

SUPPLEMENTARY MATERIALS

Kindysheva, S., Gavisova, A., Shevtsova, M., Tskhovrebova, L., Biryukova, D., Starodubtseva, N., Ivanec, T., & Frankevich, V. (2022) Identification of Androgen Deficiency in Infertility and Reduced Ovarian Reserve Based on HPLCMS/MS and IHLA Measurements. Biomedical Chemistry: Research and Methods, 5(4), e00182. DOI: 10.18097/BMCRM00182

Table S1: MRM transitions for substances and its internal standards.

Compound	m/z ion= precursor	m/z ion= product	DP, V	EP, V	CE, V	CXP, V
Aldosterone	361.1	342.9	60	8	23	25
Cortisol	363.1	121.1	36	10	31	14
Corticosterone	347.1	329.1	21	10	21	36
DHEAS	367.1	96.9	-100	-10	-20	-11
Estradiol	255.1	159.1	46	10	25	16
Testosterone	289.0	109.0	26	10	33	6
17- α -OH-Progesterone	331.1	97.0	31	10	29	12
17- α -OH-Pregnenolone	315.1	297.1	131	10	15	10
Androsten-3,17-dione	287.0	97.1	31	10	27	10
Dihydrotestosterone	291.1	255.1	176	10	21	30
Androsterone	273.1	255.5	66	10	19	24
Pregnenolone	299.2	281.1	26	10	17	6
DHEA	271.1	253.1	171	10	17	30
Progesterone	315.1	109.0	120	10	31	12
11-Deoxycortisol	347.1	109.0	21	10	33	12
Aldosterone(IS)	368.2	350.1	56	10	23	34
Cortisol(IS)	367.0	121.2	16	10	31	10
DHEAS(IS)	372.1	96.8	-125	-10	-40	-13
Estrone(IS)	273.9	256.1	136	10	19	20
Testosterone(IS)	292.0	100.0	16	10	29	10
17- α -OH-Progesterone(IS)	339.0	100.0	71	10	31	10
Androsten-3,17-dione(IS)	290.0	100.1	1	10	27	10
Dihydrotestosterone(IS)	294.0	258.2	51	10	19	22
DHEA(IS)	276.1	258.0	96	10	17	8
Progesterone(IS)	324.1	100.1	46	10	29	12
11-Deoxycortisol(IS)	352.1	100.0	26	10	31	12

Where DP – Declustering Potential.

EP – Entrance Potential.

CE – Collision Energy.

CXP – collision Cell Exit Potential.

Table S2: The retention time of the steroids hormones and its internal standards.

Compound name	t _R , min	IS Name	t _R IS, min
Aldosterone	4.9±0.1	Aldosterone(IS)	4.6±0.1
Cortisol	5.2±0.1	Cortisol(IS)	5.2±0.1
DHEAS	6.0±0.1	DHEAS(IS)	6.0±0.1
Corticosterone	6.1±0.1	17- α -OH-Progesterone(IS)	7.7±0.1
11-Deoxycortisol	6.3±0.1	11-Deoxycortisol(IS)	6.2±0.1
Estradiol	6.8±0.1	Estrone(IS)	7.4±0.1
Testosterone	7.1±0.1	Testosterone(IS)	7.0±0.1
11-Deoxycorticosterone	7.4±0.1	11-Deoxycortisol(IS)	6.2±0.1
17- α -OH-Pregnenolone	7.4±0.1	17- α -OH-Progesterone(IS)	7.7±0.1
Androsten-3,17-dione	7.5±0.1	Androsten-3,17-dione(IS)	7.5±0.1
DHEA	7.5±0.1	DHEA(IS)	7.5±0.1
17- α -OH-Progesterone	7.8±0.1	17- α -OH-Progesterone(IS)	7.7±0.1
Dihydrotestosterone	8.1±0.1	Dihydrotestosterone(IS)	7.5±0.1
Androsterone	8.9±0.1	11-Deoxycortisol(IS)	6.2±0.1
Progesterone	9.4±0.1	Progesterone(IS)	9.4±0.1
Pregnenolone	9.4±0.1	Progesterone(IS)	9.4±0.1

Table S3: Steroid hormones concentrations in the points of the calibration curve.

Compound name	Cal1	Cal12	Cal2	Cal23	Cal3	Cal34	Cal4
Cortisol, ng/ml	2.72	8.155	13.59	63.4	113.3	226.6	339.8
11-Deoxycortisol, ng/ml	0.088	0.265	0.442	2.061	3.680	7.34	11.0
Corticosterone, ng/ml	0.52	1.56	2.6	12.125	21.65	43.31	64.96
DHEAS, μ g/ml	0.055	0.166	0.276	1.290	2.30	4.61	6.91
Testosterone, ng/ml	0.043	0.13	0.216	1.01	1.8	3.61	5.41
11-Deoxycorticosterone, ng/ml	0.099	0.298	0.50	2.31	4.13	8.26	12.4
Androsten-3,17-dione, ng/ml	0.172	0.516	0.86	4.01	7.16	14.32	21.48
OH-Progesterone, ng/ml	0.399	1.194	1.99	9.31	16.62	33.25	49.9
DHEA, ng/ml	0.047	0.352	0.66	2.56	4.46	10.55	16.64
Progesterone, ng/ml	0.189	0.566	0.943	4.40	7.86	15.72	23.58
Androsterone, ng/ml	0.087	0.262	0.436	2.03	3.63	7.26	10.89

Table S4: Steroid hormones concentrations for QC levels.

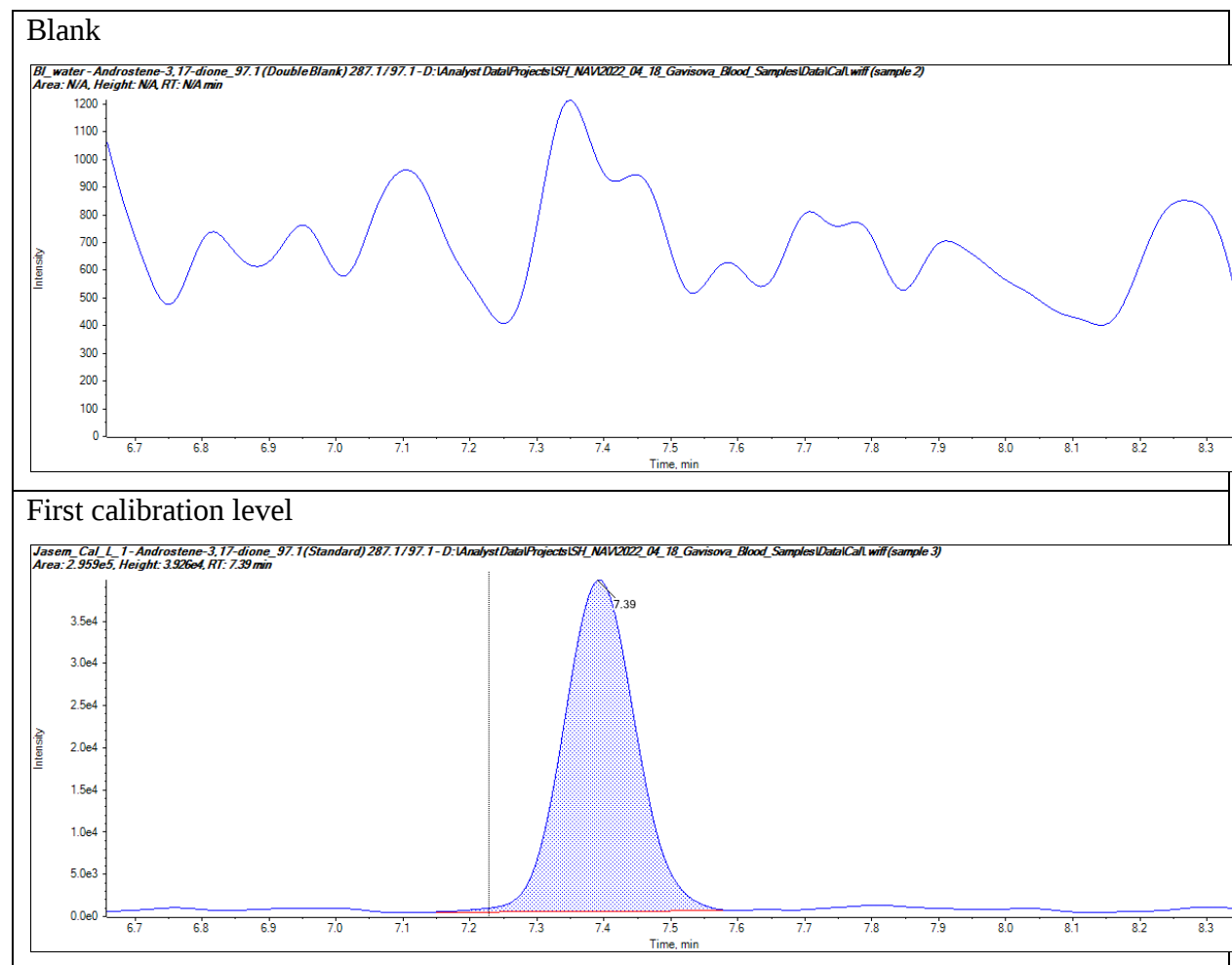
Compound name	QC _{1B}	QC ₁	QC _{2B}	QC ₂
Cortisol, ng/ml	7.55	22.65	75.33	113.0
11-Deoxycortisol, ng/ml	0.245	0.736	2.43	3.65
Corticosterone, ng/ml	1.443	4.33	14.67	22.0
DHEAS, µg/ml	0.154	0.461	1.53	2.30
Testosterone, ng/ml	0.120	0.36	1.2	1.8
11-Deoxycorticosterone, ng/ml	0.276	0.829	2.74	4.11
Androsten-3,17-dione, ng/ml	0.447	1.43	4.73	7.10
17- α -OH-Progesterone, ng/ml	0.275	0.826	2.73	4.10
DHEA, ng/ml	0.397	1.190	3.53	5.33
Progesterone, ng/ml	0.523	1.57	5.27	7.9
Androsterone, ng/ml	0.242	0.73	2.4	3.6

Table S5: The chromatography parameters.

Time, min.	Rate (µl/min)	Eluent A (%)	Eluent B (%)
0	500	95	5
2	500	95	5
2.1	500	80	20
11	500	40	60
11.10	500	5	95
11.11	800	5	95
16.00	800	5	95
16.10	800	95	5
16.11	500	95	5
20.00	500	95	5

Figure S1: Chromatograms of the blank entered after QC High in the Multiple Reaction Monitoring regime for the analyzed steroid hormones.

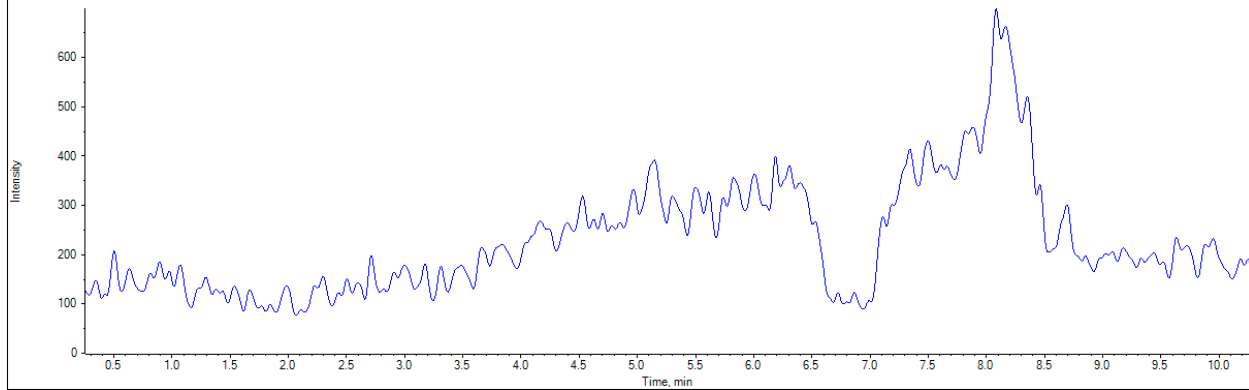
1) Androstene-3,17-dione



2) Cortisol

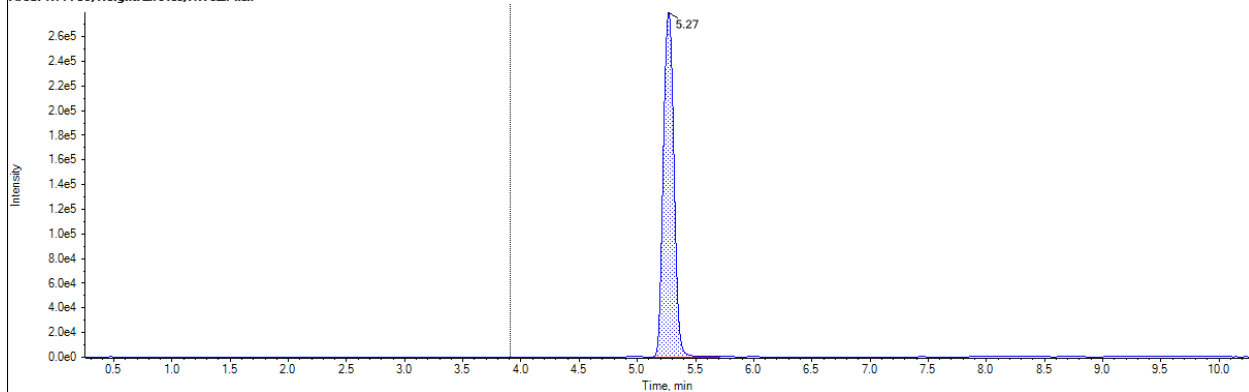
Blank

BL_water - Cortisol_121.1 (Double Blank) 363.2/121.1 - D:\Analyst Data\Projects\SH_NAV2022_04_18_Gavisova_Blood_Samples\Data\Cal.wiff (sample 20)
Area: N/A, Height: N/A, RT: N/A min



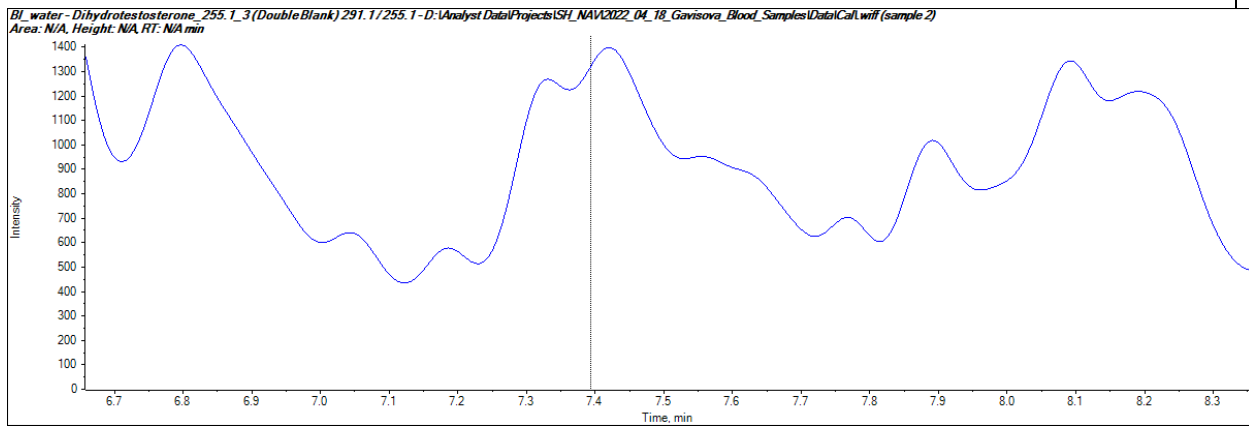
First calibration level

Jasem_QC_L_2.5_01 - Cortisol_121.1 (Quality Control) 363.2/121.1 - D:\Analyst Data\Projects\SH_NAV2022_04_18_Gavisova_Blood_Samples\Data\Cal.wiff (sample 31)
Area: 1.777e6, Height: 2.791e5, RT: 5.27 min

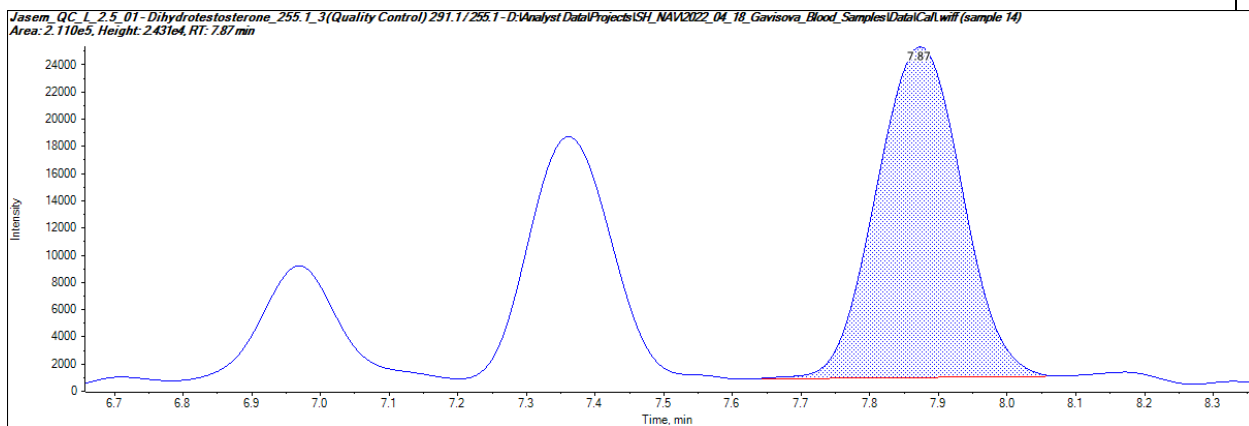


3) Dihydrotestosterone

Blank



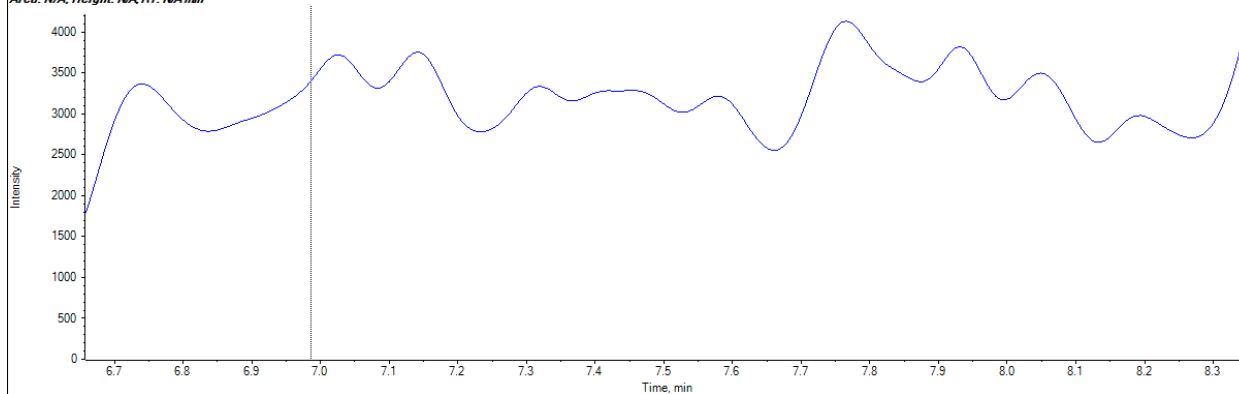
First calibration level



4) Testosterone

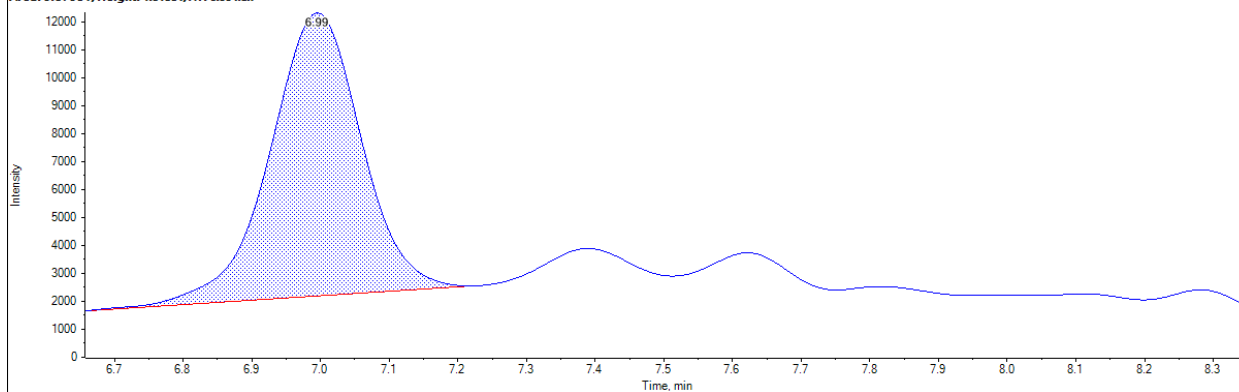
Blank

BL_water - Testosterone_97.1 (Double Blank) 289.2/97.1 - D:\Analyst Data\Projects\SH_NAV\2022_04_18_Gavisova_Blood_Samples\Data\Cal.wiff (sample 2)
Area: N/A, Height: N/A, RT: N/A min



First calibration level

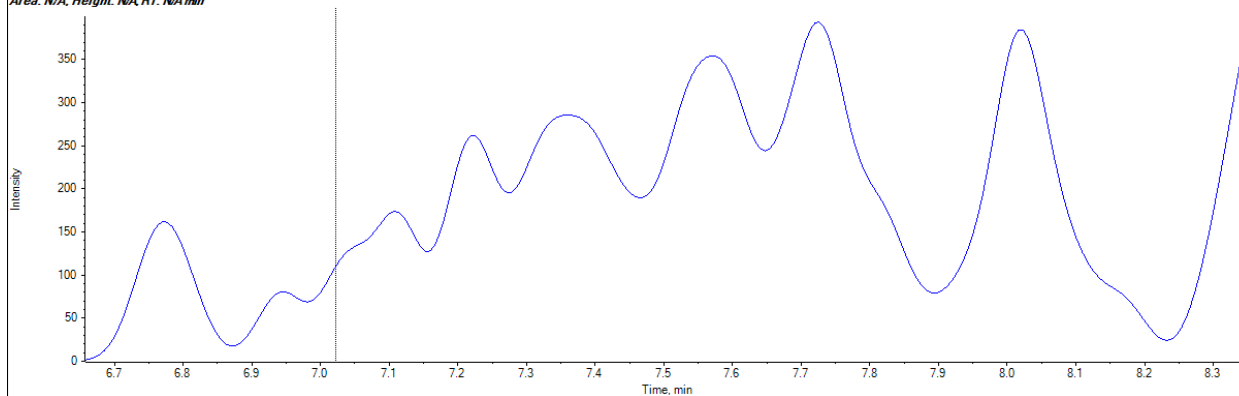
Jasem_Cal_L_1- Testosterone_97.1 (Standard) 289.2/97.1 - D:\Analyst Data\Projects\SH_NAV\2022_04_18_Gavisova_Blood_Samples\Data\Cal.wiff (sample 3)
Area: 9.379e4, Height: 1.013e4, RT: 6.99 min



5) 17- α -OH-Progesterone

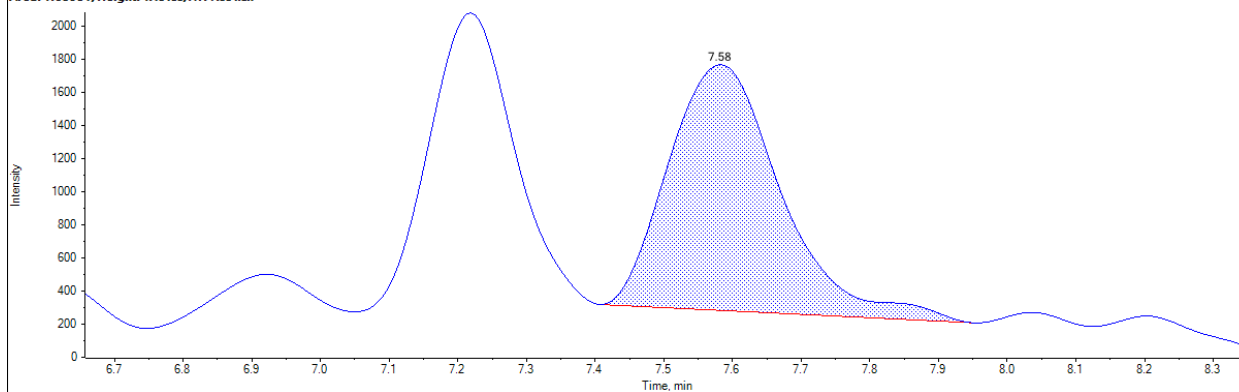
Blank

BL_water - 17-alpha-Hydroxyprogesterone_97(Double Blank) 332.2/97.0 - D:\Analyst Data\Projects\SH_NAV\2022_04_18_Gavisova_Blood_Samples\Data\Cal\wif (sample 2)
Area: N/A, Height: N/A, RT: N/A min



First calibration level

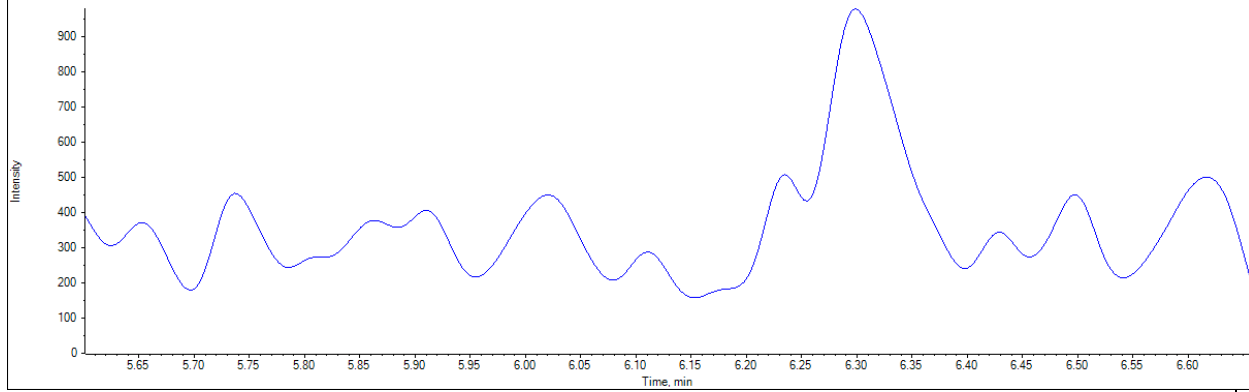
Jasem_Cal_L_1- 17-alpha-Hydroxyprogesterone_97(Standard) 332.2/97.0 - D:\Analyst Data\Projects\SH_NAV\2022_04_18_Gavisova_Blood_Samples\Data\Cal\wif (sample 3)
Area: 1.689e4, Height: 1.48e3, RT: 7.58 min



6) 11-Deoxycortisol

Blank

BL_water - 11-Deoxycortisol_109.1 (Double Blank) 347.2/109.1 - D:\Analyst Data\Projects\SH_NAV2022_04_18_Gavisova_Blood_Samples\Data\Cal.wiff (sample 2)
Area: N/A, Height: N/A, RT: N/A min



First calibration level

Jasem_QC_L_2.5_01 - 11-Deoxycortisol_109.1 (Quality Control) 347.2/109.1 - D:\Analyst Data\Projects\SH_NAV2022_04_18_Gavisova_Blood_Samples\Data\Cal.wiff (sample 14)
Area: 5.793e5, Height: 9.513e4, RT: 6.23 min

