

Supporting Information

A Comprehensive Urinary Steroid Analysis Strategy for Routine and Research using Two-Dimensional Gas Chromatography – Time of Flight Mass Spectrometry

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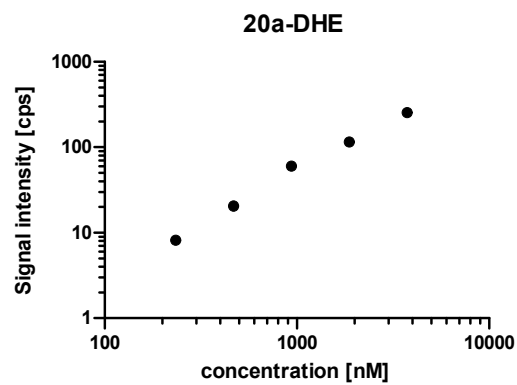
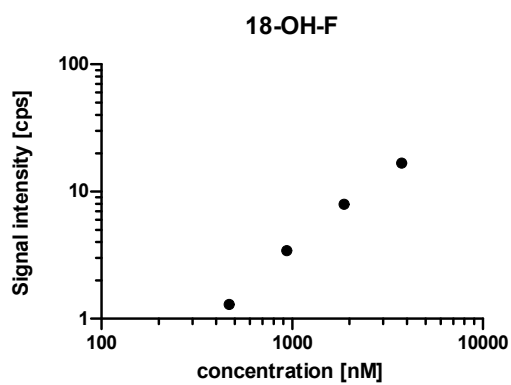
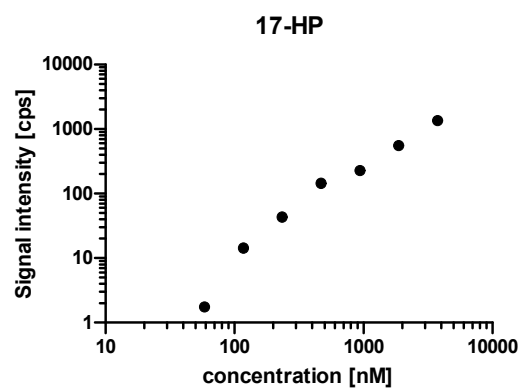
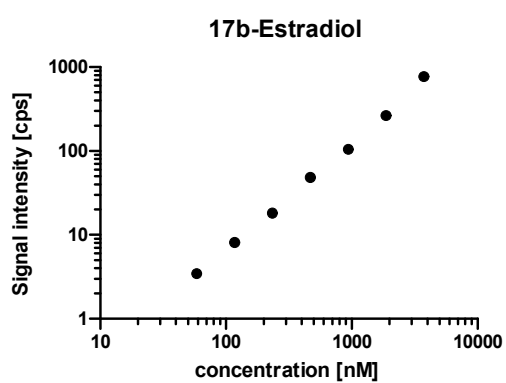
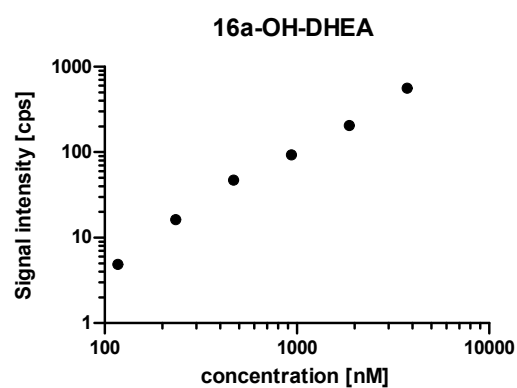
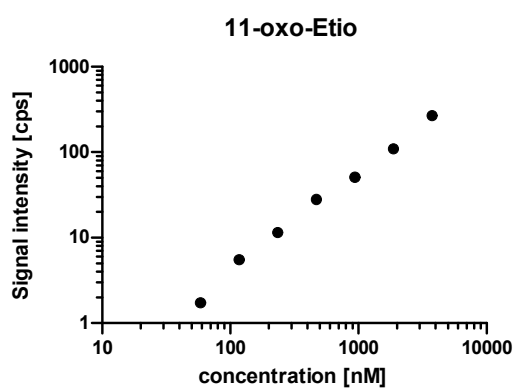
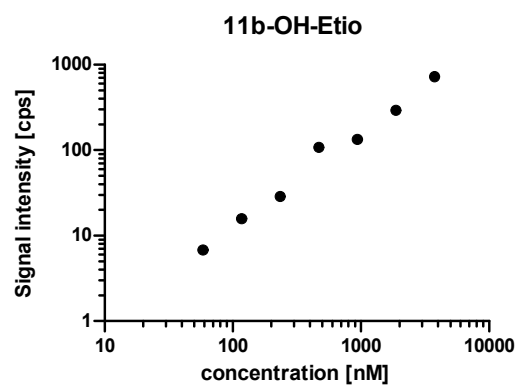
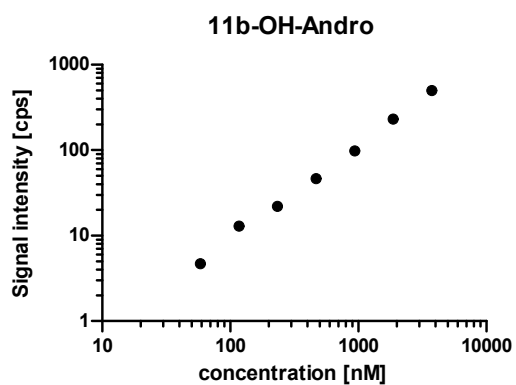
Supplementary Figure S1: Matrix-free calibration curves for 40 steroids in MeOH.

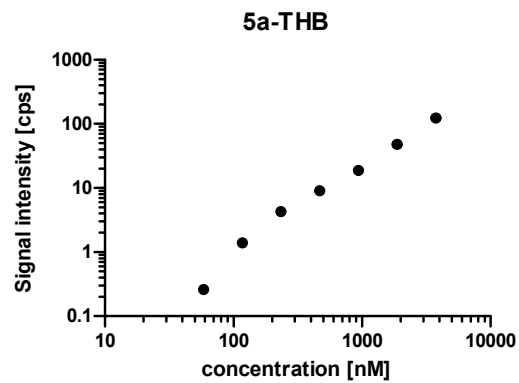
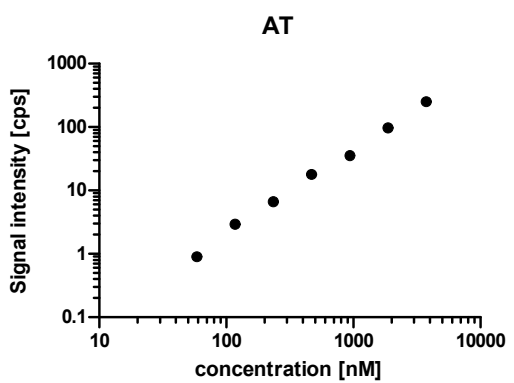
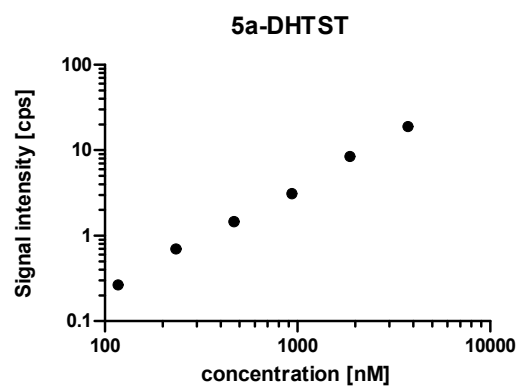
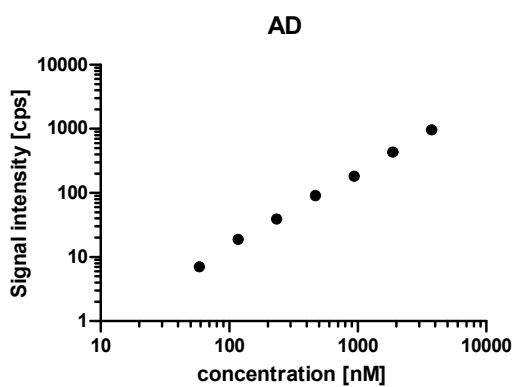
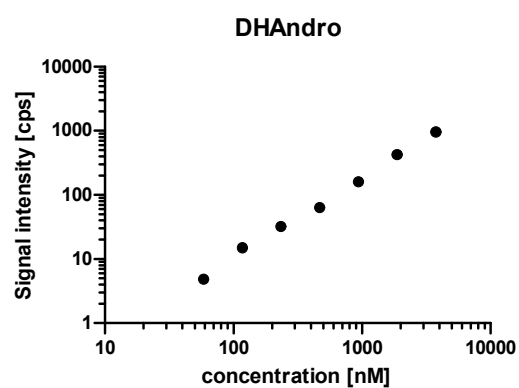
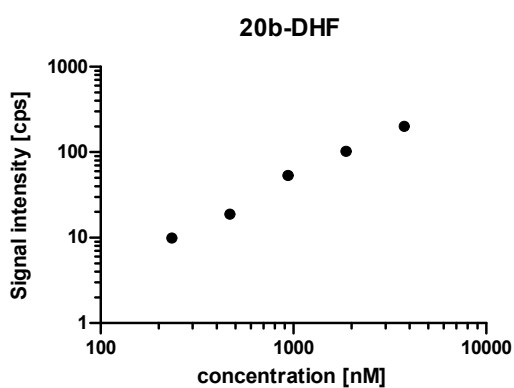
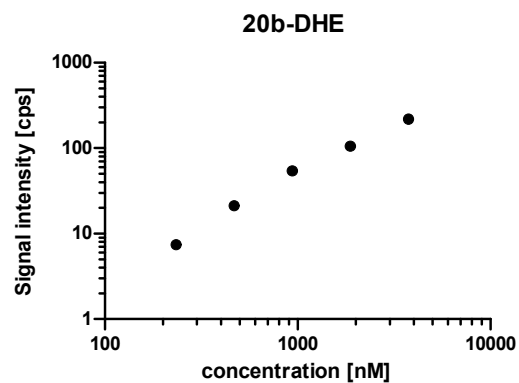
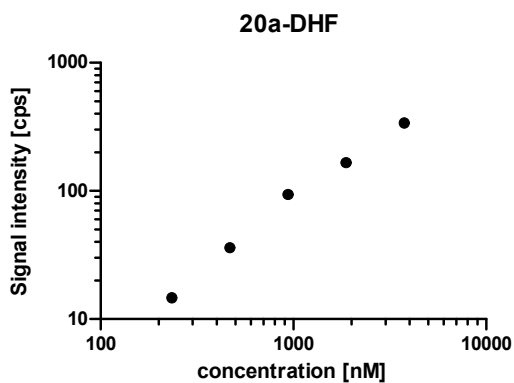
Supplementary Figure S2: Correlation of slopes of calibration curves in SFUM and MeOH. Slope and regression coefficient of determination R^2 indicate that instrument response is identical in both matrices.

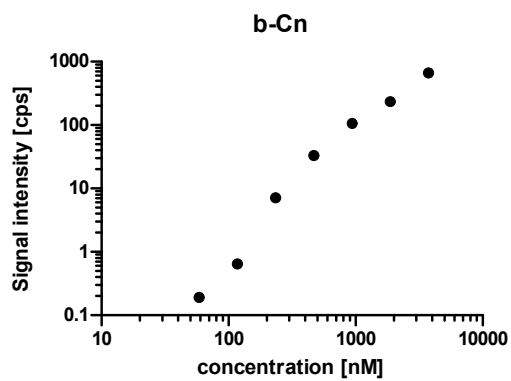
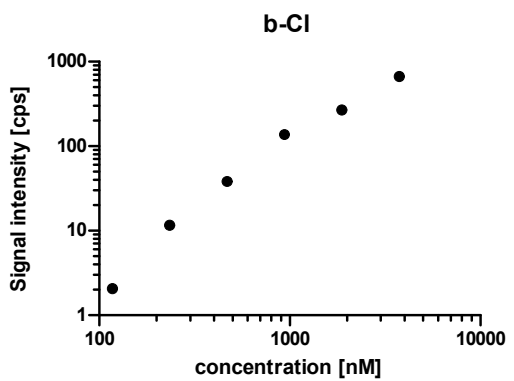
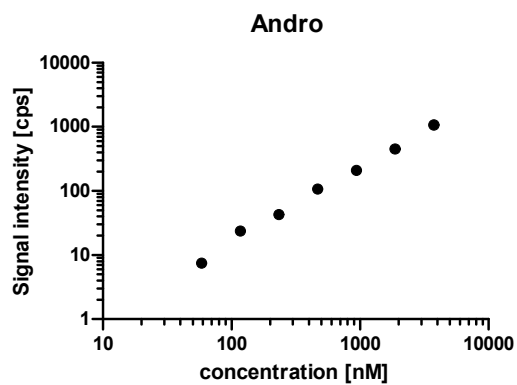
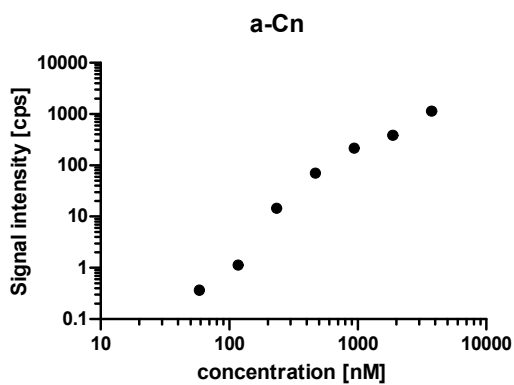
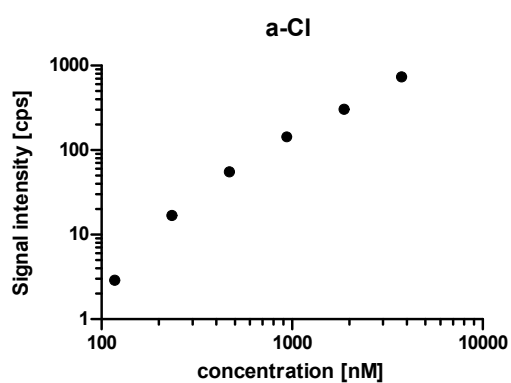
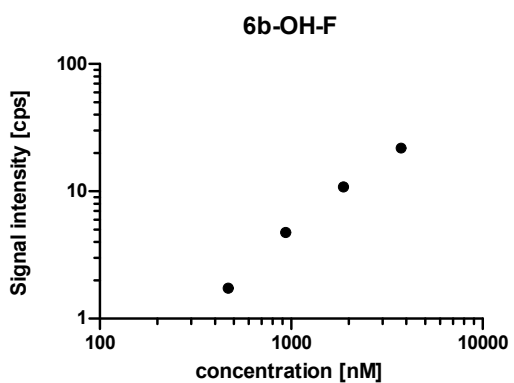
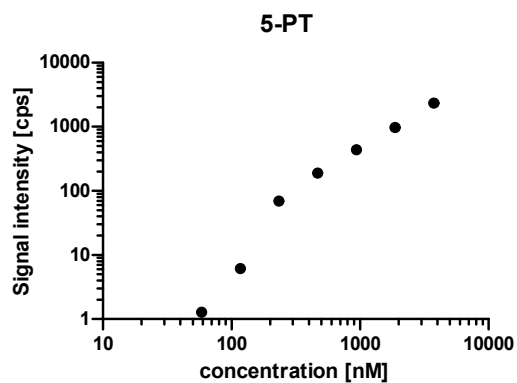
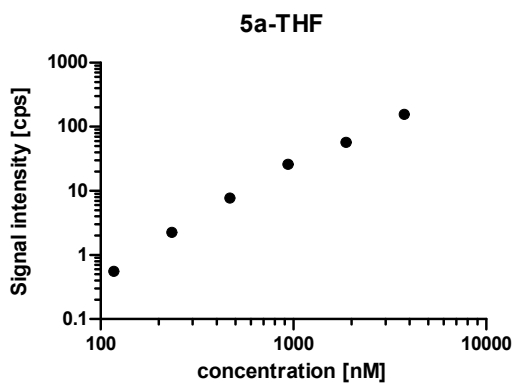
Supplementary Figure S3: Total ion chromatogram of steroids extracted from rat urine.

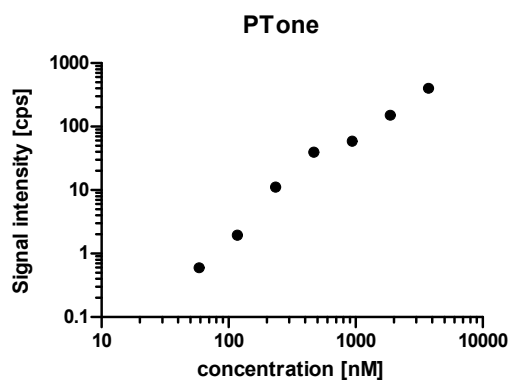
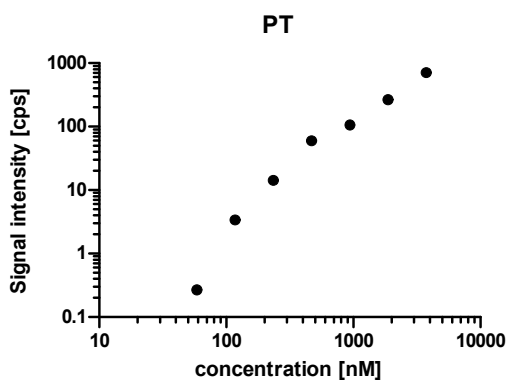
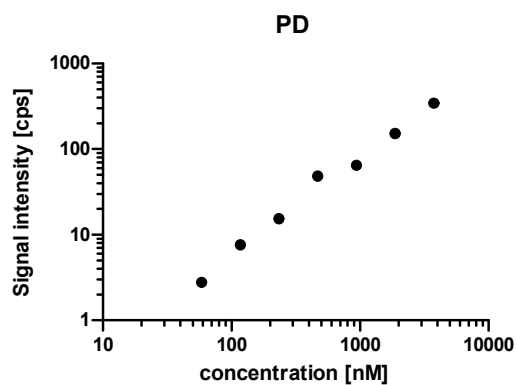
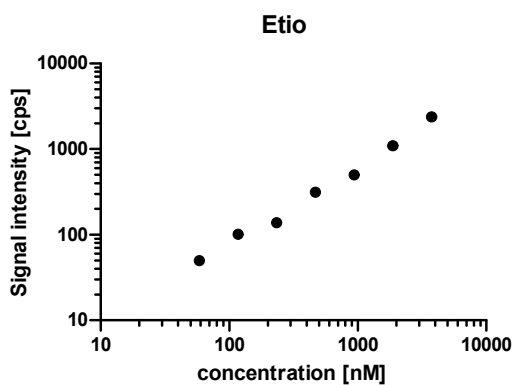
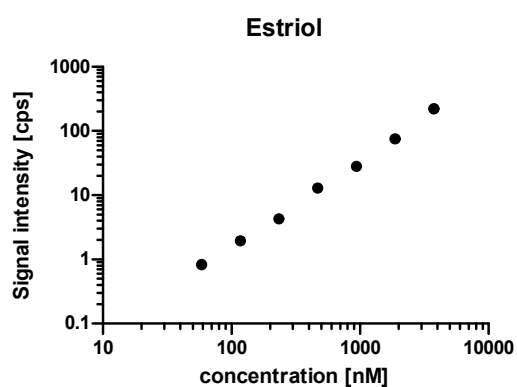
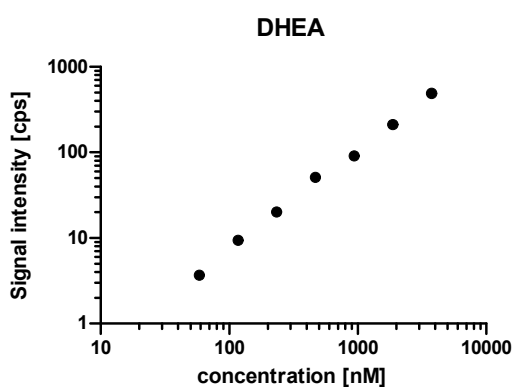
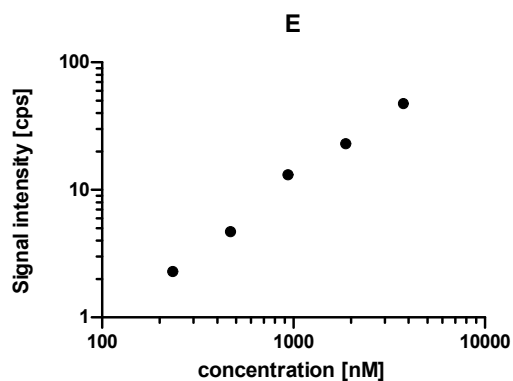
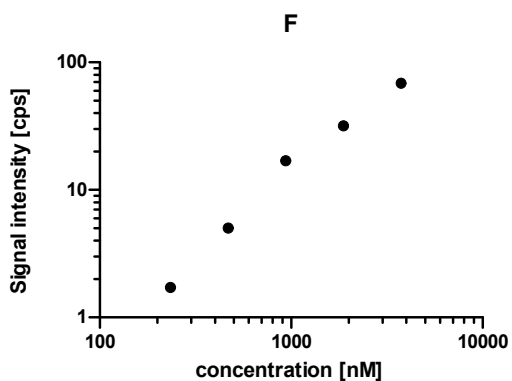
Supplementary Table 1: Urinary steroid metabolites in mouse (M) and rat (R). Steroids #1-11 were already published by Shackleton et al. (The corticosteroid metabolic profile of the mouse. Steroids (73), 2008, 1066–1076) and now re-validated using GCxGC-TOF MS. Steroids #12-56 represent newly identified steroid candidates assigned to steroid classes (androsten-, pregnan-, pregnen-, cholic acid-type). For each steroid the exact mass, the proposed sum formula, the mass deviation in ppm and the retention time in the first (GC1) and second (GC2) dimension and the species in which the compound was detected are reported.

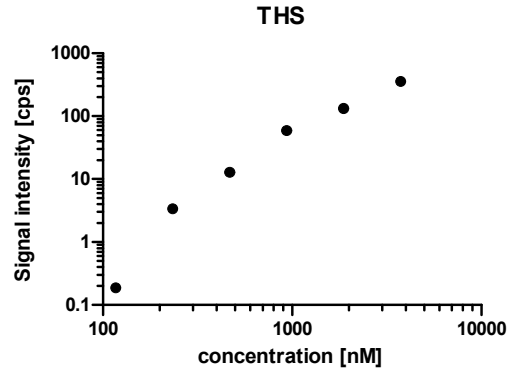
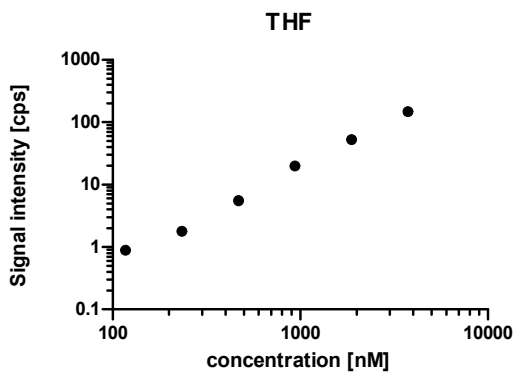
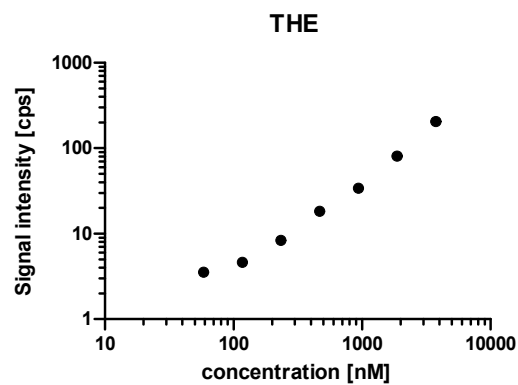
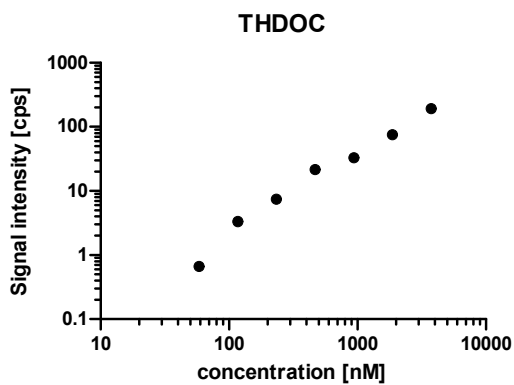
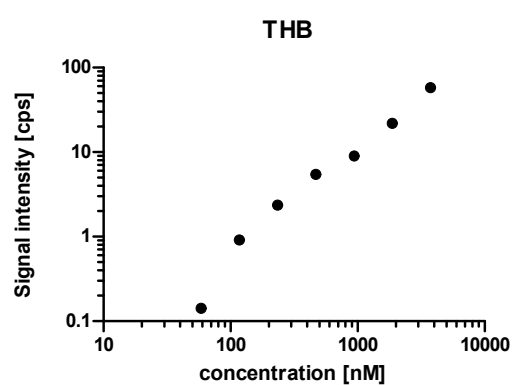
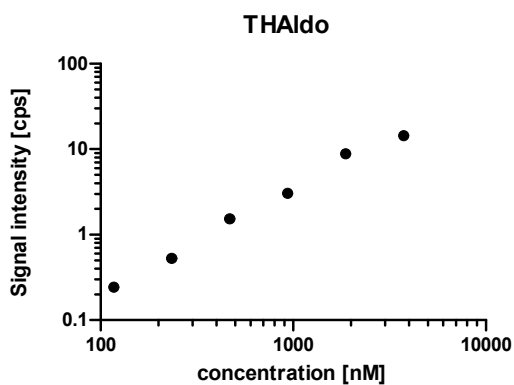
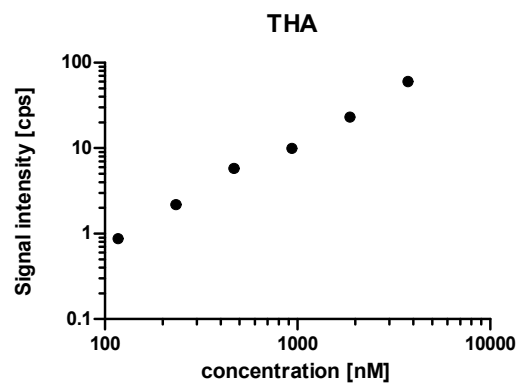
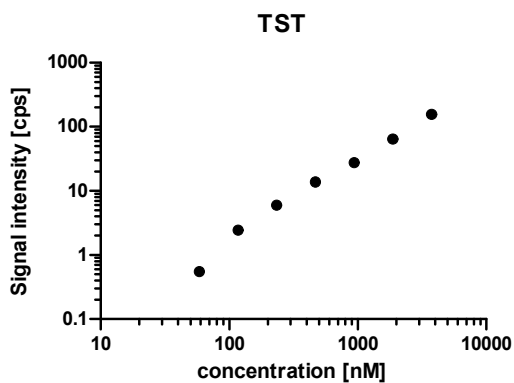
Supplementary Figure S1.



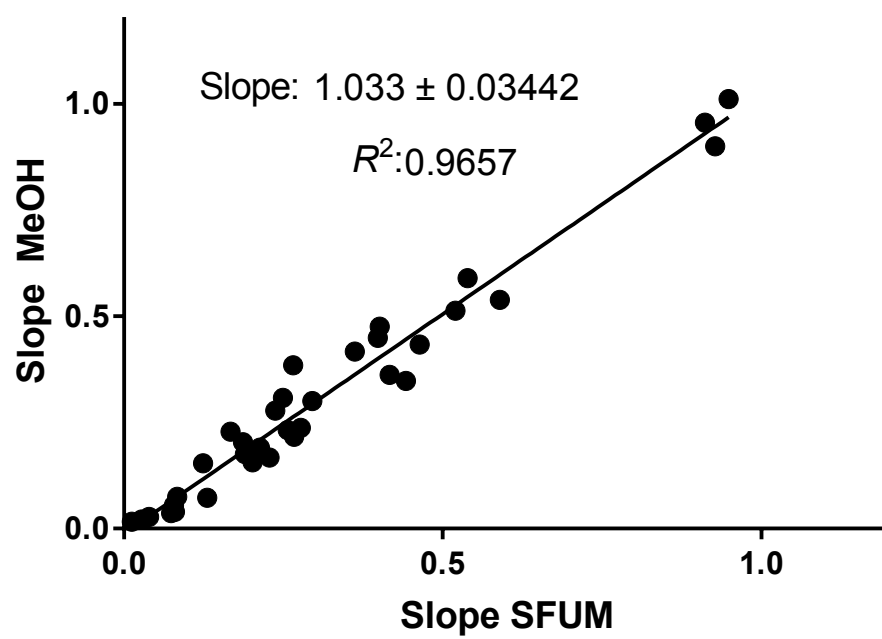




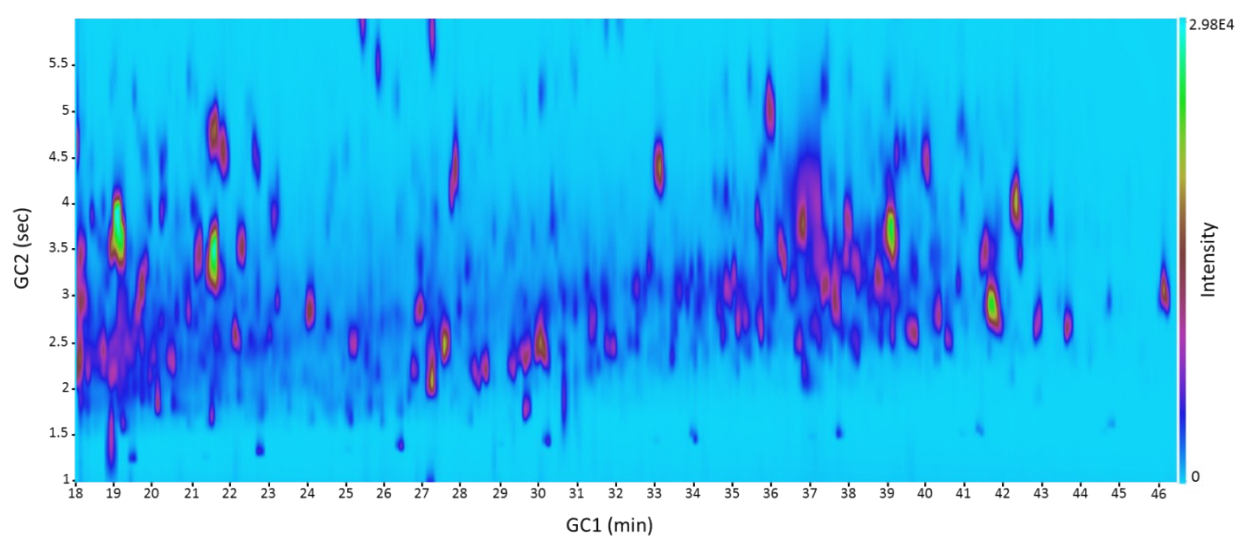




Supplementary Figure S2.



Supplementary Figure S3.



Supplementary Table S1

#	m/z	GC1 (min)	GC2 (sec)	Formula	Mass error (ppm)	Name/Type	Species
1	654.3999	38.2	3.5	C33H66O5Si4+	2.6	1b-OH-HHA	M, R
2	519.3138	34.9	4.0	C27H47N2O4Si2+	7.1	1b-OH-THA	M, R
3	595.3829	32.3	3.8	C31H61NO4Si3+	-4.9	3b-5a-THB	M, R
4	429.2227	35.6	5.8	C23H33N2O4Si+	5.2	6a-OH-THA	M
5	666.3842	41.1	3.6	C33H64NO5Si4+	-3.5	6b-OH-20-DHB	M, R
6	636.3798	40.7	4.8	C32H60N2O5Si3+	-1.0	6b-OH-B	M, R
7	607.3569	41.5	4.9	C31H57NO5Si3+	5.0	6b-OH-DHA	M, R
8	593.3781	42.2	4.4	C31H59NO4Si3+	5.8	20-DHB	M, R
9	578.3508	41.5	3.5	C30H56NO4Si3+	-0.7	OH-20-DHB	M, R
10	551.3762	46.1	2.7	C30H59O3Si3+	-0.9	OH-HHA	M, R
11	625.3897	41.7	2.9	C32H65O4Si4+	-9.2	OH-HHB	M, R
12	402.2371	19.6	4.6	C27H34OSi+	-0.7	Pregnan	M, R
13	446.2875	29.1	3.4	C22H46O5Si2+	-0.7	Androsten	R
14	522.3454	27.5	2.5	C31H50N2OSi2+	-0.4	Androsten	R
15	545.2323	19.6	2.1	C29H41N2OSi4+	6.0	Cholesterol	M
16	632.4419	46.1	2.7	C31H68N2O5Si3+	-1.8	Cholesterol	M
17	632.4435	45.8	2.7	C31H68N2O5Si3+	0.8	Cholesterol	M
18	652.4106	35.1	2.7	C33H64N2O5Si3+	-1.8	Pregnan	R
19	652.4130	37.4	3.1	C33H64N2O5Si3+	2.0	Pregnan	R
20	530.2344	31.9	6.0	C27H42O5Si3+	1.7	Androsten	M
21	385.3320	36.1	5.5	C22H49OSi2+	0.9	Cholesterol	M, R
22	516.3382	40.4	4.3	C28H52N2OSi3+	0.0	Cholic acid	M
23	369.1366	23.3	3.1	C23H23NSi2+	0.7	Cholic acid	M
24	471.1493	30.8	5.5	C27H25NO5Si+	-0.7	Cholesterol	M
25	503.2814	37.4	4.8	C27H47O3Si3+	-2.7	Pregnen	M
26	653.3950	35.8	3.3	C36H61N2O3Si3+	-5.3	Pregnen	M
27	608.3532	28.8	3.7	C35H56O3Si3+	0.1	Pregnen	R
28	629.4184	42.2	4.2	C34H65N2OSi4+	2.5	Pregnen	M
29	490.3147	39.6	4.7	C27H48NO3Si2+	-4.2	Pregnen	M
30	594.3476	34.8	4.1	C30H56NO5Si3+	2.6	Pregnen	R
31	562.3168	37.0	0.2	C29H54O3Si4+	4.2	Pregnen	R
32	634.3708	38.7	3.6	C33H62O4Si4+	-1.9	Pregnen	R
33	576.2983	35.3	4.3	C29H50NO5Si3+	-1.4	Pregnen	M
34	636.3779	37.7	4.3	C32H60N2O5Si3+	-4.1	Pregnen	M
35	518.2396	29.9	5.3	C25H44NO3Si4+	0.7	Pregnen	M
36	533.2924	24.2	3.6	C28H49O4Si3+	-1.7	Pregnen	M
37	429.1796	22.5	5.5	C22H31NO4Si2+	2.4	Pregnen	M
38	474.3293	31.1	6.0	C27H46N2O3Si+	4.4	Pregnen	M, R
39	488.3012	40.9	4.0	C27H46N2O3Si+	0.2	Pregnen	R
40	564.3583	37.6	3.0	C29H56N2O3Si3+	-1.9	Pregnen	R
41	386.2978	41.1	5.0	C20H42N2O3Si+	4.9	Pregnen	M
42	475.3100	39.1	3.7	C27H45NO4Si+	-2.7	Pregnen	R
43	490.2928	35.3	4.3	C27H46O4Si2+	-0.1	Pregnan	M
44	562.3073	41.5	3.5	C28H50N2O4Si3+	-0.1	Pregnan	R

45	506.3111	39.6	4.7	C27H48NO4Si2+	-1.1	Pregnan	R
46	609.3760	35.6	3.9	C31H59NO5Si3+	9.6	Pregnan	R
47	578.3101	37.7	4.6	C29H54O4Si4+	1.2	Pregnan	M
48	578.3527	35.8	4.5	C30H56NO4Si3+	2.7	Pregnan	R
49	578.3461	40.6	4.5	C30H58O3Si4+	0.5	Pregnan	R
50	638.4052	31.7	2.5	C33H66O4Si4+	3.0	Pregnen	R
51	535.3552	41.7	2.9	C25H57NO5Si3+	2.4	Pregnan	R
52	445.2957	41.7	4.4	C26H45O2Si2+	1.0	Cholesterol	R
53	610.3768	28.6	2.2	C31H60NO5Si3+	-0.9	Pregnen	R
54	639.3774	35.3	3.3	C35H61NO2Si4+	0.0	Pregnan	M
55	625.3690	36.3	3.4	C34H57N2O3Si3+	3.0	Pregnan	R
56	428.3098	40.4	4.3	C27H44O2Si+	-1.6	Cholic acid	M