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Supporting information

Development, optimization and validation of modified QuEChERS

based UPLC-MS/MS for simultaneous determination of nine steroid

hormones in milk powder and milk

4 Figure and 1 Table.



2 Fig. S1. Chemical structure, names, and abbreviation of the 9 steroid hormones3 analyzed.





Fig. S2. UPLC–MS/MS quantitative and qualitative ion chromatograms of for each
compound in a milk powder sample spiked at 0.01 mg/L; HC-d5, E1-d2, E2-13C2, E3d3 and DES-d8 (IS) chromatograms.



11 Fig. S3. UPLC–MS/MS quantitative and qualitative ion chromatograms of for each

12 compound in a milk sample spiked at 0.01 mg/L.



15 Fig. S4. Total ion chromatogram (TIC) of 9 compounds (1: E3; 2: HC; 3: DXMS; 4:

16 E2; 5: EE; 6: E1; 7: DES; 8: OHP; 9: HEX).

Sample	No.	Concentration of compound								
		OHP	HC	DXMS	E2	E3	E1	DES	HEX	EE
Milk powder	1	0.92 ± 0.04	2.68 ± 0.41	n.d.	n.d.	n.d.	0.71 ± 0.05	n.d.	n.d.	n.d.
	2	0.85 ± 0.06	3.35 ± 0.38	n.d.	n.d.	n.d.	0.46 ± 0.03	n.d.	n.d.	n.d.
	3	1.05 ± 0.06	3.16 ± 0.36	<loq< td=""><td>n.d.</td><td>n.d.</td><td>0.70 ± 0.05</td><td>n.d.</td><td>n.d.</td><td>n.d.</td></loq<>	n.d.	n.d.	0.70 ± 0.05	n.d.	n.d.	n.d.
	4	n.d.	0.24 ± 0.03	n.d.	<loq< td=""><td>n.d.</td><td>0.43 ± 0.03</td><td>n.d.</td><td>n.d.</td><td>n.d.</td></loq<>	n.d.	0.43 ± 0.03	n.d.	n.d.	n.d.
	5	n.d.	1.01 ± 0.04	n.d.	n.d.	n.d.	0.48 ± 0.04	n.d.	n.d.	n.d.
	6	0.79 ± 0.02	1.76 ± 0.10	n.d.	<loq< td=""><td>n.d.</td><td>0.62 ± 0.05</td><td>n.d.</td><td>n.d.</td><td>n.d.</td></loq<>	n.d.	0.62 ± 0.05	n.d.	n.d.	n.d.
	7	0.94 ± 0.06	1.45 ± 0.12	n.d.	n.d.	n.d.	0.71 ± 0.04	n.d.	n.d.	n.d.
Milk	1	<loq< td=""><td>0.04 ± 0.005</td><td>n.d.</td><td>n.d.</td><td>n.d.</td><td>0.21 ± 0.03</td><td>n.d.</td><td>n.d.</td><td>n.d.</td></loq<>	0.04 ± 0.005	n.d.	n.d.	n.d.	0.21 ± 0.03	n.d.	n.d.	n.d.
	2	<loq< td=""><td>n.d.</td><td>n.d.</td><td><loq< td=""><td>n.d.</td><td>0.21 ± 0.03</td><td>n.d.</td><td>n.d.</td><td>n.d.</td></loq<></td></loq<>	n.d.	n.d.	<loq< td=""><td>n.d.</td><td>0.21 ± 0.03</td><td>n.d.</td><td>n.d.</td><td>n.d.</td></loq<>	n.d.	0.21 ± 0.03	n.d.	n.d.	n.d.
	3	0.33 ± 0.03	n.d.	n.d.	<loq< td=""><td>n.d.</td><td>0.27 ± 0.02</td><td>n.d.</td><td>n.d.</td><td>n.d.</td></loq<>	n.d.	0.27 ± 0.02	n.d.	n.d.	n.d.
	4	0.30 ± 0.01	n.d.	n.d.	<loq< td=""><td>n.d.</td><td>0.24 ± 0.01</td><td>n.d.</td><td>n.d.</td><td>n.d.</td></loq<>	n.d.	0.24 ± 0.01	n.d.	n.d.	n.d.

17 Table S1. Analysis of real milk powder and milk samples using the UPLC–MS/MS combined with modified QuEChERS method (n=3).

18 Note: n.d.: not detected.