Supporting Information

Rapid determination of sulfamethoxazole and trimethoprim illegally added into health products using excitation-emission matrix fluorescence coupled with second-order calibration method

Lu-Zhu Chen, Hai-Long Wu*, Wan-Jun Long, Tong Wang, Gao-Yan Tong, Ru-Qin Yu

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, P. R. China

* Author for correspondence:

Tel.: (+86) 731 88821818; Fax: (+86) 731 88821818

E-mail: hlwu@hnu.edu.cn (Hai-Long Wu)

LEGENDS TO TABLES AND FIGURES

Table S1 Optimum parameters for TMP and SMZ in the LC-MS/MS method

Table S2 Predicted results for the calibration set using the SWATLD

Table S3 The concentration ranges of calibration sets, regression equations and correlation coefficients (R^2) related to SWATLD-EEMs method and LC-MS/MS method, respectively

Table S4 Quantitative results of all spiked samples using LC-MS/MS method

Fig.S1 The deduction of Rayleigh scattering and Raman scattering in EEM fluorescence data: (a) raw data with Rayleigh scattering and first order Raman scattering; (b) gapped data with scattering being removed in the regions; (c) prosthetic data fitted by an interpolation method.

Fig.S2 3-D landscapes of the excitation-emission matrix fluorescence: (a)UA02; (b)UB02; (c) UC02; (d)PA02; (e)PB02 and (f) PC02.

Fig.S3 Typical extracted MRM ion chromatogram. (a) TMP (290.7 → 122.8) (b)SMZ (253.8 → 107.9) (c) Sample C

Fig.S4 3-D landscapes of the excitation-emission matrix fluorescence for Sample C

Table S1 Optimum parameters for TMP and SMZ in the LC-MS/MS method

Compound	Precursor ion (m/z)	Product ion (m/z)	Dwell time (ms)	Fragmentor (V)	Collision Energy (V)	Polarity
TMP	290.8	122.8	180	170	35	Positive
		110.0	180	170	35	Positive
SMZ	253.8	107.9	240	135	18	Positive
		156.9	240	135	18	Positive

Table S2 Predicted results for the calibration set using the SWATLD

	TMP	SMZ
R^2	0.9974	0.9948
RRMSEC [a]	3.9 %	5.5 %
RMSEC [b] (ng mL^{-1})	12.8	7.6
AVG ± S.D.% [c]	101.9±6.9	101.1±7.4
LOF [d]	3.4	4.9

[a] RRMSEC represents relative root mean square error of calibration. *RRMSEC*=$\sqrt{\sum_{n=1}^{N}(x_n-\hat{x}_n)^2/(N_c-1)}/\bar{x}\times 100\%$, x_n and $\hat{x}_n$ are the spiked SWATLD resolved concentration in *n*th calibration sample, respectively. N_c represents the number of calibration samples. $\bar{x}$ is the mean concentration in calibration samples.

[b] RMSEC, the root-mean-square error of prediction, which is calculated as RMSEC=$\sqrt{\sum_{n=1}^{N}(c_{pre}-c_{act})^2/N}$, here I is the number of samples, c_{act} and c_{pred} are the actual and predicted concentrations of the analytes of interest, respectively.

[c] the average recovery rates (mean ± standard deviation)

[d] LOF is calculated as $LOF(\%) = 100 \times \sqrt{\sum(d_i-\hat{d}_i)^2/\sum(d_i)^2}$ where $\hat{d}_i$ are the estimated concentrations of a target analyte in the calibration samples using ATLD method, and d_i are their corresponding values of nominal concentrations in the calibration samples.

Table S3 The concentration ranges of calibration sets, regression equations and correlation coefficients (R^2) related to SWATLD-EEMs method and LC-MS/MS method, respectively

Method	Analyte	c (ng mL^{-1})	Regression equation [a]	R^2
SWATLD-EEMs	TMP	60-600	y=76.79x-0.40×10^3	0.994
	SMZ	25-250	y=77.55x+1.05×10^3	0.997
LC-MS/MS	TMP	40-800	y=9.50×10^3x+10.55×10^5	0.995
	SMZ	40-800	y=1.93×10^3x+4.75×10^3	0.996

[a] x is concentration (ng mL^{-1}) and y is corresponding response intensity.

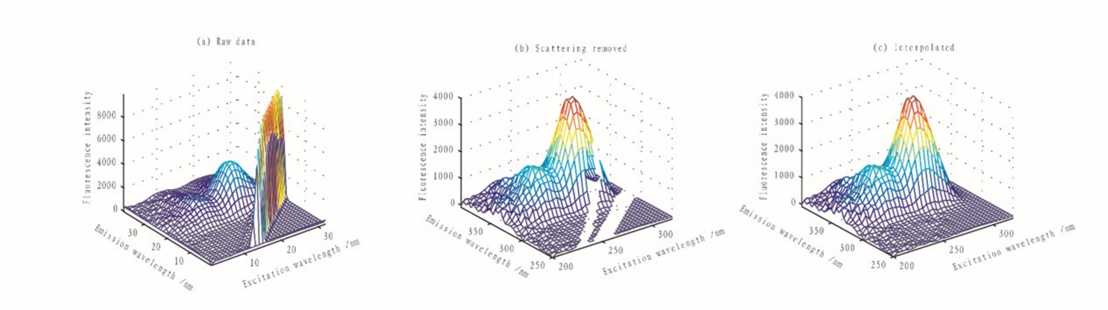
Table S4 Quantitative results of all spiked samples using LC-MS/MS method

Sample	Spiked/ng mL^{-1}		Predicted/ng mL^{-1}	
	TMP	SMZ	TMP	SMZ
		Sample A		
PA01	100.0	240.0	105.0[105.0] [a]	250.4[104.3]
PA02	200.0	150.0	209.9[105.5]	152.7[101.8]
PA03	400.0	90.0	395.4[98.9]	86.1[95.8]
AVG ± S.D.% [b]			103.1±3.7	100.6±4.4
		Sample B		
PB01	100.0	240.0	103.5[103.5]	260.2[108.4]
PB02	200.0	150.0	195.3[97.7]	145.2[96.8]
PB03	400.0	90.0	407.5[101.2]	86.0[95.6]
AVG ± S.D.%			100.8±2.9	100.3±7.1
		Sample C		
PC01	100.0	240.0	213.6[98.3]	249.9[104.1]
PC02	200.0	150.0	307.5[96.3]	158.3[105.5]
PC03	400.0	90.0	498.3[95.8]	93.0[103.3]
AVG ± S.D.%			96.8±1.3	104.3±1.1
t-test [c]			0.91	0.93

[a] values in the square brackets are recoveries, %

[b] the average recovery rates (mean ± standard deviation)

[c] *t*-test = $\frac{|\overline{X}_d|\sqrt{n}}{S_d}$, where $\overline{X}_d$ is the mean of the differences between the prediction concentration values obtained from two methods, S_d is the standard deviation of these differences, and *n* is the total numbers of prediction samples. At the 95% confidence level, the critical value for *t* is 2.31.



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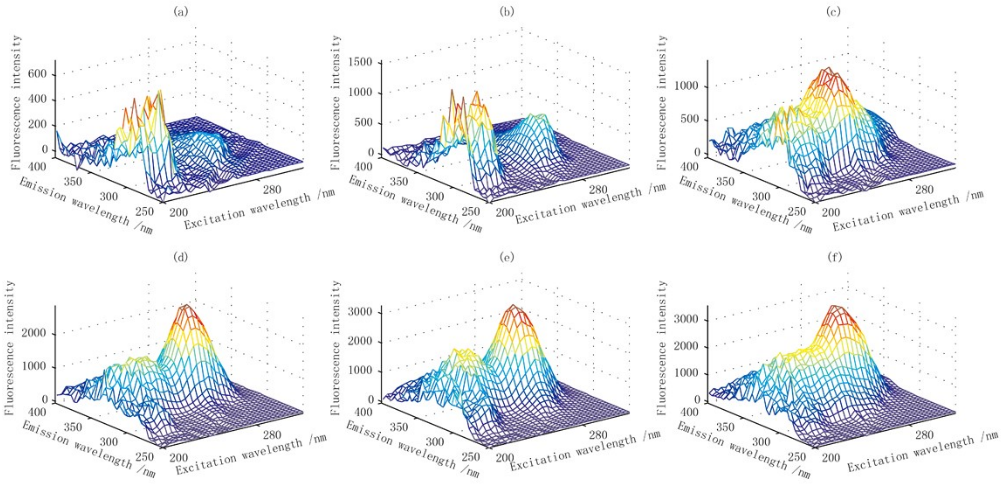

Fig. S2 3-D landscapes of the excitation-emission matrix fluorescence: (a) UA02; (b)UB02; (c) UC02; (d)PA02; (e)PB02and (f) PC02.

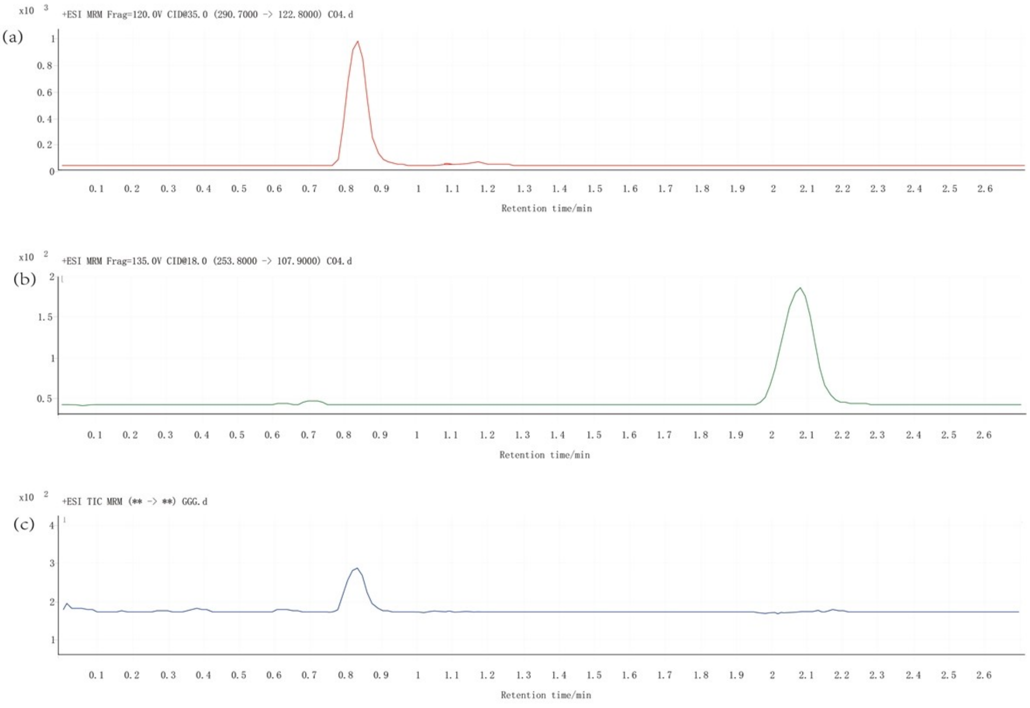

Fig. S3 Typical extracted MRM ion chromatogram. (a) TMP (290.7 → 122.8) (b)SMZ (253.8 → 107.9) (c) Sample C

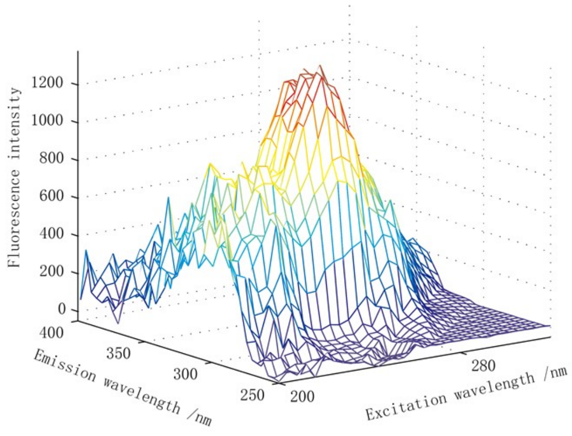

Fig. S4 3-D landscapes of the excitation-emission matrix fluorescence for Sample C