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Utilizing UPLC Separation to Enable the Analysis of 19 Steroid Hormones in Serum for Clinical Research

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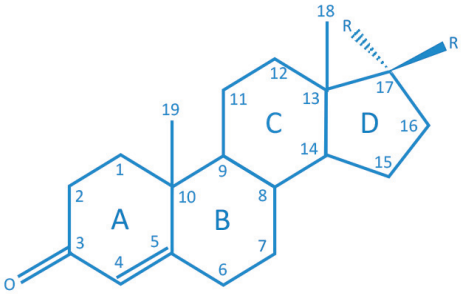

A method for the analysis of 19 steroid hormones has been successfully developed, utilizing UPLC to separate isobaric steroid hormones, allowing for low level quantification using the Xevo TQ-XS from only 200 µL of serum.

GOAL

To successfully develop a UPLC™-MS/MS method for the low-level quantification of 19 steroid hormones using the Xevo™ TQ-XS Tandem Mass Spectrometer, while separating isobaric steroid hormones to minimize mass detection interference.

BACKGROUND

Steroid hormones play a central role in many biochemical processes, which include controlling metabolism, inflammation, immune functions, salt and water balance, development of sexual characteristics, and the ability to withstand illness and injury. Enzymes that form part of the steroid biosynthesis pathway are pivotal in these metabolic processes and their dysfunction can be examined for clinical research through steroid hormones in the pathway.

Measurement of these steroids by immunoassay is prone to analytical interference as a result of cross reactivity of assay antibodies with structurally related steroid hormones and synthetic derivatives. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) can provide analytically sensitive, accurate, and precise measurement of these steroid hormones.

Figure 1. Waters ACQUITY UPLC I-Class System and Xevo TQ-XS Mass Spectrometer.

THE SOLUTION

Here we describe a clinical research method for the extraction and analysis of 19 steroid hormones by UPLC-MS/MS from serum. Chromatographic separation utilizing Waters™ ACQUITY™ UPLC BEH Technology™ enables isobaric steroid hormones to be resolved, allowing for selective quantification at low levels using the Xevo TQ-XS Tandem Mass Spectrometer.

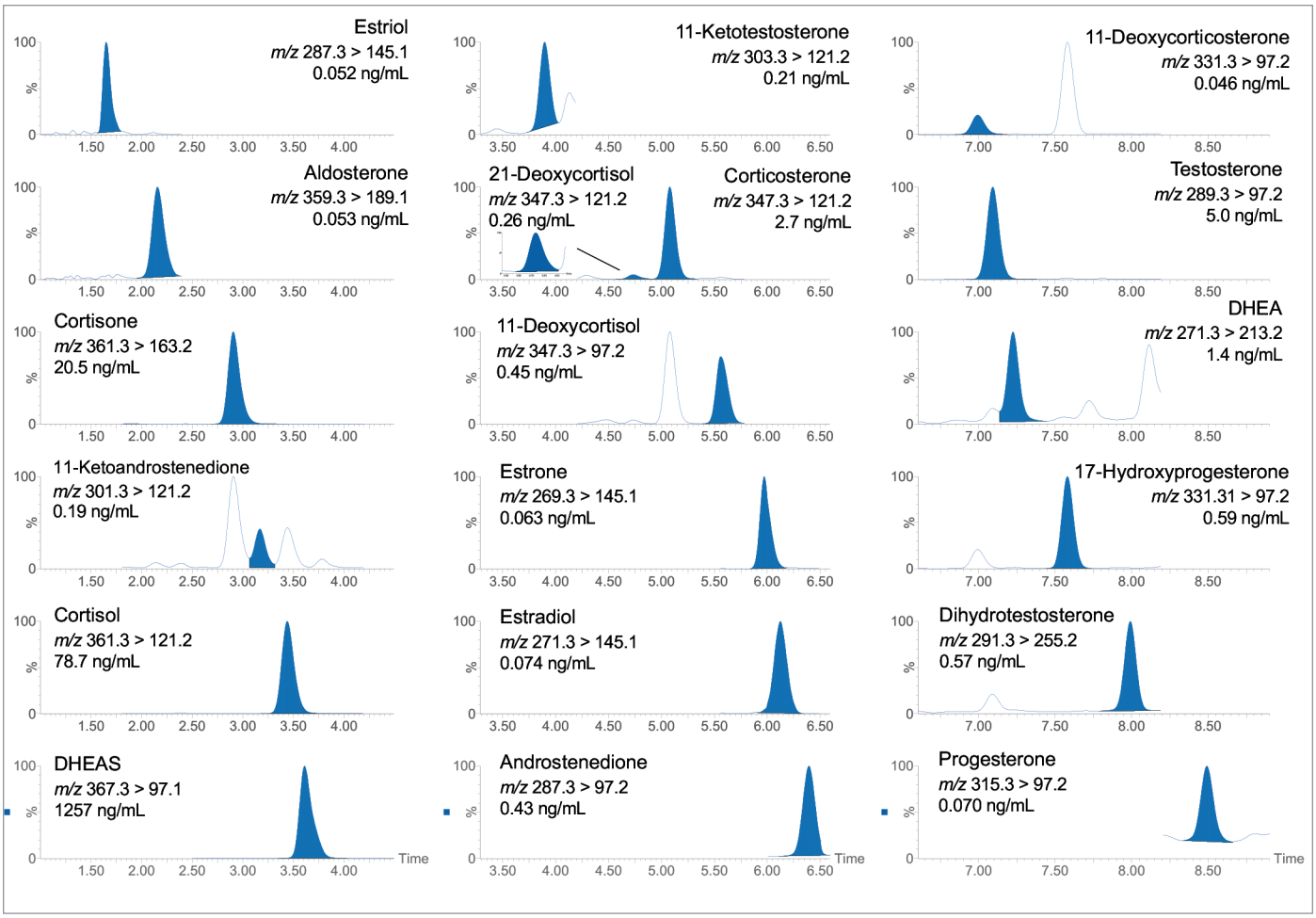

Figure 2. Chromatogram of an extracted pooled serum sample supplemented with a low concentration of each steroid hormone in the panel (peak highlighted in blue). Chromatogram legend includes MRM transitions and calculated concentration.

SAMPLE PREPARATION AND ANALYSIS

Stable labeled internal standards were added to 200 µL of calibrator, QC, or sample prior to liquid/liquid extraction using an ethyl acetate/hexane solution.

Following centrifugation, the top organic layer was transferred to an 800-µL round well collection plate and evaporated to dryness. Samples were reconstituted in 100 µL of a water/methanol/ammonium fluoride solution.

An ACQUITY UPLC I-Class PLUS System was used to separate steroid hormones, including the isobaric species using an ACQUITY UPLC BEH C_8 Column (1.7 µm, 2.1 x 50 mm, p/n: 186002877) and an ACQUITY UPLC BEH C_8 VanGuard™ (1.7 µm, 2.1 x 5 mm, p/n: 186003978) with a water, methanol, and ammonium fluoride gradient, and analyzed on a Waters Xevo TQ-XS Mass Spectrometer.

RESULTS

Separation of all isobaric steroids, such as aldosterone, cortisone, and cortisol, and 21-deoxycortisol, corticosterone, and 11-deoxycortisol was achieved using the ACQUITY UPLC BEH C_8 Column technology described. An example chromatogram is shown in Figure 2 for a pooled serum sample, demonstrating a runtime of <10 minutes injection-to-injection.

Over five days, all calibration curve correlation coefficients (r2) were >0.99 for all steroid hormones across the concentration ranges shown in Table 1.

Total precision and repeatability of the method was assessed by extracting and quantifying stripped serum samples using five replicates fortified at low, mid, and high concentrations across five days (n=25). A serum pool was also supplemented at a low concentration and five replicates were quantified across five days (n=25). All results are shown in Table 1.

Analytical sensitivity was assessed by extracting 10 replicates of stripped serum samples spiked at low to high concentrations over three days (n=30). A precision of <20 %CV was obtained at the concentrations listed in Table 1.

Extraction recovery was evaluated by spiking stripped serum samples at mid and high concentrations before and after extraction and results compared.

Steroid hormone	Calibration (ng/mL)	Precision		Analytical sensitivity			Extraction recovery	
		Range (ng/mL)	%CV	Conc. (ng/mL)	%CV	S:N (ptp)	Range (ng/mL)	(%)
Estriol	0.010–50.0	0.030–37.4	≤7.7	0.011	13.3	20.4	0.050–35.0	≥69.3
Aldosterone	0.010–2.5	0.016–1.1	≤12.8	0.011	13.1	12.5	0.024–1.1	≥59.7
Cortisone	1.0–500	3.2–382	≤2.9	0.056	9.6	81.4	5.3–387	≥75.8
11-Ketoandrostenedione	0.050–10.0	0.036–28.7	≤18.4	0.017	14.2	8.2	0.063–30.1	≥74.2
Cortisol	0.10–100	0.32–79.5	≤2.6	0.027	6.4	13.9	0.52–79.6	≥63.1
DHEAS	10.0–10,000	33.4–7772	≤4.7	5.9	4.5	14.4	56.0–7154	≥0.3
11-Ketotestosterone	0.010–10.0	0.031–43.2	≤6.4	0.011	12.8	8.7	0.047–40.5	≥71.8
21-Deoxycortisol	0.050–50.0	0.16–35.4	≤10.7	0.031	6.3	11.2	0.27–37.0	≥75.4
Corticosterone	0.10–100	0.33–77.6	≤6.1	0.021	13.9	11.0	0.52–78.3	≥73.4
11-Deoxycortisol	0.050–50.0	0.16–36.4	≤4.9	0.014	7.4	59.0	0.027–37.1	≥70.7
Estrone	0.010–2.5	0.016–1.1	≤10.7	0.010	13.1	12.9	0.026–1.1	≥66.4
Estradiol	0.010–2.5	0.016–1.3	≤11.1	0.011	17.5	13.6	0.025–1.3	≥67.0
Androstenedione	0.010–50.0	0.030–36.4	≤6.6	0.007	8.4	13.3	0.059–37.7	≥67.6
11-Deoxycorticosterone	0.005–2.5	0.016–1.2	≤2.8	0.003	8.8	15.2	0.028–1.2	≥76.1
Testosterone	0.010–50.0	0.030–35.2	≤8.6	0.010	9.7	12.4	0.051–35.6	≥65.7
DHEA	0.10–50.0	0.12–37.4	≤15.2	0.010	8.7	14.1	0.56–36.3	≥70.4
17-Hydroxyprogesterone	0.010–50.0	0.030–34.2	≤8.1	0.011	13.8	16.9	0.054–33.7	≥68.9
5α-Dihydrotestosterone	0.010–2.5	0.71–1.1	≤9.6	0.012	8.0	11.1	0.11–1.1	≥71.1
Progesterone	0.2–50.0	0.030–34.6	≤8.1	0.010	13.4	13.5	0.10–38.9	≥67.3

Table 1. Performance test data for 19 steroid hormones in serum.

SUMMARY

A selective UPLC-MS/MS clinical research method has successfully been developed for the analysis of 19 serum steroid hormones. Chromatographic separation utilizing Waters ACQUITY UPLC BEH Technology enabled all isobaric steroid hormones in the panel to be resolved in under 10 minutes, allowing for selective quantification at low levels using the Xevo TQ-XS Tandem Mass Spectrometer from only 200 µL of serum.

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