Supporting information

Automatic Magnetic Solid Phase Extraction for Rapid and High-Throughput Determination of Neonicotinoid Insecticides and Their Metabolites in Serum, Breast Milk and Urine Samples

Kaiqin Huang[a,b,1], Jing Yi[c,1], Guocheng Liu[b], Yangyang Liu[b], Kaixin Jiang[b], Zhuowen Li[b], Yanji Qu[d], Meiqing Lin[b], Shengtao Ma[a,b*]

[a] School of Public Health, Guangzhou Medical University, Guangzhou 511436, P. R. China

[b] Guangdong-Hong Kong-Macao Joint Laboratory for Contaminants Exposure and Health, Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, School of Environmental Science and Engineering, Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou 510006, P. R. China

[c] Department of Obstetrics and Gynecology, Guangdong Women and Children Hospital, Guangzhou 511400, P. R. China

[d] Global Health Research Center, Guangdong Cardiovascular Institute, Guangdong Provincial People's Hospital (Guangdong Academy of Medical Sciences), Southern Medical University, Guangzhou 510100, P. R. China

***Corresponding author:**

Dr. Shengtao Ma, E-mail: mast@gzhmu.edu.cn

Table S1. Parameters of UHPLC-MS/MS analysis for target analytes

Compound	Precursor ion (m/z)	Product ion (m/z)	Declustering potential (V)	Collision energy (V)	Retention time (min)
DIN	203.1	129.0	32	17	7.905
	203.1	157.2	26	12	
ACE	223.2	126.2	52	30	8.879
	223.2	89.9	64	48	
N-dm-ACE	209.2	126.0	48	24	8.627
	209.2	90.0	57	44	
CLO	250.3	169.2	32	19	8.683
	250.3	132.0	28	20	
IMI	256.1	209.2	45	21	8.816
	256.1	175.1	45	25	
Of-IMI	254.2	171.2	57	24	8.325
	254.2	151.9	58	20	
5-OH-IMI	273.3	225.0	64	18	8.339
	273.3	191.2	63	24	
THM	292.1	211.1	23	16	8.402
	292.1	132.0	44	31	
THD	253.1	126.1	66	28	9.194
	253.1	90.1	64	54	
DIN-d_3	206.1	132.1	32	17	7.905
	206.1	160.2	26	12	
ACE-d_3	226.3	125.9	52	30	8.879
CLO-d_3	253.1	172.2	32	19	8.669
	253.1	132.0	28	20	
IMI-d_4	260.1	213.1	45	21	8.802
	260.1	179.3	45	25	
THM-d_3	295.2	214.3	23	16	8.402
	295.2	131.9	44	31	
THD-d_4	257	125.8	66	28	9.187

Table S2. Effect of washing solvent on the matrix effect of p-NEOs and m-NEOs in bovine milk

	IMI	ACE	CLO	THM	THD	DIN	N-dm-ACE	Of-IMI	5-OH-IMI
H_2O	-43.73%	-18.58%	-50.00%	-12.08%	-51.35%	-7.34%	-15.00%	-58.48%	-34.57%
5% MeOH	-48.45%	-17.97%	-54.24%	-11.68%	-52.82%	-6.64%	-14.71%	-57.59%	-38.29%
10% MeOH	-50.42%	-17.21%	-54.24%	-12.75%	-50.82%	-6.64%	-15.88%	-63.29%	-40.36%
15% MeOH	-46.61%	-14.47%	-52.83%	-10.07%	-46.78%	-5.48%	-11.41%	-58.15%	-32.51%
20% MeOH	-37.32%	-12.64%	-55.80%	-8.99%	-45.15%	-3.16%	-10.10%	-57.25%	-31.19%
30% MeOH	-39.50%	-11.12%	-52.69%	-8.05%	-36.98%	-2.32%	-9.29%	-49.71%	-29.70%
40% MeOH	-46.12%	-20.71%	-56.08%	-12.48%	-46.78%	-5.67%	-15.73%	-58.98%	-37.22%

Table S3. Effect of washing solvent on the matrix effect of p-NEOs and m-NEOs in fetal bovine serum

	IMI	ACE	CLO	THM	THD	DIN	N-dm-ACE	Of-IMI	5-OH-IMI
H_2O	-57.99%	-32.62%	-65.60%	-36.40%	-64.10%	-33.85%	-34.81%	-57.91%	-38.74%
5% MeOH	-59.93%	-33.02%	-65.17%	-32.02%	-70.87%	-24.62%	-39.49%	-64.36%	-42.43%
10% MeOH	-60.43%	-29.81%	-64.30%	-24.12%	-70.67%	-10.38%	-35.05%	-49.83%	-34.05%
15% MeOH	-64.96%	-26.20%	-63.28%	-16.67%	-72.88%	-5.38%	-31.07%	-51.80%	-41.17%
20% MeOH	-67.05%	-26.20%	-66.04%	-21.05%	-74.07%	-8.85%	-27.10%	-50.00%	-34.05%
30% MeOH	-63.31%	-18.97%	-55.01%	-13.16%	-73.04%	-0.38%	-24.07%	-51.28%	-27.75%
40% MeOH	-55.04%	-15.35%	-55.88%	-22.81%	-70.74%	6.92%	-17.99%	-36.63%	-27.75%

Table S4. Effect of washing solvent on the matrix effect of p-NEOs and m-NEOs in pooled urine samples

	IMI	ACE	CLO	THM	THD	DIN	N-dm-ACE	Of-IMI	5-OH-IMI
H_2O	-44.60%	-37.57%	-44.65%	-46.86%	-40.29%	-14.58%	-34.00%	-68.12%	-47.99%
5% MeOH	-52.58%	-46.65%	-40.28%	-50.08%	-46.80%	-17.17%	-29.63%	-75.01%	-41.40%
10% MeOH	-49.66%	-34.02%	-50.38%	-51.79%	-47.60%	-14.51%	-32.81%	-70.43%	-40.67%
15% MeOH	-50.57%	-36.28%	-44.77%	-46.49%	-47.60%	-12.27%	-27.64%	-72.99%	-36.42%
20% MeOH	-49.75%	-22.48%	-43.87%	-48.01%	-48.00%	-15.62%	-22.87%	-67.73%	-31.00%
30% MeOH	-52.76%	-33.63%	-46.34%	-45.99%	-48.80%	-8.88%	-20.09%	-69.53%	-39.06%
40% MeOH	-48.93%	-36.75%	-34.22%	-46.49%	-43.20%	-12.83%	-8.95%	-59.55%	-26.75%

Table S5. Compare the consumption of sample, organic solvent, and time of extraction methods previously used for breast milk, human serum and urine NEO analysis with this study[a]

Matrix	Extraction method	Sample amount	Organic solvent	Extraction time[b]	Reference
urine	SPE with HLB cartridges	2 mL	5 mL	14 min per sample	1
urine	SPE with Bond Elut PCX cartridges	1 mL	2 mL	> 30 min per sample	2
urine	Automatic SPE with ISOLUTE® HYDRO DME+400 mg plate	100 µL	0.7 mL	< 10 min for one batch of samples (96-well plate)	3
serum and urine	SPE with Extrelut® NT3 cartridge	1 mL	30 mL	> 15 min per sample	4
breast milk	LLE	0.5 mL	1.8 mL	> 12 min per sample	5
breast milk	QuEChERS	5 g	11.5 mL	> 18 min per sample	6
breast milk	LLE	100–200 µL	4 mL	> 60 min per sample	7
Serum, breast milk and urine	Automatic MSPE	200 µL	1.4 mL	< 30 min for one batch of samples (*n*=32)	this study

[a]SPE, solid-phase extraction; LLE, liquid-liquid extraction; MSPE, magnetic solid phase extraction. [b]We calculated the time of some extraction procedures used as the authors stated in the literatures, but the time of some extraction procedures used and how many samples were treated in a batch was not clarify, therefore, we only provided the information based on the minimum time required for one sample.

Reference:

[1] Q. Zhang, X. Wang, Z. Li, H. Jin, Z. Lu, C. Yu, Y.F. Huang and M. Zhao, Simultaneous determination of nine neonicotinoids in human urine using isotope-dilution ultra-performance liquid chromatography-tandem mass spectrometry, *Environ. Pollut.*, 2018, 240, 647-652. https://doi.org/10.1016/j.envpol.2018.04.144.

[2] J. Ueyama, H. Nomura, T. Kondo, I. Saito, Y. Ito, A. Osaka and M. Kamijima, Biological monitoring method for urinary neonicotinoid insecticides using LC-MS/MS and its application to Japanese adults, *J. Occup. Health*, 2014, 56, 461-468. https://doi.org/10.1539/joh.14-0077-OA.

[3] Y. Nishihama, S.F. Nakayama and T. Isobe, A simultaneous, high-throughput and sensitive method for analysing 13 neonicotinoids and metabolites in urine using a liquid chromatography-tandem mass spectrometry, *MethodsX*, 2023, 10, 102129. https://doi.org/10.1016/j.mex.2023.102129.

[4] T. Yamamuro, H. Ohta, M. Aoyama and D. Watanabe, Simultaneous determination of neonicotinoid insecticides in human serum and urine using diatomaceous earth-assisted extraction and liquid chromatography-tandem mass spectrometry, *J. Chromatogr B*, 2014, 969, 85-94. https://doi.org/10.1016/j.jchromb.2014.06.008.

[5] L. Lachat and G. Glauser, Development and validation of an ultra-sensitive UHPLC-MS/MS method for neonicotinoid analysis in milk, *J. Agric. Food Chem.*, 2018, 66, 8639-8646. https://doi.org/10.1021/acs.jafc.8b03005.

[6] N. Anand, A. Kundu and S. Ray, A validated method for the determination of neonicotinoid, pyrethroid and organochlorine residues in human milk, *Chromatographia*, 2018, 81, 315-325. https://doi.org/10.1007/s10337-017-3436-6.

[7] T.L. Pedersen, J.T. Smilowitz, C.K. Winter, S. Emami, R.J. Schmidt, D.H. Bennett, I. Hertz-Picciotto and A.Y. Taha, Quantification of nonpersistent pesticides in small volumes of human breast milk with ultrahigh performance liquid

chromatography coupled to tandem mass spectrometry, *J. Agric. Food Chem.*, 2021, 69, 6676-6689. https://doi.org/10.1021/acs.jafc.0c05950.

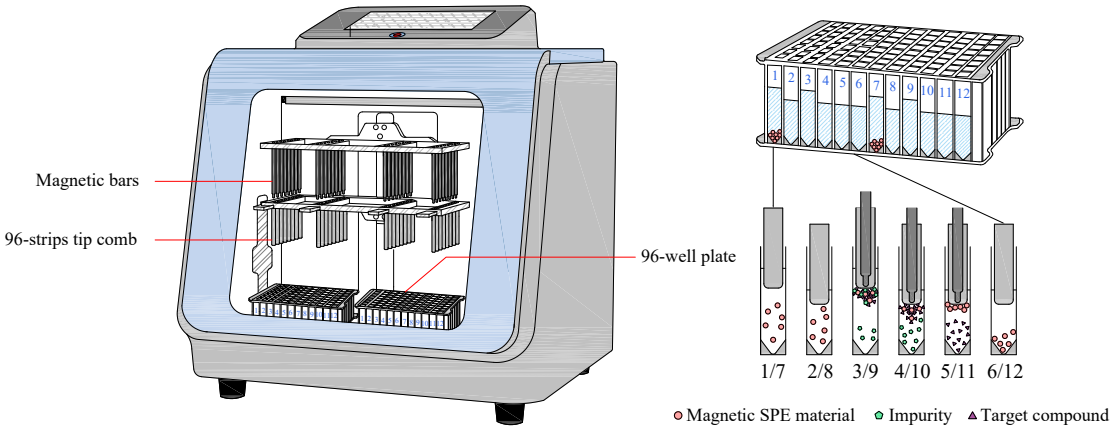

Figure S1. Schematic representation of an automatic MSPE system. 1/7: Magnetic SPE material activation; 2/8: Material equilibrium; 3/9: Extraction; 4/10: Washing; 5/11: Elution; 6/12: Waste discharge.

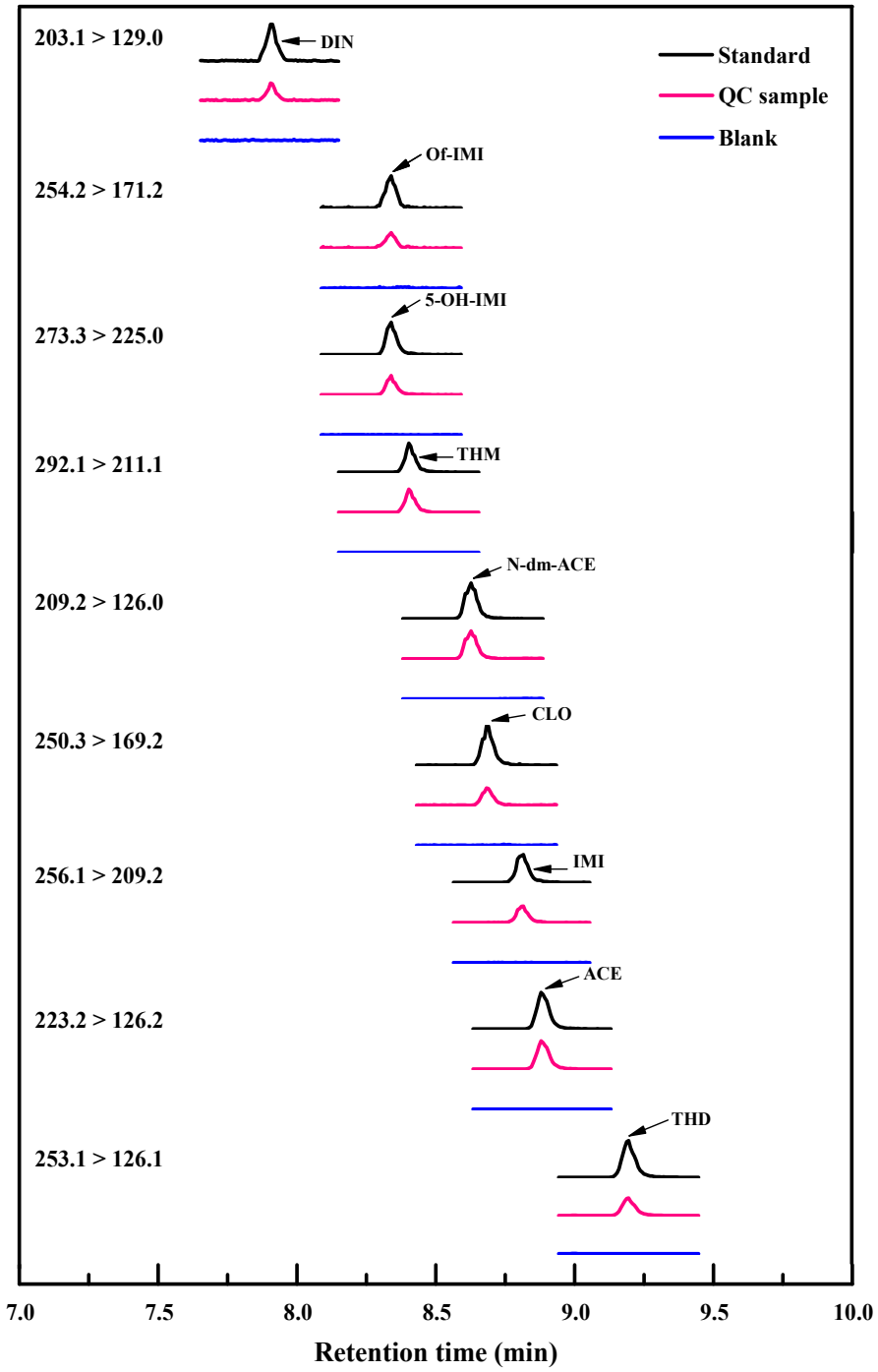

Figure S2. UHPLC-MS/MS typical MRM ion-chromatograms of target analytes resulting from a standard solution of 1 ng/mL (black line), a spiked (1 ng/mL) QC sample of bovine milk (red line), and a QC sample of breast milk (blue line).

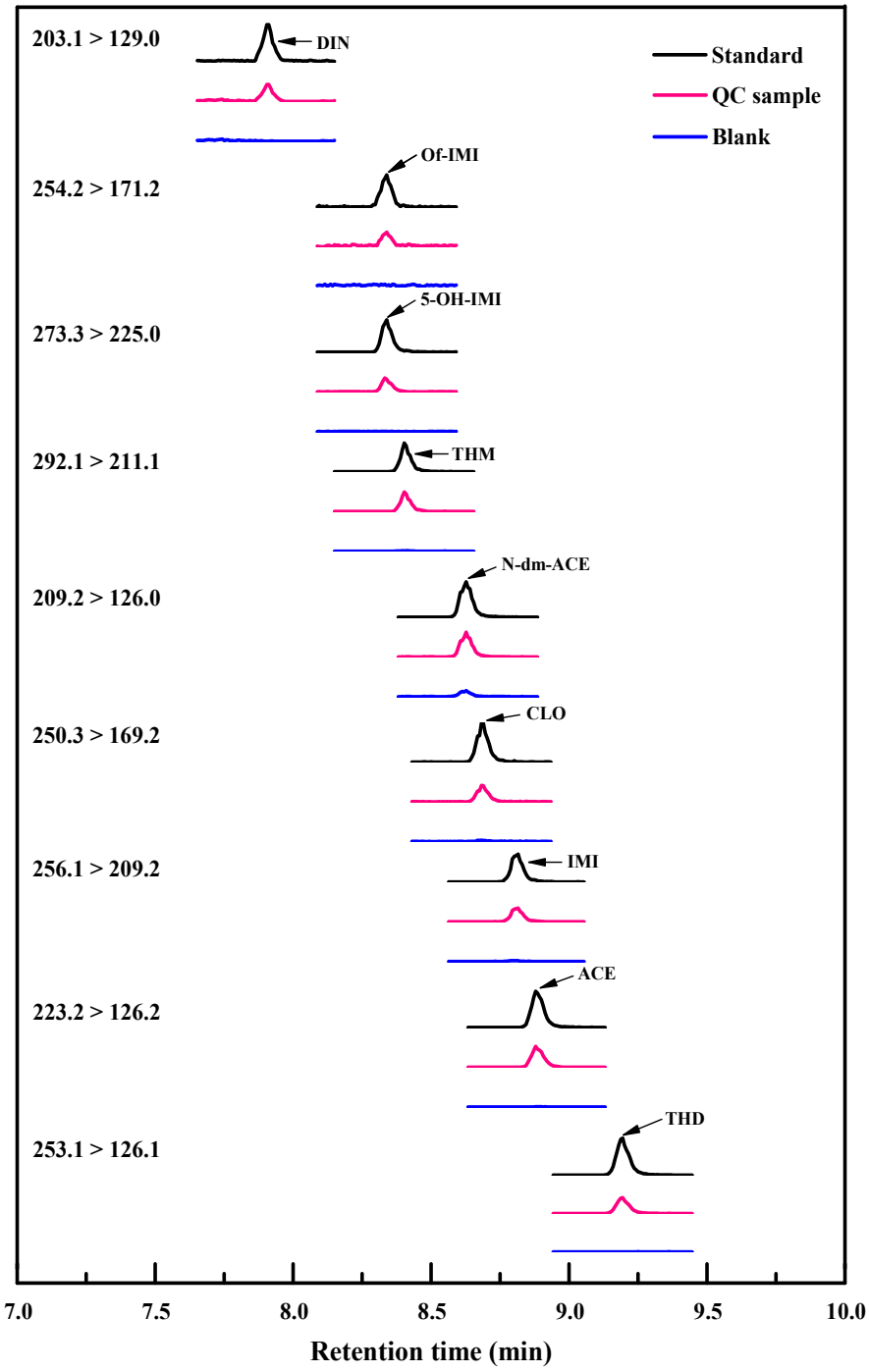

Figure S3. UHPLC-MS/MS typical MRM ion-chromatograms of target analytes resulting from a standard solution of 1 ng/mL (black line), a spiked (1 ng/mL) QC sample of fetal bovine serum (red line), and a QC sample of fetal bovine serum (blue line).

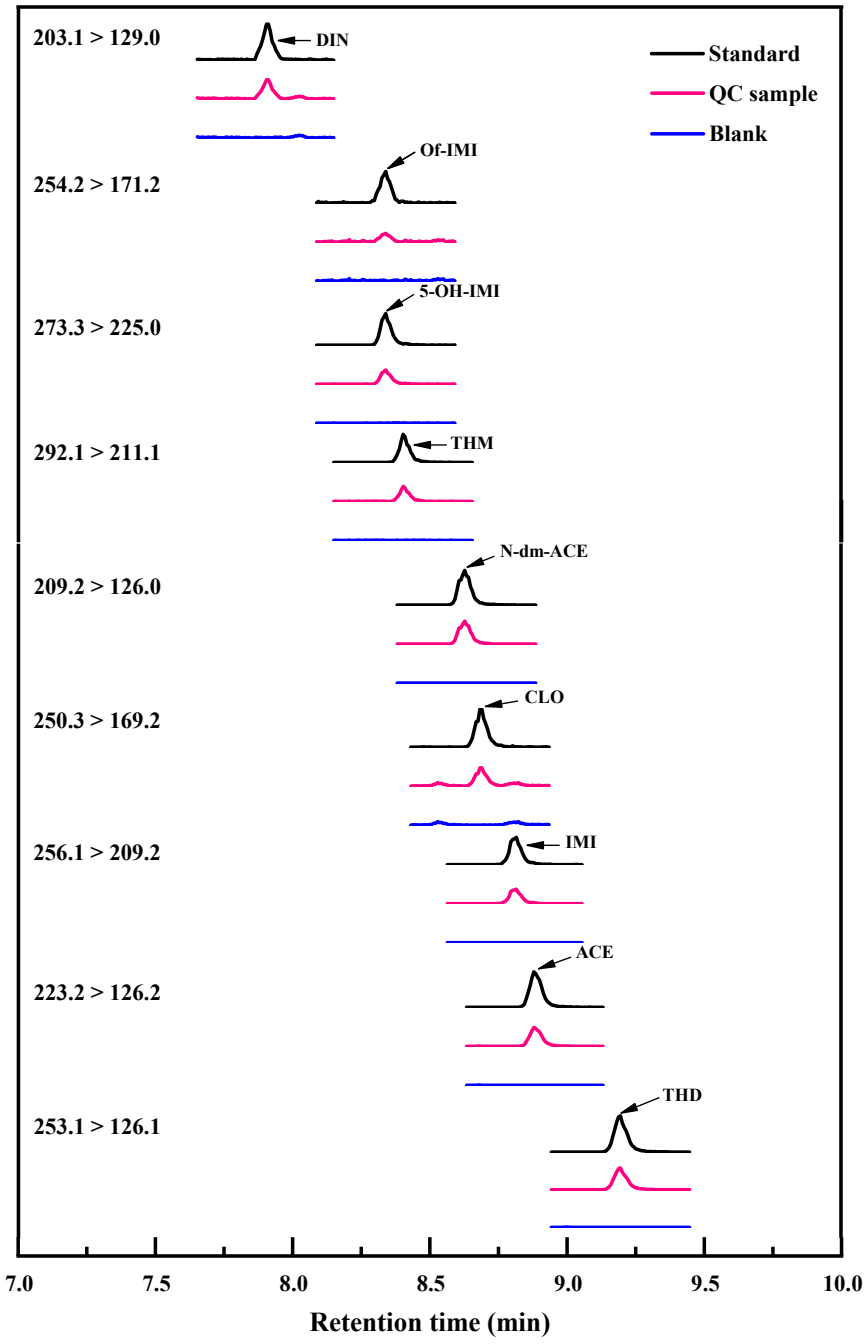

Figure S4. UHPLC-MS/MS typical MRM ion-chromatograms of target analytes resulting from a standard solution of 1 ng/mL (black line), a spiked (1 ng/mL) QC sample of pooled urine (red line), and a QC sample of pooled urine (blue line).

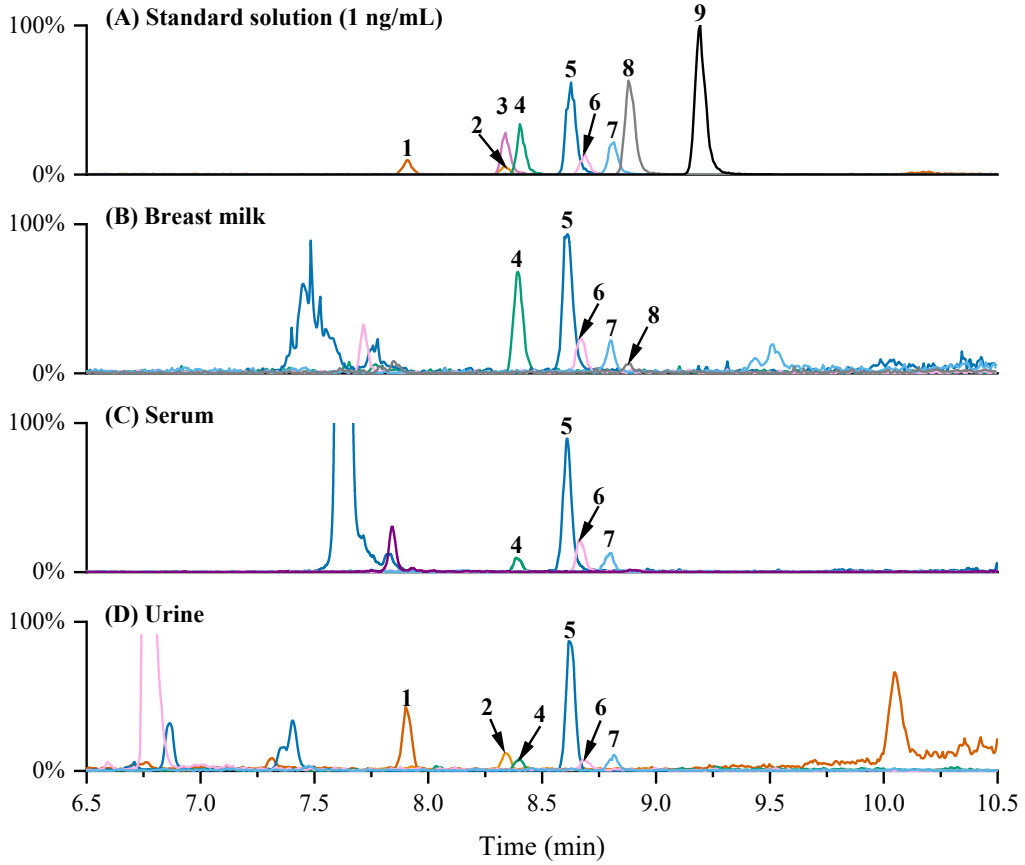

Figure S5. The LC-MS/MS chromatographs of analytical standards and representative real samples. 1: DIN; 2: Of-IMI; 3: 5-OH-IMI; 4: THM; 5: N-dm-ACE; 6: CLO; 7: IMI; 8: ACE; 9: THD.