Supplementary Information:

Ambient in-situ analysis and imaging of both hydrophilic and hydrophobic thin layer chromatography plates by electrostatic spray ionization mass spectrometry

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S1. Molecular structures of the used six dyes and six drugs

**Dye molecular structures:**

- (methylene blue - Cl)$^+$
  - $m/z$ 284
- (rhodamine B - Cl)$^+$
  - $m/z$ 443
- (rhodamine 6G - Cl)$^+$
  - $m/z$ 443
- (fluorescein + H)$^+$
  - $m/z$ 333
- (sudan III + H)$^+$
  - $m/z$ 353
- (crystal violet - Cl)$^+$
  - $m/z$ 372

**Drug molecular structures:**

- (aspirin + H)$^+$
  - $m/z$ 181
- (caffeine + H)$^+$
  - $m/z$ 195
- (4-Acetamidophenol + H)$^+$
  - $m/z$ 152
- (LOM + H)$^+$
  - $m/z$ 352
- (ENR + H)$^+$
  - $m/z$ 360
- (FLE + H)$^+$
  - $m/z$ 370

**Figure S1.** Molecular structures of the used six dyes and six drugs. LOM: lomefloxacin; ENR: enrofloxacin; FLE: fleroxacin.
S2. Mass spectra of six dyes from HPRP C18 plates

**Figure S2.** (a), (b), (c) and (d) individual ESTASI-MS spectra of methylene blue (1.7 nmole), rhodamine B (1.1 nmole), sudan III (1.4 nmole) and crystal violet (1.3 nmole), respectively. (e), (f) and (g) mass spectra obtained at $x=7.5$ mm and $y=3$ mm, $x=4.5$ mm and $y=4$ mm, $x=5.5$ mm and $y=7$ mm, respectively, of Figure 4. (Dye + H)$^+$: singly protonated dye; (Dye - Cl)$^+$: dechlorinated dye; (Ang I + 3H)$^{3+}$: triply protonated Ang I.
S3. Quantification of rhodamine 6G on HPRP C18 plates

Figure S3. Rel. IC chromatography of rhodamine 6G by TLC-ESTASI-MS. The sample spots were developed from \( \mu \)L of rhodamine 6G solutions with different concentrations: (a) 0.01 mg/mL, (b) 0.05 mg/mL, (c) 0.1 mg/mL, (d) 0.15 mg/mL and (e) 0.2 mg/mL. Rel. IC was calculated by dividing the IC of singly protonated rhodamine 6G (\( m/z \) 432.7 to \( m/z \) 433.8) by that of triply protonated Ang I (\( m/z \) 442.9 to \( m/z \) 443.7).
**S4. Recovery rate of rhodamine 6G from HPRP C18 plates by ESTASI-MS**

Two calibration curves were obtained for rhodamine 6G using angiotensin I (Ang I) as internal standard by ESTASI-MS. The concentration of Ang I was fixed at 3 μM, while the concentration of rhodamine 6G ranged from 0.01 μg mL\(^{-1}\) to 0.1 μg mL\(^{-1}\) and 0.1 μg mL\(^{-1}\) to 10 μg mL\(^{-1}\), for the two calibration curves, respectively. Rhodamine 6G solutions were mixed with Ang I in 1% acetic acid, 75% methanol and 24% water, and delivered by the wetting capillary at 1 μL min\(^{-1}\) to a HPRP C18 plate for ESTASI-MS analysis. The HPRP C18 plate was used only as the substrate of ESTASI-MS without any sample to mimic the condition of TLC-ESTASI-MS. The MS signal was recorded for 3 min. The ion current (IC) ratio between rhodamine 6G (\(m/z\) 432.7 to \(m/z\) 433.8) and Ang I (\(m/z\) 442.9 to \(m/z\) 443.7) was calculated, averaged and plotted as a function of the concentration of rhodamine 6G to obtain the two calibration curves, as shown in Figure S4(a) and S4(b). The error bar shows the standard deviation from three parallel tests.

3 μM Ang I in 1% acetic acid, 75% methanol and 24% water was delivered by the wetting capillary at 1 μL min\(^{-1}\) to extract sample continuously from the central position of a developed band of 1 μL, 10 μg mL\(^{-1}\) rhodamine 6G on a HPRP C18 plate for ESTASI analyses. The MS signal was recorded until the signal of rhodamine 6G became very weak (< 3 times of background level). As shown in Figure S4(c), the signal of rhodamine 6G from the fixed position can last for more than 2 mins, which indeed demonstrates that only a small amount of sample is consumed to generate one mass spectrum. Rel. IC chromatography of rhodamine 6G, in Figure S4(c), is the ration between rhodamine 6G and Ang I. With the two calibration curves in Figure S4(a) and S4(b), the corresponding concentration of rhodamine 6G extracted into the droplets formed at the end of the wetting capillary can be calculated for each time point on the Rel. IC chromatography to obtain the concentration chromatography of rhodamine 6G as shown in Figure S4(c). Considering the flow rate of 1 μL min\(^{-1}\), the integrated peak area of the concentration chromatography was calculated, which indicated the recovered amount of rhodamine 6G from the HPRP C18 plate.

The amount of rhodamine 6G at the sampling point could be calculated based on the sampling area (360 μm in diameter), the total area of sample band and the total loading amount of rhodamine 6G. With the recovered amount and the amount of rhodamine 6G at the sampling point, the mean recovery rate was measured as 78 ±
3.5% based on three parallel tests. Figure S4(c) and (d) show a representative result of the experiment.

Figure S4. (a) and (b) calibration curves of rhodamine 6G using Ang I (3 μM) as internal standard by ESTASI-MS on a HPRP C18 plate. Rel. IC of rhodamine 6G to Ang I is plotted as a function of rhodamine 6G concentration, ranging from 0.01 μg mL⁻¹ to 0.1 μg mL⁻¹ and 0.1 μg mL⁻¹ to 10 μg mL⁻¹, respectively. (c) Rel. IC chromatography of rhodamine 6G recovered from dried sample spot of 1 μL of 10 μg mL⁻¹ rhodamine 6G on a HPRP C18 plate. (d) Concentration chromatography of rhodamine 6G obtained from the Rel. IC chromatography with the two calibration curves. The ESTASI was performed with the extraction solvent of 1% acetic acid, 75% methanol and 24% water under the flow rate of 1 μL/min.
S5. TLC-ESTASI-MS SRM analysis of ENR

TLC-ESTASI-MS/MS was performed on precursor ions at \( m/z \) 360 by CID with normalized collision energy as 25 and isolation width as ± 1. The sample spot was 1 \( \mu \)L, 10 \( \mu \)g/mL ENR aqueous solution developed on a silica gel TLC plate followed with silane modification. Extraction solvent composed of 75% water, 24% methanol and 1% acetic acid was delivered at 1 \( \mu \)L/min for TLC-ESTASI-MS. Characteristic fragmentation pattern of ENR is shown as Figure S5 (b), including mainly two fragments at \( m/z \) 342 and \( m/z \) 316, respectively, corresponding the neutral loss of \( \text{H}_2\text{O} \) and \( \text{CO}_2 \). From a blank silica gel TLC plate with silane modification under the same instrumental parameter, no fragment was observed at \( m/z \) 316 (absolute intensity = 0) and a very weak signal was observed at \( m/z \) 342 (absolute intensity = 15) (Figure S5a). Therefore, fragment ion at \( m/z \) 316 was selected as the diagnostic ion of ENR for SRM analysis. Figure S5 (c) and (d) show the TLC-ESTASI-MS SRM spectra of sample spots from 1 \( \mu \)L, 5 \( \mu \)g/mL and 1 \( \mu \)g/mL ENR aqueous solution developed on a silica gel TLC plate with silane modification, respectively.

Figure S5. ESTASI-MS/MS full scan spectra of: (a) a blank silica gel TLC plate with silane modification and (b) 1 \( \mu \)L, 1 mg/mL ENR aqueous solution developed on a silica gel TLC plate followed with silane modification. (c) and (d) ESTASI-MS SRM spectra of sample spots from 1 \( \mu \)L, 5 \( \mu \)g/mL and 1 \( \mu \)g/mL ENR aqueous solution, respectively, developed on a silica gel TLC plate followed with silane modification, ESTASI-MS was performed with the extraction solvent of 75% water, 24% methanol and 1% acetic acid under 1 \( \mu \)L/min. Normalized collision energy of CID: 25; isolation width of precursor ion: ± 1; SRM range: \( m/z \) 316 ± 16.
S6. Mass spectra of six drugs from silane-modified silica plates.

Figure S6. (a), (b), (c) and (d) individual ESTASI-MS spectra of aspirin (2.7 nmole), caffeine (2.6 nmole), FLE (1.3 nmole) and LOM (1.4 nmole), respectively. (e), (f) and (g) mass spectra obtained at $x = 7$ mm, $x = 23$ mm and $x = 14$ mm, respectively, of Figure 8. (Drug + H)$^+$: singly protonated drug; (Ang I + 3H)$^{3+}$: triply protonated Ang I.