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% H₂O/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% H₂O/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% H₂O/1% acetic acid by 384-well plate ESTASI-MS.
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 $\mu$L of analyte solution in 50% MeOH/49% H$_2$O/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$_2$O/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% H₂O and 1% acetic acid contained (a) 0 or (b) 30 mM NaCl.

**Calculation of half maximal inhibitory concentration (IC₅₀).**

IC₅₀ could be deduced from the Cheng-Prusoff equation (IC₅₀ = Kᵢ + K[S]/Kₘ) with the Michaelis constant of Kₘ, inhibition constant of Kᵢ and the substrate concentration [S]. The Kₘ of tyrosinase for tyrosine is 0.5 mM,¹ the concentration of tyrosine [S] in our case was 0.3 mM, and Kᵢ is estimated around 0.4 mM from the fitted curve for the equation of Kₐₚ₉₄ent = Kₘ (1 + [I]/Kᵢ) reported in a literature,² where Kₘ/Kᵢ was determined as 1.25. Thus, the IC₅₀ is calculated as ~0.5 mM when using 0.3 mM of tyrosine.

**References:**