

Supplementary Table S5. Testing of extraction efficiency and efficiency of the methanolysis step using available steroid sulfates.

Steroid/sulfate														Extraction efficiency		Efficiency of methanolysis step, corrected to IS [%]
	Unconjugated steroid						Sulfated steroid									
	Diethylether layer			Polar layer			Diethylether layer			Polar layer						
Injected steroid [ng]	Injected IS (D6-DHEA) [μl]	MW sulfated steroid/MW steroid	Peak area, steroid [AUC]	Peak area, IS [AUC]	Peak area ratio (steroid/IS)	Peak area, steroid [AUC]	Peak area, steroid [AUC]	Peak area corrected to MWs [AUC]	Peak area, steroid [AUC]	Peak area corrected to MWs [AUC]	Peak area, IS [AUC]	Peak area ratio corrected to MWs (steroid/IS)	Unconjugated steroid [%]	Sulfated steroid [%]		
Androsterone	20	20	1.41	14944357	8801862	1.70	2140798	55249	77730	5441067	7655018	3880859	1.97	87	99	116
Epiandrosterone	20	20	1.41	13952600	9379142	1.49	1129634	2235	3144	2818461	3965283	3130178	1.27	93	100	85
Etiocholanolone	20	20	1.41	17330257	9379142	1.85	1806954	3542	4983	4508911	6343571	3130178	2.03	91	100	110
Pregnanolone	20	20	1.37	4802104	5681583	0.85	1161327	4494	6162	1829402	2508237	3021745	0.83	81	100	98
Epipregnanolone	20	20	1.37	6941351	6715295	1.03	2179828	9243	12673	1981282	2716475	2951234	0.92	76	100	89
DHEA	20	20	1.41	12402619	9334823	1.33	845542	1319	1859	3298765	4650342	3610621	1.29	94	100	97
Estradiol (disulfate)	2	20	1.87	3168922	6309477	0.50	47475	28	52	881558	1646439	3623213	0.45	99	100	90
													Minimum	76	99	85
													Maximum	99	100	116
													Mean	88	100	98
													SD	7.2	0.3	10.5