Chemometrics-assisted liquid chromatography-full scan-mass spectrometry for simultaneous determination of multi-class estrogens in infant milk powder

Xiao-Dong Sun, Hai-Long Wu*, Zhi Liu, Li-Xia Xie, Yong Hu, Huan Fang, Tong Wang, Rong Xiao, Yu-Jie Ding, Ru-Qin Yu

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

To whom correspondence should be addressed:

* Corresponding author : Hai-Long Wu

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

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

E-mail: hlwu@hnu.edu.cn

SUPPLEMENTARY DATA

Summary

This supplementary data file includes additional information and results as described in the main article including:

Table S.1

Concentrations of seven estrogens designed in calibration sets (C01-08) and prediction sets (P01-03), respectively.

Table S.2

Retention time, dwell time, fragmentor voltage (FV), collision energy (CE), qualitative-quantitative ion pairs and statistical parameters of seven estrogens in LC-MS/MS method.

Table S.3

Quantitative results of seven estrogens in two kinds of spiked infant samples using LC-MS/MS method.

Table S.4

Comparison of proposed method with other methods used for the quantification of estrogens in dairy and milk products.

Fig. S.1 The optimization of extraction efficiencies of seven estrogens in two infant samples, respectively: (A) and (B) three different solvents were tested for extraction efficiencies; Concentrations were as follows (all in ng mL^{-1}): E3, 100.0; BPA, 100.0; β-E2, 100.0; α-E2, 100.0; EE2, 100.0; E1, 100.0; DES, 100.0.

Fig. S.2 Calibration curves based on ATLD method resolved relative concentration of each analyte versus its nominal concentration in calibration samples.

Fig. S.3 (A) The ion current chromatograms of estrogens, and (B) corresponding mass spectrograms using LC-MS/MS method in multi-reaction monitoring (MRM) mode.

Fig. S.4 The ion current chromatograms of spiked sample P02 for infant milk sample A using LC-MS/MS method in multi-reaction monitoring (MRM) mode.

Table S.1

Concentrations of seven estrogens designed in calibration sets (C01-08) and prediction sets (P01-03), respectively.

Sample	Analytical concentration (ng mL^{-1})						
	E3	BPA	β-E2	α-E2	EE2	E1	DES
C01	60	140	250	80	330	370	480
C02	120	260	490	140	85	270	420
C03	180	380	190	200	390	170	360
C04	240	500	430	260	150	70	300
C05	300	80	130	320	450	420	240
C06	360	200	370	380	210	320	180
C07	420	320	70	440	510	220	120
C08	480	440	310	500	270	120	60
P01	80	180	250	90	100	140	220
P02	140	270	85	160	300	80	150
P03	200	90	170	230	200	200	75

Table S.2

Retention time, dwell time, fragmentor voltage (FV), collision energy (CE), qualitative-quantitative ion pairs and statistical parameters of seven estrogens in LC-MS/MS method.

Compounds	Retention time (min)	Dwell time (s)	Fragmentor voltage (V)	$M\text{-}H^+$ (m/z)	Quantifier (m/z)	Qualifier (m/z)	LOD (n≥3) (ng mL^{-1})[a]	LOQ (n≥3) (ng mL^{-1})[a]
Estriol	2.47	200	135	287.2	171.1(36)[b]	145.1(44)	0.92	2.76
Bisphenol A	4.19	200	100	227.1	211.1(17)	133.0(30)-	1.17	3.51
17β-Estradiol	4.89	200	150	271.1	183.1(40)	144.9(40)	0.44	1.32
17α-Estradiol	5.38	200	150	271.2	183.1(45)	144.9(40)	0.58	1.75
17α-Ethinyl estradiol	5.69	200	135	295.2	145.1(36)	159.1(45)	1.53	4.60
Estrone	6.08	200	135	269.2	145.1(38)	159.1(22)	0.12	0.37
Diethylstilbestrol	6.23	200	140	267.1	237.1(24)	222.0(32)	0.09	0.27

[a] The limit of detection is expressed as LOD = 3.3 S_0, the limit of quantification is expressed as LOQ = 10 S_0, S_0 is the standard deviation of three blank samples.

[b] the collision energy is in bracket, the unity is ev.

Table S.3

Quantitative results of seven estrogens in two kinds of spiked infant samples using LC-MS/MS method.

Samples	Predict concentration (ng mL^{-1}) [spiked recovery %]						
	E3	BPA	β-E2	α-E2	EE2	E1	DES
Infant milk A							
P01	79.5 [99.4]	181.0 [100.6]	257.8 [103.1]	79. 9 [88.8]	96.4 [96.4]	141.9 [101.3]	221.0 [100.5]
P02	138.2 [98.7]	272.0 [100.7]	86.7 [101.9]	175.8 [109.9]	305.1 [101.7]	80.7 [100.8]	150.2 [100.1]
P03	196.7 [98.4]	96.1 [106.8]	169.1 [99.5]	213.9 [93.0]	194.9 [97.4]	204.4 [102.2]	70.6 [94.1]
AVG.±S.D. (%)[a]	98.8±0.5	102.7±3.5	101.5±1.9	97.2±11.1	98.5±2.8	101.4±0.7	98.2±3.6
Infant milk B							
P01	78.1 [97.6]	178.7 [99.3]	250.0 [100.0]	77.6 [86.3]	94.8 [94.8]	142.1 [101.5]	218.3 [99.2]
P02	133.6 [95.4]	260.6 [96.5]	81.8 [96.3]	137.8 [86.1]	308.2 [102.7]	79.4 [99.3]	145.2 [96.8]
P03	195.4 [97.7]	87.4 [97.1]	156.6 [92.1]	216.8 [94.2]	199.2 [99.6]	199.0 [99.5]	77.3 [103.1]
AVG.+S.D. (%)	96.9±1.3	97.6±1.4	96.1±3.9	88.9±4.6	99.0±4.0	100.0±1.2	99.7±3.2
T-test	0.13	1.74	2.06	5.09	1.08	0.14	1.98

[a] Recovery: mean value with relative standard deviation.

Table S.4

Comparison of proposed method with other methods used for the quantification of estrogens in dairy and milk products.

Method	Analytes	Recovery (%)	LOD	Analysis time (min)	Reference
LC-DAD	17β-E2, E1, DES	87.6–94.2	60-170 ng L^{-1}	18.0 min	[2]
LC-ESI-MS/MS	E1, 17β-E2, EE2, E3	72-117	2-70 ng L^{-1}	25.0 min	[39]
LC-ESI-MS/MS	E1, 17β-E2, 17α-E2, E3	N.A.[a]	15-75 ng L^{-1}	10.0 min	[40]
UPLC-Q-TOF-MS	DES, DS, E1, 17β-E2, 17α-E2, 17α-EE2, E3, equilin, HEX	82-99	0.11-0.30 μg Kg^{-1}	13.0 min	[41]
LC-ESI-MS/MS	**E3, BPA, 17β-E2, 17α-E2, 17α-EE2, E1, DES**	**91.2-104.2**	**1.75-62.3 ng L^{-1}** [b]	**7.5 min**	**This work**

[a] N.A.-Non-available.

[b] LOD is calculated as follows: LOD = LOD_m / n, LOD_m is the detection limits calculated by ATLD method, n is the enrichment factor. In the present study, the values of LOD_m are in the range of 0.07-2.49 ng mL^{-1} and n is 40.

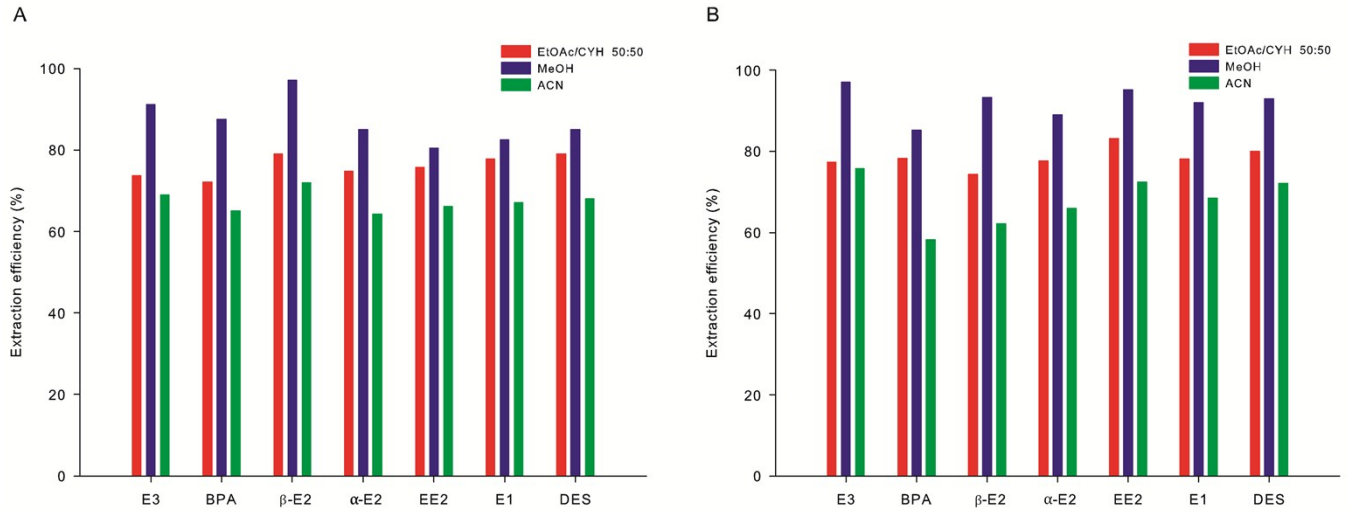


Fig. S.1 The optimization of extraction efficiencies for seven estrogens in two infant samples, respectively: (A) and (B) three different solvents were tested for extraction efficiencies. Concentrations were as follows (all in ng mL^{-1}): E3, 100.0; BPA, 200.0; β-E2, 100.0; α-E2, 100.0; EE2, 100.0; E1, 100.0; DES, 100.0.

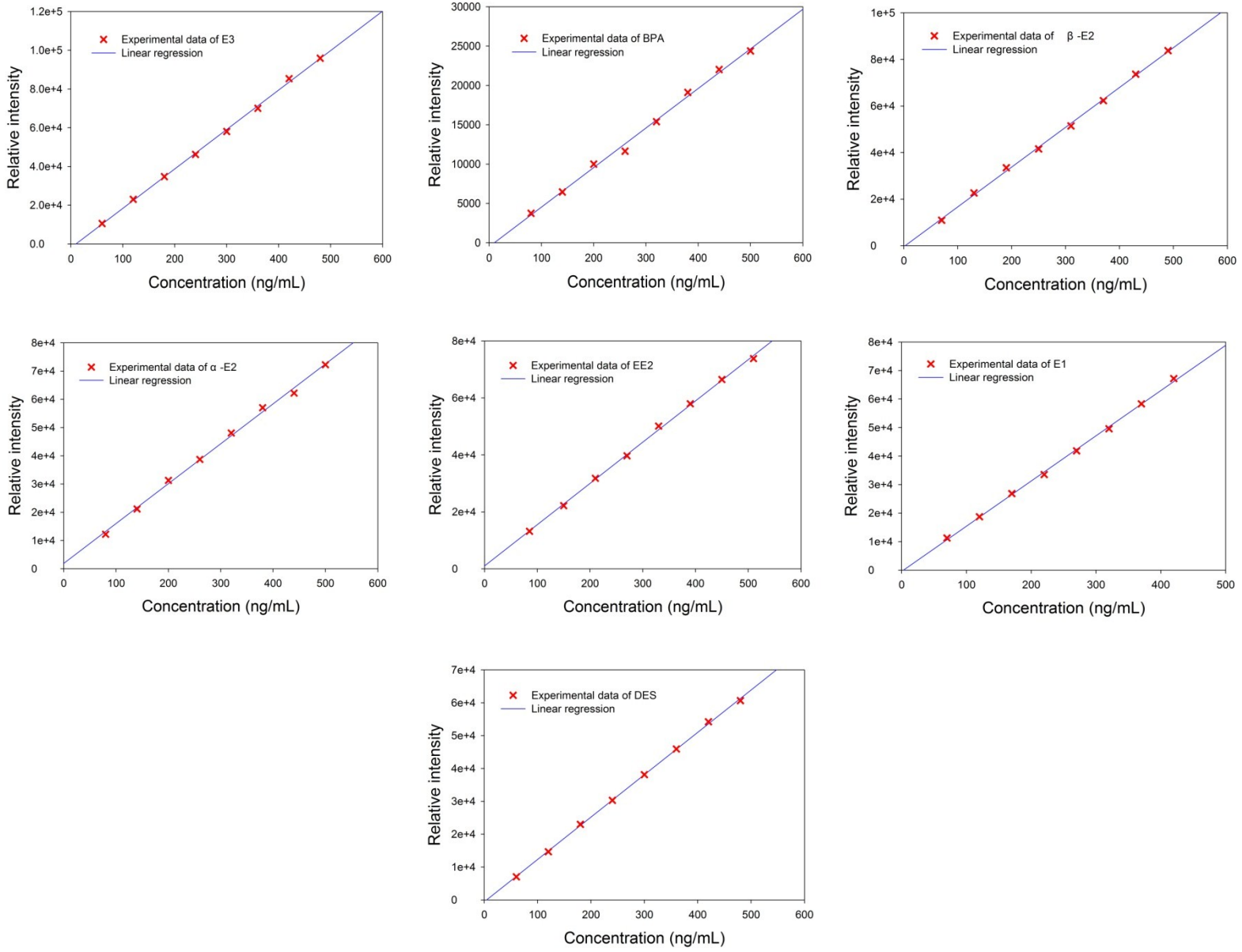
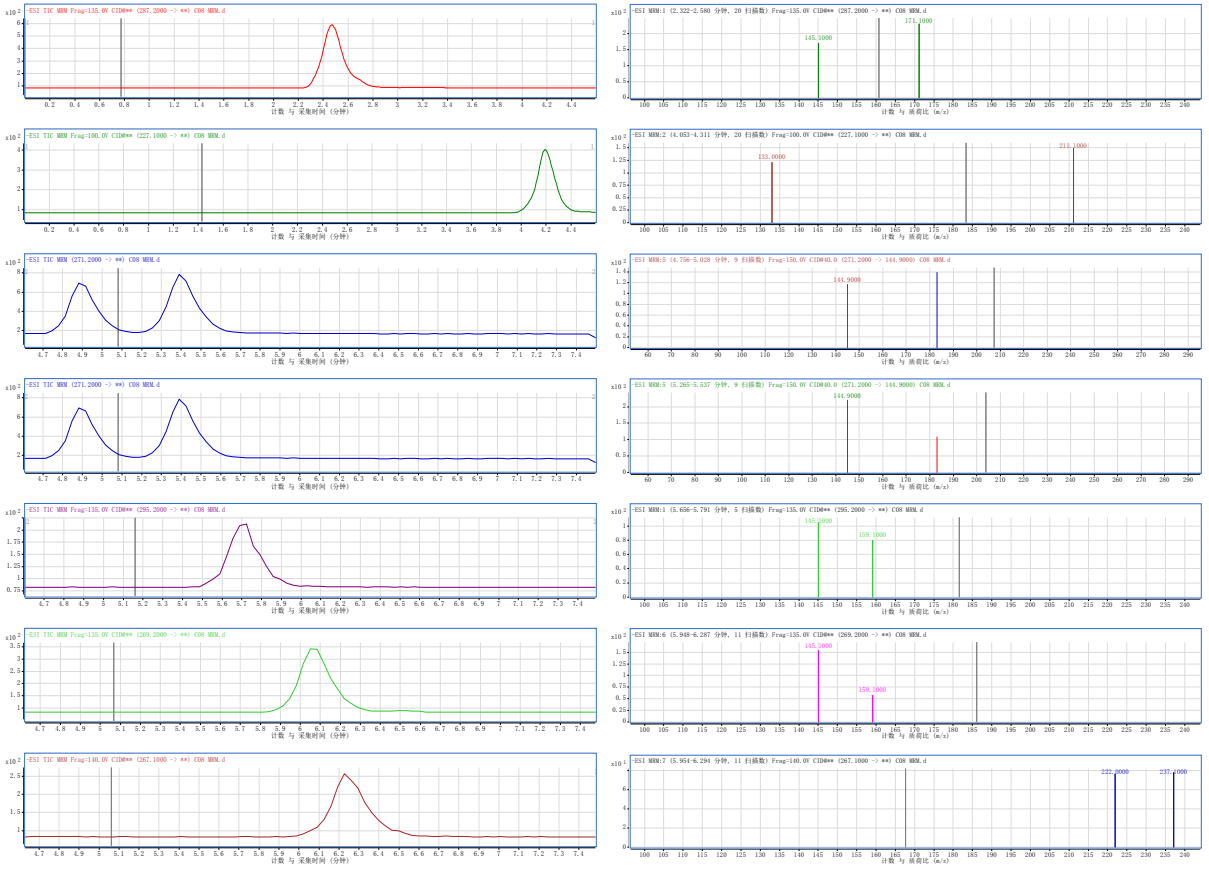


Fig. S.2 Calibration curves based on ATLD method resolved relative concentration of each analyte versus its nominal concentration in calibration samples.



(A) (B)

Fig. S.3 (A) The ion current chromatograms of estrogens, and (B) corresponding mass spectrograms using LC-MS/MS method in multi-reaction monitoring (MRM) mode.

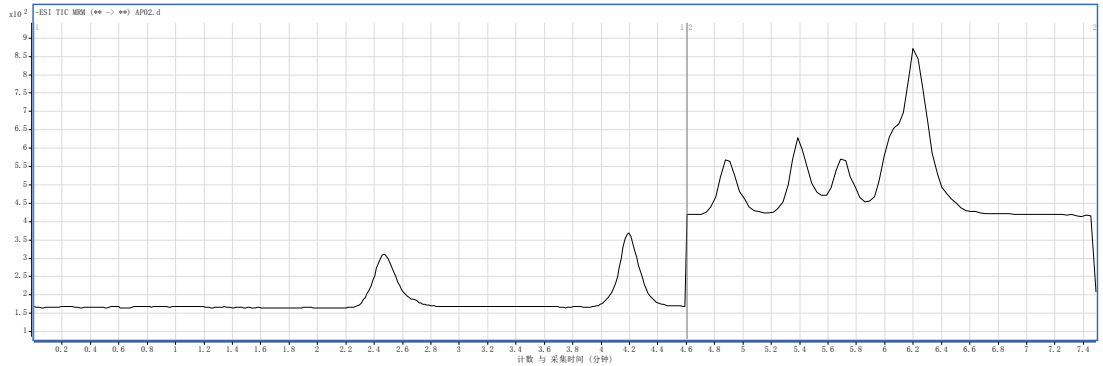


Fig. S.4 The ion current chromatograms of spiked sample P02 for infant milk sample A using LC-MS/MS method in multi-reaction monitoring (MRM) mode.

References:

2. P. Wang, Y. Xiao, W. Liu, J. Wang and Y. Yang, *Food Chem.*, 2015, **172**, 385-390.

39. J. Tso and D. S. Aga, *J. Chromatogr. A*, 2010, **1217**, 4784-4795.

40. C. Farke, E. Rattenberger, S. U. Roiger and H. H. Meyer, *J. Agric. Food Chem.*, 2011, **59**, 1423-1427.

41. W. He-Xing, Z. Ying and Q.-W. JIANG, *Chin. J. Anal. Chem.*, 2011, **39**, 1323-1328.