Supporting Information

Direct analysis of urinary 1-hydroxypyrene by using extractive electrospray ionization ion trap tandem mass spectrometry

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1. Schematic diagram of the homemade EESI source.

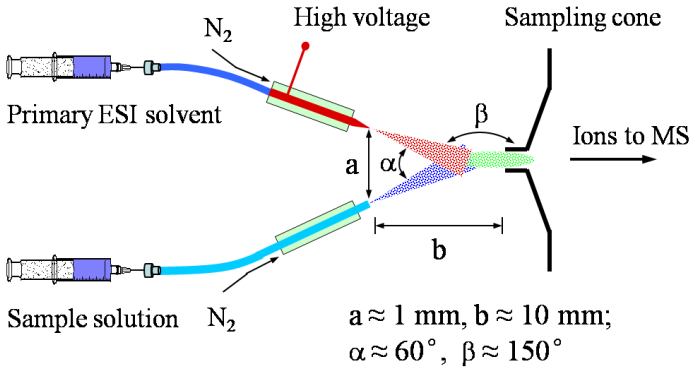
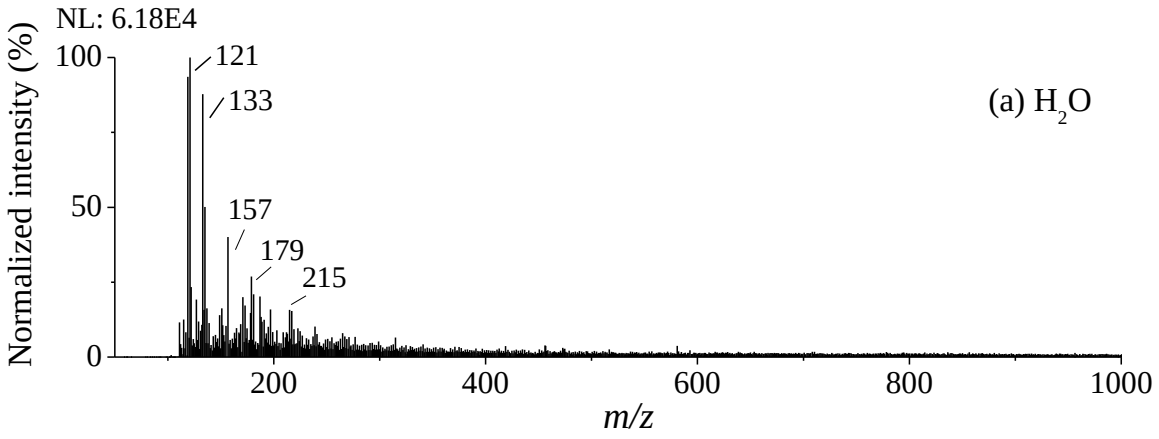
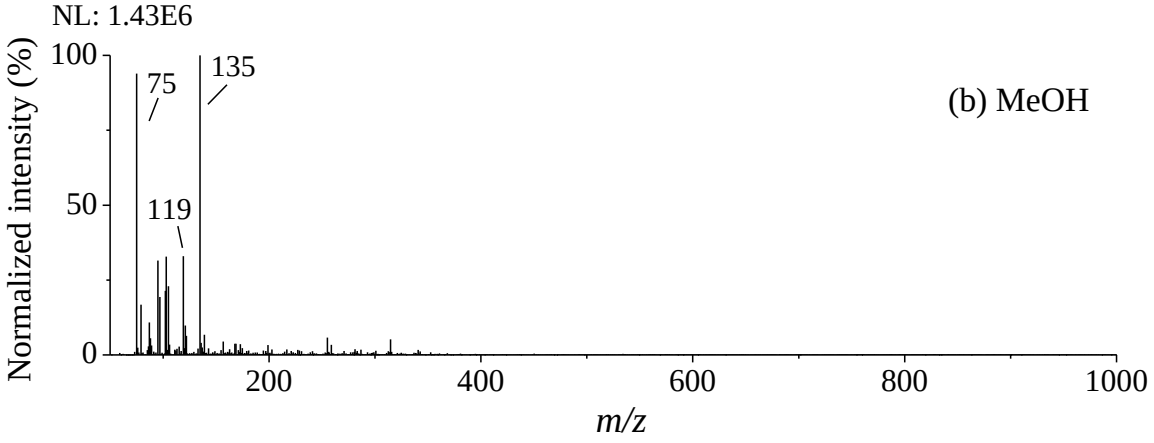
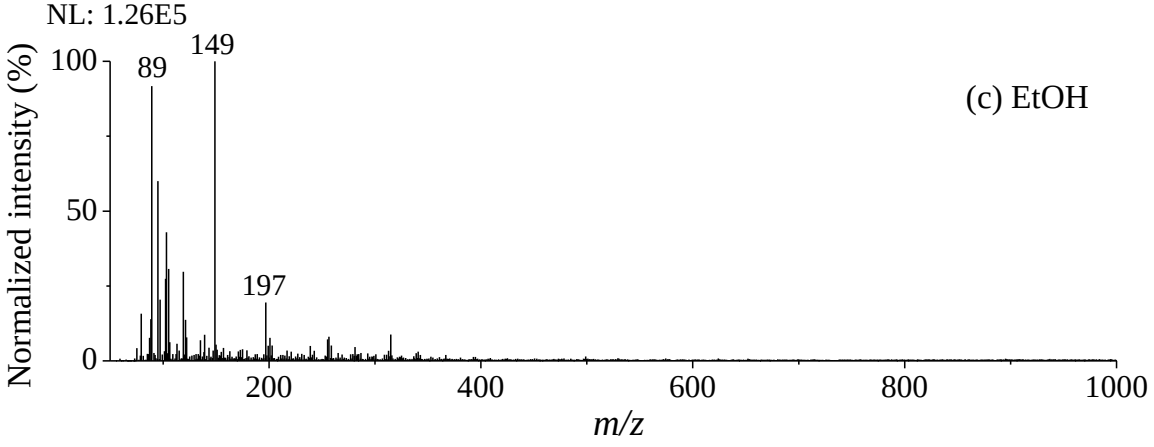


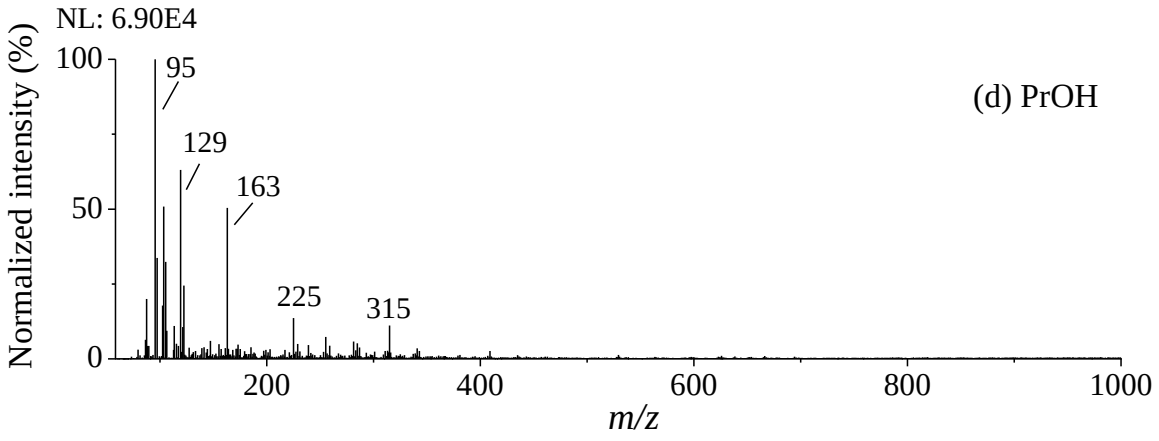
Fig. S1 Schematic diagram of the homemade EESI source.

2. EESI-MS fingerprints of the urine sample containing 0.23 μM of 1-OHP.









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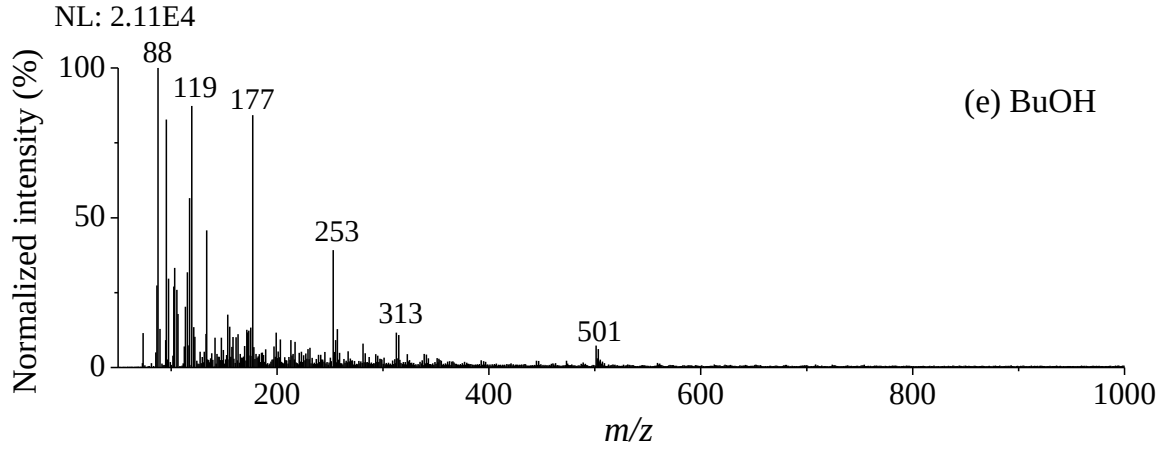

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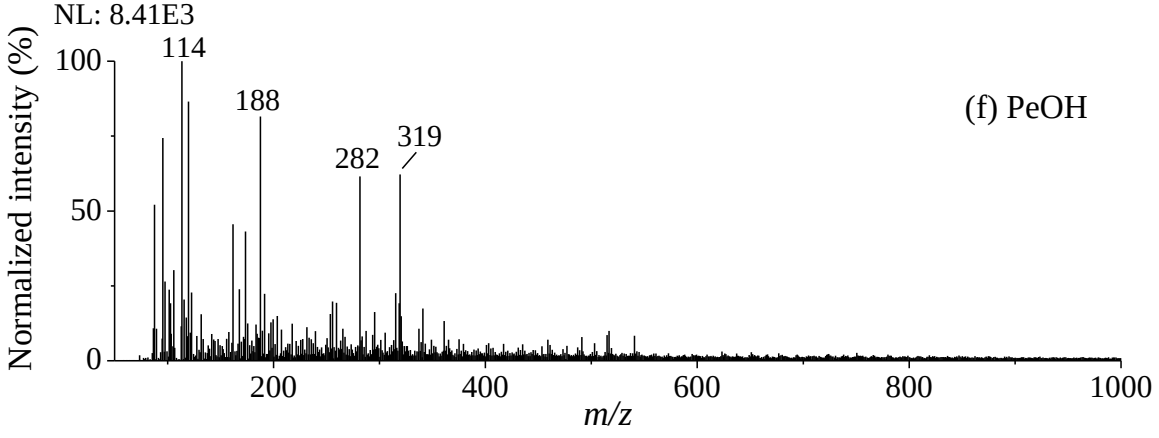

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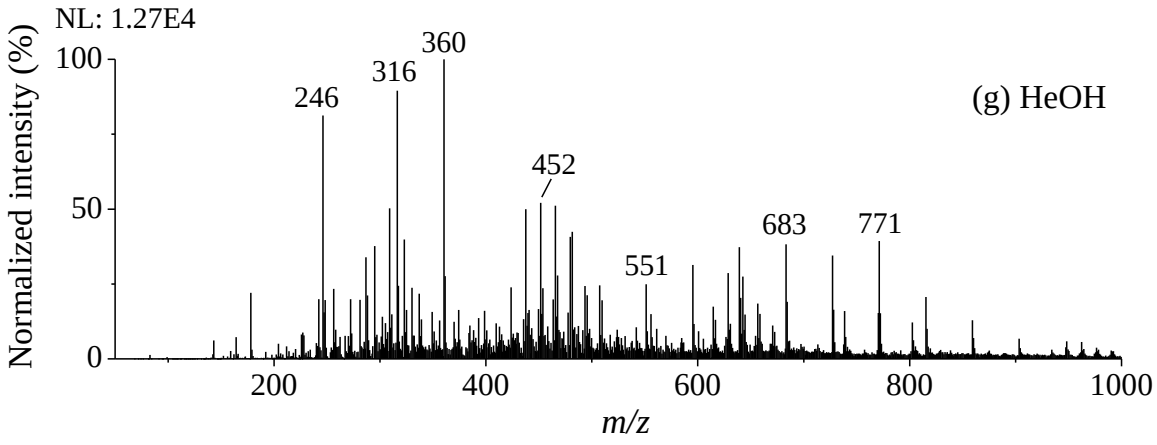

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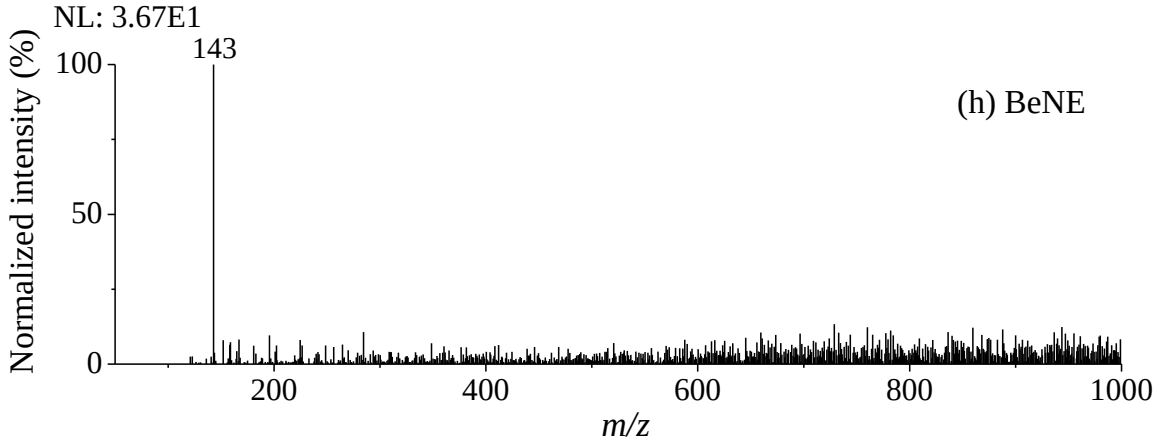

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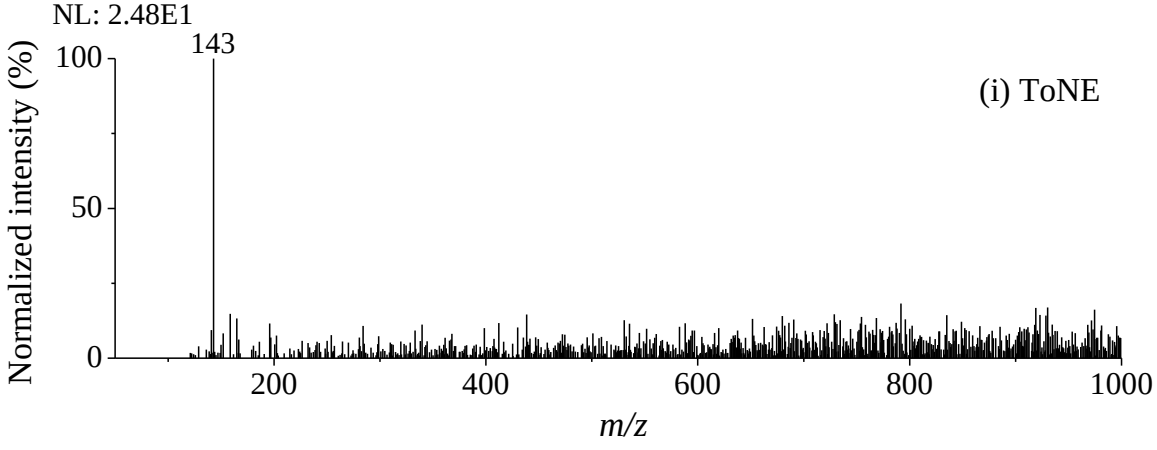

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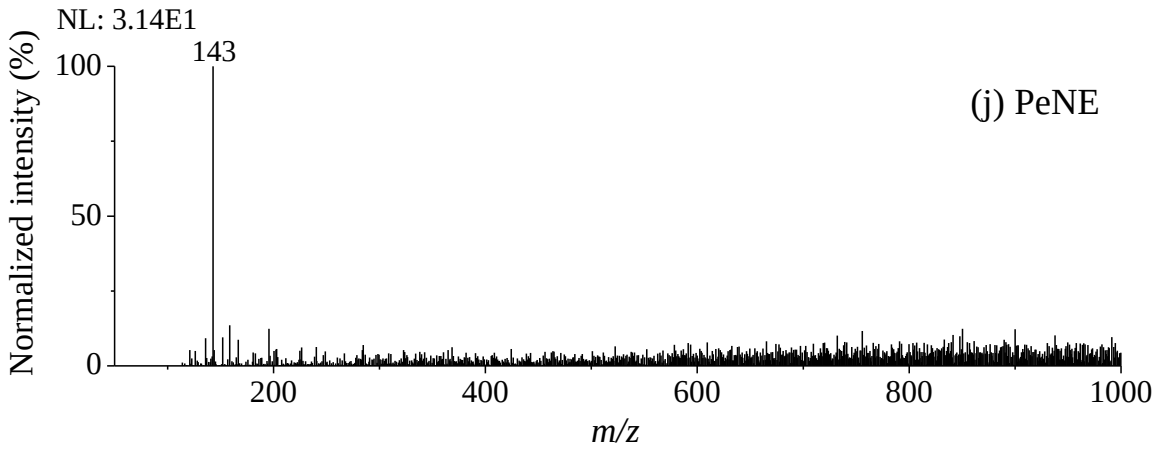


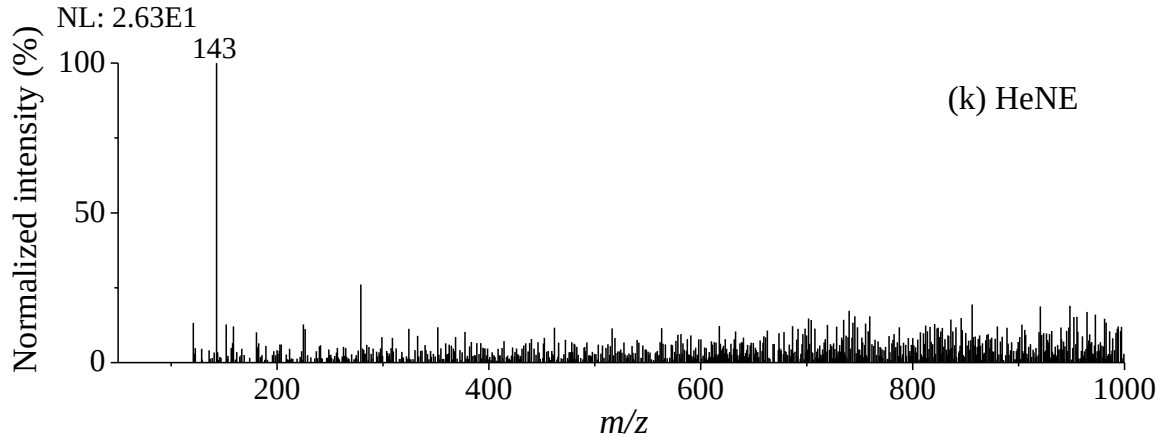
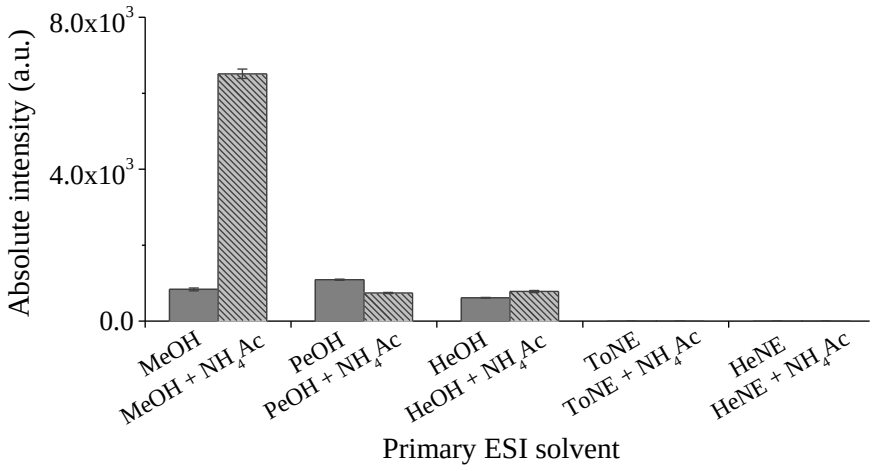


Fig. S2 EESI-MS fingerprints of the urine sample spiked with 2.29 μM of 1-OHP when eleven solvents of different polarities were used as primary ESI solvent, respectively, including (a) water, (b) MeOH, (c) EtOH, (d) PrOH, (e) BuOH, (f) PeOH, (g) HeOH, (h) BeNE, (i) ToNE, (j) PeNE and (k) HeNE.

46 **3. Effect of NH_4Ac in primary ESI solvent on 1-OHP ionization efficiency.**



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48 **Fig. S3** Signal intensities obtained by EESI-MS/MS when five solvents of different

49 polarities containing 1.0×10^{-4} M of NH_4Ac were applied as primary ESI solvent,

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63 **4. Optimization of ESI voltage, primary ESI solvent flow rate, sample flow rate and**

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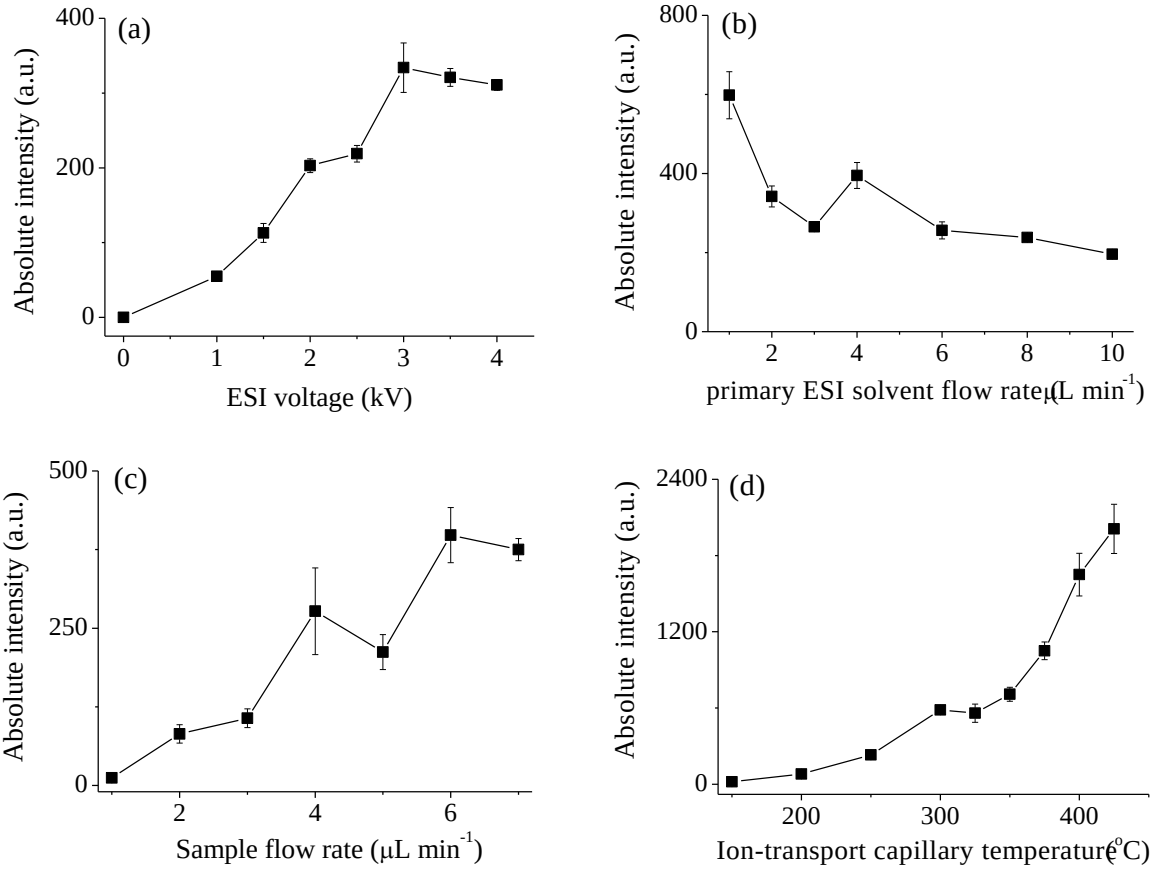

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67 **Fig. S4** Signal intensity variation with (a) ESI voltage, (b) primary ESI solvent flow rate, (c)

68 sample flow rate and (d) ion-transport capillary temperature.

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5. Signal intensity variation with 1-OHP concentration in ultrapure water.

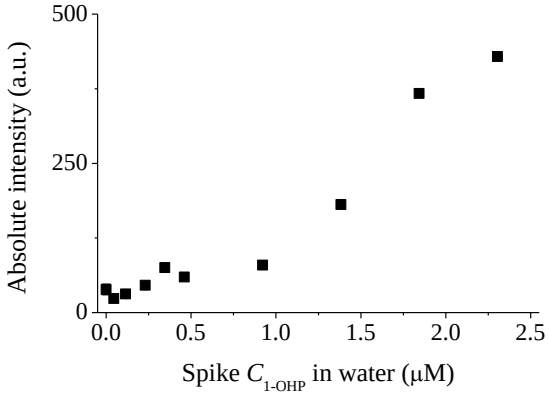

Fig. S5 Signal intensity variation with 1-OHP concentration ($C_{1\text{-OHP}}$) in ultrapure water; inset: linear relationship between signal intensity and $C_{1\text{-OHP}}$ ranging from 0.92–2.29 μM. The error bars are the SD of the mean value obtained from six independent measurements.

6. Standard addition curves used for quantifying 1-OHP in urine and hydrolyzed urine samples.

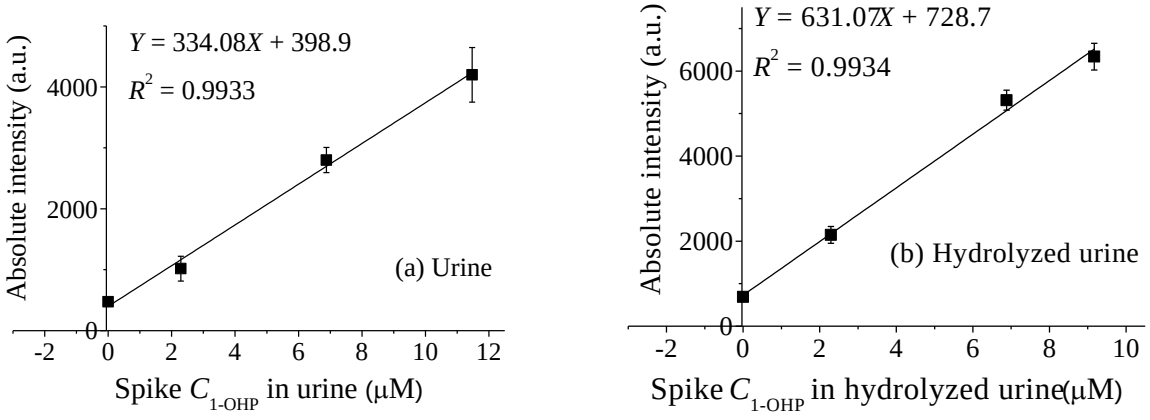

Fig. S6 Standard addition curves used for quantifying 1-OHP in (a) urine and (b) hydrolyzed urine samples containing 2.29 μM of 1-OHP.

6. Preparation of spiked samples

Table S1 Preparation of spiked urine samples.

Spiked (Spd) Level	$C_{1\text{-OHP}}$ (μM)	Preparation
Spd-1	45.83	100 μL of 1-OHP stock solution + 900 μL of urine
Spd-2	36.66	80 μL of 1-OHP stock solution + 920 μL of urine
Spd-3	27.49	60 μL of 1-OHP stock solution + 940 μL of urine
Spd-4	22.91	50 μL of 1-OHP stock solution + 950 μL of urine
Spd-5	18.33	40 μL of 1-OHP stock solution + 960 μL of urine
Spd-6	9.17	20 μL of 1-OHP stock solution + 980 μL of urine
Spd-7	4.58	10 μL of 1-OHP stock solution + 990 μL of urine
Std-8	3.44	7.5 μL of 1-OHP stock solution + 992.5 μL of urine
Std-9	2.29	5 μL of 1-OHP stock solution + 995 μL of urine

Table S2 Preparation of spiked hydrolyzed urine samples.

Spd Level	$C_{1\text{-OHP}}$ (μM)	Preparation
Spd-1	45.83	100 μL of 1-OHP stock solution + 900 μL of hydrolyzed urine
Spd-2	34.40	75 μL of 1-OHP stock solution + 925 μL of hydrolyzed urine
Spd-3	22.91	50 μL of 1-OHP stock solution + 950 μL of hydrolyzed urine
Spd-4	11.47	25 μL of 1-OHP stock solution + 975 μL of hydrolyzed urine
Spd-5	4.58	100 μL of Spd-1 + 900 μL of hydrolyzed urine
Std-6	3.44	100 μL of Spd-2 + 900 μL of hydrolyzed urine
Std-7	2.29	100 μL of Spd-3 + 900 μL of hydrolyzed urine
Std-8	1.15	100 μL of Spd-4 + 900 μL of hydrolyzed urine
Std-9	0.46	100 μL of Spd-5 + 900 μL of hydrolyzed urine
Std-10	0.23	100 μL of Spd-7 + 900 μL of hydrolyzed urine
Std-11	0.05	100 μL of Spd-9 + 900 μL of hydrolyzed urine

Table S3 Preparation of spiked ultrapure water samples.

Spd Level	$C_{1\text{-OHP}}$ (μM)	Preparation
Spd-1	2.29	5 μL of 1-OHP stock solution + 995 μL of ultrapure water
Spd-2	1.83	800 μL of Spd-1 + 200 μL of ultrapure water
Spd-3	1.38	600 μL of Spd-1 + 400 μL of ultrapure water
Spd-4	0.92	400 μL of Spd-1 + 600 μL of ultrapure water
Spd-5	0.46	200 μL of Spd-1 + 800 μL of ultrapure water
Std-6	0.34	150 μL of Spd-1 + 850 μL of ultrapure water
Std-7	0.23	100 μL of Spd-1 + 900 μL of ultrapure water
Std-8	0.11	50 μL of Spd-1 + 950 μL of ultrapure water
Std-9	0.05	100 μL of Spd-5 + 900 μL of ultrapure water

126 **Table S4** Preparation of spiked solutions [a] used for matrix effect studies.

Spd Level	$C_{1\text{-OHP}}$ (μM)	Preparation
Spd-1	11.45	25 μL of 1-OHP stock solution + 975 μL of each solution
Spd-2	2.29	200 μL of Spd-1 + 800 μL of each solution

127 [a] Solutions used for studying matrix effect include 100% hydrolyzed urine, hydrolyzed
128 urine: water (75:25, V/V), hydrolyzed urine: water (50:50, V/V) and 100% water.
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7. RSDs (n = 6) of working curves for urine, hydrolyzed urine and ultrapure water samples.

Table S5 RSDs (n = 6) of working curves for urine, hydrolyzed urine and ultrapure water samples.

Urine		Hydrolyzed urine		Ultrapure water	
$C_{1\text{-OHP}}$ (μM)	RSD (%)	$C_{1\text{-OHP}}$ (μM)	RSD (%)	$C_{1\text{-OHP}}$ (μM)	RSD (%)
0 (Blank)	/ [a]	0 (Blank)	3.1	0 (Blank)	22.9
2.29	6.2	0.05	11.5	0.05	11.2
3.44	4.0	0.23	10.4	0.11	12.9
4.58	2.6	0.46	4.2	0.23	12.4
9.17	9.7	1.15	3.5	0.34	9.5
18.33	3.9	2.29	1.7	0.46	7.2
22.91	3.0	3.44	1.5	0.92	6.0
27.49	8.0	4.58	2.3	1.38	7.2
36.66	7.0	11.47	2.2	1.83	7.7
45.83	3.3	22.91	6.4	2.29	5.5

[a] RSD cannot be calculated, for signal intensities of six measurements were all zero.

8. RSDs (n = 6) of the standard addition curves for quantifying 1-OHP in urine and hydrolyzed urine samples.

Table S6 RSDs (%) (n = 6) of standard addition curves for quantifying 1-OHP in urine and hydrolyzed urine samples (**Fig. S5**).

Urine		Hydrolyzed urine	
$C_{1\text{-OHP}}$ [a] (μM)	RSD (%)	$C_{1\text{-OHP}}$ [b] (μM)	RSD (%)
0	5.8	0	9.0
2.29	19.9	2.29	9.2
6.88	7.3	6.88	4.4
11.47	10.7	9.17	4.9

[a] Spike concentrations in urine; [a] spike concentrations in hydrolyzed urine.