Supporting information

Electrostatic Spray Ionization from 384-well Microtiter Plates for Mass Spectrometry Analysis based Enzyme Assay and Drug Metabolism Screening

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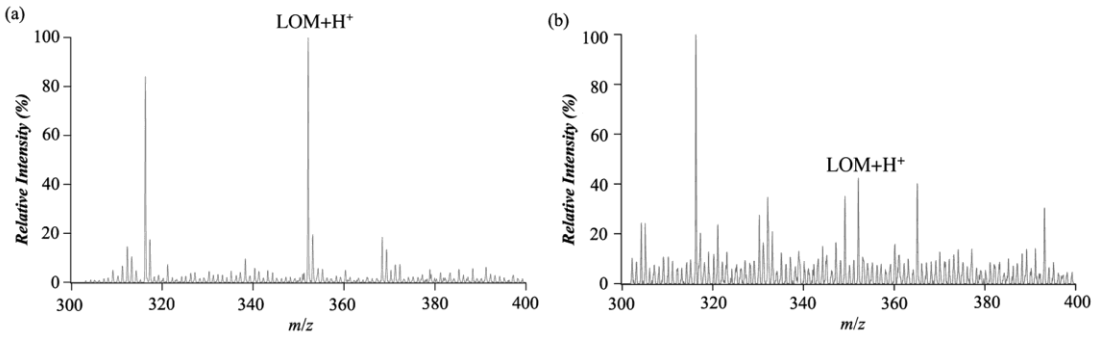

Figure SI-1: Mass spectra of (a) 1.4 μM and (b) 140 nM of LOM in 50% MeOH/49% H_2O/1% acetic acid by 384-well plate ESTASI-MS.

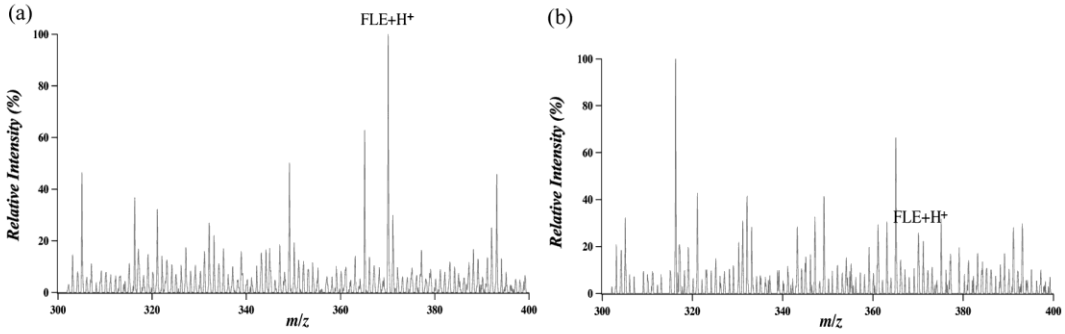

Figure SI-2: Mass spectra of (a) 1.4 μM and (b) 140 nM of FLE in 50% MeOH/49% H_2O/1% acetic acid by 384-well plate ESTASI-MS.

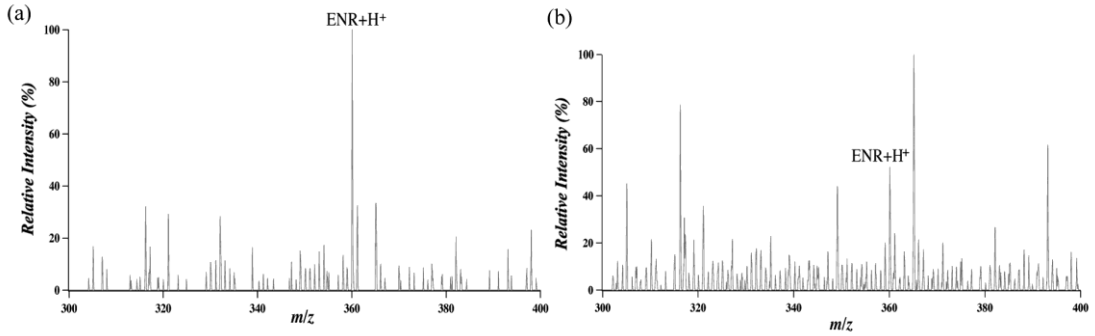

Figure SI-3: Mass spectra of (a) 1.4 μM and (b) 140 nM of ENR in 50% MeOH/49% H_2O/1% acetic acid by 384-well plate ESTASI-MS.

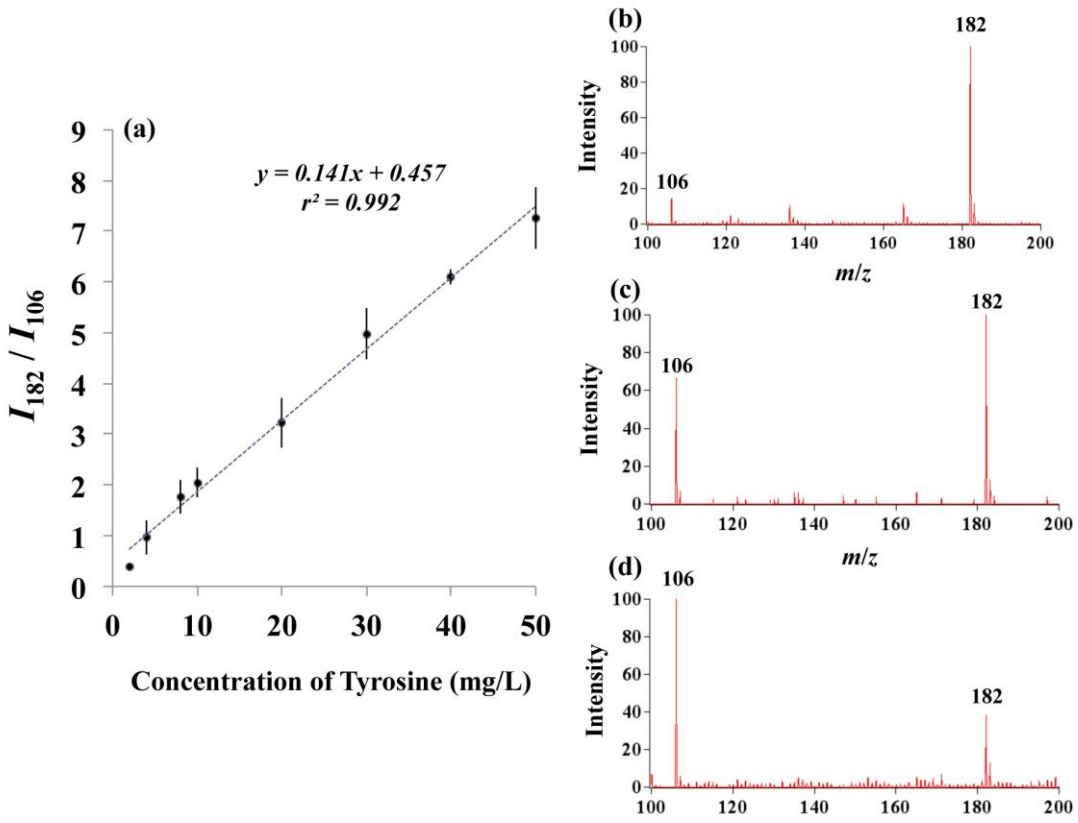

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Figure SI-4: (a) Liner curve fitted for tyrosine quantification with internal standard calibration method. I_{182}/I_{106} : relative ion intensities between tyrosine (I_{182}) and serine (I_{106}). (b), (c) and (d) Mass spectra for 50 mg/L, 8 mg/L and 2 mg/L of tyrosine, respectively. The analyses were performed with direct ESTASI-MS from a 384-well plate, each well containing 10 μL of analyte solution in 50% MeOH/49% H_2O/1% acetic acid. The internal standard of serine was always kept at 10 mg/L. Error bar shows standard deviation (n=3).

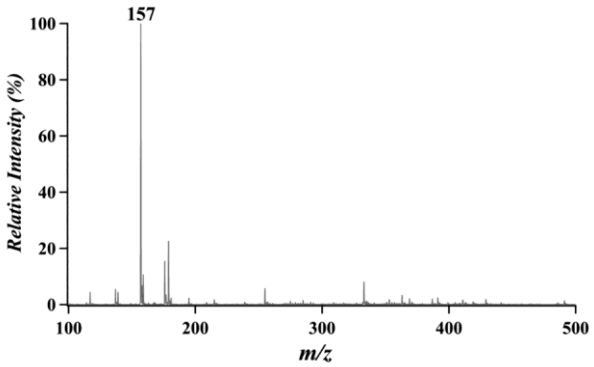

Figure SI-5: Mass spectrum of 2 mM cupferron in 50% MeOH/49% H_2O/1% acetic acid by direct infusion ESI-MS.

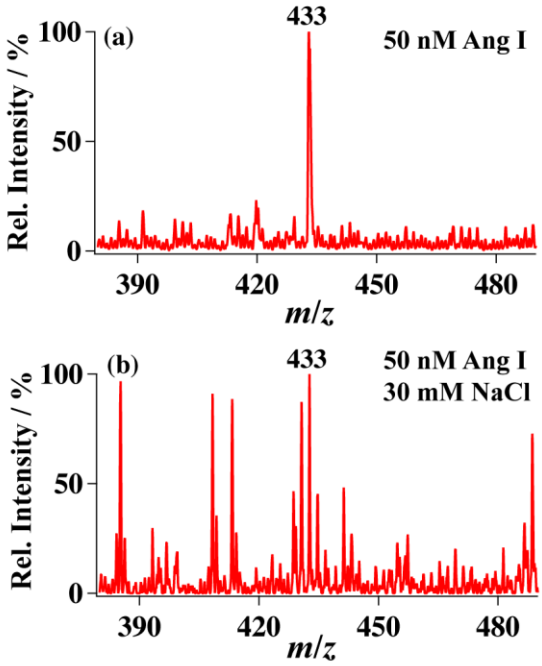

Figure SI-6: The test of ESTASI tolerance to salt by analysing 10 μL 50 nM Ang I in the wells of a 384-well plate. The buffer of 50% methanol, 49% H_2O and 1% acetic acid contained (a) 0 or (b) 30 mM NaCl.

Calculation of half maximal inhibitory concentration (IC_{50}).

IC_{50} could be deduced from the Cheng-Prusoff equation ($IC_{50} = K_i + K_i[S]/K_m$) with the Michaelis constant of K_m, inhibition constant of K_i and the substrate concentration [S]. The K_m of tyrosinase for tyrosine is 0.5 mM,[1] the concentration of tyrosine [S] in our case was 0.3 mM, and K_i is estimated around 0.4 mM from the fitted curve for the equation of $K_{apparent} = K_m (1 + [I]/K_i)$ reported in a literature,[2] where K_m/K_i was determined as 1.25. Thus, the IC_{50} is calculated as ~0.5 mM when using 0.3 mM of tyrosine.

References:

(1) Espin, J. C.; Jolivet, S.; Wichers, H. J. *J. Agric. Food Chem.* **1999**, *47*, 3495-3502.
(2) Xie, L. P.; Chen, Q. X.; Huang, H. A.; Liu, X. D.; Chen, H. T.; Zhang, R. Q. *Int. J. Biochem. Cell Biol.* **2003**, *35*, 1658-1666.