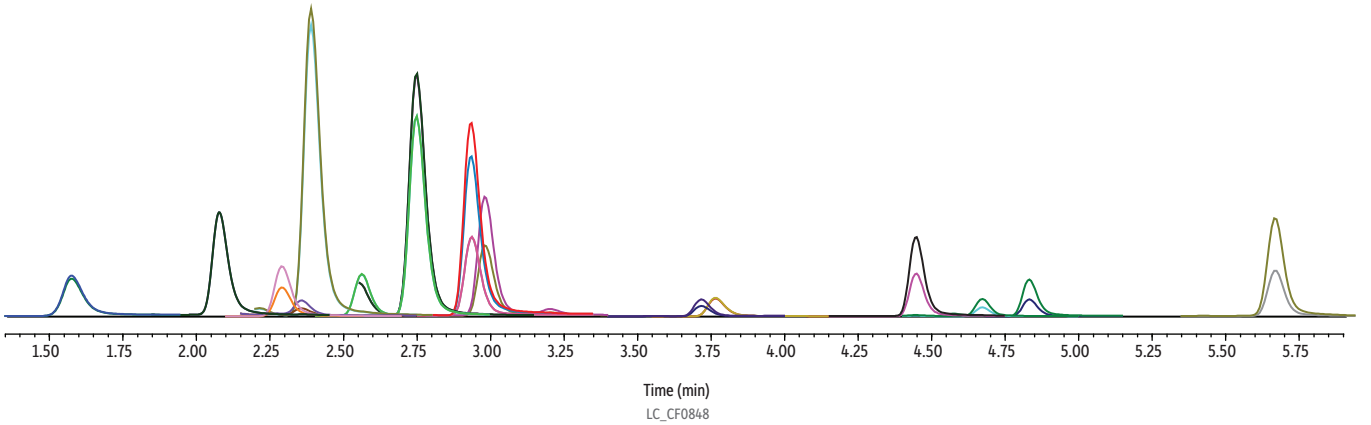
Sixteen Steroid Hormones in Serum on Force Inert C18



Peaks	tR (min)	Conc. (ng/dL)	Precursor	Product 1	Product 2	Polarity
1. Aldosterone*	1.57	1250	452.1	109.9	325.1	+
2. Cortisone*	2.08	2000	452.1	109.9	-	+
3. Estrone (E1)*	2.29	125	362.1	252.2	238.2	+
4. Estradiol (E2)*	2.36	125	364.1	110.1	128.0	+
5. Cortisol*	2.39	2000	454.1	110.1	142.95	+
6. Corticosterone*	2.56	1250	438.2	110.1	120.3	+
7. 11-Deoxycortisol*	2.75	1250	438.2	110.1	311.3	+
8. DHT*	2.92	1250	382.2	110.1	255.2	+
9. 11-Deoxycorticosterone*	2.94	250	422.2	110.1	-	+
10. DHEA*	2.98	1250	380.2	110.1	271.2	+
11. 21-Deoxycortisol	3.71	1250	347.1	311.3	121.1	+
12. DHEAS	3.76	50000	367.0	97.1	79.7	-
13. Androstenedione	4.49	1250	287.1	97.1	109.2	+
14. Testosterone	4.67	250	289.1	97.1	109.1	+
15. 17-Hydroxyprogesterone	4.83	1250	331.1	97.1	109.2	+
16. Progesterone	5.66	1250	315.1	97.1	109.2	+

*Analyte was analyzed as an FMP-TS derivative [M+92].

Column Force Inert C18 (cat.# 9634212-T)
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 1.8 µm
Pore Size: 100 Å
Temp.: 60 °C
Standard/Sample
Diluent: 60:40 Water:methanol, both acidified with 0.1% formic acid
Inj. Vol.: 12 µL
Mobile Phase
A: Water, 2 mM ammonium formate, 0.1% formic acid
B: Methanol, 2 mM ammonium formate, 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	60	40
6.00	0.4	10	90
6.01	0.4	0	100
7.00	0.4	0	100
7.01	0.4	60	40
8.00	0.4	60	40

Max Pressure: 550 bar
Detector Shimadzu 8045 MS/MS
Ion Source: Electrospray
Ion Mode: ESI+/ESI-
Mode: Scheduled MRM
Instrument Shimadzu Nexera X2

Sample Preparation

Two hundred microliters of sample was added to a microcentrifuge tube. Each sample was spiked with 10 µL of internal standard solution and vortexed. Two hundred microliters of HPLC grade water was added to each sample. Samples were briefly vortexed and then centrifuged at 3200 RPM for one minute to mix.

Using a Resprep VM-96 Vacuum Manifold (cat.# 25858), 400 µL of each sample was loaded into a 400 mg Resprep SLE 96-Well Plate (cat.# 28305) with a 2 mL collection plate underneath. Light vacuum was applied to initiate loading the sample into the sorbent bed. With the vacuum turned off, the sample was allowed to absorb into the sorbent for five minutes. The samples were eluted with three 700 µL aliquots of ethyl acetate. Each aliquot was allowed to flow under gravity for five minutes. After the third aliquot was added, light vacuum was applied to remove any remaining extraction solvent.

Samples were dried down under a stream of nitrogen. The derivatization solution was prepared fresh immediately prior to use by dissolving 2-fluoro-1-methylpyridinium p-toluenesulfonate (FMP-TS) in acetonitrile containing 1% triethylamine at a concentration of approximately 5 mg/mL. Once dried, 50 µL of the derivatization solution was added to each sample. The plate was covered with a well plate sealing mat (cat.# 26499), briefly vortexed, and then incubated at 50 °C for 15 minutes. After incubation, the reaction was quenched by adding 50 µL of methanol to each sample and vortexing.

The samples were dried down under a stream of nitrogen. Samples were reconstituted with 100 µL of 60:40 water:methanol, both with 0.1% formic acid (v/v). The plate was covered, briefly vortexed, and centrifuged at 3200 RPM for one minute. The plate was moved to the LC-MS/MS instrument and then injected for analysis.

Notes

The flow was directed to waste before one minute and after six minutes.

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