Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

for

Magnetic ZrO_2 based solid-phase extraction strategy for selective enrichment and profiling of glycosylated compounds in rice

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Preparation of magnetic ZrO_2 particles

The magnetic Fe_3O_4 particles were homemade according to the method described in our previous work.[1] Briefly, $FeCl_3 \cdot 6H_2O$ (5.4 g), was dissolved in ethylene glycol (160 mL) under magnetic stirring for 30 min. Then sodium acetate (14.4 g) and polyethylene glycol (4.0 g) were added to the solution. After stirring for another 30 min, the resultant solution was transferred into a 200 mL Teflon lined stainless-steel autoclave. The autoclave was sealed and heated at 200°C for 24 h, and then the magnetic microspheres were collected with the help of external magnet, followed by 4 times washing with ethanol and deionized water. The product was dried under vacuum at 60 °C for 8 h and stored for further use.

The prepared Fe_3O_4 microspheres (0.5 g) were dispersed in a solution containing 1.5 g cetyl trimethyl ammonium bromide (CTAB), 400 mL deionized water, 7.5 mL concentrated $NH_3 \cdot H_2O$ solution (25%) and 300 mL ethanol. The mixture was stirred continuously for 30 min to form a homogenized dispersion. Then, an aqueous solution of $ZrOCl_2$ (3.1 g dissolved in the minimum volume of water) was added drop-wise and stirred for 6 h. The product was collected by external magnet and washed repeatedly with ethanol and deionized water. The obtained particles were dispersed in 250 mL of acetone and refluxed at 80 °C for 60 h. The resultant particles were then washed with deionized water, separated through magnetic decantation and dried under vacuum at 60 ^oC for 12 h.

Preparation of magnetic boronate affinity (BA) particles

The magnetic BA particles were homemade according to the method developed in our

lab.[2] Briefly, Fe_3O_4 (120 mg) was homogeneously dispersed in a mixture of ethanol (467 mL), deionized water (139 mL) and $NH_3 \cdot H_2O$ solution (15 mL) and stirred vigorously for 30 min. Under continuous mechanical stirring, tetraethoxysilane (TEOS, 6.0 mL) was slowly added to this dispersion, and after stirring for 8 h at room temperature, silica was formed on the surface of Fe_3O_4 through hydrolysis and condensation of TEOS. The particles were washed by ethanol and deionized water several times, and then dried under vacuum at 60 °C for 8 h. Then nonporous silica magnetic spheres were converted into magnetic mesoporous silica spheres according to pseudomorphic transformations by hydrothermal reaction. Typically, nonporous silica magnetic spheres (0.400 g of SiO_2) were added to a mixture of CTAB (0.243 g), water (9.6 mL), and NaOH (0.054 g) and stirred at room temperature for 30 min. The hydrothermal reaction was carried out in a Teflon-lined autoclave at 130 °C for 24 h. The products were washed with ethanol and deionized water several times. The purified microspheres were dispersed in 50 mL of NH_4NO_3 ethanol solution (0.2 wt.%) and heated at 60 °C for 30 min to remove the template CTAB. This step was repeated six times to completely remove CTAB. Microspheres were washed with deionized water for 2 times and magnetic mesoporous silica spheres were obtained.

Then thiol modified magnetic mesoporous silica spheres were prepared by adding 30 μL 3-mercaptopropyl-trimethoxysilane, 1.0 g of magnetic mesoporous silica spheres in 10 mL of toluene in a 20 mL hydrothermal reactor at 130 °C for 15 h. The resulting material was washed with ethanol followed by water. After drying for 6 h at 60 °C under vacuum, the modified magnetic mesoporous silica spheres were obtained.

Finally Thiol–Ene Click Chemistry was used to bind boronic acid group on the particles. Briefly, 2.2 g Vinylbenzeneboronic acid and 70 mg 2, 2-azobisisobutyronitrile were dissolved in 90 mL DMF, and then 500 mg thiol modified magnetic mesoporous silica spheres were added. The mixture was heated at 70 °C for 24 h under nitrogen. The obtained magnetic BA particles were washed by ethanol and deionized water several times, and then dried under vacuum at 60 °C for further use.

Adsorption capacity evaluation of magnetic ZrO_2 and magnetic BA adsorbents

The adsorption capacity of magnetic ZrO_2 and magnetic BA adsorbents was evaluated simply according to a previous method with minor modification.[3] 40 mg adsorbents were incubated with 1 mL of adenosine (0.5 mg/mL) with a sampling solution containing 0.5% ammonium hydroxide and vortex mixing was applied to reach adsorption equilibration. After transferred to a 1.5 mL centrifuge tube with the help of a magnet, the supernatant was lyophilized to dryness, and then dissolved in 100 μL of MeOH/H_2O (5/95, v/v) for further analysis.

Table S1. Parameters of multiple reaction monitoring (MRM) mode for analysis of CKs standards and compounds used to optimize the conditions of magnetic ZrO_2-based magnetic solid-phase extraction (MSPE).

Compounds	Precursor ion	Product ion	DP (V)	EP (V)	CE (V)	CXP (V)
trans-zeatin (tZ)	220.1	136.0	80	5	25	10
trans-zeatin-riboside (tZR)	352.2	136.0	85	5	27	8
trans-zeatin 9-glucoside (tZ9G)	382.1	136.0	105	5	30	8
cytidine (C)	244.1	112.0	24	6	22	5
uridine (U)	245.0	113.0	30	5	20	4
guanosine (G)	284.2	152.2	28	6	20	5
adenosine (A)	268.2	136.0	24	6	24	5
1-methyladenosine (m1A)	282.1	150.0	25	10	25	3
5-methyluridine (5mU)	259.2	127.2	20	6	20	4
N^6-methyladenosine (m^6A)	282.1	150.0	30	7	25	4
*N*4-Acetylcytidine (ac4C)	286.1	154.1	15	4	25	5

Parameters: declustering potential (DP), entrance potential (EP), collision energy (CE), collision cell exit potential (CXP)

Table S2. The extraction efficiency of magnetic ZrO_2 and magnetic boronate affinity (BA) adsorbents to *cis*-diol and non-*cis*-diol models.

Analyte [a]		Extraction efficiency [b] (%, n=3)	
		Magnetic ZrO_2	Magnetic BA
cis-diol	Adenosine (A)	85.7±1.8	21.3±0.9
	Uridine (U)	83.3±0.2	13.2±0.6
	Cytidine (C)	84.3±0.6	11.4±2.2
	Guanosine (G)	89.0±1.2	33.4±1.8
non-*cis*-diol	2'-deoxyadenosine (dA)	0	0
	2'-O-methyladenosine (Am)	0	0

[a] 1μg for each in 1 mL sampling solution.

[b] Acquired on HPLC-UV system.

Table S3. The extraction efficiency of different adsorbents to 3 CKs standards.

Analyte [a]	Extraction efficiency [b] (%, n=3)		
	TiO_2	Magnetic ZrO_2	Magnetic BA
trans-zeatin (tZ)	0	0	0
trans-zeatin 9-glucoside (tZ9G)	54.7±12	71.6±0.6	0
trans-zeatin-riboside (tZR)	28.2±7.0	63.0±1.2	28.4±7.0

[a] 1 ng for each in 1 mL sampling solution.

[b] Acquired on LC-MS/MS system.

Table S4. Extraction performance and the LOD of our MSPE-LC-MS strategy under the optimum condition.

Time (min)	**Analyte**	**LOD [a] (pg/g)**	**Recoveries [b] (%, n=3)**
6.3	cytidine (C)	1.2	93.1±1.6
8.0	uridine (U)	88.2	96.8±2.0
15.0	guanosine (G)	13.0	95.5±7.3
15.1	5-methyluridine (5mU)	39.7	89.7±5.8
17.4	1-methyladenosine (m1A)	0.7	110.8±9.5
22.1	adenosine (A)	0.1	120.5±12.1
29.1	N^6-methyladenosine (m^6A)	0.7	115.7±12.6
30.0	*trans*-zeatin 9-glucoside (tZ9G)	1.2	115.8±11.4

[a] LOD: amount of analytes at a signal-to-noise ratio of 3.

[b] Acquired on LC-HRMS system.

Table S5. The information of the detected compounds.

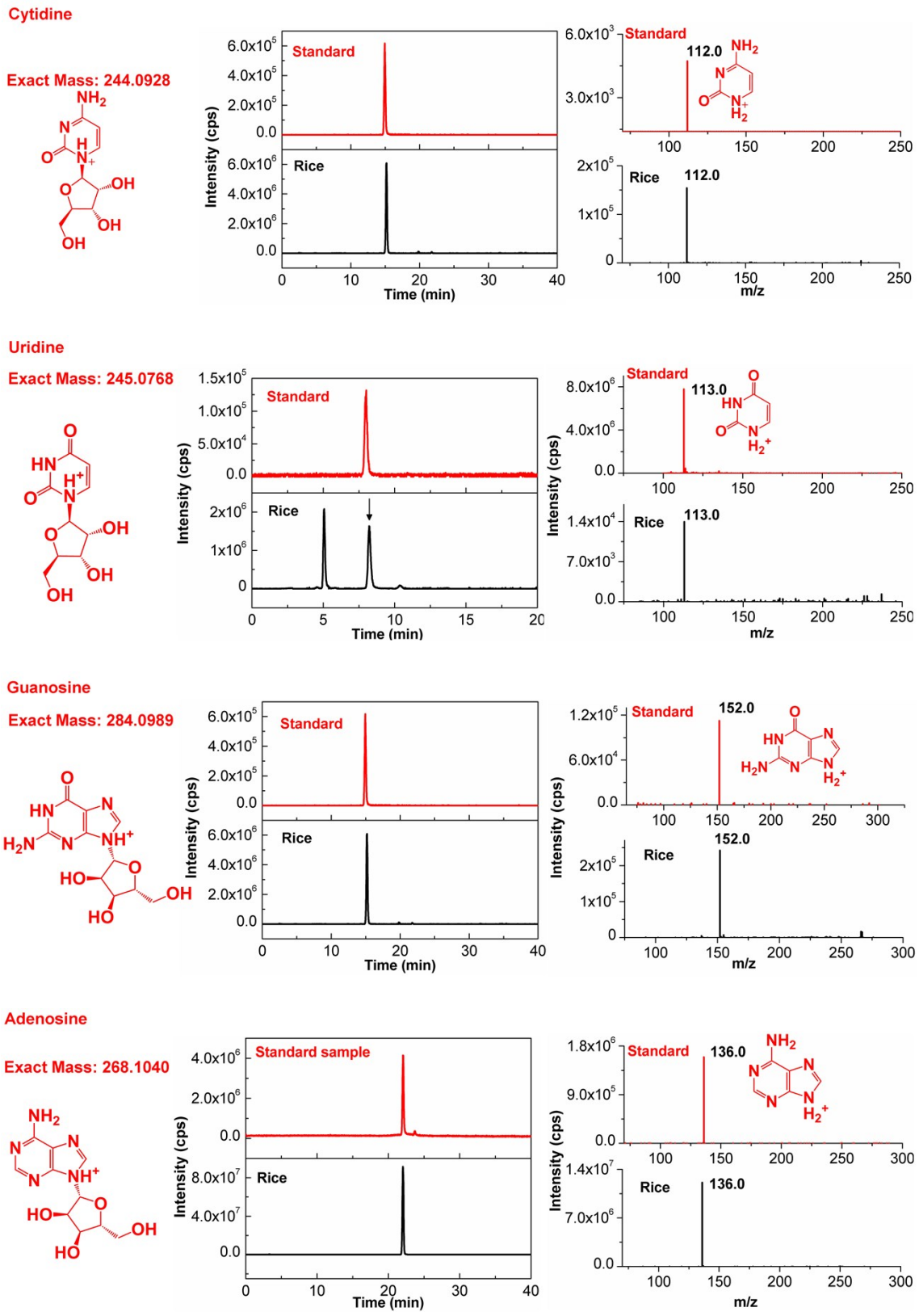
NO.	Time min	Experimental m/z	Ratio Cd-treated/Cd-free	Prospective formulas	Theoretical m/z	Theoretical mass M	Error (mD)	Name
1	**5.6**	**255.0974**	**2.03**	**C11H14N2O5**	**255.0975**	**254.0903**	**-0.1**	**Nicotinamide riboside**
2	**4.4**	**256.0816**	**0.43**	**C11H13NO6**	**256.0816**	**255.0743**	**0**	**Nicotinate D-ribonucleoside**
3	13.3	257.1131	1.70	C11H16N2O5	257.1132	256.1059	-0.1	1-(beta-D-Ribofuranosyl)-1,4-dihydronicotinamide
4	5.8	244.0928	0.63	C9H13N3O5	244.0928	243.0855	0	Cytidine
5	8.3	245.0768	0.64	C9H12N2O6	245.0768	244.0695	0	Uridine
6	15.2	284.0988	0.82	C10H13N5O5	284.0989	283.0917	-0.1	Guanosine
7	22.1	268.1038	1.33	C10H13N5O4	268.1040	267.0968	-0.2	Adenosine
8	35	298.0966	0.86	C11H15N5O3S	298.0968	297.0896	-0.2	5’-Deoxy-5’-(methylthio)adenosine
9	7.4	399.1440	1.07	C15H22N6O5S	399.1445	398.1372	0.5	S-Adenosylmethionine
10	19.5	298.1145	0.94	C11H15N5O5	298.1146	297.1073	-0.1	1-Methylguanosine
11	40.6	493.1332	1.11	C23H24O12	493.1341	492.1268	-0.9	Eupalitin 3-galactoside Quercetin 3,4'-dimethyl ether 7-glucoside Betuletol 3-galactoside Eupalitin 3-glucoside Betuletol 3-glucoside Quercetin 3,3'-dimethyl ether 7-glucoside Quercetin 3,7-dimethyl ether 4'-glucoside Ombuin 3-galactoside Ombuin 3-glucoside Caryatin 7-glucoside Quercetin 3,7-dimethyl ether 5-glucoside Betuletol 7-glucoside Licoagroside A 3',8-Dimethoxyapigenin 7-glucoside 3',4',5-Trihydroxy-3,7-dimethoxyflavone 5-glucoside 3',7-Dimethoxy-4',5,8-trihydroxyflavone 8-glucoside Aurantio-obtusin beta-D-glucoside Malvidin 3-O-glucoside Prudomenin Rhamnazin 4'-glucoside Rhamnazin 3-glucoside
12	**45.7**	**493.1335**	**2.03**	**C23H24O12**	**493.1341**	**492.1268**	**-0.6**	Isopyrenin Tricin 7-glucoside Tricin 5-glucoside Tricin 4'-glucoside

								Dillenetin 7-glucoside Homotectorigenin 7-O-glucoside Europinidin 3-galactoside Europinidin 3-glucoside Iristectorigenin A 7-O-glucoside Iristectorigenin B 7-O-glucoside 5,2',6'-Trihydroxy-6,7-dimethoxyflavone 2'-glucoside 6-Hydroxyluteolin 6,3'-dimethyl ether 7-glucoside Lagotiside Rhamnazin 3-galactoside Isothymusin 8-glucoside Hypolaetin 7,3'-dimethyl eter 4'-glucoside 6-Hydroxyluteolin 6,4'-dimethyl ether 7-glucoside 6-Hydroxyluteolin 7,3'-dimethyl ether 6-glucoside 5,2',3'-Trihydroxy-7,8-dimethoxyflavone 3'-glucoside 8-Hydroxyluteolin 8,3'-dimethyl ether 7-glucoside Oenin
13	28.1	565.1543	1.52	C26H28O14	565.1552	564.1479	-0.9	6-beta-D-Glucopyranosyl-8-beta-D-ribopyranosylapigenin
14	30.6	565.1541	1.10	C26H28O14	565.1552	564.1479	-1.1	
15	34.5	565.1539	1.01	C26H28O14	565.1552	564.1479	-1.3	Apigenin 6-C-glucoside 8-C-arabinoside Isocorymboside Schaftoside Neocorymboside 6-C-Galactopyranosyl-8-C-xylopyranosylapigenin 6-C-Xylopyranosyl-8-C-galactopyranosylapigenin Vicenin 1
16	35.0	565.1544	1.28	C26H28O14	565.1552	564.1479	-0.8	Corymboside 6-C-beta-D-Glucopyranosyl-8-C-beta-D-apiofuranosylapigenin Vicenin 3 Isoschaftoside Neoschaftoside Neoisoschaftoside Isovitexin 2"-O-glucoside Meloside A Vitexin 6"-O-glucoside 6-C-Galactosylapigenin 6"-O-galactoside

18	38.0	625.1752	1.65	C28H32O16	625.1763	624.1690	-1.1	Isovitexin 6"-O-glucoside 2"-O-beta-L-galactopyranosylvitexin Flavosativaside 8-C-Glucopyranosylgenistein 4'-O-glucoside Vitexin 4'-O-galactoside Vitexin 7-O-glucoside Saponarin Vitexin 4'-O-glucoside Neosaponarin Isoscoparin 2"-O-glucoside Scoparin 2"-O-glucoside Scoparin 6"-O-glucoside Isoscoparin 4'-O-glucoside Isoscoparin 7-O-glucoside Knoutinoside Chrysoeriol 7-O-8-C-bisglucoside Swertiajaponin 3'-O-glucoside Scoparin 2"-glucoside
19	42.7	741.2012	1.14	C36H36O17	741.2025	740.1952	-1.3	Pelargonidin 3-O-β-D-glucoside 5-O-(6-coumaroyl-β-D-glucoside) Vitexin 7-O-glucoside 2"-p-coumarate
20	43.5	741.2019	1.06	C36H36O17	741.2025	740.1952	-0.6	Isovitexin 7-O-(6'"-O-E-p-coumaroyl)glucoside Pelargonidin 3-[6-((Z)-p-coumaroyl)glucoside]-5-glucoside Bisdemalonylmonardaein
21	42.9	771.2126	1.07	C37H38O18	771.2131	770.2058	-0.5	Peonidin 3-(6"-p-coumarylglucoside)-5-glucoside 6'"-O-Sinapoylsaponarin
22	42.7	801.2231	1.33	C38H40O19	801.2237	800.2164	-0.6	Malvidin 3-O-(6-O-(Z)-p-coumalonyl-beta-glucopyranoside)-5-O-beta-glucopyranoside Tibouchinin
23	41.1	538.2275	1.49	C26H32O11 $[M+NH4]^+$	538.2283	520.1945	-0.8	(7'S,8'S)-4,7'-Epoxy-3,8'-bilign-7-ene-3',5-dimethoxy-4',9,9'-triol 4'-glucoside (-)-Pinoresinol glucoside
24	**43.6**	**510.2324**	**2.14**	**C25H32O10 $[M+NH4]^+$**	**510.2334**	**492.1995**	**-1.0**	**Palmatoside G**
25	**24.0**	**355.1497**	**0.23**	**C16H19NO7 $[M+NH4]^+$**	**355.1500**	**337.1162**	**-0.3**	**Indole-3-acetyl-myo-inositol Indole-3-acetyl-beta-1-D-glucoside**

								1H-Indol-3-ylacetyl-myo-inositol
26	**2.8**	**335.0738**	**0.47**	**C9H19O11P**	**335.0738**	**334.0665**	**0**	**1-(sn-Glycero-3-phospho)-1D-myo-inositol**
27	16.6	332.1331	0.85	C14H21NO8	332.1340	331.1267	-0.9	5'-O-beta-D-Glucosylpyridoxine
28	26.4	772.2662	1.67					
29	29.0	772.2659	1.51					
30	44.9	754.2540	1.43					
31	31.0	712.2437	1.39					
32	43.2	712.2433	1.39					
33	37.9	700.2804	1.65					
34	31.3	568.2958	1.01					
35	38.7	552.3008	0.91					
36	40.0	552.3004	1.25					
37	27.0	453.2477	0.91					
38	15.7	391.0999	0.59					
39	3.3	353.0843	1.03					
40	3.7	353.0844	0.55					
41	38.0	325.1391	0.57					
42	**44.6**	**323.1383**	**0.08**					
43	27.2	316.1500	0.58					
44	44.0	307.1761	0.92					

Figure S1. Comparison of the retention times and fragment ions of glycosylated compounds with commercial standards.



9

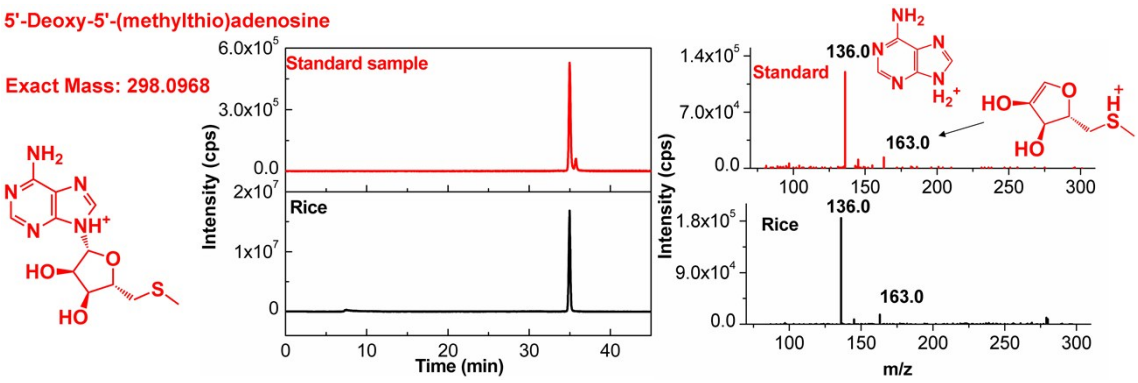
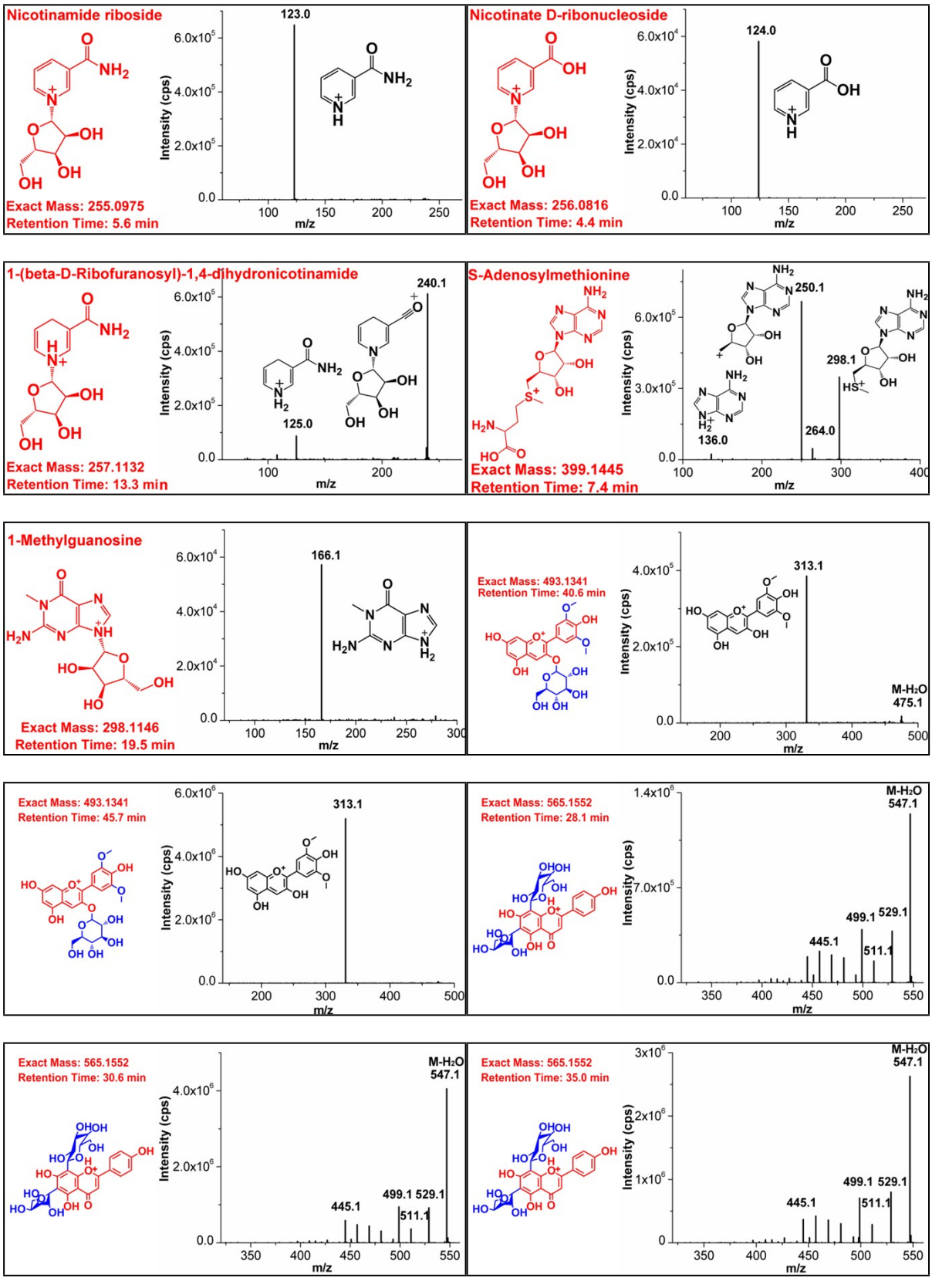
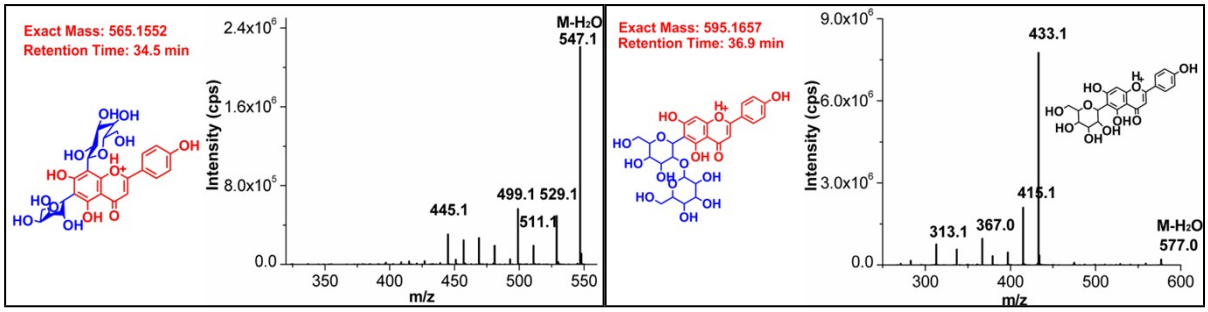
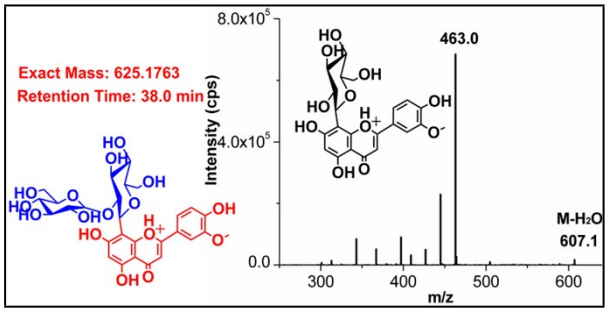
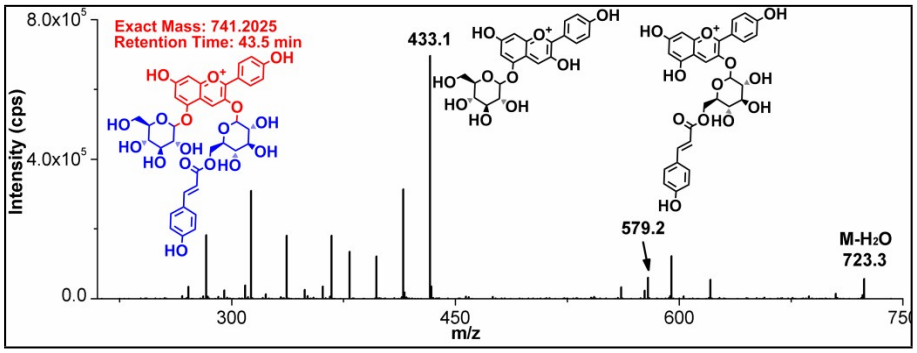

5'-Deoxy-5'-(methylthio)adenosine
Exact Mass: 298.0968
Standard sample
Rice
Intensity (cps)
Time (min)
Standard
Rice
136.0
163.0
m/z

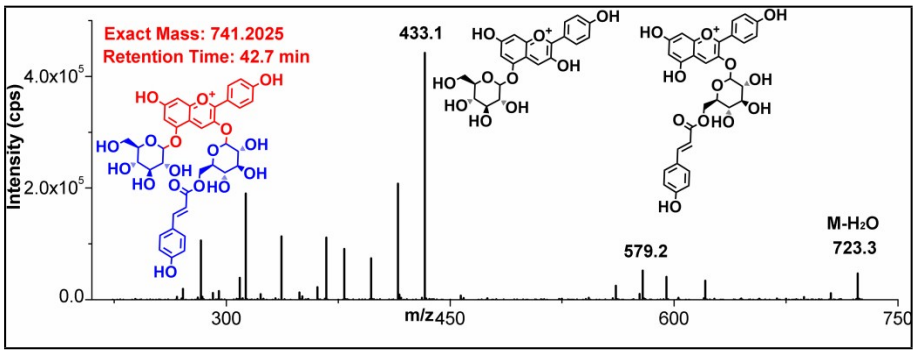
Figure S2. MS/MS fragmentation and their possible structure of the 22 identified compounds.

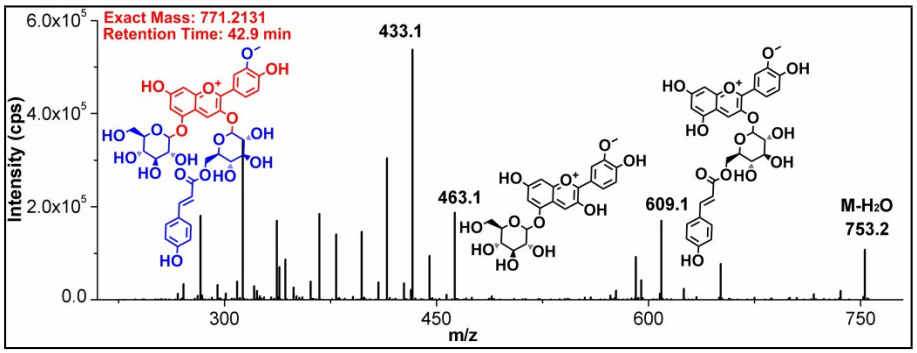


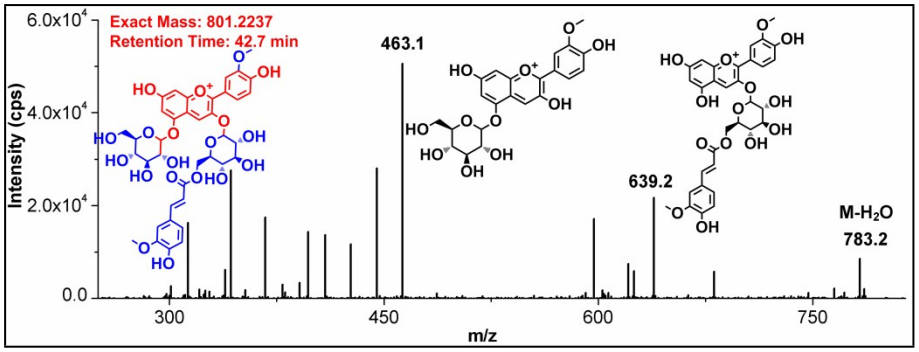

Exact Mass: 565.1552
Retention Time: 34.5 min
Intensity (cps)
m/z
M-H2O
547.1
445.1
499.1 529.1
511.1
Exact Mass: 595.1657
Retention Time: 36.9 min
433.1
313.1
367.0
415.1
M-H2O
577.0

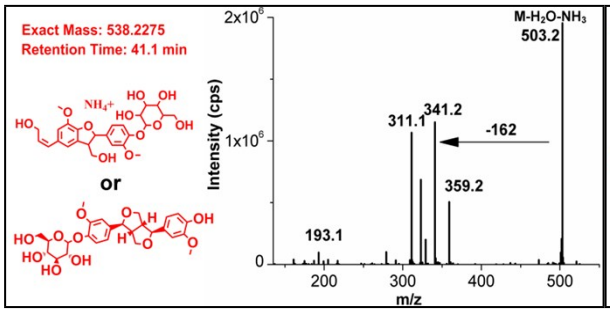

Exact Mass: 625.1763
Retention Time: 38.0 min
Intensity (cps)
m/z
463.0
M-H2O
607.1

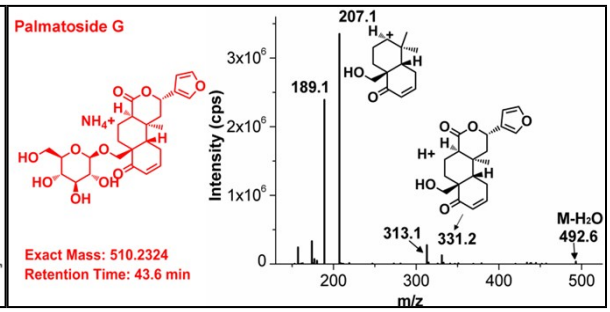

Exact Mass: 741.2025
Retention Time: 43.5 min
Intensity (cps)
m/z
433.1
579.2
M-H2O
723.3

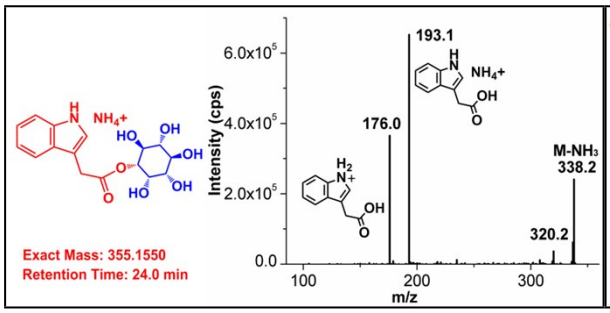

Exact Mass: 741.2025
Retention Time: 42.7 min
Intensity (cps)
m/z
433.1
579.2
M-H2O
723.3

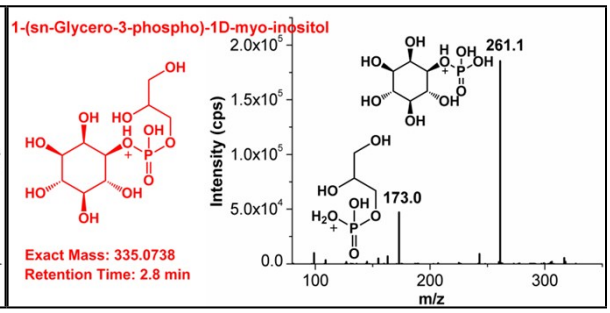

Exact Mass: 771.2131
Retention Time: 42.9 min
Intensity (cps)
m/z
433.1
463.1
609.1
M-H2O
753.2


Exact Mass: 801.2237
Retention Time: 42.7 min
463.1
639.2
M-H2O
783.2
Intensity (cps)
m/z


Exact Mass: 538.2275
Retention Time: 41.1 min
or
M-H2O-NH3
503.2
311.1
341.2
-162
359.2
193.1
Intensity (cps)
m/z


Palmatoside G
Exact Mass: 510.2324
Retention Time: 43.6 min
207.1
189.1
313.1
331.2
M-H2O
492.6
Intensity (cps)
m/z


Exact Mass: 355.1550
Retention Time: 24.0 min
193.1
176.0
M-NH3
338.2
320.2
Intensity (cps)
m/z


1-(sn-Glycero-3-phospho)-1D-myo-inositol
Exact Mass: 335.0738
Retention Time: 2.8 min
261.1
173.0
Intensity (cps)
m/z

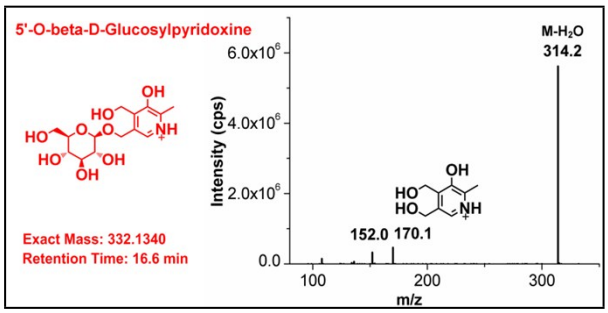
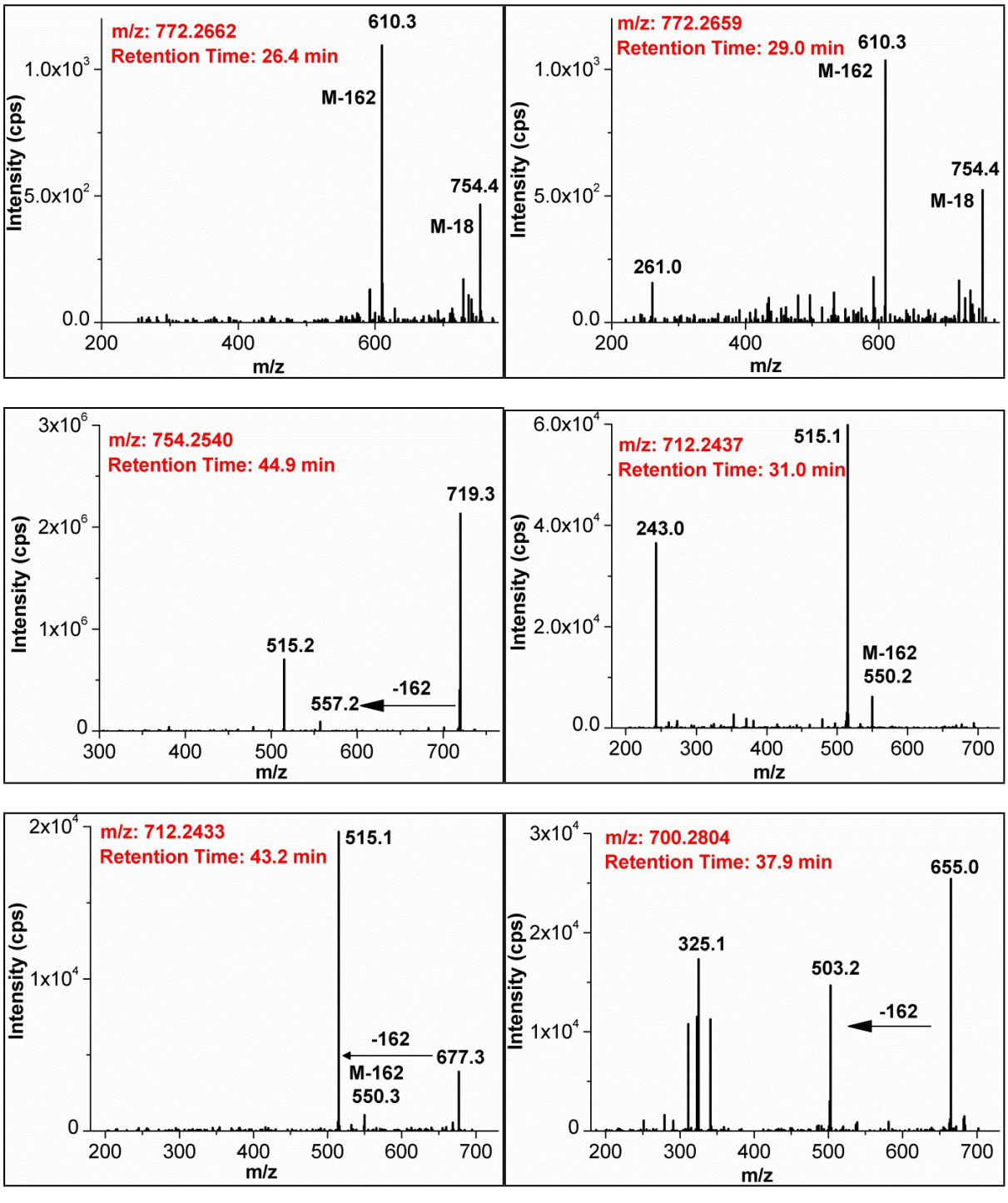
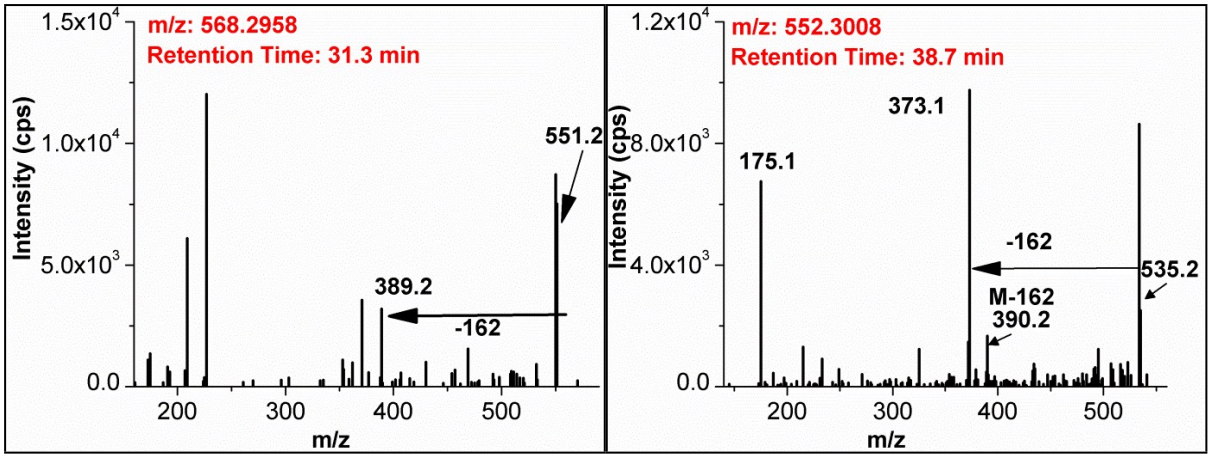
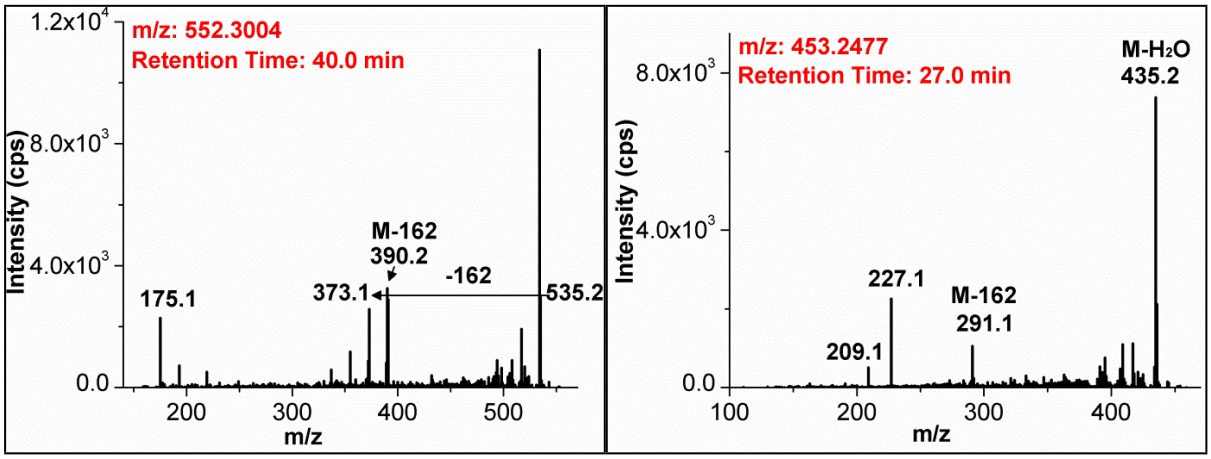
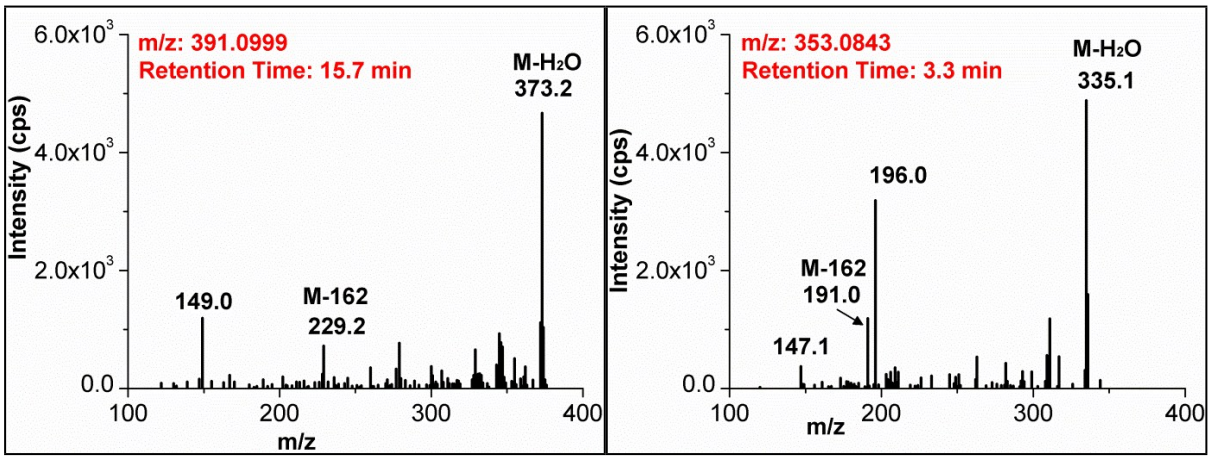

5'-O-beta-D-Glucosylpyridoxine
Exact Mass: 332.1340
Retention Time: 16.6 min
M-H2O
314.2
152.0
170.1
Intensity (cps)
m/z

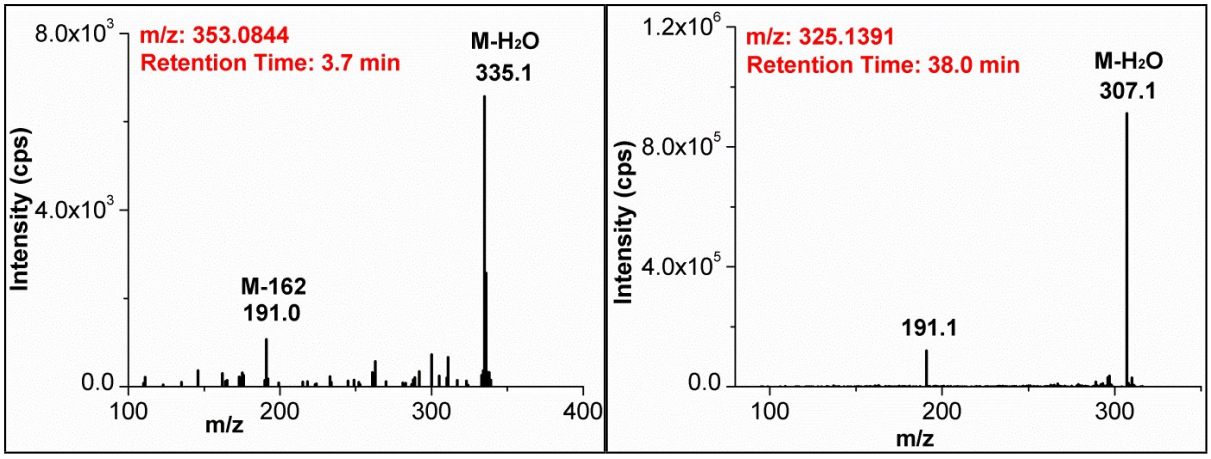
Figure S3 MS/MS fragmentation of the 17 unknown compounds.

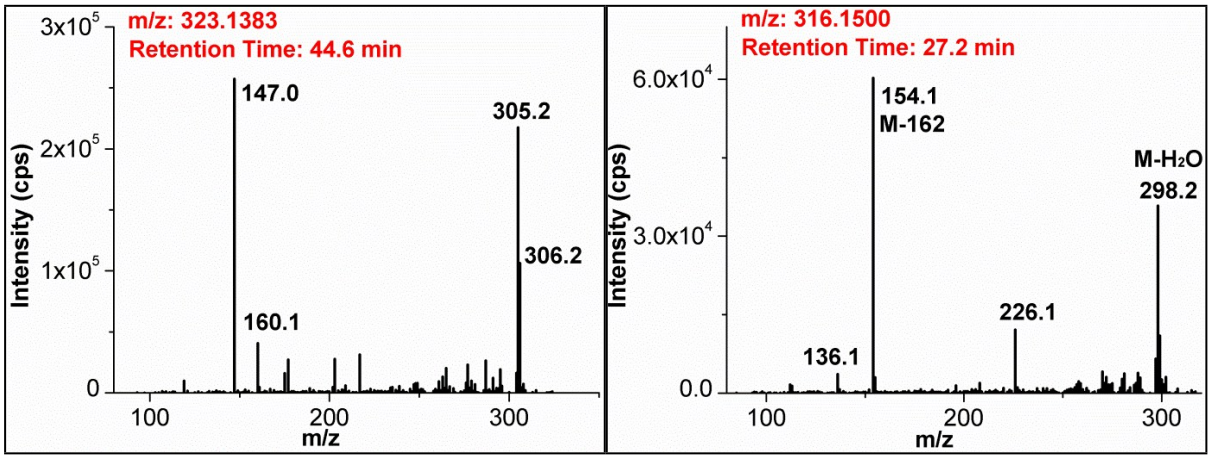


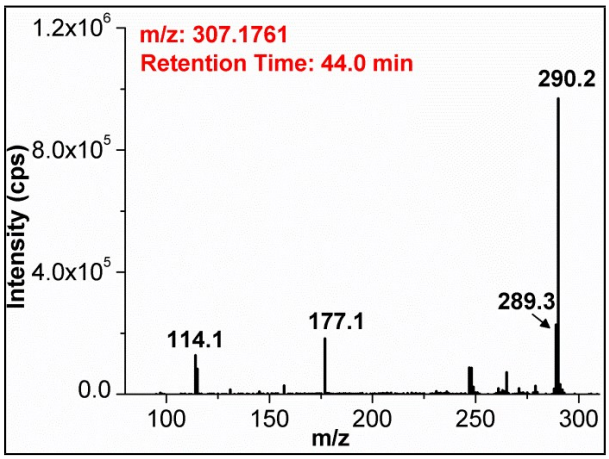

m/z: 568.2958
Retention Time: 31.3 min
Intensity (cps)
m/z
551.2
389.2
-162
m/z: 552.3008
Retention Time: 38.7 min
373.1
175.1
-162
535.2
M-162
390.2


m/z: 552.3004
Retention Time: 40.0 min
Intensity (cps)
m/z
M-162
390.2
-162
175.1
373.1
535.2
m/z: 453.2477
Retention Time: 27.0 min
M-H₂O
435.2
227.1
M-162
291.1
209.1


m/z: 391.0999
Retention Time: 15.7 min
Intensity (cps)
m/z
M-H₂O
373.2
149.0
M-162
229.2
m/z: 353.0843
Retention Time: 3.3 min
M-H₂O
335.1
196.0
M-162
191.0
147.1


m/z: 353.0844
Retention Time: 3.7 min
M-H2O
335.1
M-162
191.0
Intensity (cps)
m/z
m/z: 325.1391
Retention Time: 38.0 min
M-H2O
307.1
191.1
Intensity (cps)
m/z


m/z: 323.1383
Retention Time: 44.6 min
147.0
305.2
306.2
160.1
Intensity (cps)
m/z
m/z: 316.1500
Retention Time: 27.2 min
154.1
M-162
M-H2O
298.2
226.1
136.1
Intensity (cps)
m/z


m/z: 307.1761
Retention Time: 44.0 min
290.2
289.3
177.1
114.1
Intensity (cps)
m/z

References

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