Supporting information

Hexagonal boron nitride nanosheets as a multifunctional background-free matrix to detect small molecules and complicated samples by MALDI mass spectrometry

Jianing Wang[a1], Jie Sun[ab1], Jiyun Wang[a], Huihui Liu[a], Jinjuan Xue[ab] and Zongxiu Nie*[abc]

a. Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China

b. University of Chinese Academy of Sciences, Beijing 100049, China

c. National Center for Mass Spectrometry in Beijing, Beijing 100190, China.

1. These authors contributed equally to this work.

*Corresponding author: Prof. Zongxiu Nie; E-mail: znie@iccas.ac.cn

Materials and reagents. h-BN nanosheets were purchased from Nanjing Xianfeng Nanotechnology Corporation. h-BN nanosheets should be stored in a dry, sealed container. Acetonitrile, methanol and formic acid were of HPLC grade and were purchased from Fischer Scientific. Amino acids were purchased from Beijing Chemical Reagents Inc. Ethinylestradiol, fatty acids, ascorbic acid, salicylic acid and 9-Aminoacridine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Peptides were purchased from Shanghai Jill biochemical. 9-Nitroanthracene was purchased from J&K Scientific. Ultrapure water used throughout the experiment was obtained from a Milli-Q system (Millipore USA). Ethinyl estradiol was dissolved in methanol at a concentration of 1mM. 9-nitroanthracene was dissolved in methanol at a concentration of 100mM and solutions at other concentrations were diluted gradually. All the water soluble analytes were dissolved in ultrapure water to form 1mM stock solutions and were placed at -20℃. Details of reagents were described in supporting information. Bacterial culture chemicals were obtained commercially: peptone tryptone, yeast extract (Oxoid Hampshire, England) and NaCl (Carl Roth, Karlsruhe, Germany). They were used without further purification.

Characterization of h-BN nanosheets. Absorption spectrum (220 nm to 500 nm) was measured by a solid UV-VIS spectrometer (UV2600, Mitsubishi). The morphology of h-BN nanosheets was investigated by transmission electron microscopy (TEM, JEM-2010, JEOL, Japan) with an accelerating voltage of 120 kV.

Sample preparation for MALDI MS. Details of reagents was described in supporting information. 9-AA was dissolved in methanol at concentration of 10

mg/mL. The h-BN nanosheets were dispersed in a mixed solvent of deionized water and methanol (v:v=1:1) with ultrasonic oscillation for 15 min to form a suspension of 0.1mg/mL. 1μL matrix solution was mixed with 1μL analytes solution. Then 1μL mixture was pipetted on the MALDI target plate and air-dried to form a thin layer of matrix and analytes mixture for further MS analysis. For each experiment, the matrix solution was freshly prepared. For fear of background interference, the MALDI target plate needed to be washed according to the standard target washing procedure provided by Bruker Daltonics and dried under nitrogen before use.

Mass spectrometer. MALDI-MS analysis was acquired in negative ion mode using a reflectron geometry MALDI-TOF mass spectrometer (Ultraflextreme, Bruker Daltonics, Bremen, Germany). The mass spectrometer was equipped with a smart beam Nd: YAG pulsed laser operated at 355 nm. The acceleration and reflection voltages were 19 and 20 KV. The laser pulse energy was adjusted from 40% to maximum output per pulse by regulating the laser power modulation from 0% to 100% at an offset of 40%. The mass spectra were acquired at an average of 100 laser shots and 1000 Hz frequency. The mass spectra were processed by FlexAnalysis v3.4.

Accurate masses were recorded on a Solarix 9.4T AS FTICR MS (Bruker Daltonics, Bremen, Germany) equipped with an MALDI source.

Mass spectrometry imaging. The wild-type male Kunming mouse (20-22g) were provided by the Experimental Animal Center of Peking University. The mice were anesthetized with carbon dioxide followed by cervical dislocation. Organs were harvested within 15 minutes. The animal experiments were performed according to

the NIH Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication, No.3040-2, revised 1999, Bethesda, MD) and were approved by the Animal Care and Use Committee of the Chinese Academy of Sciences. The fresh tissue was frozen in liquid nitrogen very quickly and was cryosectioned by a Leica CM1950 cryostat (Leica Biosystems, Nussloch, Germany) to form 2 or 3 μm thickness sections. The tissue cryosections were then transferred or imprint onto a polished stainless steel plate (Bruker Daltonics, Bremen, Germany) and were desiccated in vacuum for 30 to 60 minutes at room temperature. The matrix solution of 0.025 mg/mL in water/methanol (50/50, v/v) was sprayed by the Bruker Image Prep device (Bruker Daltonics, Bremen, Germany). The parameters for matrix deposition were: 80 cycles, each cycle was composed of 1s of spraying at 30% power, 15s of incubation and 60s of drying. The imaging data was acquired in negative ion mode with laser spot size setting at 50 μm, step width 100 μm and summed up by 200 shots at a laser repetition rate of 1000 Hz without intra-spot rastering. Imaging data were processed by FlexImaging v3.0. The bin width is ±0.15 Da.

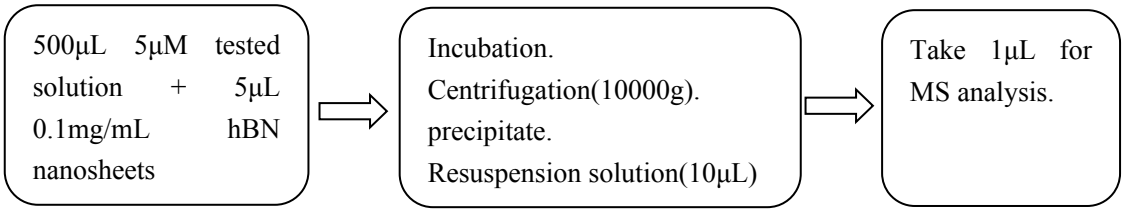
***E. Coli* extraction preparation.** The E.coli DH5α were grown in LB (Luria-Bertani) medium (5 g/L NaCl, 5 g/L yeast extract, 10 g/L tryptone) at 37℃ with 150rpm shaking speed, and harvested in the stationary phase with the final optical density at 600nm (OD_{600nm}) was 0.8-1.0. Aliquots of 100 μl of broth cultured bacteria were washed with 1x PBS buffer solution (500 μl, pH=7.4) for three times and the bacteria pellets were collected by centrifugation at 8000x*g* for 10min for further treatment.

The bacteria pellets were washed with deionized water and suspended in 80%

methanol/ water to 30 μL. The suspending liquid was incubated at room temperature for 5 min with intermittent vortexing. The extract was collected by centrifugation at 10,000x*g* for 10 min.

h-BN as an adsorbent. 5μL h-BN nanosheets suspension (0.1mg/mL) was added to 495μL of analytes solution (5μM) and then incubated in rotary vibrator for a period (12 hours or 1 minute). After that, we centrifuged the mixture at a rotating speed of 10000x*g* for 15 min and then removed the supernatant. Finally, the obtained precipitate was resuspended to 10μL (deionized water: methanol v: v=1:1) and pipetted 1μL for MALDI-TOF MS detection (Scheme S1).

Aerosol sample collection and extraction. The sampling was carried out on the roof of a 10-story building on the campus of Institute of Chemistry, Chinese Academy of Sciences (ICCAS) in Beijing. An aerosol sampler (Series 20-800, Thermo Fisher Scientific) was used to collect particles with an aerodynamic diameter of 0.4-2.1μm. Aerosol particles were adsorbed on Whatman microglass fiber filters. The aerosol sample was collected from December 9 to 10, 2015 for 24h at a flow rate of 20L/min. The aerosol sample was Soxhlet extracted for 24h with dichloromethane (DCM). The extract was concentrated to 5mL by vacuum rotary evaporation at 30℃ and then dried by flow nitrogen. The final extract was redissolved by DCM to 200μL for further enrichment or detection.



Scheme S1. Method roadmap of solid phase extraction (SPE) prior to MALDI-TOF MS analysis with hBN nanosheets as absorbent and matrix.

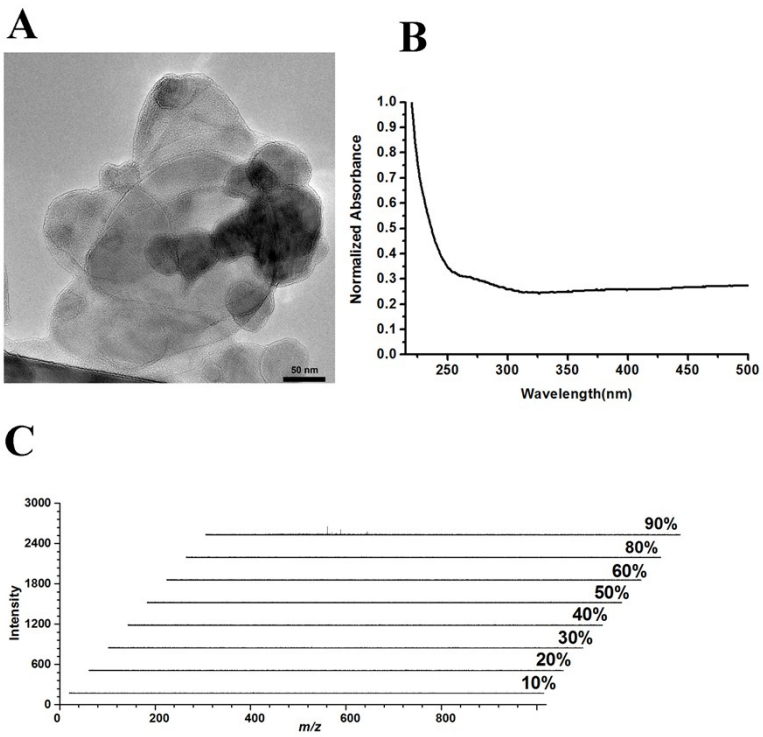


Figure S1. A: TEM photograph of h-BN nanosheets, bar scale: 50nm; B: UV-vis spectrum of h-BN; C: LDI-TOF mass spectra of h-BN nanosheets in negative ion mode in the range of *m/z* 0 to 1000 with laser energy from 10% to 90%.

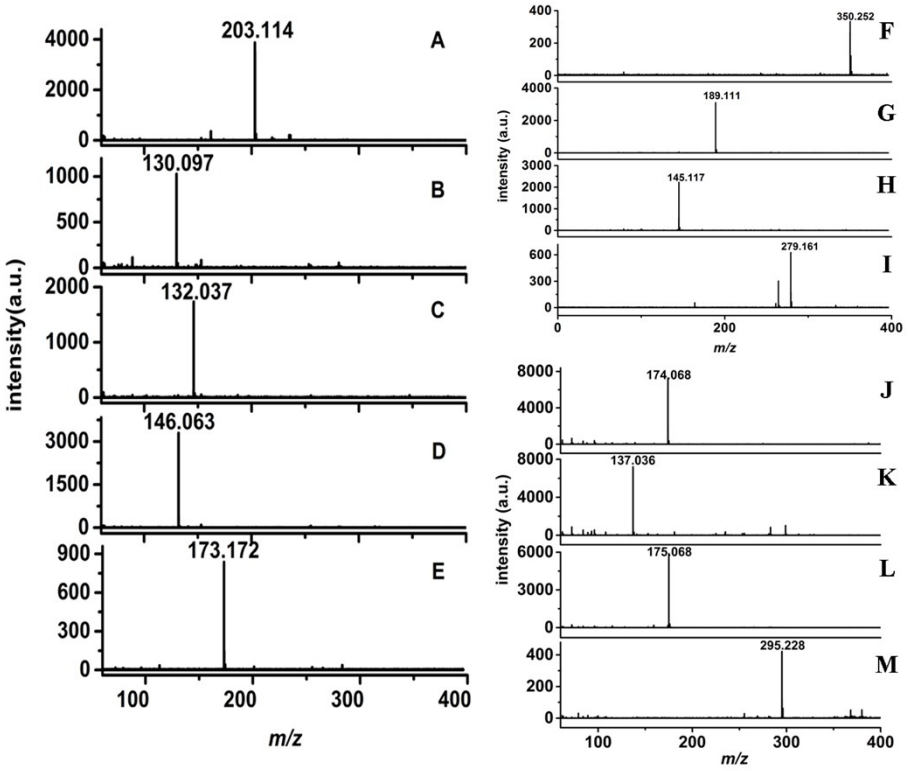


Figure S2. MALDI-TOF mass spectra of amino acids with matrix of h-BN nanosheets: A: Trp (*m/z* 203.114), B: Leu (*m/z* 130.097), C: Asp (*m/z* 132.037), D: Glu (*m/z* 146.063), E: Arg (*m/z* 173.172), F: Gly-Leu-Tyr (*m/z* 350.252), G: Gly-Asp (*m/z* 189.111), H: Gly-Ala (*m/z* 145.117), I: Asp-Phe (*m/z* 279.161), J: N-acetylaspartic acid (*m/z* 174.068); K: salicylic acid (*m/z* 137.036); L: ascorbic acid (*m/z* 175.068); M: Ethinyl estradiol (*m/z* 295.228) in negative ion mode. The amount of each analyte is 500 pmol.

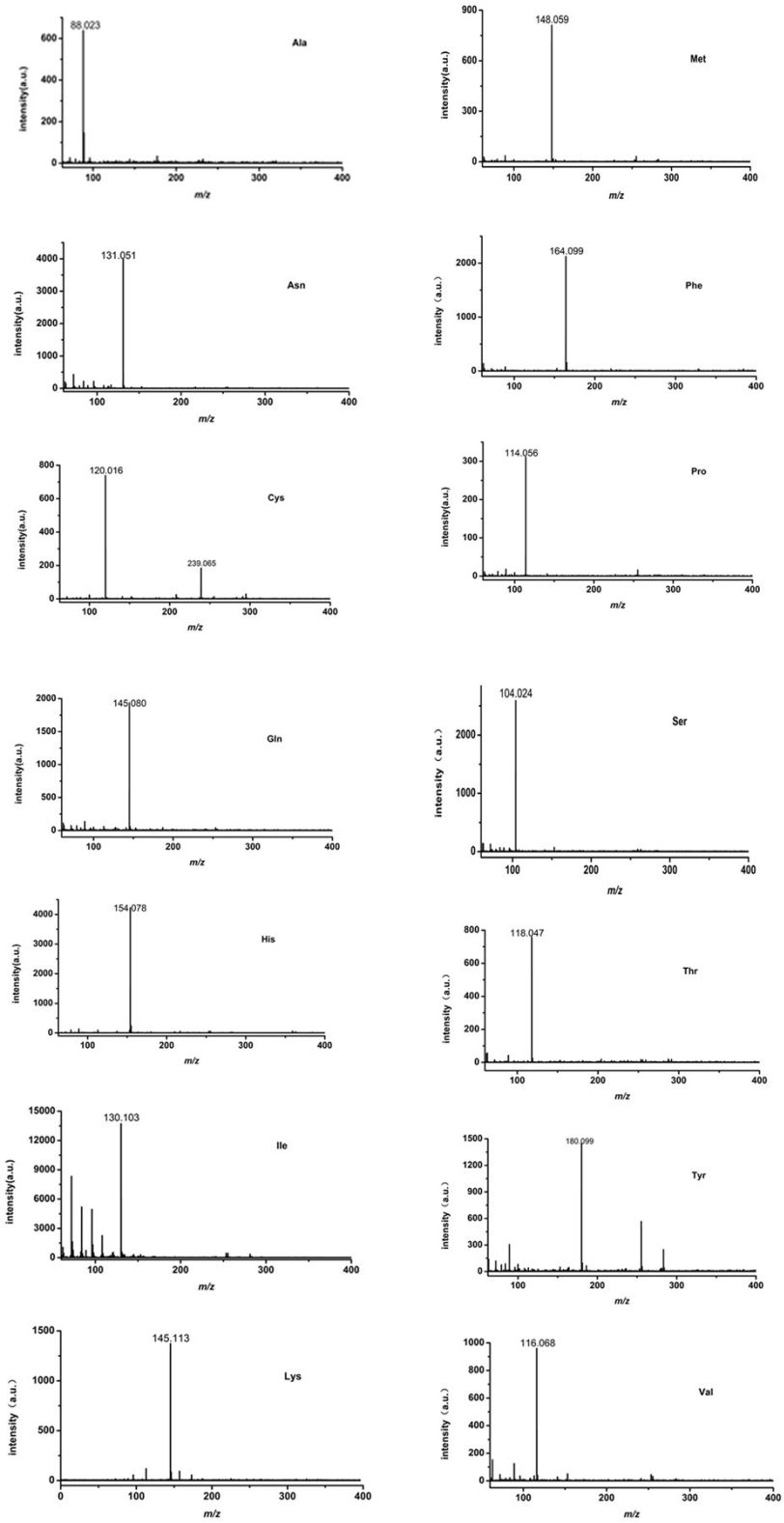


Figure S3. MALDI-TOF mass spectra of amino acids with a matrix of hBN nanosheets in negative ion mode. The amount of each analyte is 500 pmol.

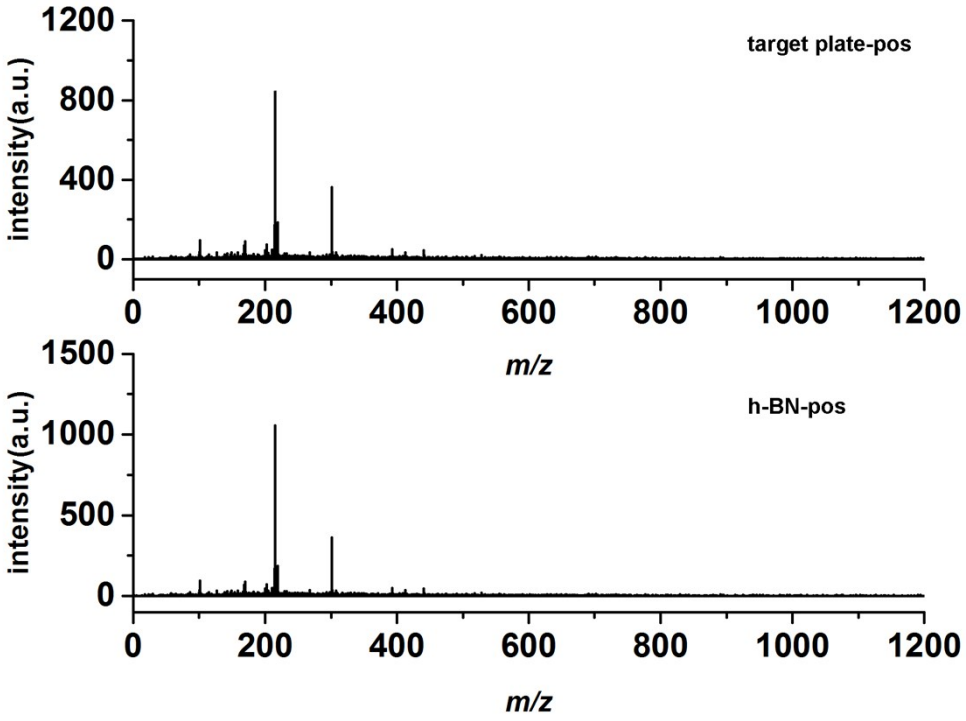


Figure S4. LDI-TOF mass spectra of stainless steel target plate (up panel) and h-BN (bottom panel) in positive ion mode. The major peaks of target plate and h-BN are the same.

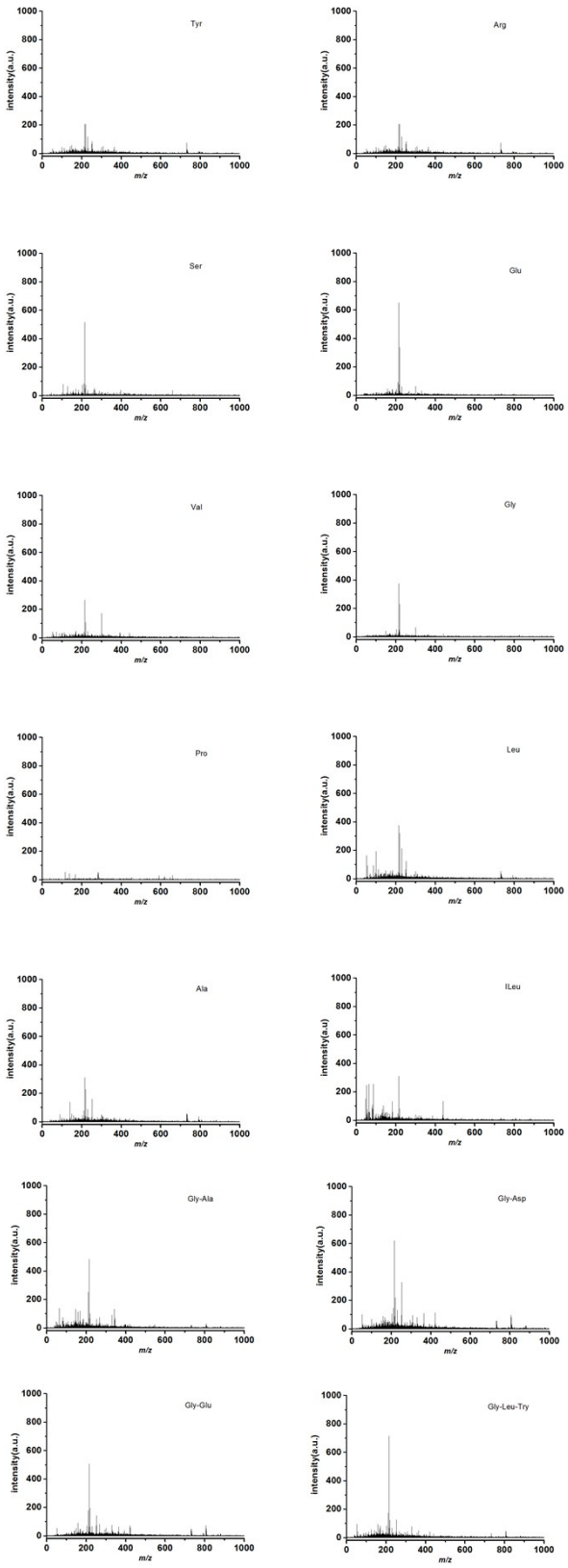


Figure S5. MALDI-TOF mass spectra of amino acids and oligopeptides with a matrix of hBN nanosheets in positive ion mode. The amount of each analyte is 500 pmol. 1% trifluoroacetic acid (TFA) is added.

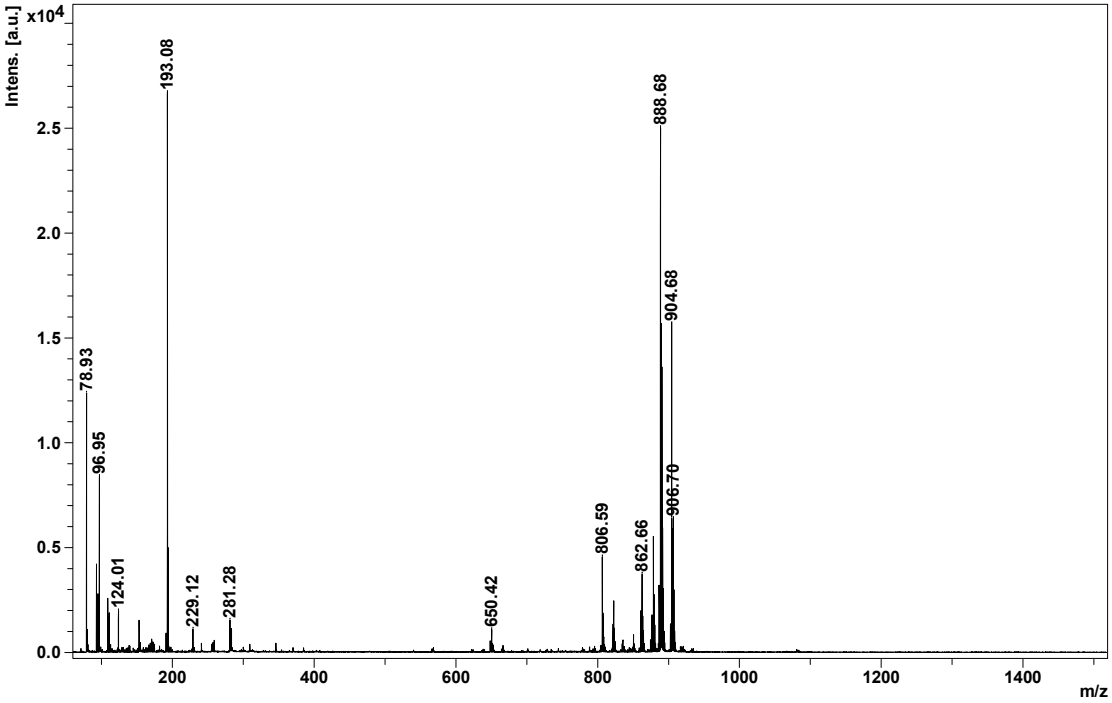


Figure S6. Metabolites in mouse brain tissue detected by MALDI-TOF MS using 9-AA as a matrix in negative ion mode.

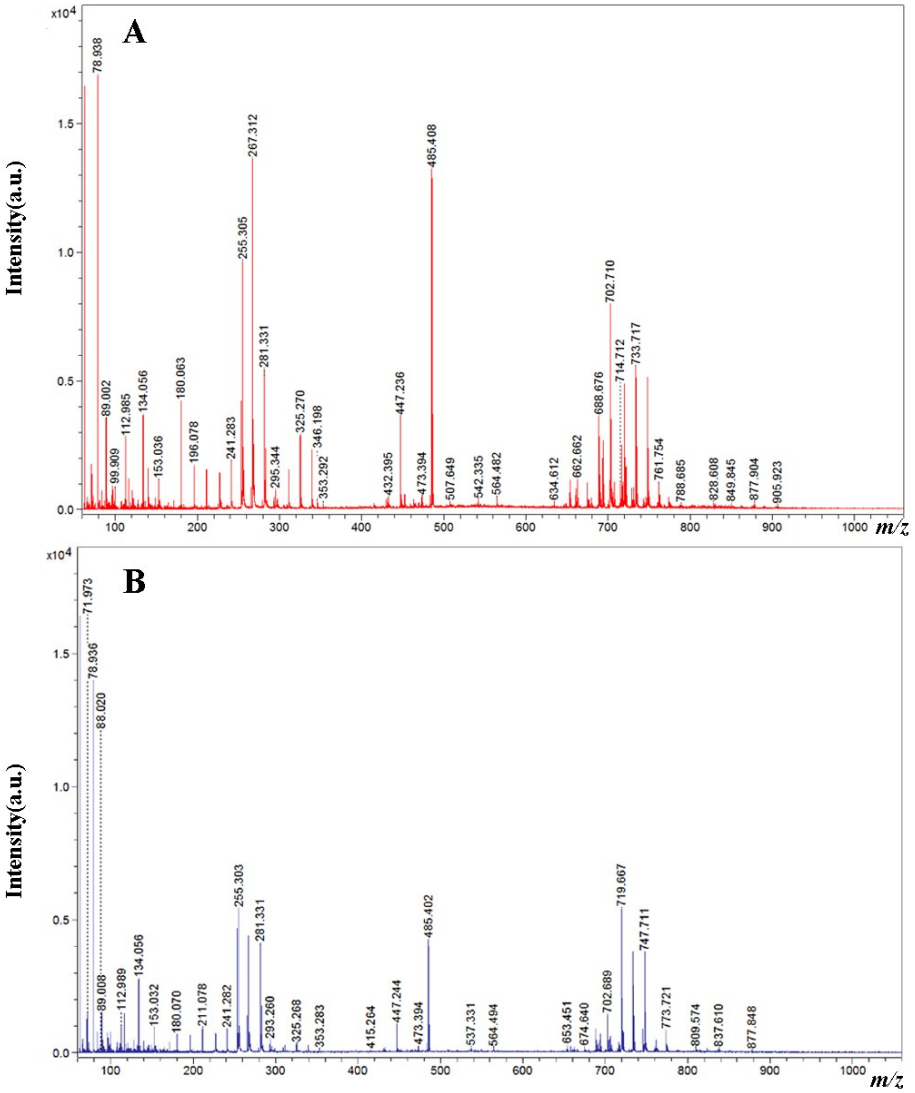


Figure S7. A: h-BN nanosheets-assisted LDI mass spectra acquired from the extraction of *E.coli* and B: extraction of *E.coli* treated by starvation for 24 hrs.

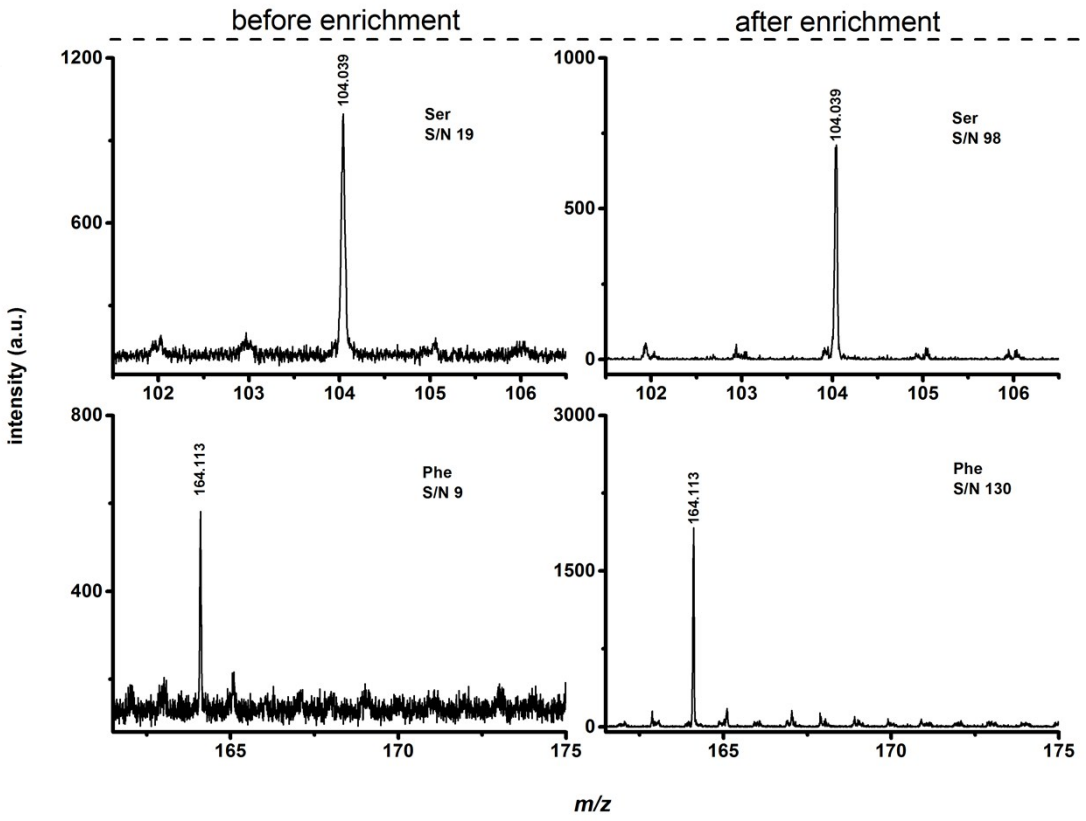


Figure S8. MALDI-TOF mass spectra of analytes before (left panel) and after (right panel) overnight enrichment with hBN nanosheets as both matrix and adsorbent. The amounts of all analytes before enrichment are 5pmol. The concentration of each analyte for adsorption is 5μM.

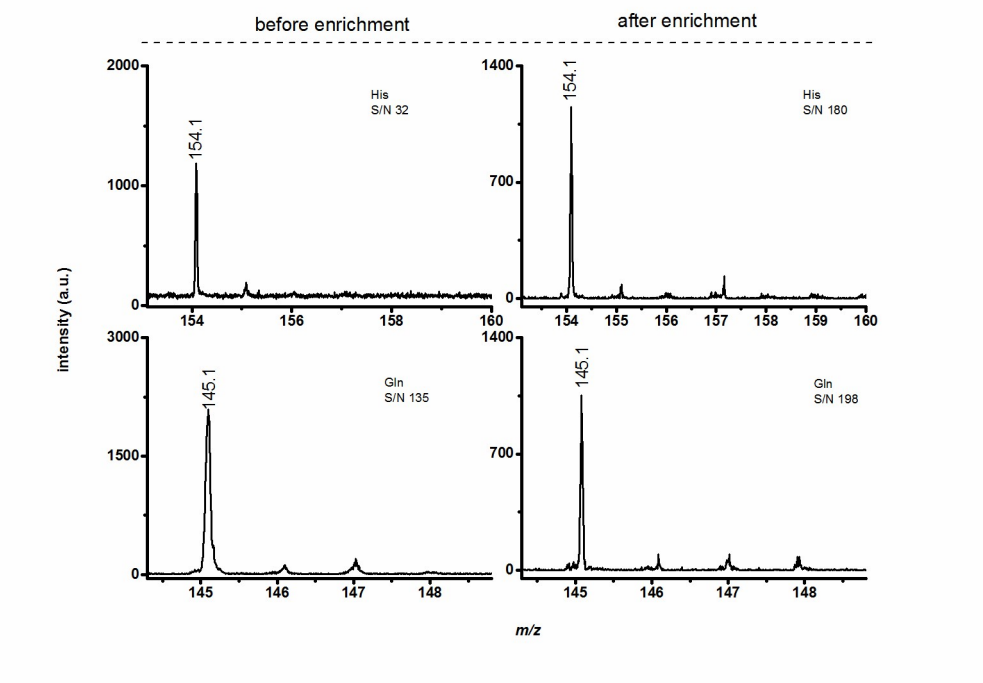


Figure S9. MALDI-TOF mass spectra of analytes (histidine and glutamine) before (left panel) and after (right panel) enrichment with hBN nanosheets as both matrix and adsorbent. The amounts of all analytes before enrichment are 5 pmol. The concentration of each analyte for adsorption is 5 μM.

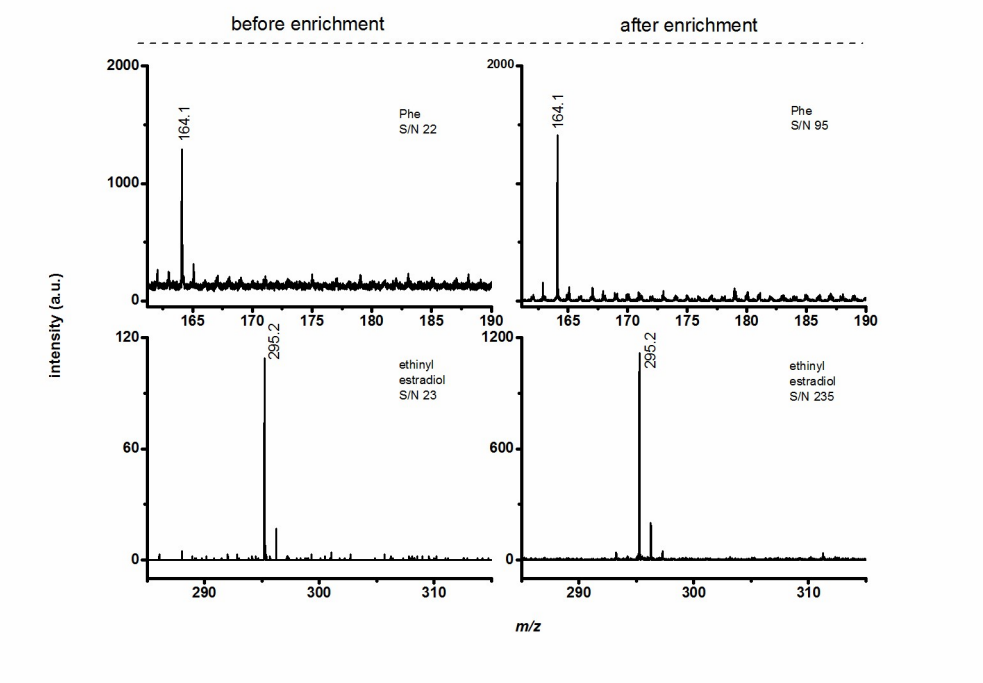


Figure S10. MALDI-TOF mass spectra of analytes before (left panel) and after (right panel) 2 min enrichment with hBN nanosheets as both matrix and adsorbent. The amounts of all analytes before enrichment are 5 pmol. The concentration of each analyte for adsorption is 5 μM.

Table S1. Sulfatide assigned in mouse brain with h-BN nanosheets as a matrix.

m/z	S/N	intensity	Molecular species	ion
778.7275	5	34	d18:1-N16:0 ST	$[M-H]^-$
806.7167	101	756	d18:1-N18:0 ST	$[M-H]^-$
808.7131	20	148	d18:0-N18:0 ST	$[M-H]^-$
822.7188	44	325	d18:1-OHN18:0ST	$[M-H]^-$
834.7574	6	46	d18:1-N20:0 ST	$[M-H]^-$
836.7355	7	55	d18:0-N20:0ST	$[M-H]^-$
848.7261	3	25	d18:1-OHN20:1ST	$[M-H]^-$
850.7669	10	74	d18:1-OHN20:0ST	$[M-H]^-$
852.7644	5	36	d18:0-OHN20:0ST	$[M-H]^-$
860.7953	14	106	d18:1-N22:1ST	$[M-H]^-$
862.7909	38	277	d18:1-N22:0ST	$[M-H]^-$
864.8070	14	100	d18:0-N22:0ST	$[M-H]^-$
874.8061	5	39	d18:1-OHN22:2ST	$[M-H]^-$
876.7812	26	185	d18:1-N23:0ST d18:1-OHN22:1ST	$[M-H]^-$
878.7921	81	588	d18:1-OHN22:0ST	$[M-H]^-$
880.7703	22	159	d18:0-OHN22:0ST	$[M-H]^-$
886.7831	29	208	d18:1-N24:2ST	$[M-H]^-$
888.4859	4	26	d18:1-N24:1ST	$[M-H]^-$
890.8215	224	1597	d18:1-N24:0ST d18:1-OHN23:1ST	$[M-H]^-$
892.3183	4	32	d18:0-N24:0ST d18:1-OHN23:0ST	$[M-H]^-$
902.8212	11	79	d18:1-N25:1ST d18:1-OHN24:2ST	$[M-H]^-$
904.8321	71	492	d18:1-N25:0ST d18:1-OHN24:1ST	$[M-H]^-$
906.8301	107	743	d18:1-OHN24:0ST	$[M-H]^-$
908.8242	19	133	d18:0-OHN24:0ST	$[M-H]^-$
916.8424	3	23	d18:1-N26:1ST	$[M-H]^-$
918.8228	4	28	d18:1-N26:0ST d18:1-OHN25:1ST	$[M-H]^-$

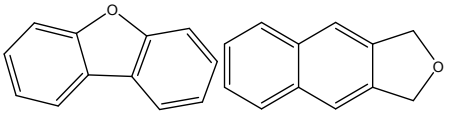
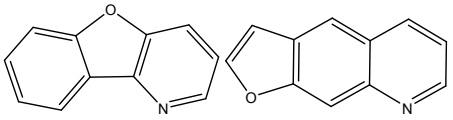
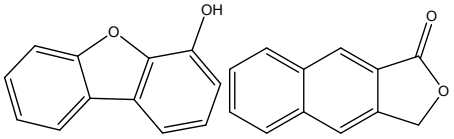
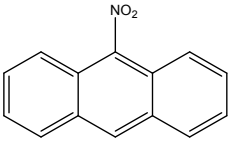
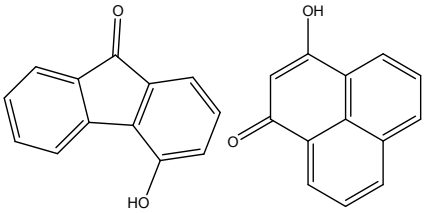
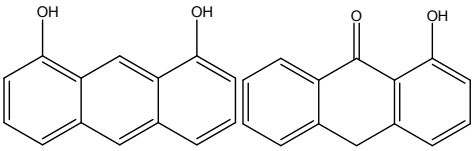
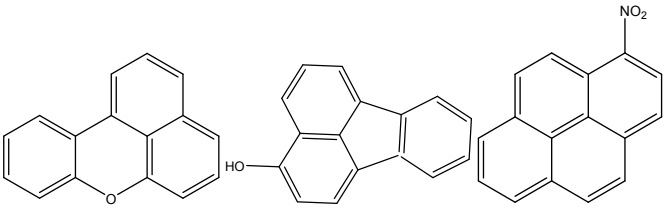
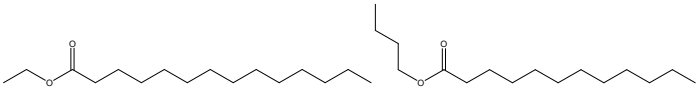
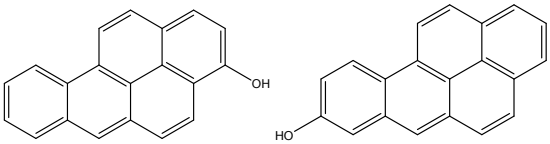
Table S2. Sulfatide assigned in mouse brain with 9-AA as a matrix.

m/z	S/N	intensity	Molecular species	ion
778.6007	4	50	d18:1-N16:0 ST	$[M-H]^-$
806.5909	109	1213	d18:1-N18:0 ST	$[M-H]^-$
808.5971	16	176	d18:0-N18:0 ST	$[M-H]^-$
822.5873	48	522	d18:1-OHN18:0ST	$[M-H]^-$
834.632	9	95	d18:1-N20:0 ST	$[M-H]^-$
836.5995	4	42	d18:0-N20:0ST	$[M-H]^-$
848.6072	3	35	d18:1-OHN20:1ST	$[M-H]^-$
850.6055	20	207	d18:1-OHN20:0ST	$[M-H]^-$
852.6201	6	66	d18:0-OHN20:0ST	$[M-H]^-$
860.6453	35	343	d18:1-N22:1ST	$[M-H]^-$
862.6558	100	985	d18:1-N22:0ST	$[M-H]^-$
864.6436	27	270	d18:0-N22:0ST	$[M-H]^-$
874.6486	9	87	d18:1-OHN22:2ST	$[M-H]^-$
876.6527	57	542	d18:1-N23:0ST d18:1-OHN22:1ST	$[M-H]^-$
878.6438	268	2533	d18:1-OHN22:0ST	$[M-H]^-$
880.6578	61	575	d18:0-OHN22:0ST	$[M-H]^-$
886.6426	39	374	d18:1-N24:2ST	$[M-H]^-$
888.6592	946	8549	d18:1-N24:1ST	$[M-H]^-$
890.6799	472	4266	d18:1-N24:0ST d18:1-OHN23:1ST	$[M-H]^-$
892.6876	136	1277	d18:0-N24:0ST d18:1-OHN23:0ST	$[M-H]^-$
902.6731	17	152	d18:1-N25:1ST d18:1-OHN24:2ST	$[M-H]^-$
904.6776	235	2022	d18:1-N25:0ST d18:1-OHN24:1ST	$[M-H]^-$
906.6881	315	2711	d18:1-OHN24:0ST	$[M-H]^-$
908.7077	50	428	d18:0-OHN24:0ST	$[M-H]^-$
916.7317	6	52	d18:1-N26:1ST	$[M-H]^-$
918.7151	5	46	d18:1-N26:0ST d18:1-OHN25:1ST	$[M-H]^-$
920.6988	5	39	d18:0-N26:0ST d18:1-OHN25:0ST	$[M-H]^-$

Table S3. Phospholipids (*m/z* 600-800) assigned in *E.coli* with h-BN as matrix.

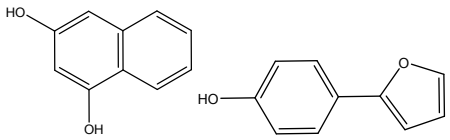
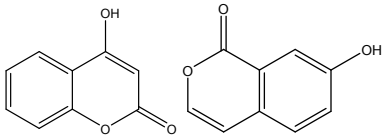
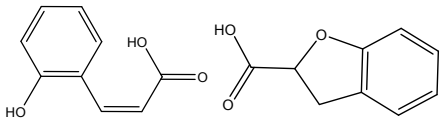
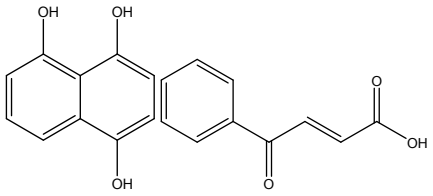
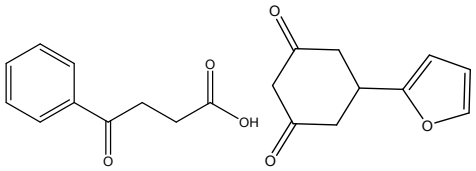
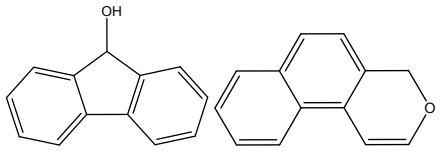
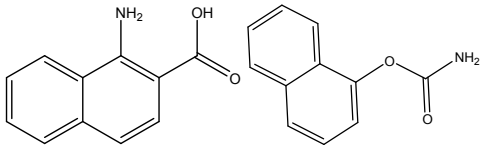
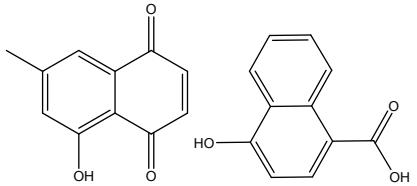
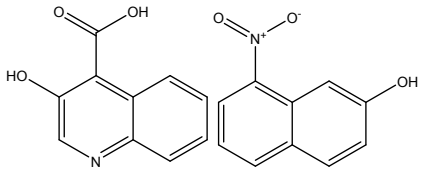
m/z	S/N	intensity	Molecular species	ion
634.612	4	296	Phosphatidylethanolamine(28:0)	$[M-H]^-$
648.619	4	253	Phosphatidylethanolamine(29:0)	$[M-H]^-$
662.662	19	1164	Phosphatidylethanolamine(30:0)	$[M-H]^-$
674.658	17	1043	Phosphatidylethanolamine(31:1)	$[M-H]^-$
676.679	6	375	Phosphatidylethanolamine(31:0)	$[M-H]^-$
688.676	61	3637	Phosphatidylethanolamine(32:1)	$[M-H]^-$
690.697	10	645	Phosphatidylethanolamine(32:0)	$[M-H]^-$
691.651	6	394	Phosphatidylethanolamine(30:1)	$[M-H]^-$
700.693	5	330	Phosphatidylethanolamine(33:2)	$[M-H]^-$
702.71	134	7966	Phosphatidylethanolamine(33:1)	$[M-H]^-$
705.687	11	668	Phosphatidylethanolamine(31:1)	$[M-H]^-$
707.689	18	1079	Phosphatidylethanolamine(31:0)	$[M-H]^-$
714.712	17	1029	Phosphatidylethanolamine(34:2)	$[M-H]^-$
719.697	83	4902	Phosphatidylglycerol(32:1)	$[M-H]^-$
721.713	28	1684	Phosphatidylglycerol(32:0)	$[M-H]^-$
728.741	13	798	Phosphatidylethanolamine(35:2)	$[M-H]^-$
730.751	14	864	Phosphatidylethanolamine(35:1)	$[M-H]^-$
733.717	96	5555	Phosphatidylglycerol(33:1)	$[M-H]^-$
742.761	6	409	Phosphatidylethanolamine(36:2)	$[M-H]^-$
745.726	8	487	Phosphatidylglycerol(34:2)	$[M-H]^-$
747.734	90	5149	Phosphatidylglycerol(34:1)	$[M-H]^-$
759.714	4	291	Phosphatidylglycerol(35:2)	$[M-H]^-$
760.687	4	282	Phosphatidylethanolamine(P-36:4/20:4)	$[M-H]^-$
761.754	17	996	Phosphatidylglycerol(35:1)	$[M-H]^-$
773.735	8	477	Phosphatidylglycerol(36:2)	$[M-H]^-$

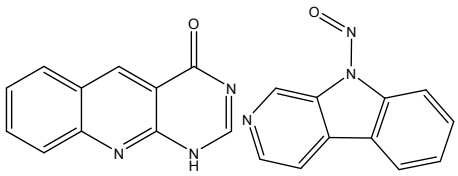
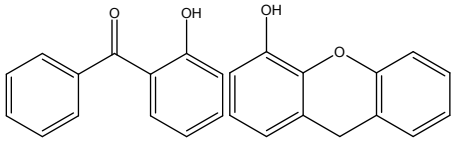
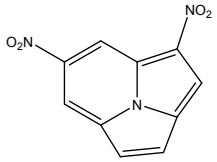
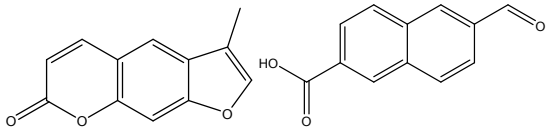
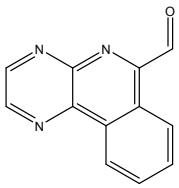
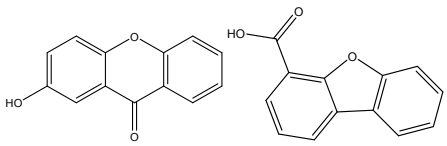
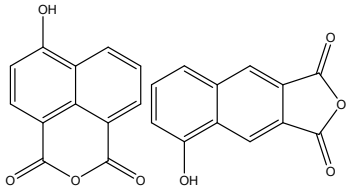
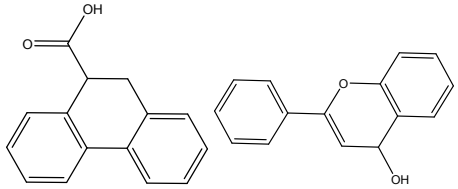
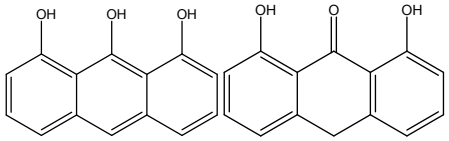
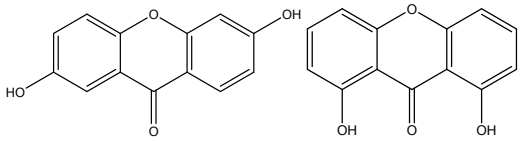
Table S4. Identification of some typical singly charged ions with relatively high intensity and high signal to noise value.

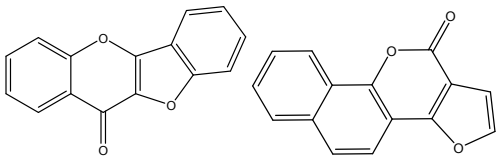
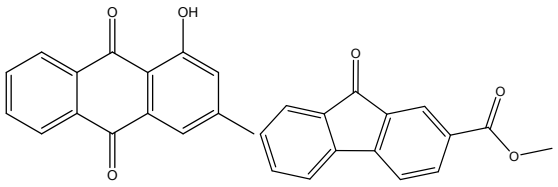
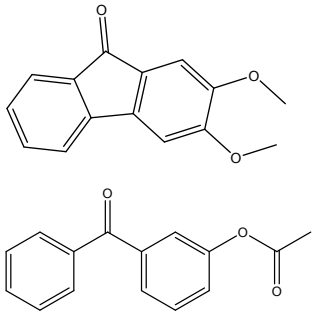
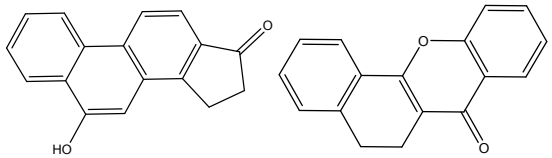
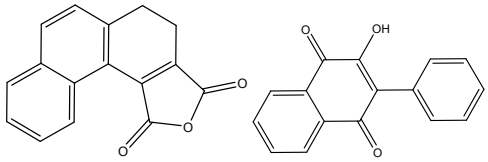
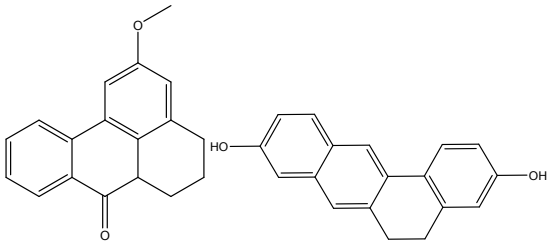
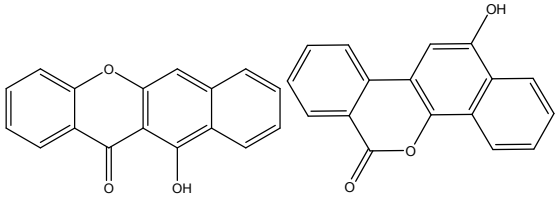
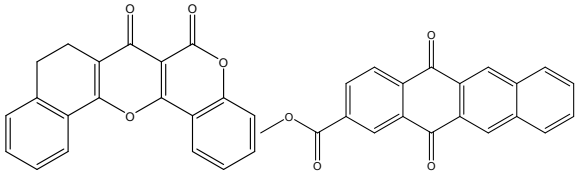
m/z	formula	DBE	Proposed structure
167.085	$C_{12}H_8O$	9	
168.099	$C_{11}H_8NO$	9	
183.077	$C_{12}H_8O_2$	9	
193.135	$C_{14}H_9NO_2$	11	
195.124	$C_{13}H_8O_2$	10	
209.144	$C_{14}H_{10}O_2$	10	
217.151	$C_{16}H_{10}O$ $C_{16}H_9NO_2$	12	
255.331	$C_{16}H_{32}O_2$	1	
267.178	$C_{20}H_{12}O$	15	

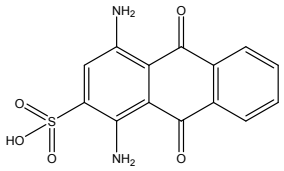
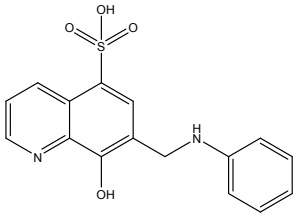
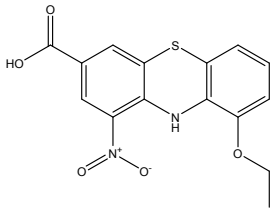
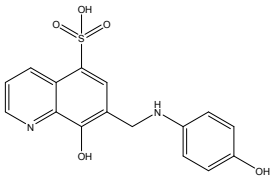
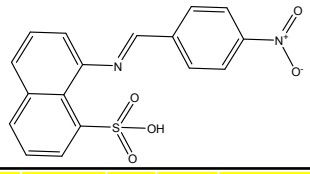
Most of the peaks are of $[M-H]^-$ ions. There are also $[M-NO]^-$ ions for $C_{14}H_9NO_2$ at *m/z* 193.135 and $C_{16}H_9NO_2$ for *m/z* 217.151. DBE: ring and double bond value.

Table S5. Assignment of singly charged ions with high intensity and high signal to noise value.

m/z	formula	DBE	Proposed structure
159.111	$C_{10}H_8O_2$	7	
161.091	$C_9H_6O_3$	7	
163.092	$C_9H_8O_3$	6	
175.109	$C_{10}H_8O_3$	7	
177.096	$C_{10}H_{10}O_3$	6	
181.108	$C_{13}H_{10}O$	9	
186.123	$C_{11}H_9NO_2$	8	
187.116	$C_{11}H_8O_3$	8	
188.122	$C_{10}H_7NO_3$	8	

196.104	$C_{11}H_7N_3O$	10	
197.095	$C_{13}H_{10}O_2$	9	
198.106	$C_{10}H_5N_3O_4$	10	
199.121	$C_{12}H_8O_3$	9	
208.134	$C_{12}H_7N_3O$	11	
211.123	$C_{13}H_8O_3$	10	
213.101	$C_{12}H_6O_4$	10	
223.146	$C_{15}H_{12}O_2$	10	
225.144	$C_{14}H_{10}O_3$	10	
227.122	$C_{13}H_8O_4$	10	

235.131	$C_{15}H_8O_3$	12	
237.143	$C_{15}H_{10}O_3$	11	
239.159	$C_{15}H_{12}O_3$	10	
247.158	$C_{17}H_{12}O_2$	12	
249.149	$C_{16}H_{10}O_3$	12	
259.173	$C_{18}H_{12}O_2$	13	
261.152	$C_{17}H_{10}O_3$	13	
315.159	$C_{20}H_{12}O_4$	15	

317.137	$C_{14}H_{10}N_2O_5S$	11	
329.177	$C_{16}H_{14}N_2O_4S$	11	
331.150	$C_{15}H_{12}N_2O_5S$	11	
345.168	$C_{16}H_{14}N_2O_5S$	11	
359.188	$C_{17}H_{16}N_2O_5S$	11	

Most of the peaks are of $[M-H]^-$ ions. There are also $[M-NO]^-$ ions for $C_{14}H_9NO_2$ at *m/z* 193.135 and $C_{16}H_9NO_2$ for *m/z* 217.151. DBE: ring and double bond value.