An on-tissue chemical derivatization method for MALDI-MSI analysis of anthraquinones in mouse tissues

Yong-An Yan [a,b,#], Si-Qi Han [a,b,#], Pian Jin [a,b], Xiao-Bo Zhao [a], Yan-Ping Shi [a*] Wei Ha [a*]

[a] CAS Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, PR China

[b] University of Chinese Academy of Sciences, Beijing 100049, P. R. China

* Corresponding Author. E-mail address:

shiyp@licp.cas.cn (Y.-P. Shi);

hawei2012@licp.cas.cn (W. Ha)

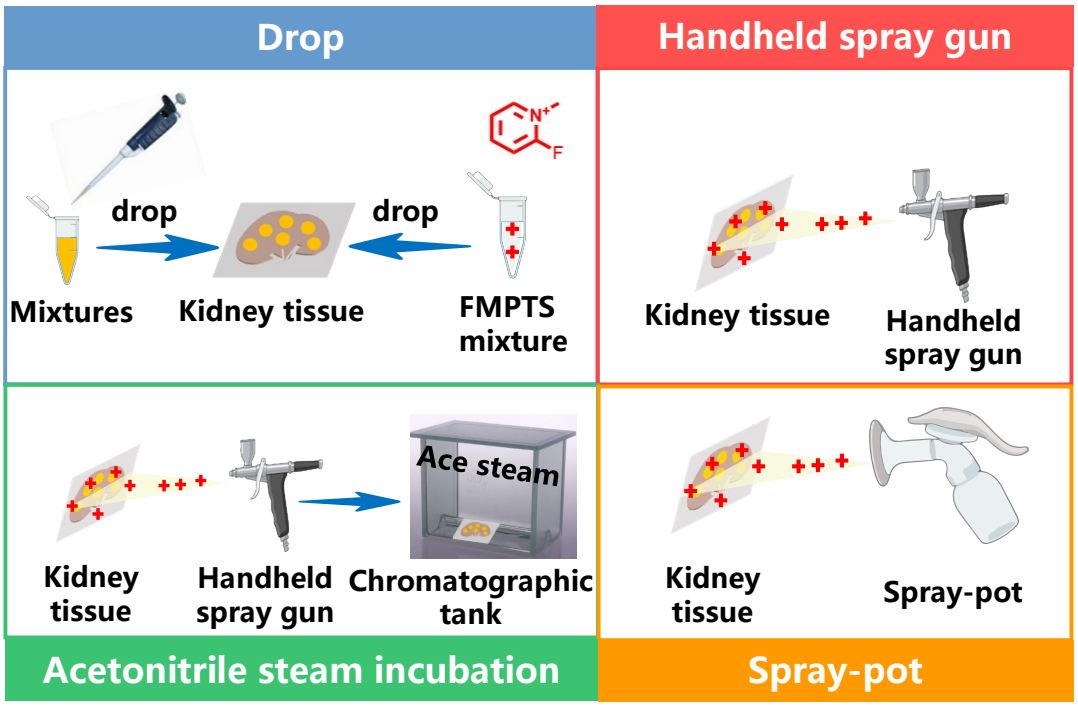

Fig. S1 Experimental schematics of the four derivatization reagent spraying modes.

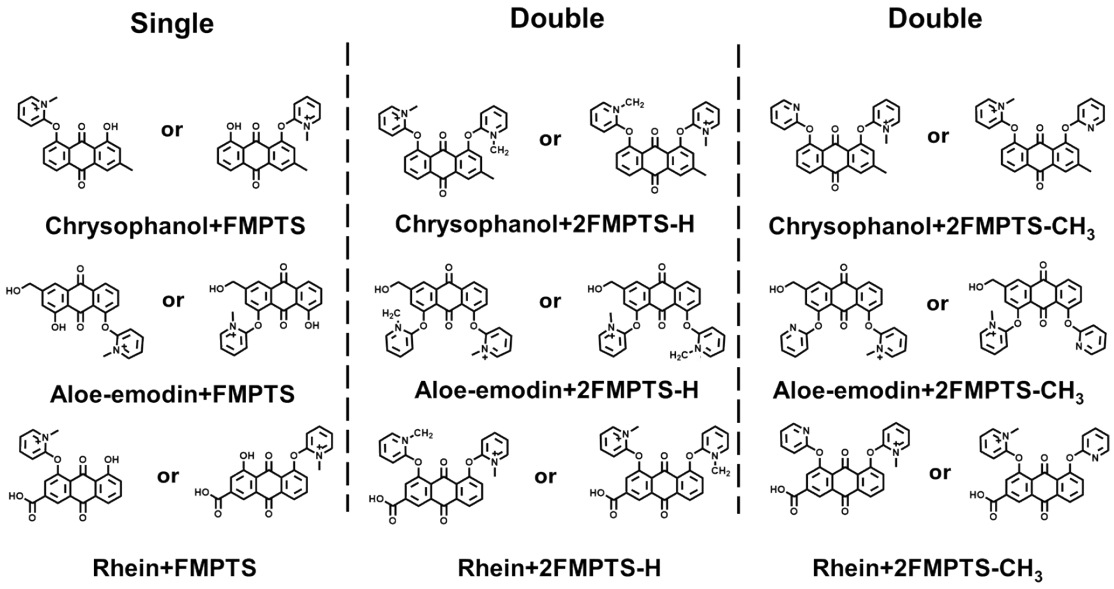

Fig. S2 Structures of $[M+FMPTS]^+$, $[M+2FMPTS-H]^+$, and $[M+2FMPTS-CH_3]^+$ derivatives of chrysophanol, aloe-emodin, rhein.

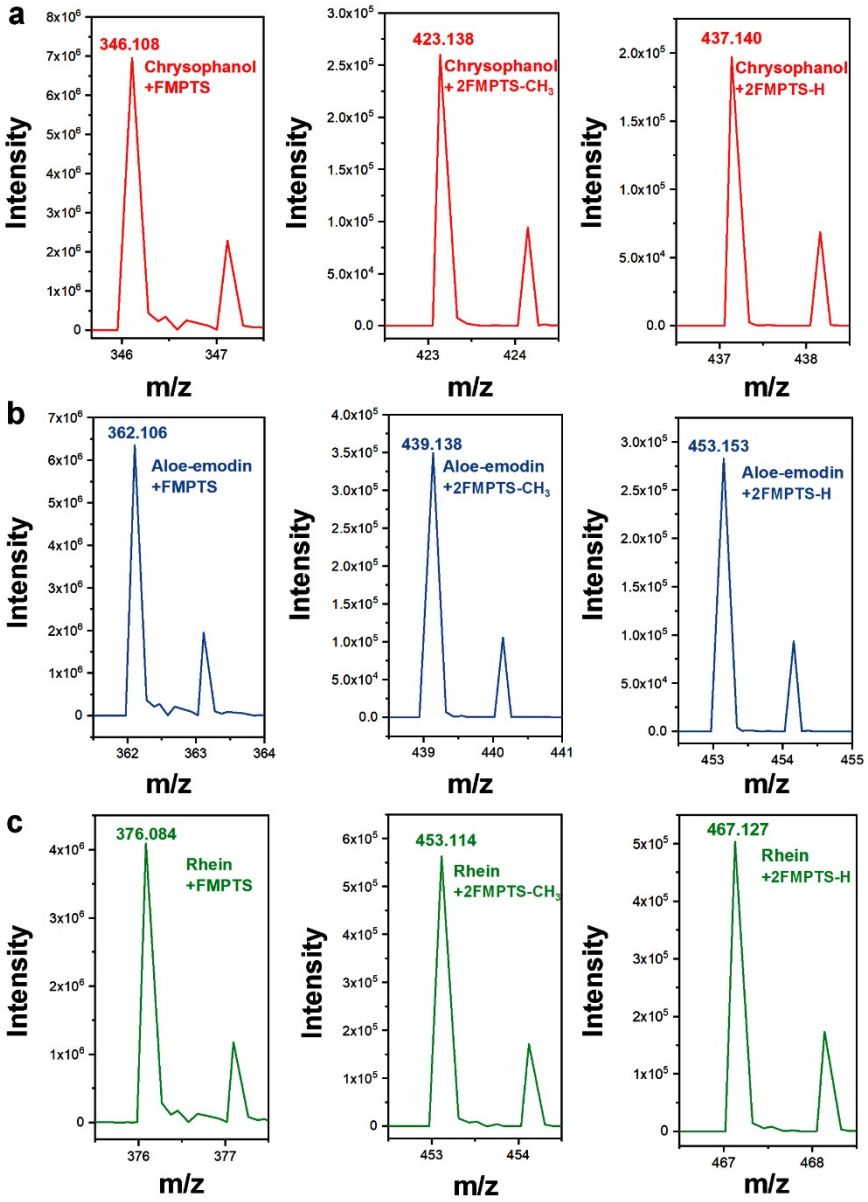

Fig. S3 MALDI-TOF-MS of $[M+FMPTS]^+$, $[M+2FMPTS-H]^+$, and $[M+2FMPTS-CH_3]^+$ derivatives of (a) chrysophanol, (b) aloe-emodin (c) rhein.

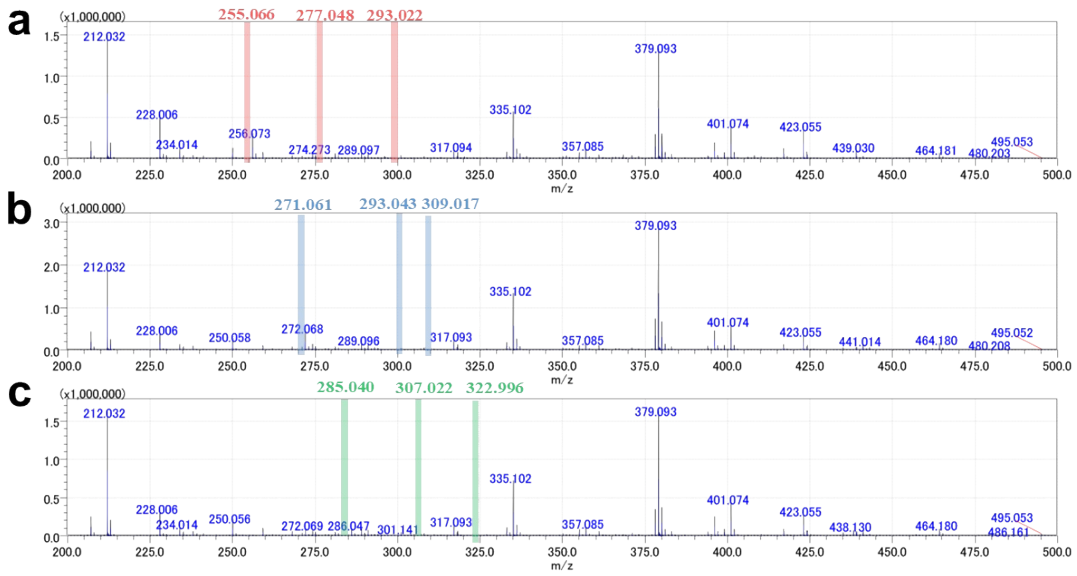

Fig. S4 MALDI-TOF-MS of (a) chrysophanol (b) aloe-emodin and (c) rhein in positive ion mode.

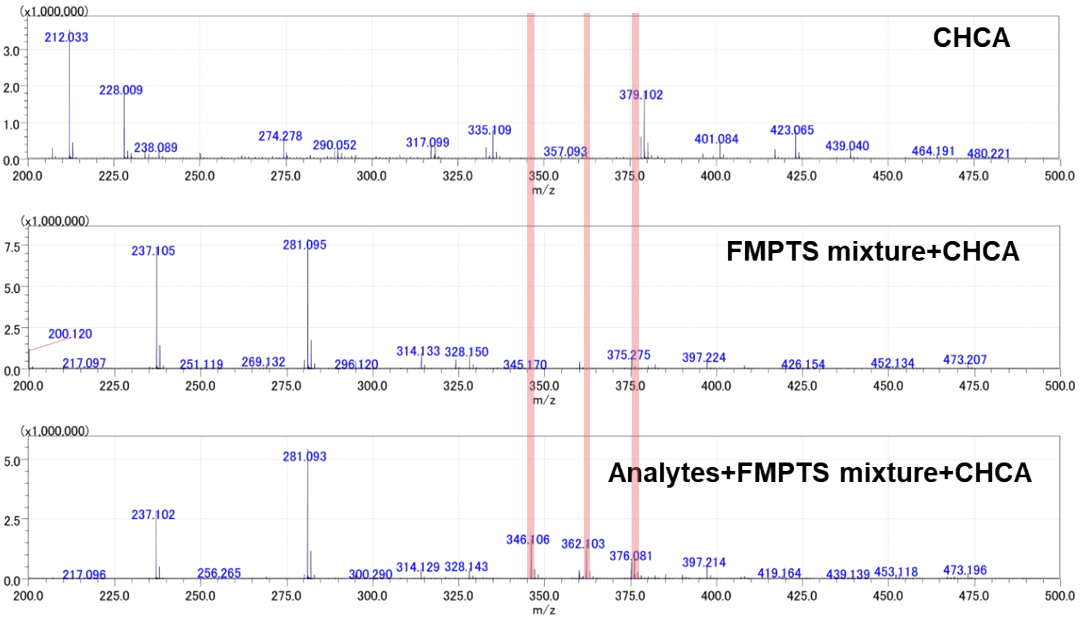

Fig. S5 MALD-TOF-MS of CHCA, FMPTS mixture and spot deposition of CHCA, derivatives of rhubarb anthraquinone mixture and spot deposition of CHCA (Chrysophanol derivatives were *m/z* 346.106, aloe-emodin derivatives were *m/z* 362.103, rhein derivatives were *m/z* 376.081).

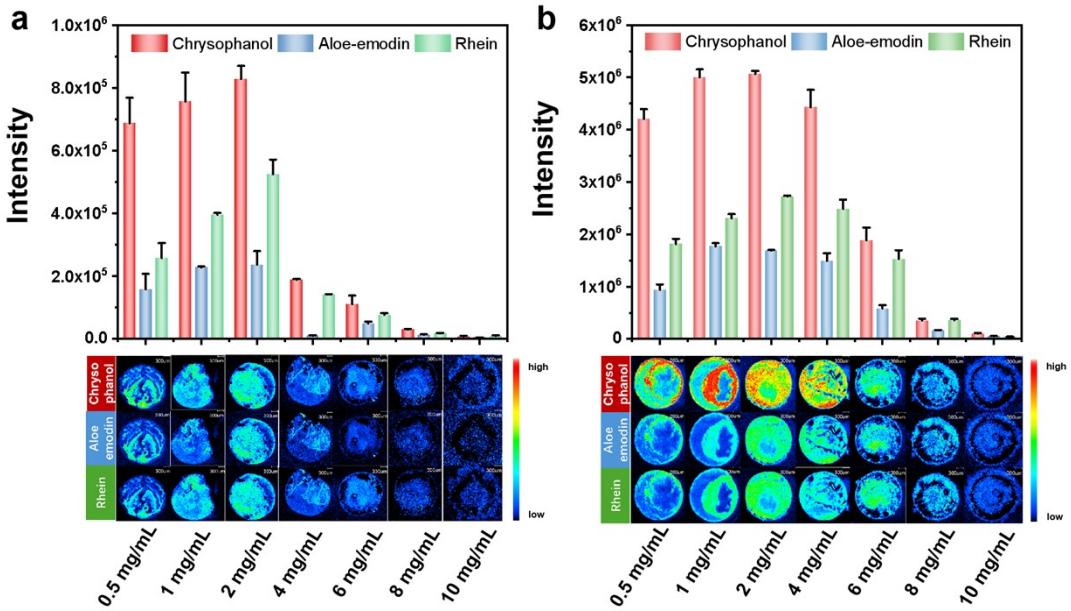

Fig. S6. Optimization of FMPTS concentration in blank kidney tissue. The concentrations of the rhubarb anthraquinone mixture were (a) 10 μg/mL and (b) 100 μg/mL, respectively.

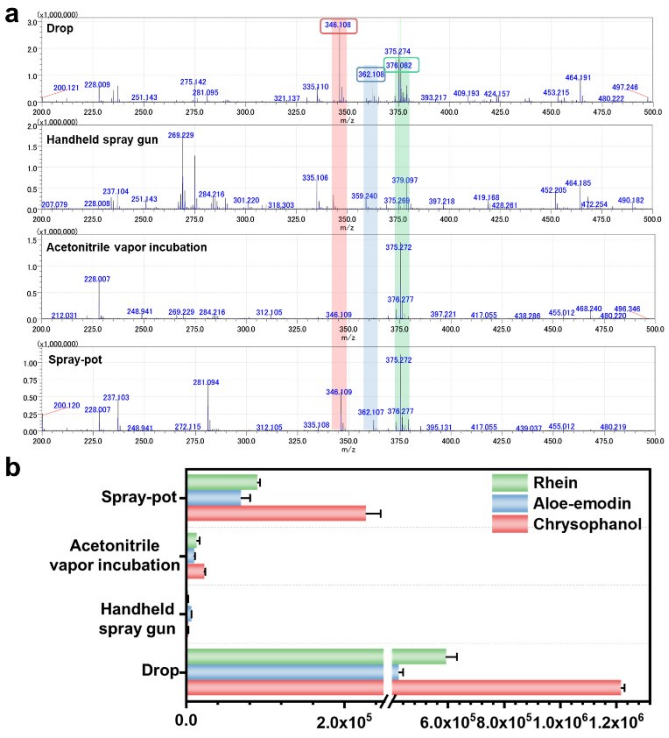

Fig. S7. (a) MALDI-TOF-MS analysis of four derivatization reagent spraying methods: drop method, handheld spray gun method, acetonitrile steam incubation method and spray-pot method. (b) Average MS intensities of different anthraquinone derivatives under four spraying methods (n=3).

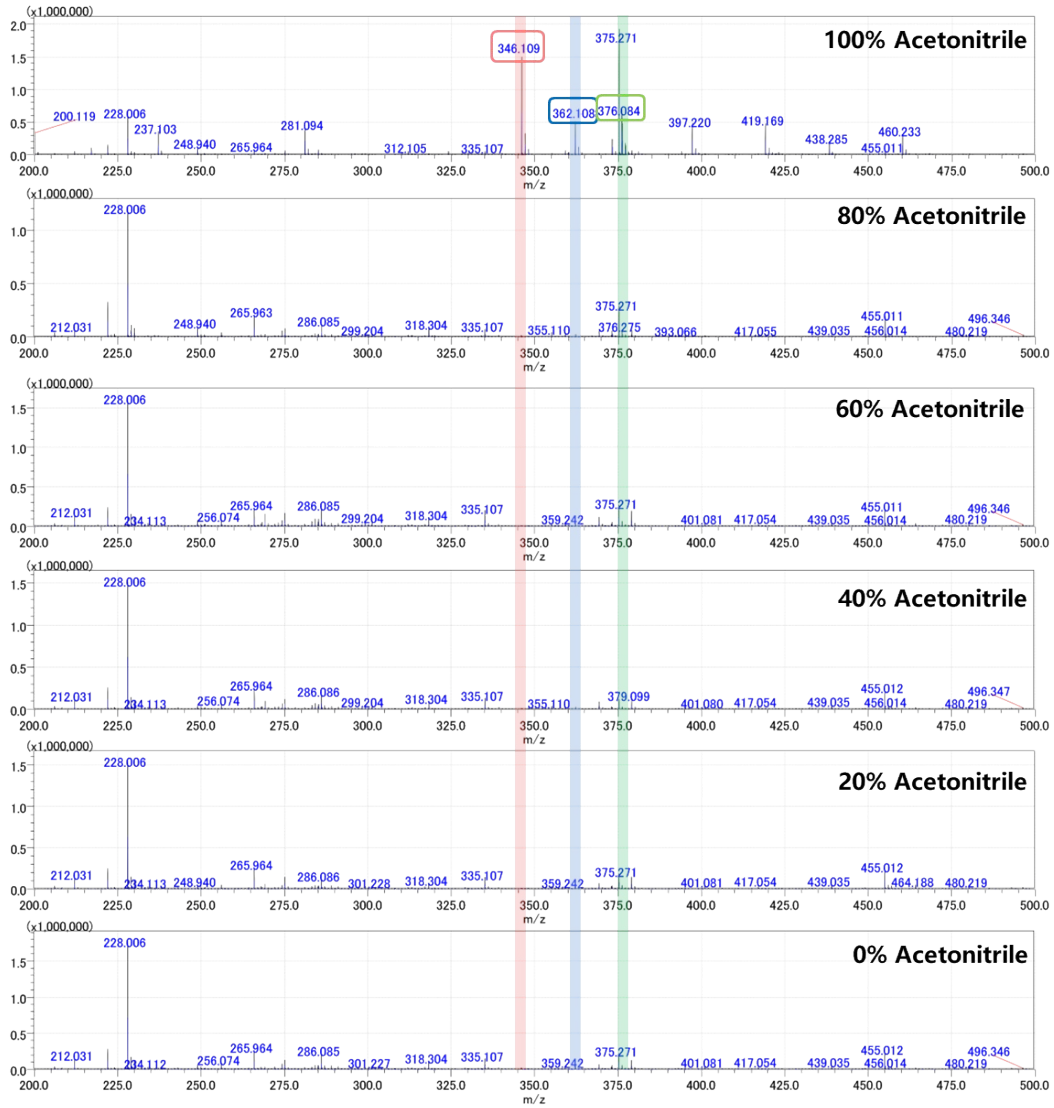

Fig. S8 Effect of water content on derivatization reaction.

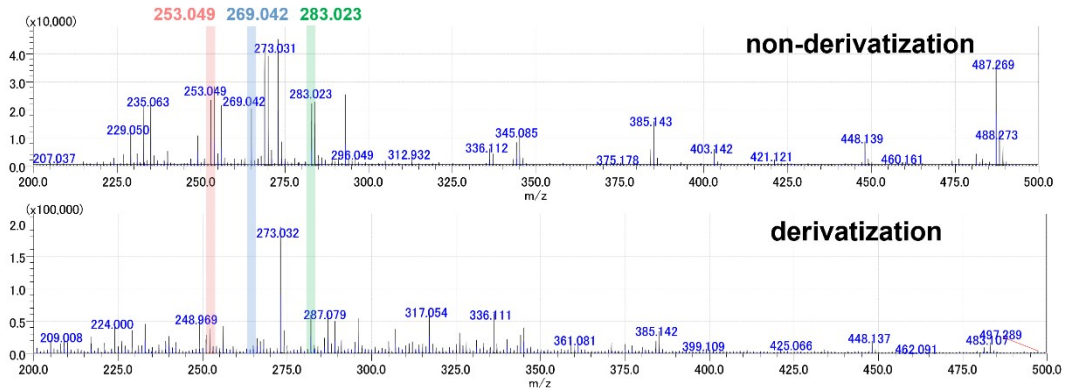

Fig. S9 MALDI-TOF-MS analysis of anthraquinone mixture (a) before and (b) after derivatization in negative ion mode using 9-AA as matrix.

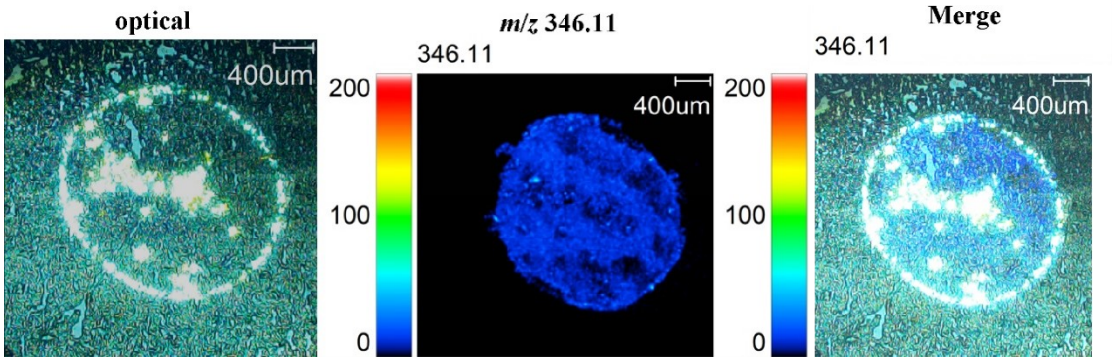

Fig. S10 Delocalization check for the drop-based derivatization workflow on kidney tissue. Left: optical image of the spot region; middle: ion image of the spot region (*m/z* 346.11); right: overlay of the optical and ion images.

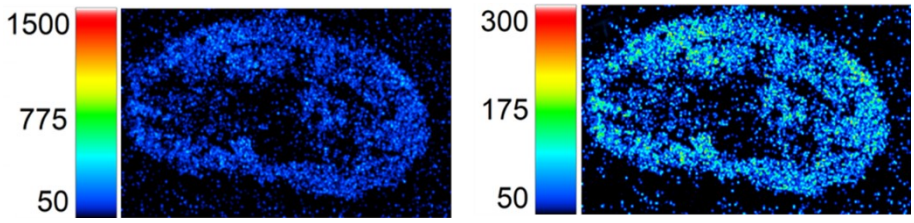

Fig. S11 Effect of intensity scaling on the visualization of rhein distribution in kidney.

Table S1 After derivatization using FMPTS, the theoretical mass spectrometry signals of amino acids.

Endogenous phenolic compounds and amino acids	M+FMPTS (*m*/*z*)	M+2FMPTS-CH_3 (*m*/*z*)	M+2FMPTS-H (*m*/*z*)
Serine	197.0921	274.1221	288.1241
Proline	207.1128	284.1428	298.1448
Threonine	211.1077	288.1377	302.1397
Aspartic acid	225.0870	302.1170	316.1190
Glutamic acid	239.1027	316.1327	330.1347
Dopamine	245.1285	322.1585	336.1605
Noradrenaline	261.1234	338.1534	352.1554
5-hydroxytryptamine	268.1445	345.1745	359.1765
Tyrosine	273.1234	350.1534	364.1554
Homovanillic acid	274.1074	351.1374	365.1394
5-Hydroxyindole-3-acetic acid	283.1077	360.1377	374.1397
Glycine	167.0815		
Alanine	181.0972		
Gamma-aminobutyric acid	195.1128		
Valine	209.1285		
Cysteine	213.0692		
Leucine	223.1441		
Lysine	238.1550		
Methionine	241.1005		
Histidine	247.1190		
Phenylalanine	257.1285		
Arginine	268.1768		
Tryptophan	296.1394		

Table S2 The signal-to-noise ratio (S/N) of FMPTS-derivatized and underivatized anthraquinone mixtures at different spiking concentrations.

Concentration of anthraquinone mixtures (μg/mL)	S/N (FMPTS-derivatized)			S/N (underivatized)		
	Chrysophanol	Aloe-emodin	Rhein	Chrysophanol	Aloe-emodin	Rhein
0.1	291	146	467	N.D.	N.D.	N.D.
1	3644	1309	5227	N.D.	N.D.	N.D.
5	9744	3297	11579	N.D.	N.D.	N.D.
10	39111	13394	54237	36	77	47
50	83789	28370	131583	737	1098	980
100	128113	41188	151308	1444	2102	5755

This signal-to-noise (S/N) calculation method has been established in previous studies[1-3]. Specifically, for each target ion at *m/z* = M, local background was estimated from two side windows **[**M − 0.5, M − 0.2**]** and **[**M + 0.2, M + 0.5**]** in the blank spectrum[3]. The intensities from the two windows were pooled, and the top 5% values were removed to mitigate occasional interference spikes. The local baseline was defined as the median intensity (I_{baseline}), and the noise level was defined as the standard deviation (σ_{noise})[2,3]. The estimated S/N* was calculated as:

$$\frac{S}{N} = \frac{I_{peak} - I_{baseline}}{\sigma_{\text{noise}}}$$

and S/N ≥ 3 was used as a commonly adopted reference criterion for detectability.

The estimated local background levels from six blank spectra (mean ± SD; threshold = $I_{\text{baseline}} + 3\sigma_{\text{noise}}$) are summarized below for reviewer verification:

FMPTS derivative)	m/z	I_{baseline}	σ_{noise}	threshold = $I_{\text{baseline}} + 3\sigma_{\text{noise}}$
Chrysophanol	346.108	24.7 ± 8.5	12.94 ± 3.54	63.49 ± 11.33
Aloe-emodin	362.106	21.7 ± 9.5	17.57 ± 4.22	74.37 ± 5.71
Rhein	376.084	10.2 ± 6.5	5.41 ± 0.91	26.39 ± 9.19

References

1. Signal-to-noise ratio: revision of Ph. Eur. general chapter Chromatographic separation techniques (2.2.46), (accessed January 11, 2026).
2. J. Zhang, E. Gonzalez, T. Hestilow, W. Haskins and Y. Huang, *CG*, 2009, **10**, 388–401.
3. S.-O. Deininger, D. S. Cornett, R. Paape, M. Becker, C. Pineau, S. Rauser, A. Walch and E. Wolski, *Anal Bioanal Chem.*, 2011, **401**, 167–181.