SUPPLEMENTARY INFORMATION for Research Article:

Profile of steroid metabolites in human breast milk in different stages of lactation.

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I. Supplementary Methodology

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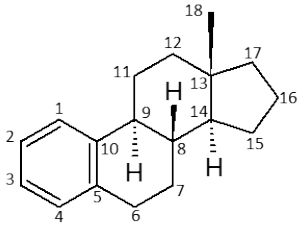
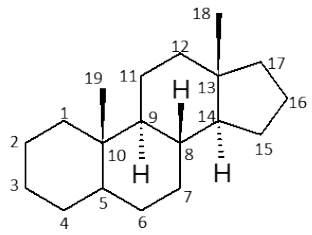
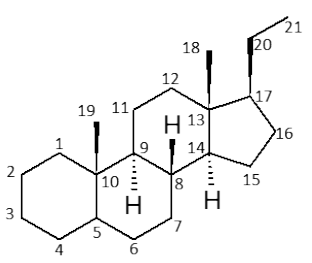
In-house sulfated steroid standards synthesis

For *in-house* synthesis of qualitative (QL) sulfated standards, next steroids were purchased: 2-hydroxy-estrone (2-OH-E1), 4-hydroxy-estrone (4-OH-E1), pregnane-5α,3α,20α-diol (PD-5α,3α,20α), pregnane-5β,3α,20α-diol (PD-5β,3α,20α), 5α-pregnan-3α-ol-20-one (alloP), pregn-5-ene-3β,20α-diol (5PD-3β20α) from Steraloids INC., (Newport, RI, USA). Sulfur trioxide pyridine complex (SO_3·py) was obtained from Sigma–Aldrich (Louis, MO, USA) and *N,N*-Dimethylformamide (DMF) was provided by Merck (Darmstadt, Germany).

The *in-house* synthesis of steroid sulfates as the ammonium salts was performed as previously described [McLeod et al., 2017] with small modifications. Briefly, 100 µg of each steroid standard was directly dissolved in 100 µL freshly prepared 5% solution of SO_3.py complex in DMF and incubated at room temperature for 2-12 h. The success of synthesis was confirmed by analysis of reaction using both LC–MS in scan mode and LC–MS/MS for CID studies. Afterward, 1 mL of Millipore water was added to stop the reaction and the purification of synthesized bis-sulfates was performed following earlier SPE described protocol [Waller CC et al., 2014]. Synthesized *in-house* sulfated steroids were defined as qualitative standards.

II. Supplementary Tables

Table 1S. Structures of breast milk steroid conjugated metabolites detected in BM within present study. The backbone structures of each steroid family are presented. The steroid carbons, subjects to metabolic modifications, are characterized for each conjugated metabolite in relation to steroid family and metabolic group. For individual structure of each conjugated metabolite detected in the present study, please refer to Supplementary Tables 2S.

Conjugated residues:			Sulfate residue (**S**)		Glucuronic acid residue (**G**)			
Estrogen Metabolites (ESTR)			**C2**	**C3**	**C4**	**C16**	**C17**	
	E1	E1-3S	-H	-O-**S**	-H	-H	=O	
		E1-3G	-H	-O-**G**	-H	-H	=O	
		2OH-E1-3S	-OH	-O-**S**	-H	-H	=O	
		4OH-E1-3S	-H	-O-**S**	-OH	-H	=O	
	E2	E2-3S	-H	-O-**S**	-H	-H	-βOH	
		E2-3G	-H	-O-**G**	-H	-H	-βOH	
		E2-17βG	-H	-OH	-H	-H	-βO-**G**	
	E3	E3-3S	-H	-O-**S**	-H	-αOH	-βOH	
		E3-3G	-H	-O-**G**	-H	-αOH	-βOH	
		E3-16αG	-H	-OH	-H	-αO-**G**	-βOH	
Androgen Metabolites								
	**4-Androsten (4A)**		**C3**	**C5**	**C16**	**C17**		
	AN	AN-S	-αO-**S**	-αH	-H	=O		
		AN-G	-αO-**G**	-αH	-H	=O		
		AD-5α3α17β-3S	-αO-**S**	-αH	-H	-βOH		
		AD-5α3α17β-SS	-αO-**S**	-αH	-H	-βO-**S**		
	Etio	Etio-S	-αO-**S**	-βH	-H	=O		
		Etio-G	-αO-**G**	-βH	-H	=O		
		AD-5β3α17β-3S	-αO-**S**	-βH	-H	-βOH		
		AD-5β3α17β -SS	-αO-**S**	-βH	-H	-βO-**S**		
	epiAN	epiAN-S	-βO-**S**	-αH	-H	=O		
		AD-5α3β17β-3S	-βO-**S**	-αH	-H	-βOH		
		AD-5α3β17β -SS	-βO-**S**	-αH	-H	-βO-**S**		
	T	T-S	=O	Δ^4	-H	-βO-**S**		
	epiT	epiT-S	=O	Δ^4	-H	-αO-**S**		
		epiT-G	=O	Δ^4	-H	-αO-**G**		
	5-Androsten (5A)		**C3**	**C5**	**C16**	**C17**		
	DHEA	DHEA-3S	-βO-**S**	Δ^5	-H	=O		
		16αOH-DHEA-3S	-βO-**S**	Δ^5	-αOH	=O		
		16βOH-DHEA-3S	-βO-**S**	Δ^5	-βOH	=O		
		16βOH-DHEA-SS	-βO-**S**	Δ^5	-βO-**S**	=O		
	5AED	5AED-3β17β-3S	-βO-**S**	Δ^5	-H	-βOH		
		5AED-3β17β-SS	-βO-**S**	Δ^5	-H	-βO-**S**		
		5AED-3α17β-3S	-αO-**S**	Δ^5	-H	-βOH		
		5AED-3α17β-SS	-αO-**S**	Δ^5	-H	-βO-**S**		
Progestogen Metabolites								
	**4-Pregnen (4P)**		**C3**	**C5**	**C16**	**C17**	**C20**	
	alloP	alloP-3S	-αO-**S**	-αH	-H	-H	=O	
		PD-5α3α20α-3S	-αO-**S**	-αH	-H	-H	-αOH	
		PD-5α3α20α-3G	-αO-**G**	-αH	-H	-H	-αOH	
		PD-5α3α20α-20S	-αOH	-αH	-H	-H	-αO-**S**	
		PD-5α3α20α-SS	-αO-**S**	-αH	-H	-H	-αO-**S**	
		PD-5α3α20α-SG	-αO-**S/G**	-αH	-H	-H	-αO-**G/S**	
	P	P-3S	-αO-**S**	-βH	-H	-H	=O	
		PD-5β3α20α-3S	-αO-**S**	-βH	-H	-H	-αOH	
		PD-5β3α20α-3G	-αO-**G**	-βH	-H	-H	-αOH	
		PD-5β3α20α-20S	-αOH	-βH	-H	-H	-αO-**S**	
		PD-5β3α20α-SS	-αO-**S**	-βH	-H	-H	-αO-**S**	
		PD-5β3α20α -SG	-αO-**S/G**	-βH	-H	-H	-αO-**G/S**	

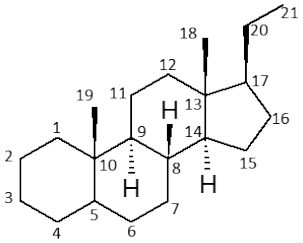
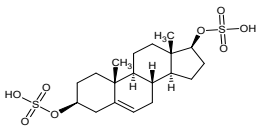
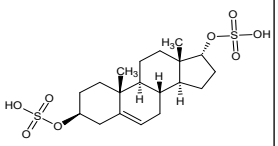
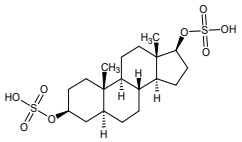
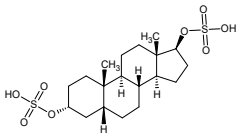
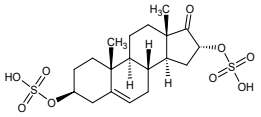
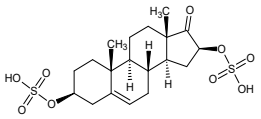
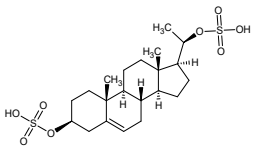
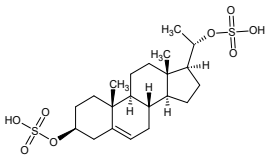
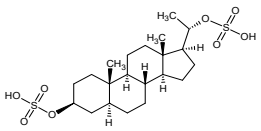
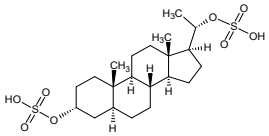
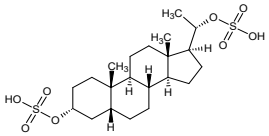
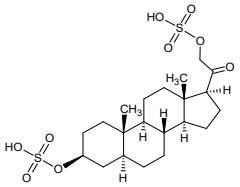
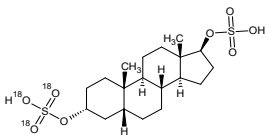
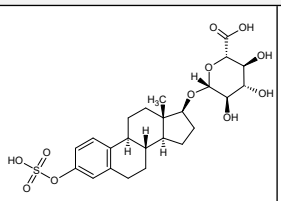
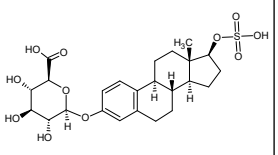
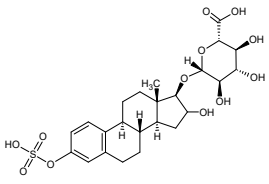
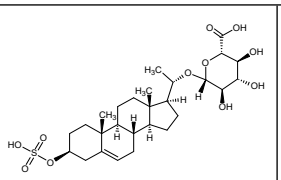
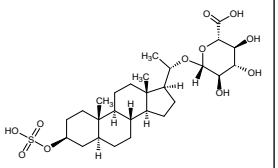
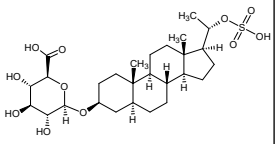
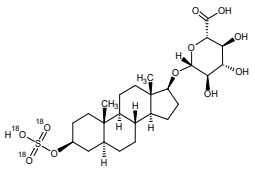
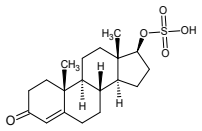
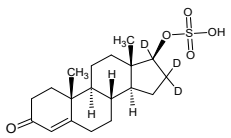
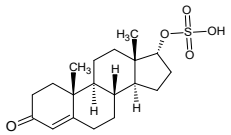
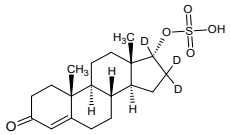
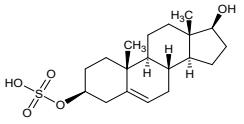
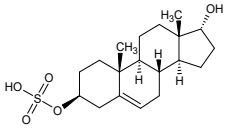
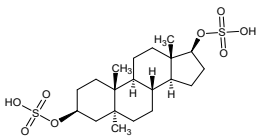
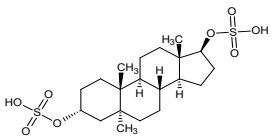
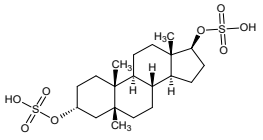
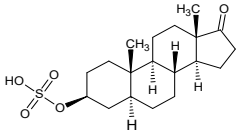
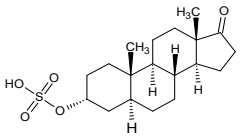
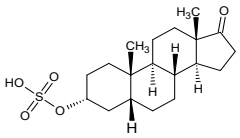
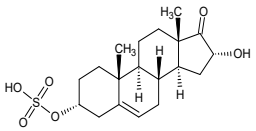
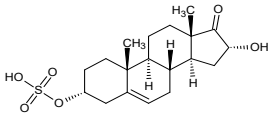
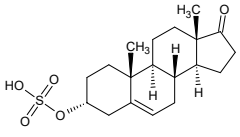
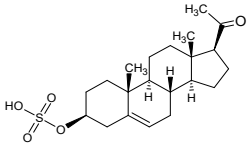
	EAP	EAP-3S	-βO-**S**	-αH	-H	-H	=O	
		PD-5α3β20α-3S	-βO-**S**	-αH	-H	-H	-αOH	
		PD-5α3β20α-20S	-βOH	-αH	-H	-H	-αO-**S**	
		PD-5α3β20α-SS	-βO-**S**	-αH	-H	-H	-αO-**S**	
		PD-5α3β20α-SG	-βO-**S/G**	-αH	-H	-H	-αO-**G/S**	
	epiP	epiP-3S	-βO-**S**	-βH	-H	-H	=O	
	5-Pregnen (5P)		**C3**	**C5**	**C16**	**C17**	**C20**	
	5P	5P-S	-βO-**S**	Δ^5	-H	-H	=O	
		5PED-3β20α-3S	-βO-**S**	Δ^5	-H	-H	-αOH	
		5PED-3β20α-20S	-βOH	Δ^5	-H	-H	-αO-**S**	
		5PED-3β20α-SG	-βO-**S/G**	Δ^5	-H	-H	-αO-**G/S**	
Corticoid Metabolites (CORT)								
	**Glucocorticoid**		**C3**	**C4/C5**	**C11**	**C17**	**C20**	**C21**
	E	E-21S	=O	Δ^4	=O	-αOH	=O	-O-**S**
		THE-3G	α-O-**G**	-βH	=O	-αOH	=O	-OH
	F	F-21S	=O	Δ^4	-βOH	-αOH	=O	-O-**S**
		F-21G	=O	Δ^4	-βOH	-αOH	=O	-O-**G**
	Mineralocorticoid		**C3**	**C4/C5**	**C11**	**C17**	**C20**	**C21**
	B	B-21S	=O	Δ^4	-βOH	-H	=O	-O-**S**
		21OH-5P-SS	-βO-**S**	Δ^5	-βOH	-H	=O	-O-**S**

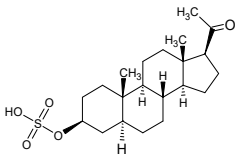
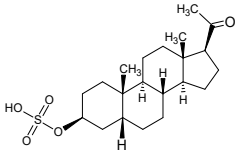
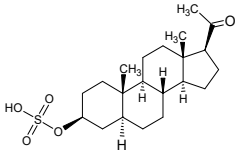
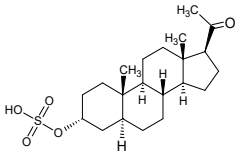
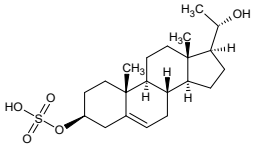
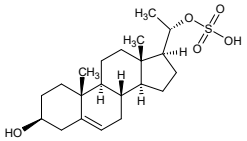
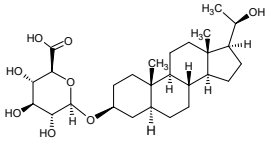
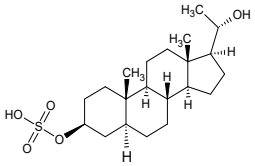
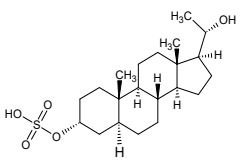
Table 2S. Structural and mass spectrometric features used for detection of conjugated steroids (QN – quantitative standards, QL - qualitatively detected and PA - putatively annotated) and corresponding internal standards (ISTD, SS – bis-sulfated, SG – sulfate-glucuronylated, S – mono-sulfated, and G – mono-glucuronylated). The origin of QN and QL standards is index-marked and disclosed in footnote of the table.

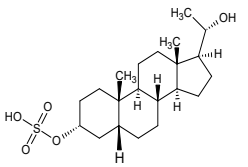
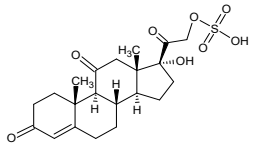
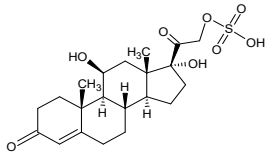
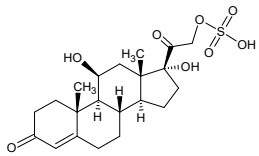
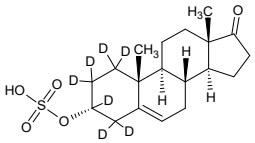
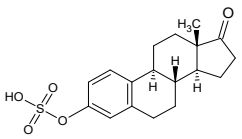
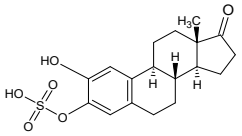
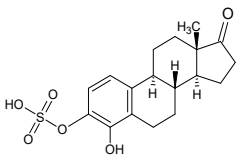
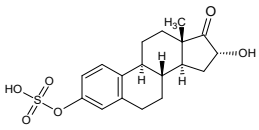
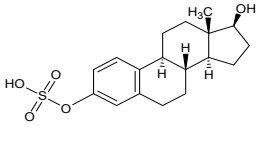
Analyte (MW)	**Acronym (type of standard)**	**Structure**	**Prec (m/z)**	**Col (eV)**	**Prod (m/z)**
Bis-sulfated steroids*					
Androst-5-ene-3β,17β-diol bis-sulfate[i] (450)	5AD-3β,17β-SS (QN)		224	15	351
Androst-5-ene-3α,17β-diol bis-sulfate[ii] (450)	5AD-3α,17β-SS (QL)		224	15	351
5α-androstane-3β,17β-diol bis-sulfatei (452)	AD-5α3β17β-SS (QN)		225	15	353
5β-androstane-3α,17β-diol bis-sulfatei (452)	AD-5β3α17β-SS (QN)		225	15	353
16α-hydroxy-dehydroepiandrosterone bis-sulfatei (464)	16αOH-DHEA-SS (QN)		231	15	367
16β-hydroxy-dehydroepiandrosterone bis-sulfatei (464)	16βOH-DHEA-SS (QN)		231	15	367
Pregn-5-ene-3β,20β-diol bis-sulfatei (478)	5PED-3β20β-SS (QN)		238	15	379
Pregn-5-ene-3β,20α-diol bis-sulfatei (478)	5PED-3β20α-SS (QL)		238	15	379

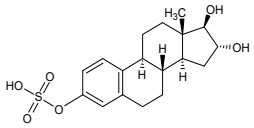
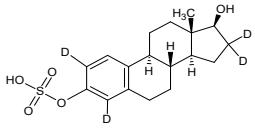
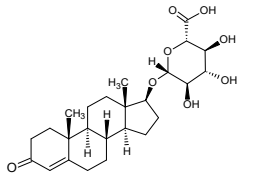
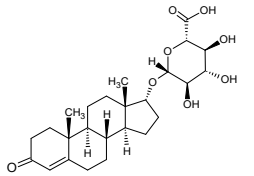
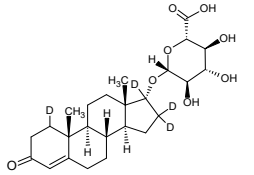
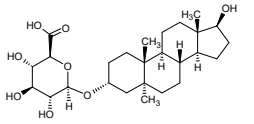
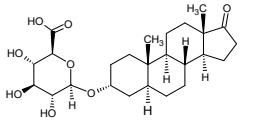
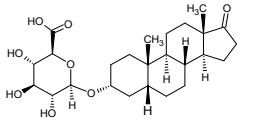
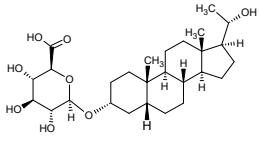
5α-pregnane-3β,20α-diol bis-sulfatei (480)	PD-5α3β20α-SS (QN)		239	15	381
5α-pregnane-3α,20α-diol bis-sulfateii (480)	PD-5α3α20α-SS (QL)		239	15	381
5β-pregnane-3α,20α-diol bis-sulfateii (480)	PD-5β3α20α-SS (QL)		239	15	381
21-hydroxy-pregnenolone-bis sulfatei (492)	21OH-5P-SS (QN)		245	15	393
17-S{18O}3-5β-androstan-3α,17β-diol 3,17-bis-sulfatei (458)	ISTD-SS		228	15	359
Sulfo- glucuronylated steroids*					
Estradiol 3-sulfate-17β-gluconidate[iii] (528)	E2-3S17βG (QN)		263	15	351
Estradiol 3-glucuronidate-17β-sulfateiii (528)	E2-3G17βS (QN)		263	15	351
Estriol sulfo-glucuronidate (544)	E3-SG (PA)		271	15	351

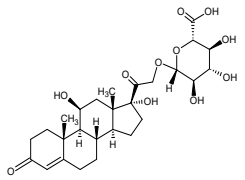
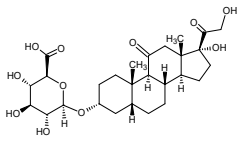
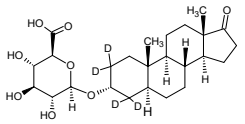
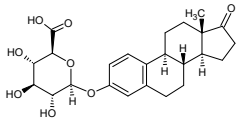
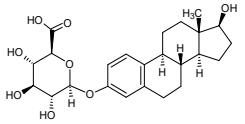
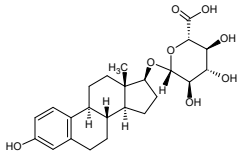
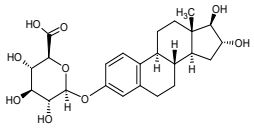
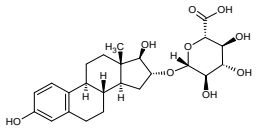
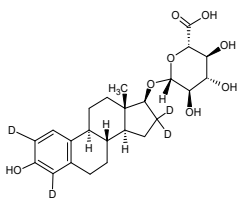
Pregn-5-ene-3β,20α-diol 3β-sulfate, 20α-glucuronideiii (574)	5PD-3β20α-3S20G (QN)		286	15	397
5α-pregnane-3β,20α-diol, 3β-sulfate, 20α-glucuronideiii (576)	PD-5α3β20α-3S20G (QN)		287	15	399
5α-pregnane-3β,20α-diol, 3β-glucuronide, 20α-sulfateiii (576)	PD-5α3β20α-3G20S (QN)		287	15	399
5α-androstanediol-3β-{18O3}sulfate, 17β-glucuronideiii (554)	ISTD-SG		276	15	377
Mono-sulfated steroids					
Testosterone sulfate[iv] (368)	T-S (QN)		367	30	97
Testosterone-d3 sulfateiv (371)	ISTD-S-1		370	30	98
Epitestosterone sulfateiv (368)	epiT-S (QN)		367	30	97
Epitestosterone-d3 sulfateiv (371)	ISTD-S-2		370	30	98
Androst-5ene-3β, 17β-diol 3-sulfateii (370)	5AED-3β17β-3S (QL)		369	30	97
Androst-5ene-3β, 17α-diol 3-sulfateii (370)	5AED-3β17α-3S (PA)		369	30	97

5α-androstane-3β, 17β-diol 3-sulfatei (372)	AD-5α3β17β-3S (QN)		371	30	97
5α-androstane-3α, 17β-diol 3-sulfatei (372)	AD-5α3α17β-3S (QN)		371	30	97
5β-androstane-3α, 17β-diol 3-sulfatei (372)	AD-5β3α17β-3S (QN)		371	30	97
Epiandorsterone sulfate[v] (370)	epiAN-S (QN)		369	35	97
Androsterone sulfateiv (370)	AN-S (QN)		369	35	97
Etiocholanolone sulfateiv (370)	Etio-S (QN)		369	35	97
16α-hydroxy-dehydroepiandrosterone 3-sulfate[vi] (383)	16αOH-DHEA-3S (QN)		383	30	97
16β-hydroxy-dehydroepiandrosterone 3-sulfateii (383)	16βOH-DHEA-3S (QL)		383	30	97
Dehydroepiandrosterone sulfatev (368)	DHEA-S (QN)		367	30	97
Pregn-5-ene-3β-ol-20-one sulfatevi (396)	5P-S (QN)		395	30	97

5β-Pregnan-3α-ol-20-one sulfatevi (398)	P-S (QN)		397	30	97
5β-pregnan-3β-ol-20-one sulfatevi (398)	epiP-S (QN)		397	30	97
5α-pregnan-3β-ol-20-one sulfatevi (398)	EAP-S (QN)		397	30	97
5α-pregnan-3α-ol-20-one sulfateii (398)	alloP-S (QL)		397	30	97
Pregn-5-ene-3β, 20-one 3-sulfatevi (398)	5PED-3β20α-3S (QN)		397	30	97
Pregn-5-ene-3β, 20-one 20-sulfateii (398)	5PED-3β20α-20S (QL)		397	30	97
5α-pregnan-3β, 20β-diol 3-sulfatevi (400)	PD-5α3β20β-3S (QN)		399	40	97
5α-pregnan-3β, 20α -diol 3-sulfateii (400)	PD-5α3β20α-3S (QL)		399	40	97
5α-pregnan-3α, 20α -diol 3-sulfateii (400)	PD-5α3α20α-3S (QL)		399	40	97

5β-pregnan-3α, 20α -diol 3-sulfateii (400)	PD-5β3α20α-3S (QL)		399	40	97
Cortisone 21-sulfate[vii] (440)	E-S (QN)		439	35	97
Cortisol 21-sulfatevii (442)	F-S (QN)		441	35	97
Corticosterone 21-sulfate (426)	B-21S (QN)		425	35	97
Dehydroepiandrosterone-d7 sulfatev (374)	ISTD-S-3		373	30	98
Estrone sulfatev (350)	E1-S (QN)		349	30	269
2-hydroxyestrone 3-sulfateii (366)	2OH-E1-3S (QL)		365	30	285
4-hydroxyestrone 3-sulfateii (366)	4OH-E1-3S (QL)		365	30	285
16-hydroxyestrone 3-sulfate (366)	16OH-E1-3S (QL)		365	30	283
Estradiol 3-sulfate (352)	E2-3S (QN)		351	30	271

Estriol 3-sulfateviii (368)	E3-3S (QN)		367	35	287
Estradiol-d4 3-sulfate (355)	ISTD-S-4		354	35	274
Mono-glucuronylated steroids					
Testosterone glucuronideiv (464)	T-G (QN)		463	30	75
Epitestosterone glucuronideiv (464)	epiT-G (QN)		463	30	75
Epitestosterone-d4 glucuronideiv (468)	ISTD-G-1		467	30	75
5α-androstane-3α, 17β-diol 3-glucuronideiv (468)	AD-5α3α17β-3G (QN)		467	30	75
Androsterone glucuronideiv (466)	AN-G (QN)		465	30	75
Etiocholanolone glucuronidevi (466)	Etio-G (QN)		465	30	75
5β-pregnan-3α, 20α -diol 3-glucuronidevi (496)	PD-5β3α20α-3G (QN)		495	30	75
Pregnanediol glucuronide-1 (496)	PD-G-1 (PA)		495	30	75

Pregnanediol glucuronide-2 (496)	PD-G-2 (PA)	PD-5a3a20a-?G	495	30	75
Cortisol 21-glucuronidevii (538)	F-G (QN)		537	30	75
Tetrahydrocortisone 3-glucuronide[viii] (540)	THE-3G (QN)		539	30	75
Androsterone-d4 glucuronide (470)	ISTD-G-2		469	35	75
Estrone glucuronidevi (446)	E1-G (QN)		445	30	269
Estradiol 3-glucuronidevi (448)	E2-3G (QN)		447	30	271
Estradiol 17β-glucuronide (448)	E2-17βG (QN)		447	30	271
Estriol 3-glucuronideviii (464)	E3-3G (QN)		463	30	287
Estriol 16a-glucuronideviii (464)	E3-16aG (QN)		463	30	287
Estradiol-d4 17β-glucuronide (451)	ISTD-G-3		450	20	113[ix]

** - bis-conjugated steroid metabolites (bis-sulfated and sulfo-glucuronidated) were detected using constant ion loss method, as described earlier [McLeod M.D. et al, 2017]. Their precursors, being dianions ($[M - 2H]^{2-}$) due to the presence of two ionizable parts on the molecule, were fragmented to generate as products mono-ions due to the loss of one ionizable part: sulfate, in case of bis-sulphates, or glucuronide, in case of sulfo-glucuronides.*

[i] - custom quantitative synthesis, as described earlier [Waller CC et al., 2017]
[ii]- *in-house* qualitative synthesis, as described earlier [McLeod MD et al., 2017]
[iii] - custom quantitative synthesis, as described earlier [Pranata A et al., 2018]
[iv] - National Measurements Institute (Sydney, Australia)
[v] - Sigma Aldrich (St Louis, MO, USA)
[vi] - Steraloids (Newport RI, USA)
[vii] - Toronto Research Chemicals (Toronto, Canada)
[viii] - kindly provided by colleagues Dr. Lorna Gilligan and Prof. Celdric Schakelton

Table 3S Participants' data and relevant breast-feeding information.

	Colostrum (n=18)	**Transion (n=17)**	**Mature (n=25)**	**Total (n=60)**
Mother´s age (Mean ± SD (range), years)	35.1 ± 5.0 (23 – 44)	33.8 ± 7.6 (20 – 50)	34.4 ± 7.1 (22 – 44)	34.4 ± 6.6 (20 – 50)
Gestation terms (Mean ± SD (range), weeks)	39.4 ± 1.4 (36 – 41.4)	37.6 ± 3.2 (30.4 – 42)	37.7 ± 4.6 (27 – 42)	38.2 ± 3.6 (27 – 42)
Age of infants (Mean ± SD (range), days)	4.2 ± 1.3 (1 – 6)	11.6 ± 3.8 (7 – 19)	32.7 ± 6.7 (20 – 51)	18.2 ± 13.6 (1 – 51)
Type of birth (n)				
On term (>37 weeks)	n = 17	n = 14	n = 20	n = 51
Preterm (<37 weeks)	n = 1	n = 3	n = 5	n = 9
Type of delivery (n)				
Vaginal	n = 12	n = 10	n = 19	n = 38
Cesarean	n = 6	n = 7	n = 6	n = 18
Sex of a newborn (n)				
Female	n = 10	n = 12	n = 9	n = 31
Male	n = 9	n = 5	n = 16***	n = 31***
Fraternal twins	n = 2	n = 0	n = 0	n = 2
Type of sample (n)				
Whole breast milk*	n = 1	n = 6	n = 5	n = 12
Foremilk**	n = 17	n = 11	n = 20	n = 48
Time of sampling (n)				
Morning (7 am – 2 pm)	n = 15	n = 12	n = 17	n = 44
Evening (after 2 pm)	n = 3	n = 5	n = 8	n = 16

*- an aliquot of whole milk (foremilk + hind milk) collected from one breast

**- an aliquot of foremilk collected from one breast

***- one delivery was of male twins

Table 4S. Parameters used in quantitative analysis of phase II conjugated steroids detected in breast milk samples. Calibrators, analytes with quantitative standards, and corresponding parameters of quantification which were used for relative quantification of resemble to them compounds (listed right below corresponding calibrator) are marked in bold letters.

Analyte	RT (min)	Calibrator	ISTD	Ranges (ng mL^{-1})	LOD (ng mL^{-1})	Recoveries (mean±SD, %)	Matrix Effect (%)		
							Colostrum	Transition	Mature
Bis-sulfated steroids									
5AED-3β17β-SS	6.56	**5AED-3β17β-SS**	ISTD-SS	**0.39 - 25**	**0.04**	**105 ± 8**	**73**	**72**	**93**
5AED-3α17β-SS*	7.43	5AED-3β17β-SS	ISTD-SS						
AD-5β3α17α-SS	8.41	**AD-5β3α17α-SS**	ISTD-SS	**0.195 - 50**	**0.01**	**107 ± 24**	**142**	**146**	**219**
AD-5α3β17β-SS	7.73	AD-5β3α17α-SS	ISTD-SS						
AD-SS	8.28	AD-5β3α17α-SS	ISTD-SS						
AD-5α3α17β-SS	8.22	AD-5β3α17α-SS	ISTD-SS						
AD-5β3α17β-SS	8.85	AD-5β3α17α-SS	ISTD-SS						
16βOH-DHEA-SS	5.66	**16βOH-DHEA-SS**	ISTD-SS	**0.195 - 50**	**0.04**	**98 ± 8**	**177**	**100**	**93**
16αOH-DHEA-SS*	6.15	16βOH-DHEA-SS	ISTD-SS						
5PED-3β20β-SS	8.68	**5PD-3β20β-SS**	ISTD-SS	**0.16 – 41.70**	**0.08**	**98 ± 8**	**177**	**100**	**93**
5PED-3β20α-SS*	7.78	5PD-3β20β-SS	ISTD-SS						
PD-5α3β20α-SS	7.26	**PD-5α3β20α-SS**	ISTD-SS	**0.39 - 50**	**0.05**	**100 ± 22**	**50**	**95**	**135**
PD-5α3α20α-SS*	7.40	PD-5α3β20α-SS	ISTD-SS						
PD-5β3α20α-SS*	8.49	PD-5α3β20α-SS	ISTD-SS						
21-OH-5P-SS*	7.40	PD-5α3β20α-SS	ISTD-SS						
Sulfo-glucuronylated steroids									
E2-3S17βG	3.27	E2-3S17βG	ISTD-SG	0.195 - 50	0.1	91 ± 8	71	68	69
E2-3G17βS	3.97	**E2-3G17βS**	ISTD-SG	**0.195 - 50**	**0.1**	**89 ± 18**	**47**	**62**	**47**
E3-SG**	2.99	E2-3G17βS	ISTD-SG						

5PED-3β20α-3S,20G	5.77	**5PD-3β20α-3S,20G**	ISTD-SG	**0.195 - 50**	**0.05**	**99 ± 9**	**66**	**102**	**124**
PD-SG_1**	5.48	5PD-3β20α-3S,20G	ISTD-SG						
PD-SG_2**	6.16	5PD-3β20α-3S,20G	ISTD-SG						
PD-SG_3**	7.72	5PD-3β20α-3S,20G	ISTD-SG						
PD-SG_4**	7.75	5PD-3β20α-3S,20G	ISTD-SG						
Mono-sulfated steroids									
T-S	9.87	**T-S**	ISTD-S_1	**0.195 - 50**	**0.02**	**101 ± 3**	**100**	**107**	**103**
epiT-S	10.29	**epiT-S**	ISTD-S_2	**0.195 - 50**	**0.02**	**100 ± 2**	**93**	**104**	**100**
epiAN-S	11.45	**epiAN-S**	ISTD-S_3	**0.195 - 50**	**0.02**	**100 ± 7**	**78**	**94**	**110**
AN-S	11.95	**AN-S**	ISTD-S_3	**0.39 - 50**	**0.02**	**99 ± 8**	**143**	**150**	**137**
Etio-S	12.13	**Etio-S**	ISTD-S_3	**0.195 - 50**	**0.02**	**106 ± 13**	**110**	**149**	**143**
DHEA-S	10.97	**DHEA-S**	ISTD-S_3	**0.39 - 100**	**0.02**	**109 ± 4**	**104**	**100**	**108**
16αOH-DHEA-3S	8.18	**16αOH-DHEA-3S**	ISTD-S_3	**0.195 - 50**	**0.01**	**97 ± 7**	**190**	**62**	**85**
16βOH-DHEA-3S*	6.79	16αOH-DHEA-3S	ISTD-S_3						
AD-5α3β17β-3S	10.00	**AD-5α3β17β-3S**	ISTD-S_3	**0.195 - 50**	**0.02**	**106 ± 13**	**89**	**80**	**75**
AD-5α3α17β-3S	10.54	AD-5α3β17β-3S	ISTD-S_3						
AD-5β3α17β-3S	10.96	AD-5α3β17β-3S	ISTD-S_3						
5AED-3α17β-3S*	9.30	AD-5α3β17β-3S	ISTD-S_3						
5AED-3β17β-3S*	9.40	AD-5α3β17β-3S	ISTD-S_3						
5P-S	13.41	**5P-S**	ISTD-S_3	**0.195 - 50**	**0.02**	**77 ± 12**	**9**	**38**	**54**
P-S	14.18	**P-S**	ISTD-S_3	**0.195 - 50**	**0.01**	**89 ± 12**	**17**	**44**	**53**
EAP-S	13.88	**EAP-S**	ISTD-S_3	**0.195 - 50**	**0.01**	**89 ± 19**	**26**	**20**	**44**
epiP-S	13.65	**epiP-S**	ISTD-S_3	**0.195 - 50**	**0.01**	**86 ± 13**	**17**	**34**	**46**

alloP-S*	14.05	epiP-S	ISTD-S_3						
PD-5α3β20β-3S	12.70	**PD-5α3β20β-3S**	ISTD-S_3	**0.195 - 50**	**0.02**	**82 ± 17**	**18**	**63**	**73**
5PED-3β20α-3S*	10.76	PD-5α3β20β-3S	ISTD-S_3						
5PED-3β.20-one-3S*	11.90	PD-5α3β20β-3S	ISTD-S_3						
PD-5 β3α20α-3S*	12.70	PD-5α3β20β-3S	ISTD-S_3						
PD-5α3β20α-20S*	12.36	PD-5α3β20β-3S	ISTD-S_3						
PD-5α3α20α-20S*	13.05	PD-5α3β20β-3S	ISTD-S_3						
PD-5 β3α20α-20S*	13.34	PD-5α3β20β-3S	ISTD-S_3						
PD-5α3β20α-20S*	11.34	PD-5α3β20β-3S	ISTD-S_3						
PD-5α3α20α-3S*	12.60	PD-5α3β20β-3S	ISTD-S_3						
E1-S	10.33	**E1-S**	ISTD-S_4	**0.39 - 50**	**0.02**	**94 ± 5**	**69**	**63**	**65**
2OH-E1-3S*	10.16	E1-S	ISTD-S_4						
4OH-E1-3S*	11.30	E1-S	ISTD-S_4						
E2-3S	9.31	**E2-3S**	ISTD-S_4	**0.195 - 25**	**0.02**	**93 ± 2**	**66**	**58**	**57**
E3-3S	5.05	**E3-3S**	ISTD-S_4	**0.39 – 6.25**	**0.002**	**99 ± 3**	**142**	**58**	**46**
B-21S	8.65	**B-21S**	ISTD-S_3	**0.39 – 6.25**	**0.002**	**97 ± 6**	**272**	**185**	**158**
E-21S	8.36	**E-21S**	ISTD-S_3	**0.39 – 12.5**	**0.002**	**107 ± 5**	**415**	**155**	**176**
F-21S	8.65	**F-21S**	ISTD-S_3	**0.39 – 12.5**	**0.002**	**107 ± 6**	**450**	**174**	**185**
Mono-glucuronylated steroids									
epiT-G	8.93	**epiT-G**	ISTD-G_1	**0.195 - 50**	**0.02**	**107 ± 8**	**98**	**101**	**109**
AN-G + **Etio-G**	9.89	**Etio-G**	ISTD-G_2	**0.195 - 50**	**0.01**	**99 ± 2**	**102**	**110**	**105**
PD-5β3α20α-3G	11.16	**PD-5β3α20α-3G**	ISTD-G_2	**0.195 - 50**	**0.04**	**99 ± 14**	**30**	**75**	**64**
PD-G_1**	10.56	PD-5β3α20α-3G	ISTD-G_2						
PD-G_2**	11.03	PD-5β3α20α-3G	ISTD-G_2						

F-21G	6.93	**F-21G**	ISTD-G_2	**0.195 – 3.13**	**0.02**	**88 ± 7**	**299**	**187**	**77**
THE-3G	6.30	**THE-3G**	ISTD-G_2	**0.195 - 50**	**0.1**	**76 ± 13**	**278**	**80**	**101**
E1-G	7.17	**E1-G**	ISTD-G_3	**0.195 - 50**	**0.04**	**95 ± 4**	**148**	**120**	**126**
E2-3G	6.33	**E2-3G**	ISTD-G_3	**0.195 - 50**	**0.04**	**101 ± 6**	**121**	**119**	**108**
E2-17βG	7.01	**E2-17βG**	ISTD-G_3	**0.195 - 50**	**0.04**	**94 ± 4**	**121**	**157**	**114**
E3-3G	2.31	**E3-3G**	ISTD-G_3	**0.195 - 50**	**0.05**	**86 ± 7**	**108**	**87**	**42**
E3-16αG	5.61	**E3-16αG**	ISTD-G_3	**0.195 - 50**	**0.04**	**90 ± 11**	**161**	**146**	**65**

*-analyte identified by available qualitative standard

**-analyte putatively annotated on the base of their LC-MS behavior

Table 5S. Concentrations of endogenous phase II steroid metabolites (nmol L^{-1}) monitored in three types of breast milks from the study, presented as Mean (calculated using concentration data where zero values were transformed to half of the lowest detected concentration for each metabolite) and concentration range (min-max) detected in a number of samples (n). Differences between breast milk groups are expressed as p-value.

Phase II steroid conjugated metabolite	**Colostrum (n=18)**	**Transition (n=17)**	**Mature (n=25)**	**p-value[i]**
ESTR				
E1-S	38.245 (0.08 – 194.33) n = 18	5.043 (0.13 – 62.94) n = 17	1.606 (0.18 – 13.74) n = 25	0.000
2OH-E1-3S	2.638 (0.08 – 14.30) n = 18	0.135 (0.03 – 0.86) n = 12	0.305 (0.03– 4.82) n = 20	0.000
4OH-E1-3S	1.152 (0.20 – 7.45) n = 17	0.042 (0.01 – 0.15) n = 9	0.190 (0.01 – 3.77) n = 12	0.000
E1-G	2.265 (0.05 – 13.90) n = 17	0.088 (0.02 – 0.72) n = 16	0.177 (0.01 – 3.86) n = 14	0.000
E2-3S	0.469 (0.00 – 3.27) n = 18	0.073 (0.002 – 0.64) n = 16	0.014 (0.003 – 0.07) n = 24	0.001
E2-3G	0.104 (0.00 – 0.31) n = 15	0.057 (0.00 – 0.11) n = 15	0.059 (0.00 – 0.15) n = 22	0.484
E2-17βG	0.080 (0.00 – 0.71) n = 9	0.028 (0.00 – 0.13) n = 6	0.030 (0.00 – 0.11) n = 11	0.612
E3-3S	13.922 (0.01 – 192.08) n = 18	0.541 (0.001 – 5.97) n = 17	0.025 (0.00 – 0.30) n = 25	0.000
E3-3G	1.535 (0.00 – 9.31) n = 17	0.052 (0.00 – 0.22) n = 12	0.069 (0.00 – 0.73) n = 5	0.000
E3-16αG	8.650 (0.05 – 54.36) n = 18	0.085 (0.02 – 0.41) n = 7	0.079 (0.11 – 1.40) n = 4	0.000
E3-SG	1.204 (0.01 – 9.85) n = 15	0.01 n = 1	0.03 n = 1	0.000
Total ESTR	70.264 (1.71 – 471.50)	6.150 (0.23 – 71.53)	2.560 (0.36 – 28.75)	0.000
4A				
T-S	1.276 (0.08 – 15.47) n = 17	0.121 (0.04 – 0.69) n = 11	0.114 (0.05 – 0.27) n = 23	0.000
epiT-S	2.494 (0.05 – 30.65) n = 18	0.189 (0.04 – 1.36) n = 17	0.095 (0.06 – 0.15) n = 25	0.000
epiT-G	0.214 (0.01 – 2.87) n = 12	0.017 (0.02 – 0.06) n = 8	0.014 (0.003 – 0.06) n = 14	0.081
AN-S	68.731 (0.65 – 447.34) n = 18	16.169 (0.29 – 196.70) n = 17	6.505 (0.36 – 14.46) n = 25	0.008
AN-G + Etio-G	3.154	0.410	0.434	0.035

	(0.16 – 43.09) n = 18	(0.12 – 2.35) n = 17	(0.10 – 1.42) n = 25	
AD-5α3α17β-3S	176.664 (3.78 – 2210.76) n = 18	15.757 (3.06 – 104.85) n = 17	7.961 (3.16 – 20.40) n = 25	0.042
AD-5α3α17β-SS	1.390 (0.12 – 7.60) n = 16	1.045 (0.10 – 1.81) n = 17	0.707 (0.19 – 3.18) n = 24	0.029
Etio-S	57.570 (0.57 – 522.82) n = 18	6.611 (1.10 – 42.17) n = 17	5.234 (0.62 – 19.15) n = 25	0.005
AD-5β3α17β-3S	0.364 (0.30 – 2.45) n = 7	0.481 (0.37 – 1.31) n = 11	0.508 (0.12 – 1.53) n = 19	0.112
AD-5β3α17β-SS	0.336 (0.13 – 7.6) n = 5	0.02 n = 1		0.008
epiAN-S	36.786 (1.74 – 157.28) n = 18	6.878 (1.10 – 63.42) n = 17	2.797 (1.06 – 6.23) n = 25	0.000
AD-5α3β17β-3S	4.811 (0.29 – 21.62) n = 17	0.953 (0.40 – 3.13) n = 12	0.970 (0.32 – 2.58) n = 24	0.009
AD-5α3β17β-SS	1.369 (0.09 – 11.31) n = 14	1.468 (0.03 – 3.82) n = 17	1.475 (0.29 – 2.75) n = 24	0.046
AD-SS	0.202 (0.11 – 1.62) n = 6	0.077 (0.02 – 0.34) n = 6	0.060 (0.04 – 0.37) n = 9	0.975
Total 4A	355.361 (14.60 – 2820.84)	50.187 (12.37 – 416.74)	26.886 (10.03 – 66.99)	0.010
5A				
DHEA-S	217.267 (8.02 – 2099.80) n = 18	42.717 (4.29 – 440.69) n = 17	18.744 (3.40 – 85.33) n = 25	0.000
16αOH-DHEA-3S	80.290 (0.12 – 1358.11) n = 18	1.096 (0.15 – 6.05) n = 17	0.454 (0.11 – 2.37) n = 25	0.000
16βOH-DHEA-3S	21.622 (0.02 – 362.01) n = 18	0.379 (0.00 – 3.52) n = 17	0.073 (0.02 – 0.37) n = 25	0.000
16αOH-DHEA-SS	(0.00 – 1.29) n = 2	-	-	
16βOH-DHEA-SS	0.727 (0.01 – 11.08) n = 15	0.031 (0.01 – 0.1) n = 11	0.026 (0.01 – 0.38) n = 10	0.005
5AED-3β17β-3S	84.708 (1.44 – 814.79) n = 18	12.434 (0.55 – 135.99) n = 17	5.936 (0.34 – 32.41) n = 25	0.003
5AED-3α17β-3S	6.612 (0.34 – 80.37) n = 18	0.302 (0.04 – 2.64) n = 13	0.218 (0.03 – 0.66) n = 24	0.000
5AED-3β17β-SS	4.245 (0.09 – 21.57) n = 18	4.674 (0.37 – 10.52) n = 17	5.103 (0.96 – 14.33) n = 25	0.146
5AED-3α17β-SS	1.409 (0.08 – 10.60) n = 15	0.190 (0.02 – 0.95) n = 16	0.255 (0.02 – 0.88) n = 25	0.133

Total 5A	416.903 (11.45 – 4738.89)	61.825 (7.45 – 596.67)	30.813 (7.17 – 109.92)	0.001
		5P		
5P-S	87.940 (0.68 – 520.15) n = 18	9.530 (0.13 – 87.37) n = 17	1.181 (0.42 – 2.61) n = 25	0.000
5PED-3β20α-3S	1.032 (0.07 – 4.33) n = 18	0.150 (0.02 – 0.93) n = 16	0.123 (0.01 – 0.34) n = 25	0.000
5PED-3β20α-20S	23.131 (0.54 – 115.51) n = 18	1.236 (0.12 – 10.55) n = 17	0.562 (0.16 – 1.62) n = 25	0.000
5PED-3β20α-SS	21.935 (0.55 – 141.26) n = 18	8.093 (0.43 – 23.44) n = 17	3.909 (0.73 – 10.57) n = 25	0.009
5PED-3βS-20αG	0.022 (0.01 – 0.11) n = 8	(0.01 – 0.03) n = 2	0.003 (0.003 – 0.02) n = 4	0.018
Total 5P	134.060 (2.11 – 666.11)	19.013 (1.47 – 122.30)	5.777 (1.44 – 14.46)	0.000
		4P		
alloP-S	239.851 (0.24 – 1784.18) n = 18	20.091 (0.07 – 170.04) n = 15	0.719 (0.01 – 4.14) n = 23	0.000
PD-5α3α20α-3S	2.979 (0.01 – 11.43) n = 17	0.300 (0.01 – 2.87) n = 14	0.018 (0.002 – 0.18) n = 19	0.000
PD-5α3α20α-20S	180.137 (0.59 – 612.25) n = 18	8.634 (0.09 – 38.20) n = 16	1.091 (0.04 – 10.23) n = 25	0.000
PD-G_2	52.060 (0.21 – 617.12) n = 18	1.271 (0.03 – 9.42) n = 12	0.399 (0.02 – 6.20) n = 25	0.000
PD-5α3α20α-SS	15.488 (0.86 – 147.21) n = 10	(0.14 – 2.47) n = 2		0.000
EAP-S	123.926 (0.08 – 517.34) n = 18	13.050 (0.05 – 126.02) n = 15	0.564 (0.01 – 2.43) n = 22	0.000
PD-5α3β20α-3S	4.197 (0.11 – 28.94) n = 17	0.087 (0.01 – 0.77) n = 14	0.023 (0.003 – 0.23) n = 24	0.000
PD-5α3β20α-20S	125.148 (0.51 – 579.28) n = 18	3.751 (0.07 – 26.18) n = 16	0.474 (0.04 – 4.54) n = 25	0.000
PD-5α3β20α-SS	49.518 (3.02 – 286.14) n = 16	2.470 (0.28 – 26.03) n = 8	0.260 (0.13 – 4.46) n = 4	0.000
P-S	47.947 (0.04 – 570.95) n = 17	4.123 (0.05 – 42.28) n = 13	0.137 (0.004 – 1.18) n = 18	0.000
PD-5β3α20α-3S	2.156 (0.02 – 8.63) n = 16	0.120 (0.01 – 1.38) n = 13	0.009 (0.002 – 0.04) n = 14	0.000
PD-5β3α20α-20S	33.676 (0.06 – 201.36) n = 18	2.262 (0.02 – 16.02) n = 16	0.177 (0.01 – 0.93) n = 25	0.000

PD-5β3α20α-3G	16.272 (0.47 – 135.85) n = 18	0.339 (0.03 – 1.44) n = 17	0.452 (0.08 – 2.39) n = 25	0.000
PD-5β3α20α-SS	0.169 (0.25 – 0.72) n = 2	5.07 n = 1		0.267
epiP-S	27.189 (0.03 – 188.48) n = 18	1.886 (0.10 – 20.26) n = 12	0.086 (0.01 – 0.53) n = 20	0.000
PD-G_1	0.318 (0.08 – 0.98) n = 13	0.200 (0.09 – 0.85) n = 9	0.186 (0.08 – 0.41) n = 22	0.416
PD-SG_1	0.049 (0.01 – 0.16) n = 9		0.02 n = 1	0.000
PD-SG_2	0.031 (0.01 – 0.26) n = 5		0.01 n = 1	0.010
PD-SG_3	0.233 (0.003 – 2.67) n = 8			0.000
PD-SG_4	0.062 (0.003 – 0.38) n = 6			0.000
Total 4P	921.398 (3.56 – 4785.89)	59.262 (0.20 – 453.06)	4.832 (0.42 – 37.44)	0.000
CORT				
E-21S	0.812 (0.005 – 11.88) n = 11	0.022 (0.01 – 0.21) n = 6	0.004 (0.004 – 0.02) n = 4	0.002
THE-3G	6.387 (0.61 – 44.31) n = 18	4.437 (0.31 – 15.25) n = 17	1.760 (0.58 – 9.03) n = 25	0.004
F-21S	4.161 (0.13 – 63.77) n = 16	0.226 (0.13 – 1.86) n = 4	0.117 (0.13 – 0.83) n = 6	0.000
F-21G	0.028 (0.00 – 0.10) n = 17	0.009 (0.00 – 0.05) n = 3	0.009 (0.00 – 0.07) n = 3	0.000
B-21S	0.355 (0.04 – 3.68) n = 13	0.048 (0.05 – 0.29) n = 7	0.069 (0.03 – 0.16) n = 22	0.014
21-OH-5P-SS	0.473 (0.003 – 4.12) n = 12	0.017 (0.002 – 0.06) n = 9	0.009 (0.04 – 0.04) n = 12	0.021
Total CORT	12.216 (0.66 – 126.21)	4.760 (0.36 – 15.27)	1.969 (0.58 – 10.12)	0.002
Total SS	97.262 (1.11 – 578.23)	18.713 (3.80 – 62.60)	12.017 (3.06 – 25.49)	0.001
Total SG	1.602 (0.00 – 10.72)	(0.00 - 0.03)	(0.00 - 0.03)	0.000
Total S	1720.251 (37.78 -10218.74)	175.436 (16.21 – 1588.79)	57.099 (18.04 – 135.73)	0.000
Total G	91.066 (2.82 – 804.56)	6.994 (1.21 – 18.76)	3.667 (1.59 – 16.64)	0.000
Total Phase II	1910.180	201.193	72.834	0.000

conjugates	(41.71 – 11602.65)	(32.78 – 1669.70)	(24.32 – 152.70)	

[i] P-value – according to Krustal-Wallis no-parametrical test for independent samples performed on 0-adjusted and log-transformed concentration data.

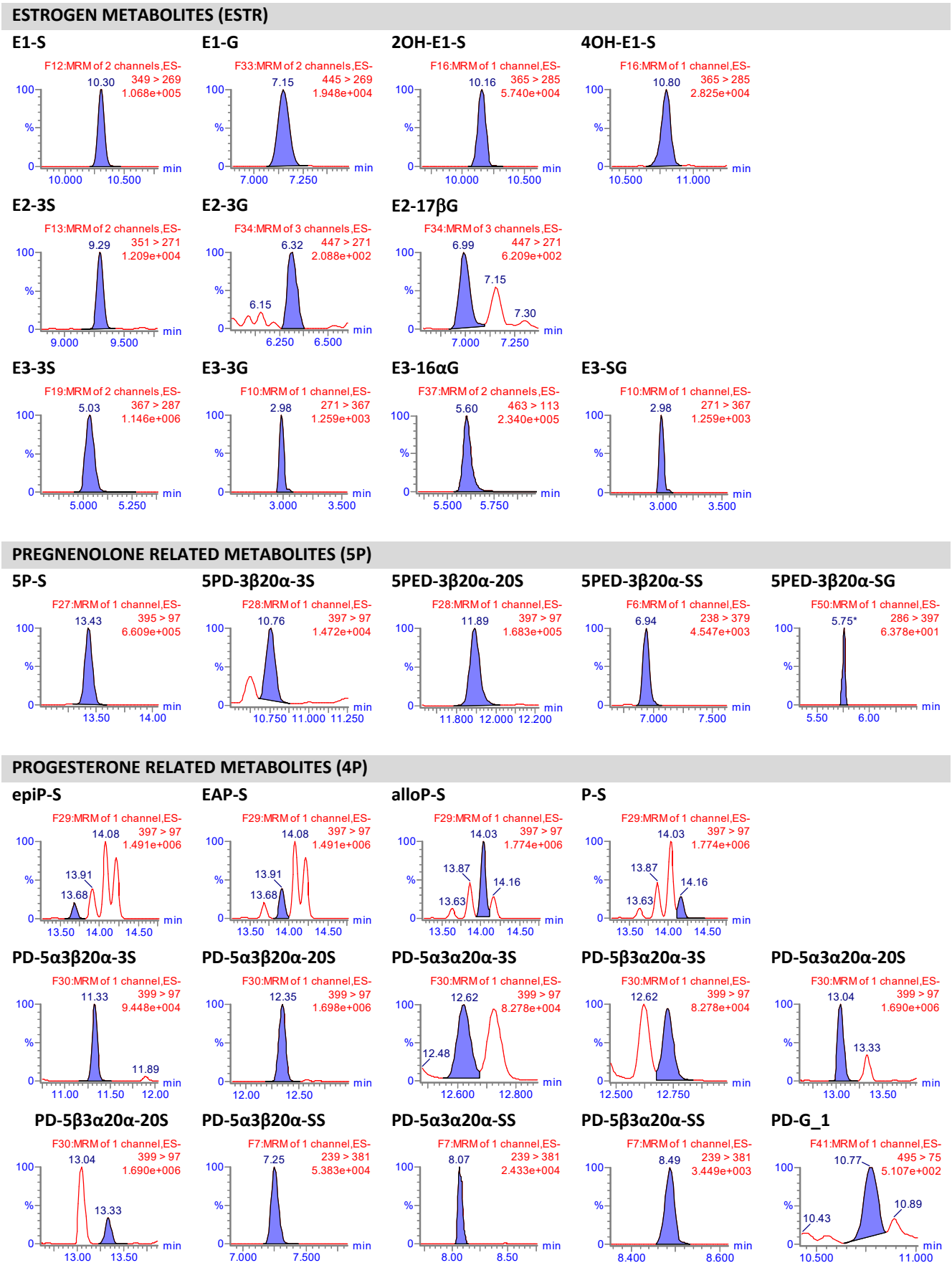
Table 6S. Approximated amounts of daily excreted steroid phase II metabolites (μmol $24h^{-1}$) during onset and full lactation periods. on the base of levels of metabolites detected in BM samples from population of lactating mothers in present study and published earlier data on BM yields [Neville. M C et al., 1988].

Postpartum periods (days)	BM type	N samples per group	BM daily Volume[i] (mL $24h^{-1}$)	Daily excreted steroid metabolites (μmol $24h^{-1}$) in BM (Mean ± SD), ranges (min-max)						
				ESTR	5P	4P	5A	4A	CORT	TOTAL
1-3	C	3	211.3	19.60 ± 18.55 (1.06 – 38.16)	54.47 ±74.87 (6.91 – 140.77)	354.71 ± 568.55 (20.02 – 1011.17)	41.50 ± 57.39 (4.91 – 107.64)	201.90 ± 341.43 (3.16 – 596.15)	2.23 ± 2.24 (0.17 – 4.61)	674.414 ± 1060.133 (56.49 – 1898.51)
4-6	C	15	567.0	37.28 ± 58.82 (0.97 – 236.73)	61.99 ± 80.46 (1.20 – 287.90)	436.44 ± 633.82 (2.19 – 2417.64)	261.38 ± 677.10 (6.50 – 2686.88)	133.45 ± 219.18 (8.33 – 877.49)	7.11 ± 17.91 (0.41 – 71.56)	937.65 ± 1643.07 (23.97 – 6578.21)
7-9	T	6	624.3	9.08 ± 17.4 (0.37 – 44.54)	19.03 ±27.90 (1.57 – 76.35)	82.34 ± 106.51 (5.134 – 282.90)	81.79 ± 142.65 (11.57 – 372.52)	58.24 ± 99.09 (13.12 – 260.23)	4.20 ± 1.71 (1.97 – 5.99)	255.58 ± 389.312 (40.95 – 1042.54)
10-14	T	6	664.6	1.55 ± 1.65 (0.27 – 4.70)	11.26 ±7.59 (5.56 – 25.74)	14.69 ± 16.94 (0.82 – 36.465)	22.00 ± 11.92 (9.30 – 41.52)	22.83 ± 14.67 (10.99 – 51.75)	1.33 ± 0.84 (0.29 – 2.44)	73.67 ± 46.51 (33.94 – 147.30)
15-23	T/M	7	703.0	0.50 ± 0.24 (0.19 – 0.85)	4.61 ± 2.99 (1.03 – 9.05)	9.78 ± 15.91 (0.41 – 44.49)	13.89 ± 14.05 (5.24 – 44.60)	15.30 ± 7.83 (8.73 – 31.90)	3.06 ± 3.75 (0.56 – 10.80)	47.14 ± 31.09 (23.30 – 95.06)
24-32	M	13	794.0	2.80 ± 6.06 (0.31 – 22.83)	4.35 ±3.09 (1.14 – 11.48)	2.36 ± 3.71 (0.54 – 9.89)	24.65 ± 17.80 (5.69 – 62.30)	22.29 ± 12.94 (7.98 – 53.20)	1.83 ± 1.95 (0.53 – 8.03)	60.82 ± 34.01 (19.66 – 120.82)
33-39	M	6	726.0	1.00 ±0.36 (0.65 – 1.53)	3.32 ± 1.73 (1.67 – 6.28)	2.36 ± 3.71 (0.54 – 9.89)	13.17 ± 5.18 (6.21 – 18.64)	17.03 ± 5.17 (11.03 – 24.92)	1.22 ± 0.58 (0.77 – 2.01)	38.10 ± 9.80 (25.49 – 47.39)
40-53	M	4	745.0	1.55 ± 2.11 (0.33 – 4.71)	5.54 ± 2.02 (3.89 – 8.07)	1.22 ± 0.35 (0.85 – 1.58)	34.61 ± 5.18 (15.25 – 81.89)	18.92 ± 3.24 (14.09 – 20.99)	1.33 ± 0.191 (1.05 –1.49)	63.17 ± 35.03 (35.95 – 114.03)

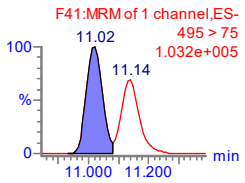
[i] Volumes of BM corresponded to a mean of BM yields reported for grouped days of lactations [Neville. M C et al., 1988]

Supplementary Figures

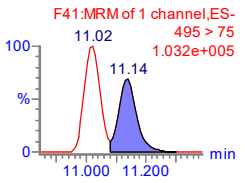
Figure 1S



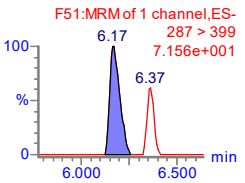
PD-G_2



PD-5β3α20α-3G



PD-SG-1



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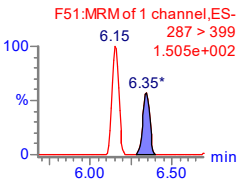

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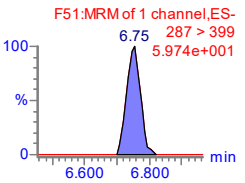
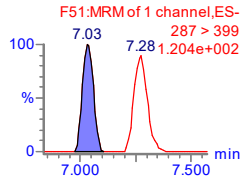
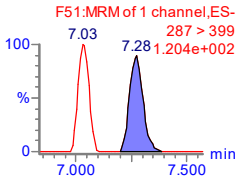


PD-SG-4



PD-SG-5*



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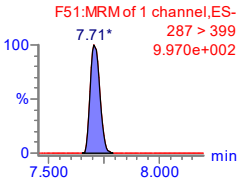
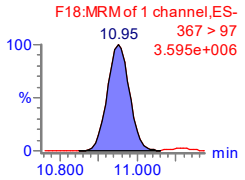
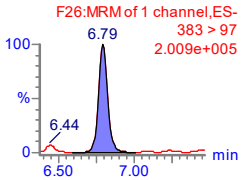


5-ANDROSTEN METABOLITES (5A)

DHEAS



16βOH-DHEA-3S



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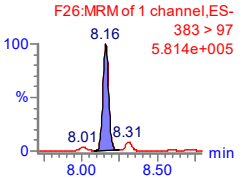
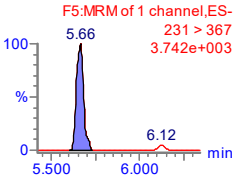
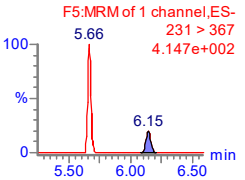


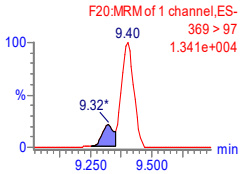
16βOH-DHEA-SS



16αOH-DHEA-SS*



5AED-3α17β-3S



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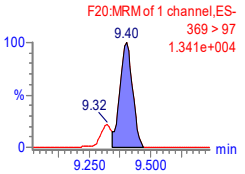

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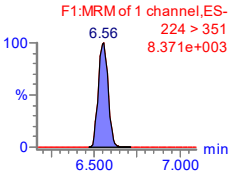

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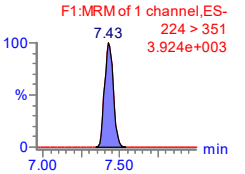
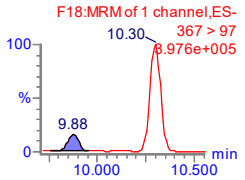
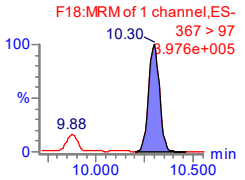


4-ANDROSTEN METABOLITES (4A)

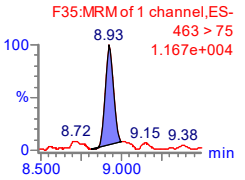
T-S



epiT-S



epiT-G



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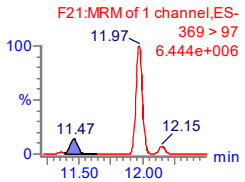
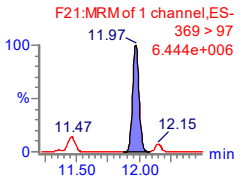
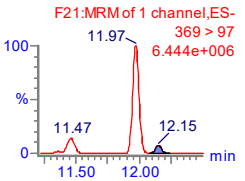


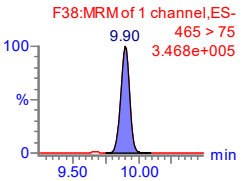
AN-S



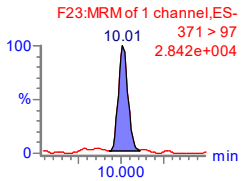
Etio-S



AN-G + Etio-G



AD-5α3β17β-3S



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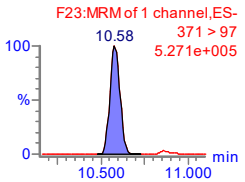

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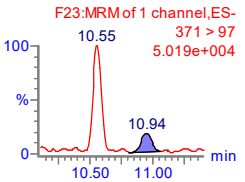
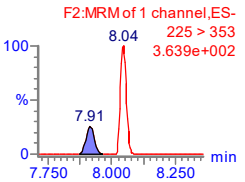
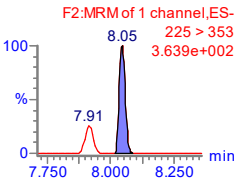


AD-SS_1



AD-5α3α17β-SS



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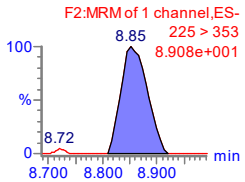
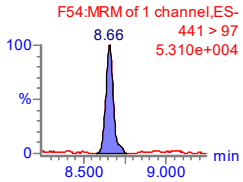
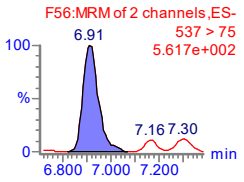


CORTICOSTEROID METABOLITES (CORT)

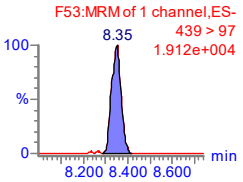
F-21S



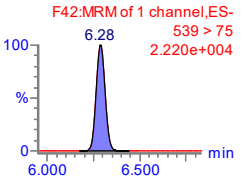
F-21G



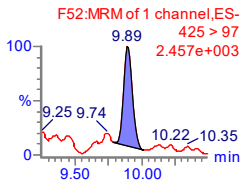
E-21S



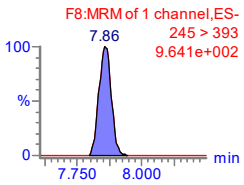
THE-3G



B-21S

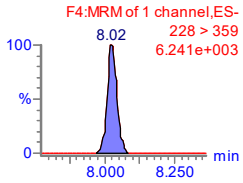


21OH-5P-SS

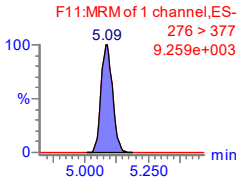


INTERNAL STANDARDS (ISTDs)

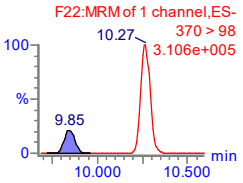
ISTD-SS (AD-5β3α17β-3S-17S$^{18}O_3$)



ISTD-SG (AD-5α3β17β-3S$^{18}O_3$-17G)



ISTD-S-1 (T-d3-S)



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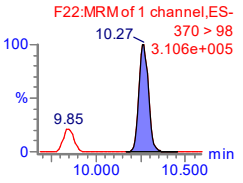
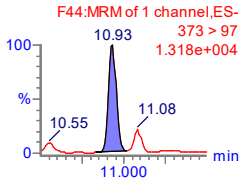
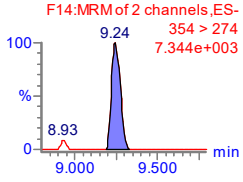


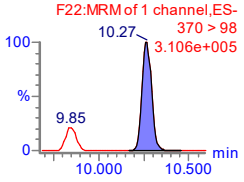
ISTD-S-3 (DHEA-d6-S)



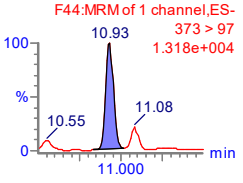
ISTD-S-4 (E2-d4-3S)



ISTD-G-1 (epiT-d4-G)



ISTD-G-2 (AN-d4-G)



ISTD-G-3 (E2-d4-17βG)

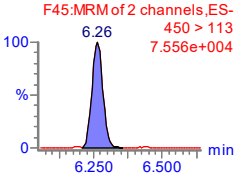


Figure 1S. Extracted chromatograms of endogenous phase II conjugated steroids detected in breast milk samples and internal standards used in the study. Metabolites are grouped by steroids families (ESTR – estrogens, 5P – pregnenolone metabolites, 4P – progesterone derived metabolites, 5A – 5-androstene metabolites, 4A – 4-androstene metabolites, and CORT – corticosteroid metabolites) and corresponding parent steroid compound (for abbreviation see Table S1), and in case of isomers are presented in order of chromatographic elution. Extracted chromatograms of used ISTDs (internal standards) are presented as separate group. * - detected only in one or a few samples.

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