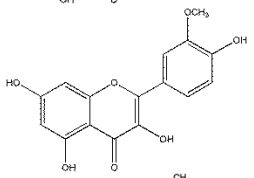
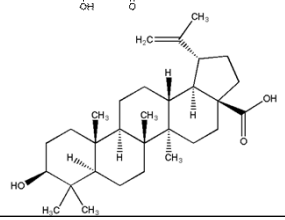
[1)] PG-1 content, w/w %.

[2)] Extractant volume per 100 mg of solid sample.

[3)] Extraction time, min.

Supplementary Table S2. Compounds found in the extract of *Flos sophorae* in the current study.

Peak No.	t_R [a]	Compound	Structure	Molecular formula	Theoretical exact mass for $[M+H]^+$ (Da)	Measured accurate mass for $[M+H]^+$ (Da)	Mass error (ppm)
1	3.10	Rutin		$C_{27}H_{30}O_{16}$	611.1612	611.1665	8.7
2	3.69	Nicotiflorin		$C_{27}H_{30}O_{15}$	595.1663	595.1704	6.9
3	3.84	Narcissin		$C_{28}H_{32}O_{16}$	625.1769	625.1677	-14.7
4	4.64	Sophorabioside		$C_{27}H_{30}O_{14}$	579.1714	579.1712	-0.3

5	4.94	Quercetin		$C_{15}H_{10}O_7$	303.0505	303.0522	5.6
6	5.74	Kaempferol		$C_{15}H_{10}O_6$	287.0556	287.0534	-7.7
7	5.94	Isorhamnetin		$C_{16}H_{12}O_7$	317.0661	317.0657	-1.3
8	8.57	Betulinic acid		$C_{30}H_{48}O_3$	457.3682	457.3541	-30.8

Supplementary Table S3. List of DESs initially prepared in the present study.

Components		Molar ratio	Note
Component A	Component B		
Choline chloride	Sucrose	1 : 1	Stable but incompatible with acid hydrolysis
Choline chloride	D-(+)-Glucose	1 : 1	Stable (ChGlu) [1]
Choline chloride	D-(-)-Fructose	1 : 1	Stable but incompatible with acid hydrolysis
Choline chloride	Citric acid	1 : 1	Unstable (precipitation)
Choline chloride	Xylitol	5 : 2	Stable (ChX) [1]
Choline chloride	Glycerol	1 : 1	Stable (ChG) [1]
Choline chloride	Adonitol	5 : 2	Unstable (precipitation)
Citric acid	D-(+)-Glucose	1 : 1	Stable (CaGlu) [1]
Citric acid	D-(-)-Fructose	1 : 1	Unstable (color change) and incompatible with acid hydrolysis
Citric acid	Adonitol	1 : 1	Stable (CaA) [1]
Citric acid	Sucrose	1 : 1	Stable but incompatible with acid hydrolysis
Citric acid	L-Proline	1 : 1	Unstable (color change)
DL-Malic acid	Sucrose	1 : 1	Stable but incompatible with acid hydrolysis
DL-Malic acid	Xylitol	1 : 1	Unstable (precipitation)
DL-Malic acid	Adonitol	1 : 1	Unstable (precipitation)
Betaine	Sucrose	2 : 1	Stable but incompatible with acid hydrolysis
Betaine	DL-Malic acid	1 : 1	Stable (BM) [1]
L-Proline	D-(+)-Glucose	5 : 3	Stable (PGlu) [1]

[1] Abbreviation for the DESs listed in Table 1 is provided in parenthesis.

Supplementary Table S4. ANOVA results of the models for quercetin, kaempferol, and isorhamnetin.

Quercetin					
Source	Sum of squares	Degree of freedom	Mean square	F value	*p*-value

Model	12166.67	9	1351.85	22.63	< 0.0001	Significant [a]
A	4976.80	1	4976.80	83.31	< 0.0001	
B	1161.97	1	1161.97	19.45	0.0013	
C	3722.28	1	3722.28	62.31	< 0.0001	
AB	101.38	1	101.38	1.70	0.2219	
AC	0.057	1	0.057	9.549E-004	0.9760	
BC	26.36	1	26.36	0.44	0.5215	
A^2	484.46	1	484.46	8.11	0.0173	
B^2	1313.47	1	1313.47	21.99	0.0009	
C^2	778.57	1	778.57	13.03	0.0048	
Residual	597.41	10	59.74			
Lack of fit	393.83	5	78.77	1.93	0.2432	Not significant
Pure error	203.58	5	40.72			
R^2	0.9532					

Kaempferol

Source	Sum of squares	Degree of freedom	Mean square	F value	*p*-value	
Model	15.97	9	1.77	7.29	0.0023	Significant
A	3.92	1	3.92	16.11	0.0025	
B	7.19	1	7.19	29.53	0.0003	
C	0.48	1	0.48	1.98	0.1896	
AB	0.22	1	0.22	0.92	0.3604	
AC	0.037	1	0.037	0.15	0.7030	
BC	0.021	1	0.021	0.086	0.7759	
A^2	1.66	1	1.66	6.82	0.0260	
B^2	0.75	1	0.75	3.09	0.1092	
C^2	2.42	1	2.42	9.96	0.0102	
Residual	2.43	10	0.24			
Lack of fit	1.23	5	0.25	1.01	0.4937	Not significant

Pure error	1.21	5	0.24			
R^2	0.8678					
Isorhamnetin						
Source	Sum of squares	Degree of freedom	Mean square	F value	*p*-value	
Model	116.19	9	12.91	8.81	0.0011	Significant
A	36.37	1	36.37	24.82	0.0006	
B	52.28	1	52.28	35.68	0.0001	
C	5.08	1	5.08	3.47	0.0922	
AB	0.67	1	0.67	0.46	0.5128	
AC	1.00	1	1.00	0.69	0.4269	
BC	0.27	1	0.27	0.18	0.6778	
A^2	12.92	1	12.92	8.82	0.0141	
B^2	1.94	1	1.94	1.32	0.2766	
C^2	8.87	1	8.87	6.05	0.0336	
Residual	14.65	10	1.47			
Lack of fit	5.81	5	1.16	0.66	0.6723	Not significant
Pure error	8.85	5	1.77			
R^2	0.8880					

[a] Significant if $p < 0.05$.

Figure captions.

Supplementary Fig. S1. Hygroscopic property of the DES (PG-1). Weight changes in the produced DES (2.5 mL) were monitored during the storage in a conical tube with a tightly closed cap (squares) or with an air permeable cover (circles) at room temperature with the average humidity at ~40% for 18 days.

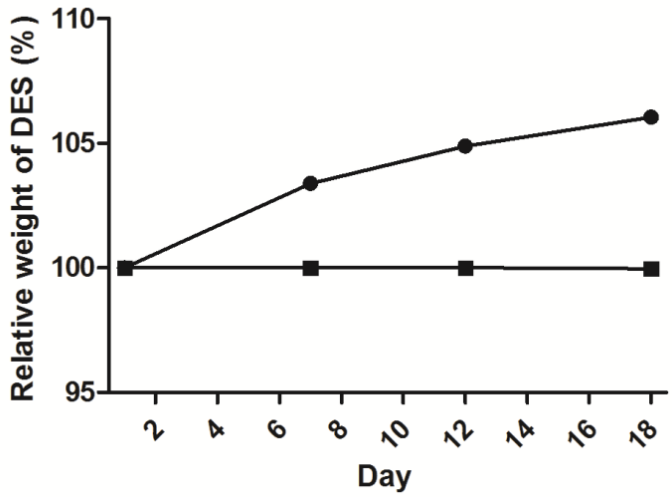
Supplementary Fig. S2. LC-UV chromatograms of the *Flos sophorae* extract in methanol (a, c) and the DES-based extractant (b, d) before (a, b) and after (c, d) acid hydrolysis. Methanol extract was prepared as described in the section 2.4 and the DES-based extract was prepared using the optimized conditions described in the section 3.7. Peak identification: 1, rutin; 2, nicotiflorin; 3, narcissin; 5, quercetin; 6, kaempferol; 7, isorhamnetin.

Supplementary Fig. S3. Extracted ion chromatograms and mass spectra for the identified compounds in the unhydrolyzed or hydrolyzed extracts (methanol or DES-based extracts) by the UHPLC-Q-TOF-MS analysis. (a, rutin; b, nicotiflorin; c, narcissin; d, sophorabioside; e, quercetin; f, kaempferol; g, isorhamnetin; h, betulinic acid).

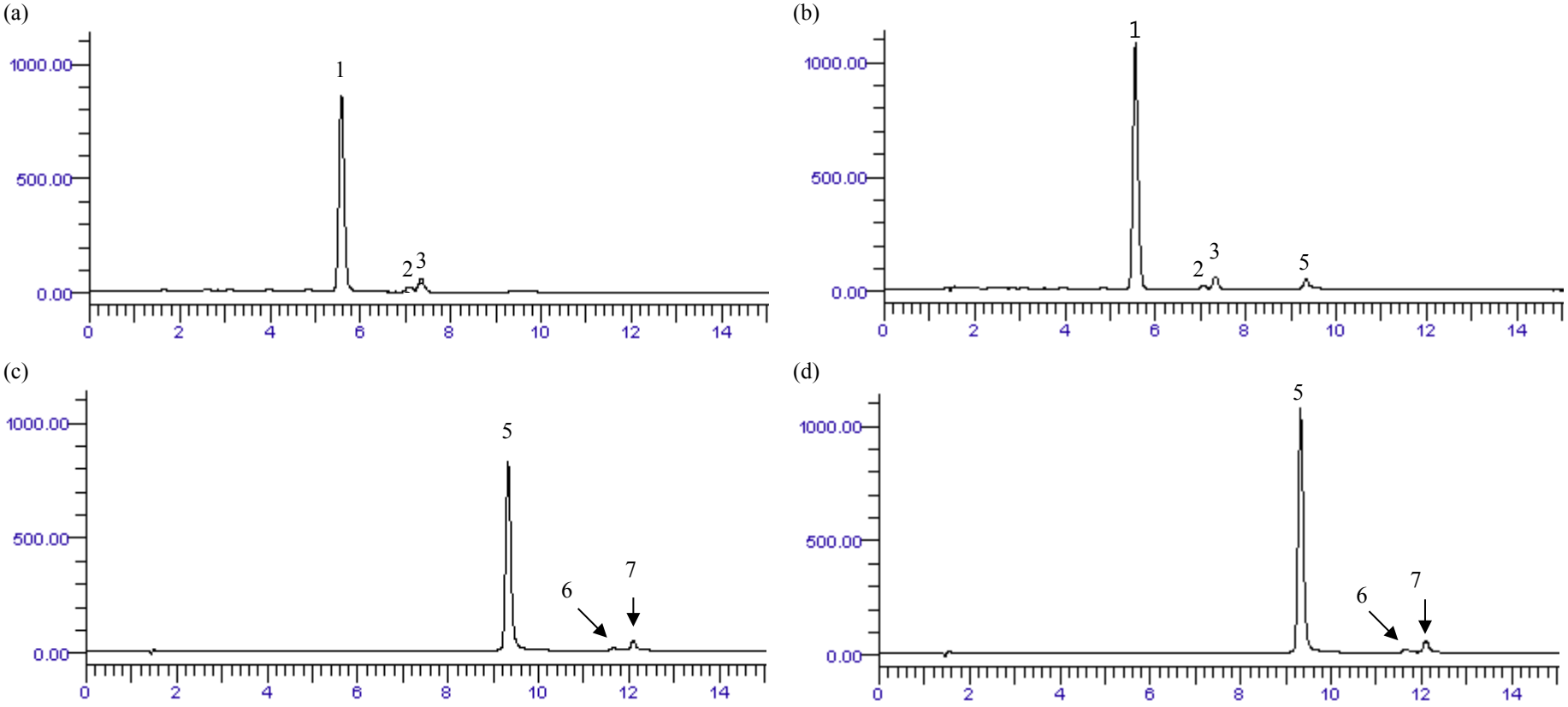
Supplementary Fig. S4. LC-UV chromatograms of rutin standard solution dissolved in 100% methanol (a, b) and the DES-based extractant (c, d) before (a, c) and after (b, d) acid hydrolysis. The DES-based extractant was 90% PG-1. Peak identification: 1, rutin; 5, quercetin.

Supplementary Fig. S5. Comparison of the flavonoid extraction efficiencies between 100% methanol and 70% aqueous methanol (n=3; error bars indicate SEM). Quercetin (gray); kaempferol (white); isorhamnetin (black). Extraction conditions were the same as described in the section 2.1.

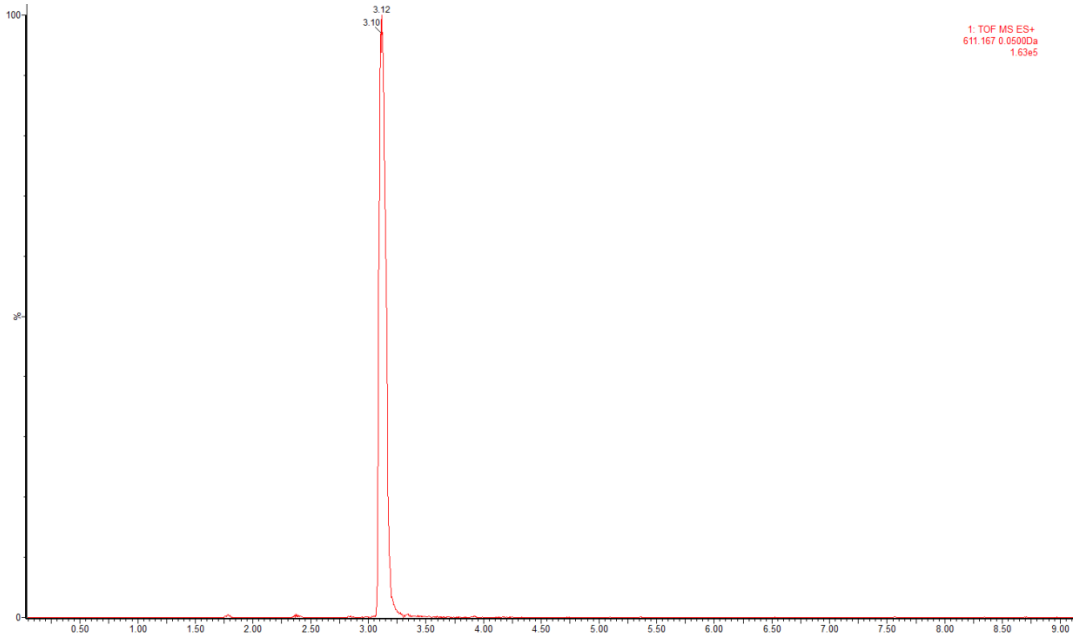
Supplementary Fig. S1.

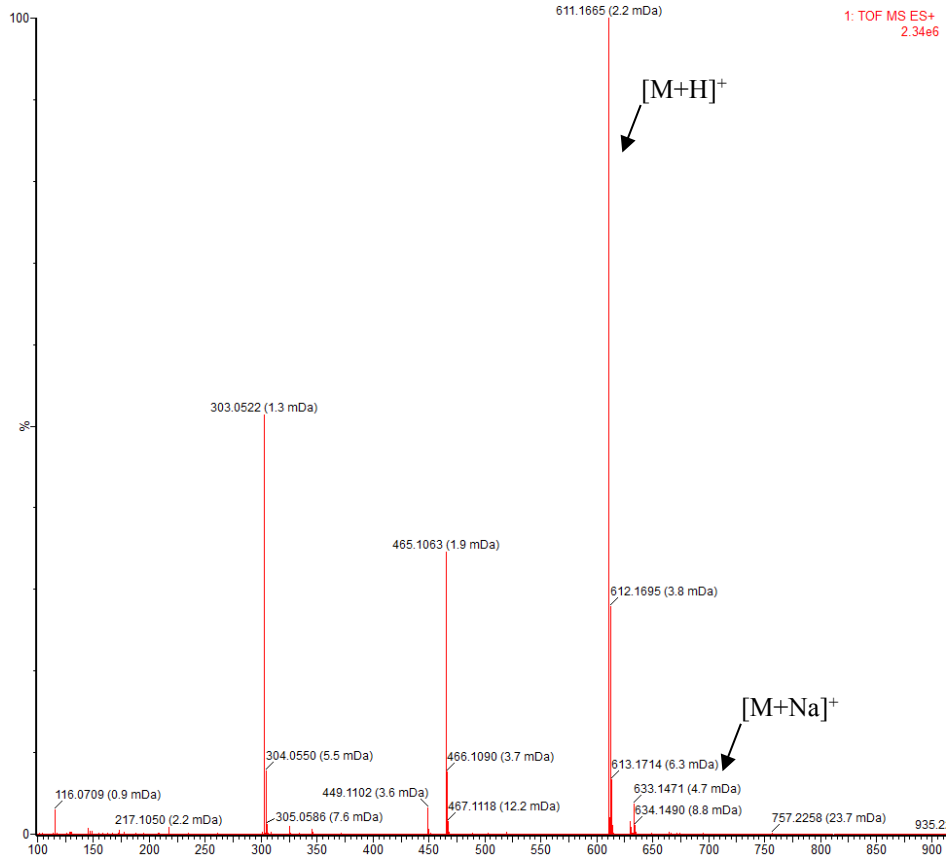


Supplementary Fig. S2.

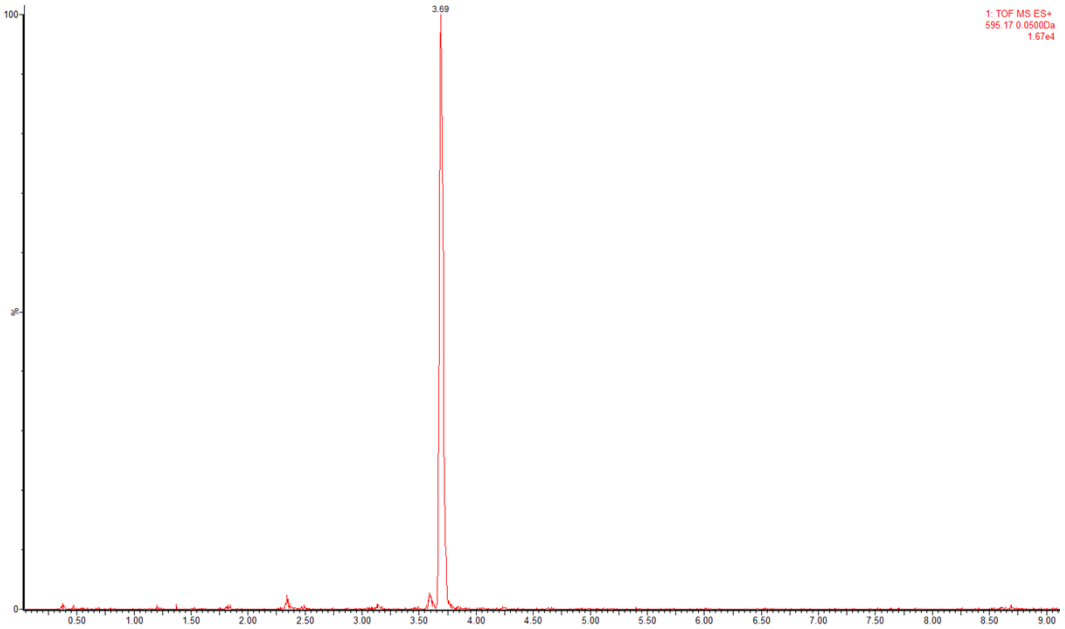


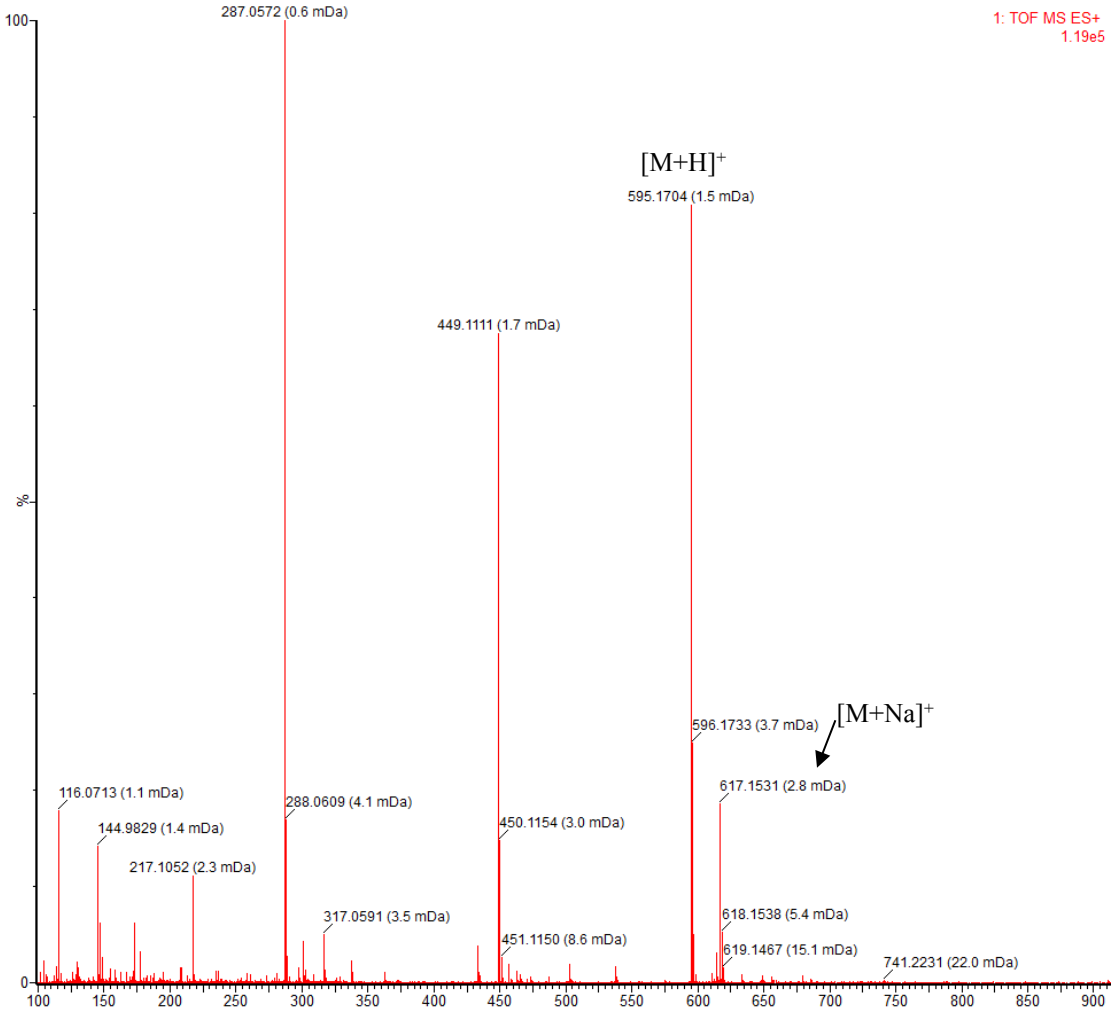
Supplementary Fig. S3(a).



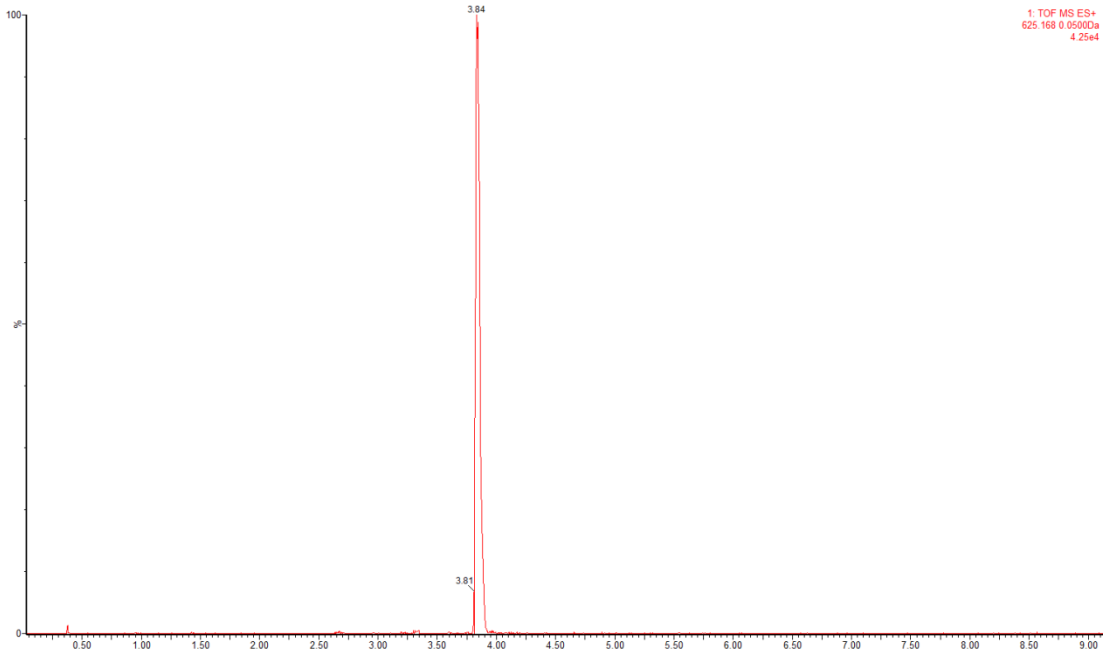


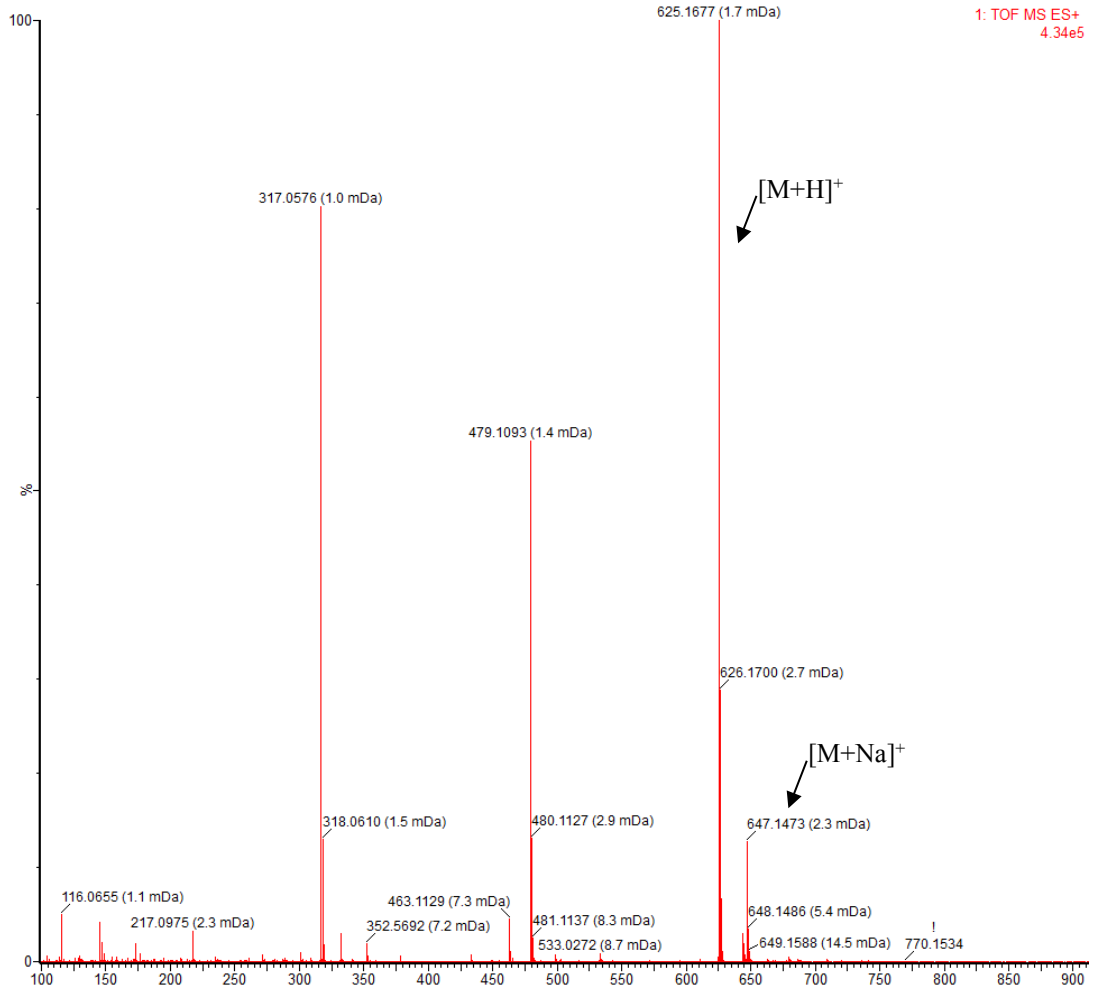
Supplementary Fig. S3(b).



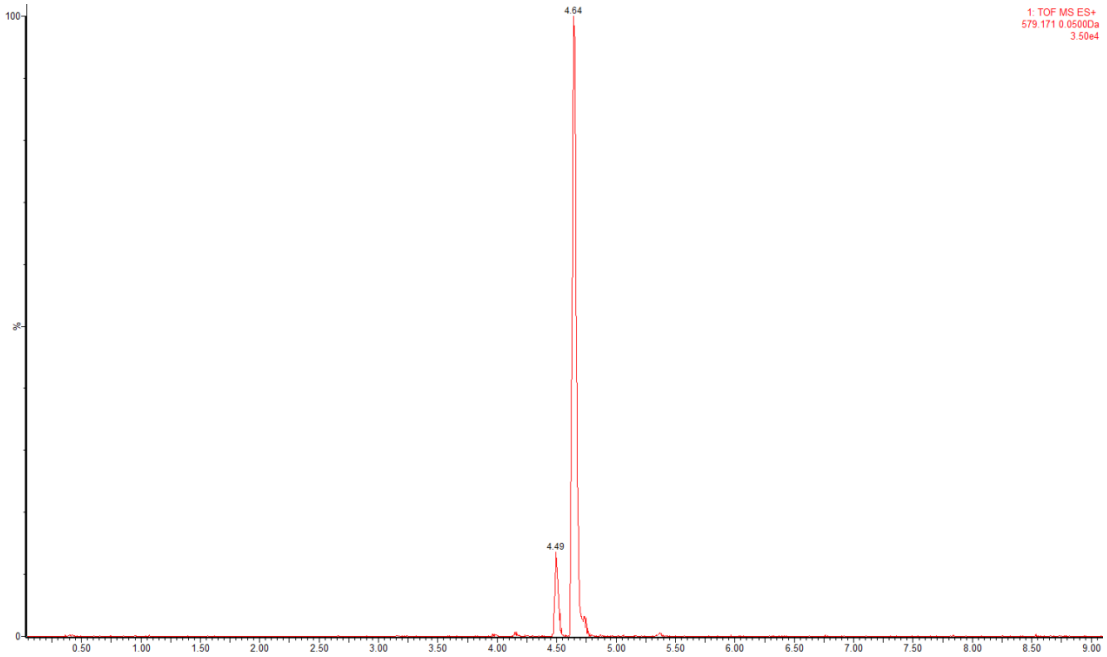


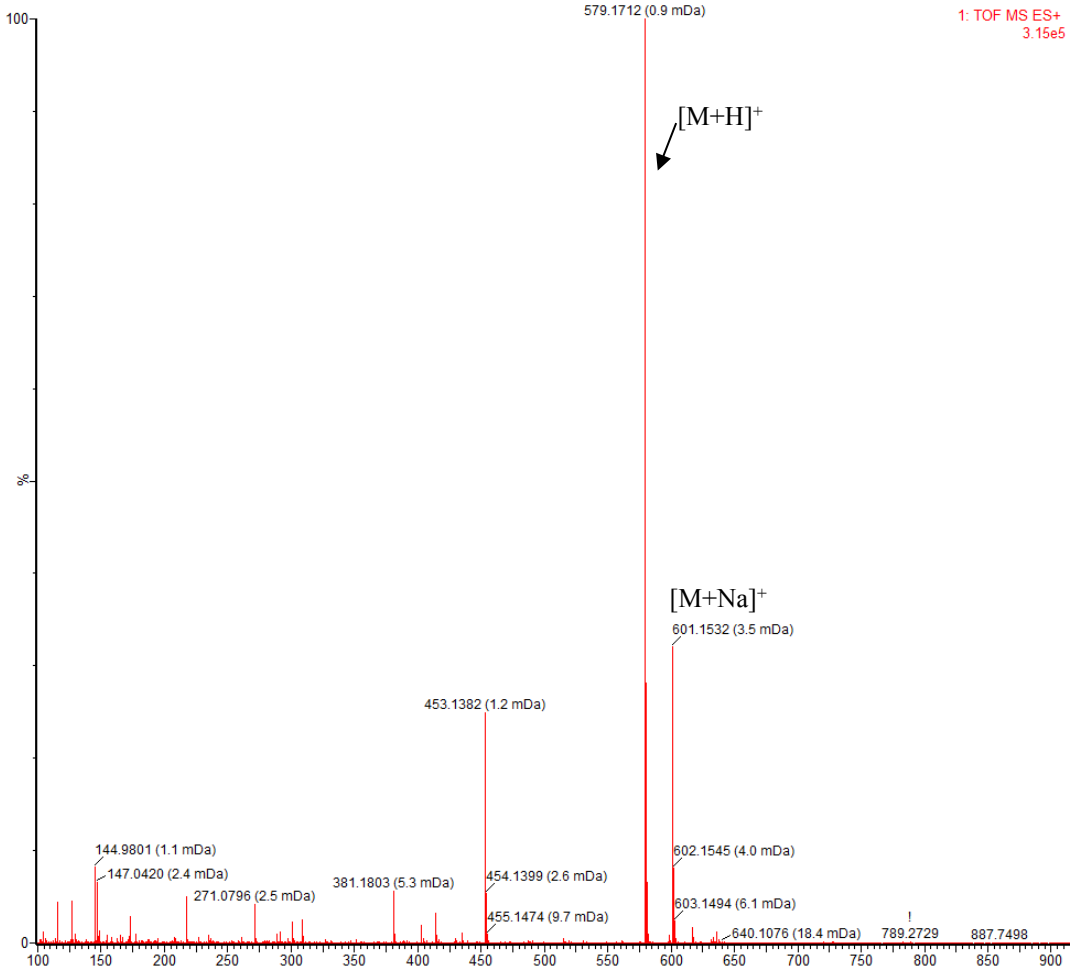
Supplementary Fig. S3(c).



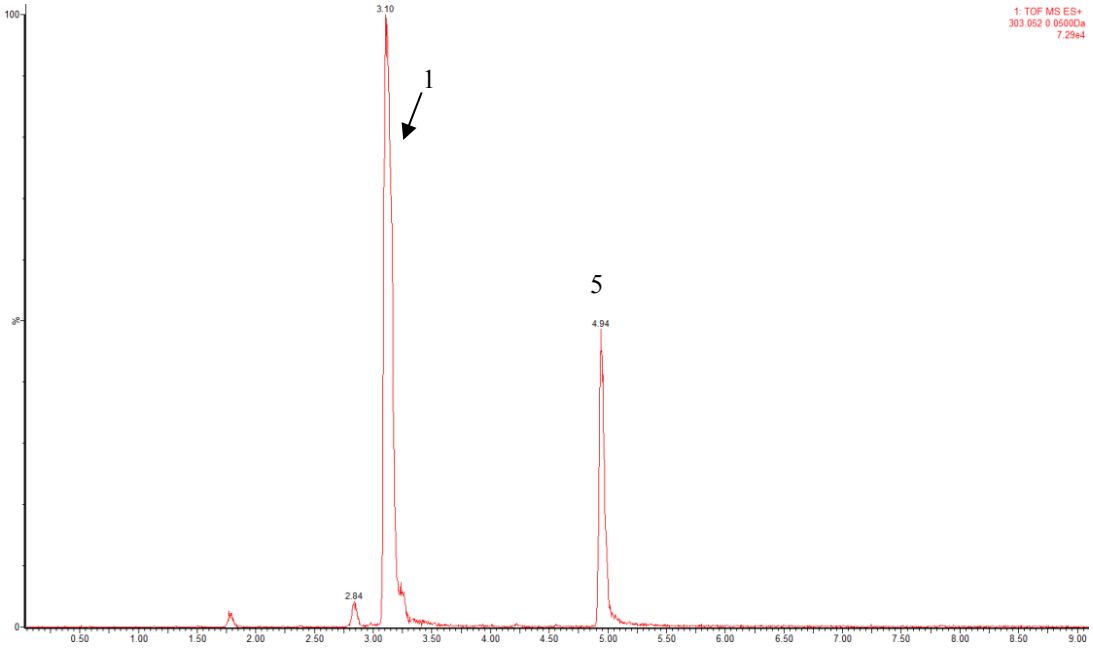


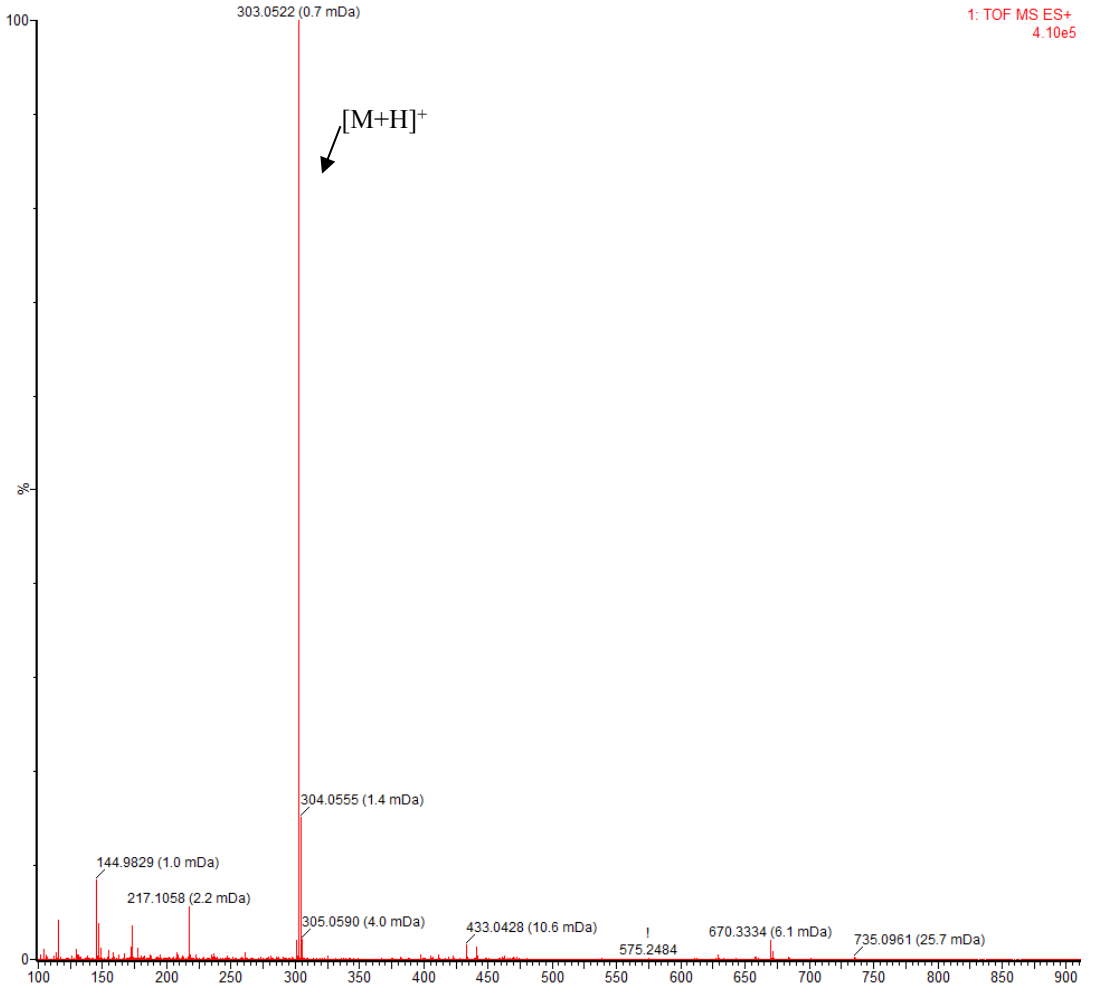
Supplementary Fig. S3(d).



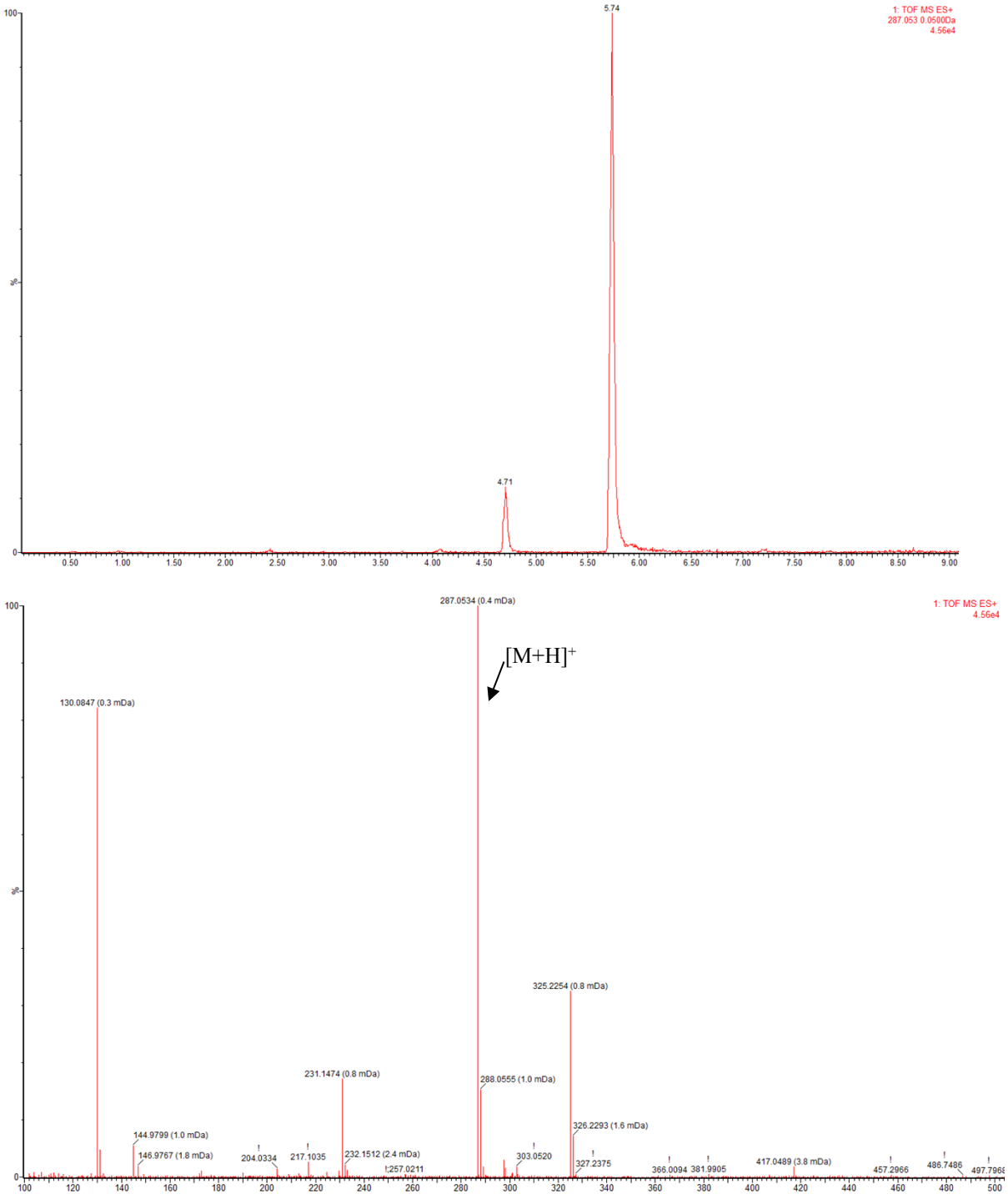


Supplementary Fig. S3(e).

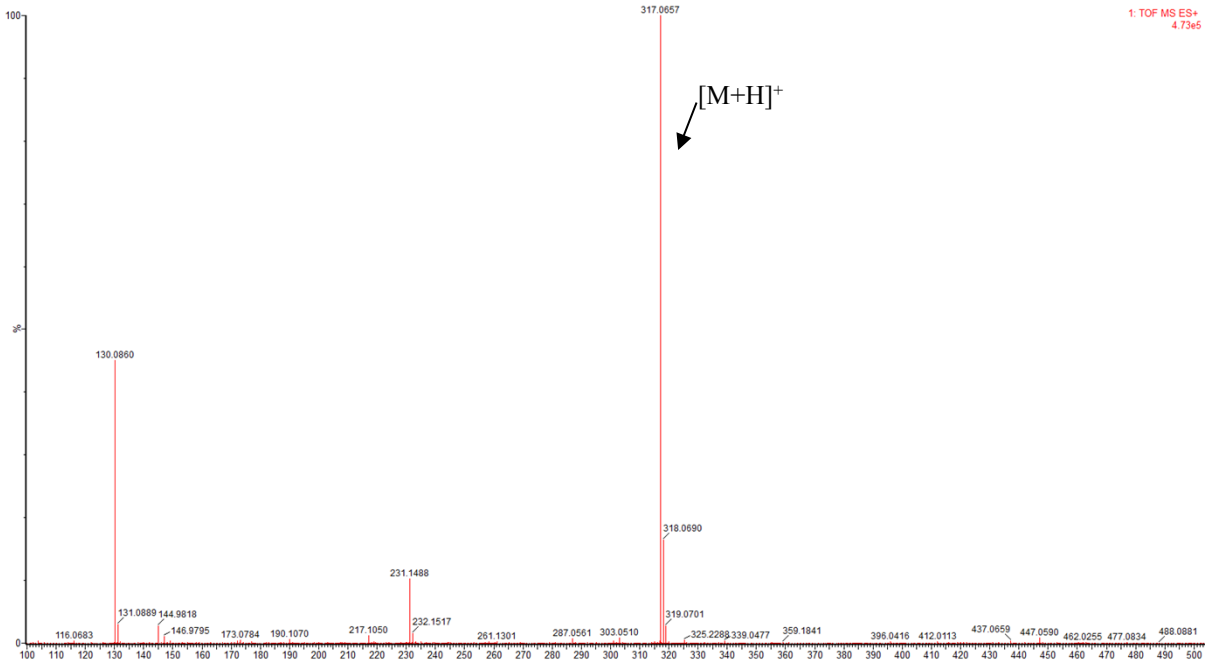




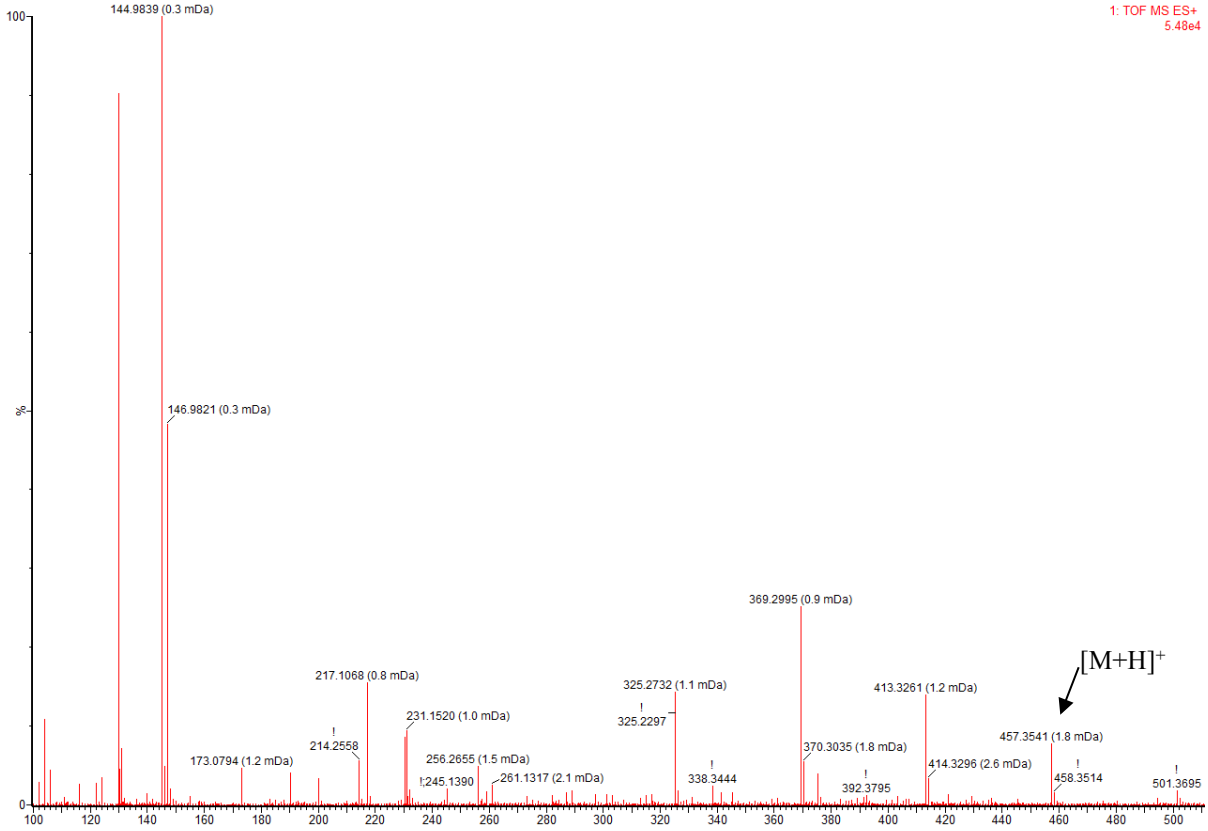
Supplementary Fig. S3(f).



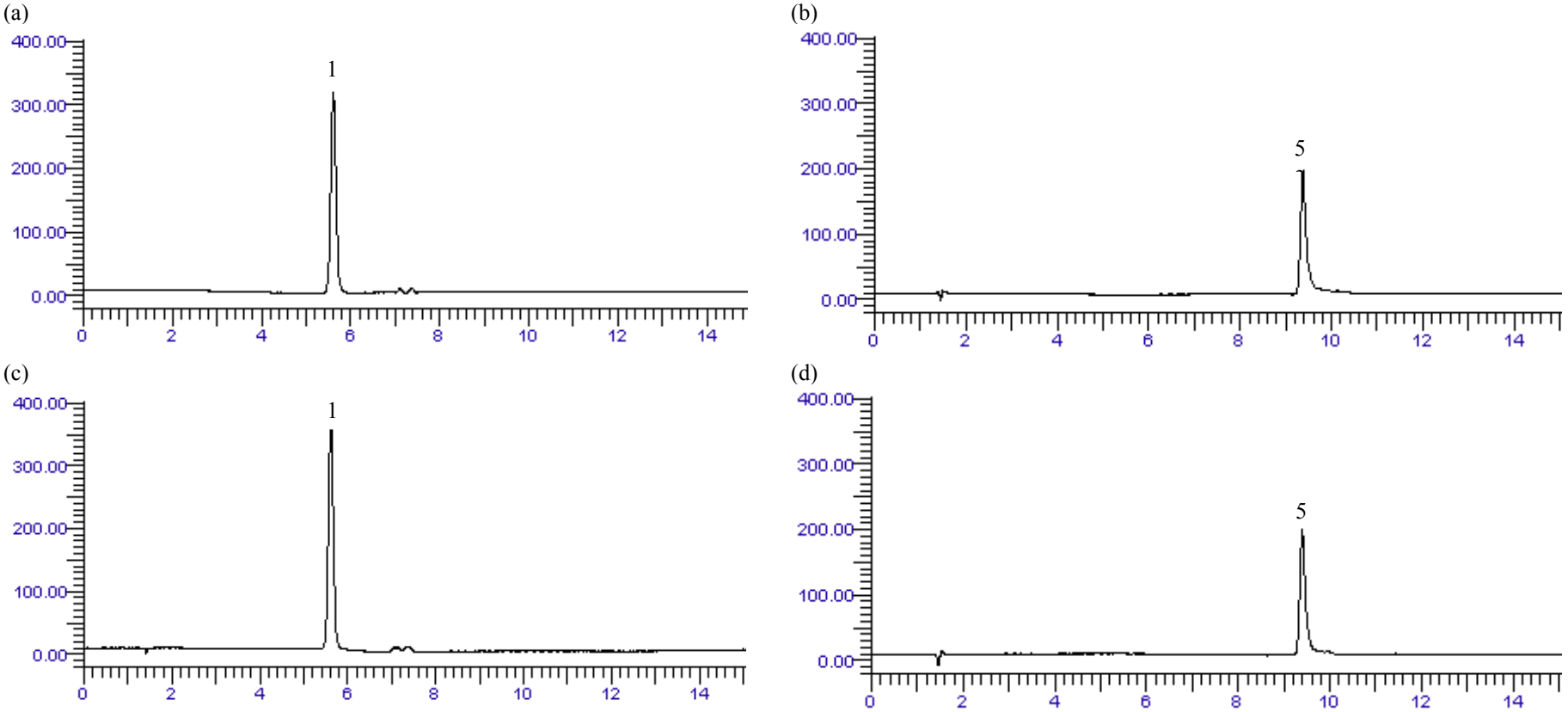
Supplementary Fig. S3(g).



Supplementary Fig. S3(h).

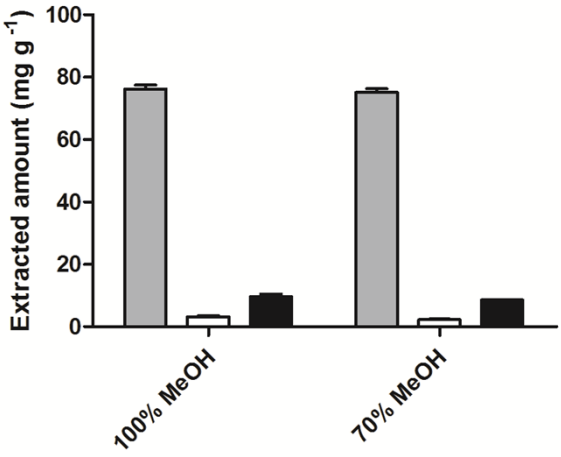


Supplementary Fig. S4.

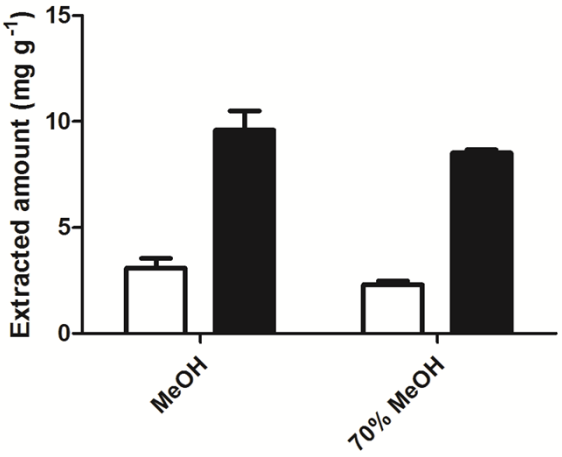


Supplementary Fig. S5.

(a)



(b)



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2. S. Benvenuti, F. Pellati, M. Melegari and D. Bertelli, *Food Chem. Toxicol.*, 2004, 69, 164-169.