Measurement of a Panel of Steroids by LC-MS/MS, Employing Rapid Polarity Switching

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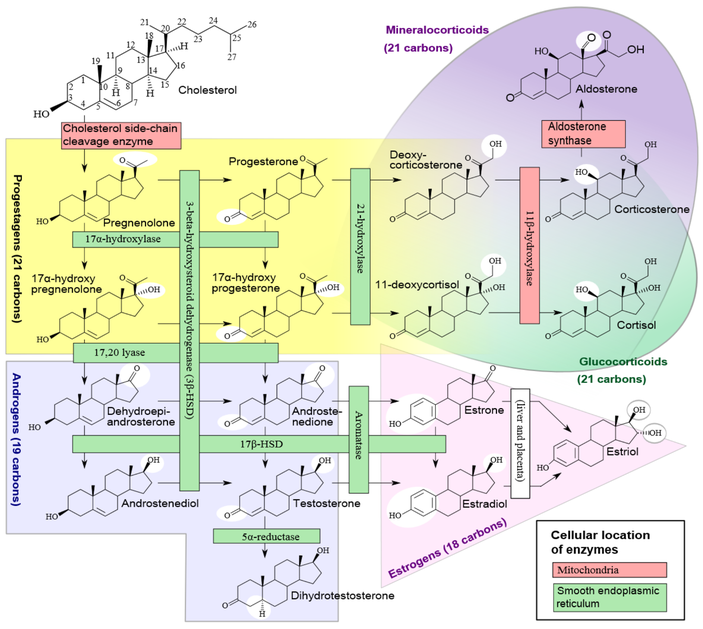
INTRODUCTION

Steroid profiling is useful for the identification of potential defects in steroid biosynthesis, since abnormal ratios of steroids may be indicative of metabolic deficiencies. Ideally, a single analytical method should provide coverage for a broad panel of steroids.



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It has been well established that liquid chromatography-tandem mass spectrometry (LC-MS/MS) provides excellent accuracy, precision and sensitivity for measurements of steroids in biological matrices compared to traditional techniques such as immunoassays, which may suffer from cross-reactivity.

However, the analysis of individual steroids by LC-MS/MS are typically accomplished under a wide range of optimized conditions (positive versus negative ionization, optimized mobile phase compositions, etc), which vary from analyte to analyte, making it challenging to simultaneously measure a broad panel of steroids. The goal of this work was to develop a sensitive method, employing rapid polarity switching, enabling the simultaneous measurement of a broad panel of steroids.

Table 1: Panel of steroids for analysis by LC-MS/MS

Aldosterone	11-Deoxycorticosterone	Pregnenolone
Androstenedione	DHEA	Pregnenolone Sulfate
Corticosterone	DHEAS	17-OH-Progesterone
Cortisol	5a-DHT	Progesterone
11-Deoxycortisol	Estradiol	Testosterone
21-Deoxycortisol	17-OH-Pregnenolone	

MATERIALS AND METHODS

Calibration standards were prepared in steroid-free serum from Golden West Biologicals using stock solutions obtained from Cerilliant Corporation (Round Rock, Texas). Table 1 lists the analytes considered in the steroid panel. Deuterated analogs were used as internal standards for 14 of the 17 analytes.

Sample Preparation:

Samples were prepared using narrow-bore extraction (NBE) SPE cartridges from SPEware Corporation (Baldwin Park, CA), and using the CEREX TouchPro system. The TouchPro system enabled computer-controlled positive pressure SPE and sample evaporation, ensuring reproducibility from run to run.

Table 2: Solid Phase Extraction sample preparation, using the SPEware TouchPro system and Maestro NBE columns.

1.	Condition Maestro NBE columns with 200uL methanol, followed by 200uL deionized water.
2.	To 100uL of sample, add 600 uL of 95:5 water:isopropanol and 50uL of internal standard. Vortex mix well, then load prepared samples onto Maestro columns.
3.	Wash columns with 300uL of 95:5 water:isopropanol.
4.	Dry columns under nitrogen gas for 8 minutes.
5.	Wash columns with 250uL of hexane.
6.	Dry columns under nitrogen for 1 minute.
7.	Elute analytes with 200uL of 80:20 hexane:ethyl acetate in high-recovery autosampler vials.
8.	Evaporate extracts to dryness under nitrogen.
9.	Reconstitute samples in 25uL MeOH. Then add 50uL deionized water.

HPLC Conditions:

Chromatographic separation was accomplished using the Shimadzu Prominence 30AD UHPLC system, with a Phenomenex Kinetex C18 column (150x3.0mm, 2.6µm), at a flow rate of 0.6 mL/min. Mobile phase A consisted of water containing 0.2mM NH_4F. Mobile phase B consisted of methanol. An 11-minute gradient allowed separation of the analytes, with no interference between isomers. Figure 2 displays an example chromatogram for a high concentration standard. Figure 3 displays the separation of critical pairs of steroid isomers.

Figure 2. LC-MS/MS analysis of 17 steroids in 11 minute run-time.

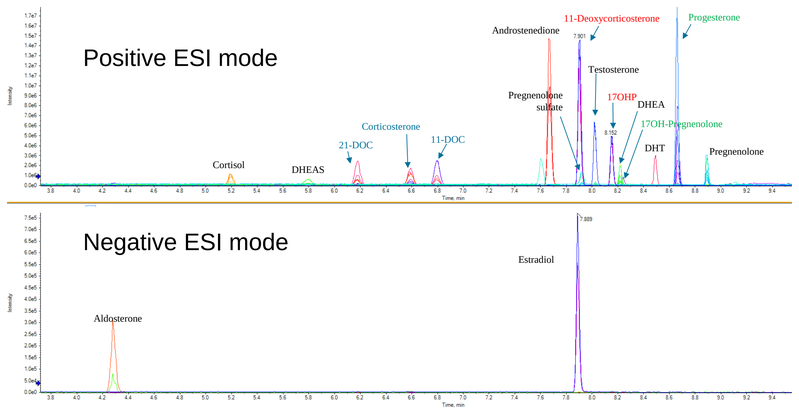
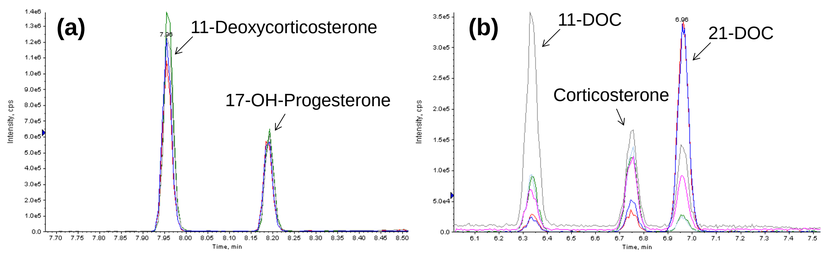


Figure 3. Baseline chromatographic separation was achieved for the critical pairs of steroid isomers, including (a) 11-Deoxycorticosterone and 17-OH-Progesterone, and (b) 21-Deoxycortisol (21-DOC), Corticosterone, and 11-Deoxycortisol (11-DOC)



MS/MS Conditions:

MS/MS detection was accomplished using the SCIEX Triple Quad™ 6500+ system equipped with IonDrive™ Turbo V source and operated in electrospray ionization mode. Multiple Reaction Monitoring (MRM) mode was employed, with 2 MRM transitions monitored per analyte.

Table 3. MRM parameters for panel of 17 steroids

Q1	Q3	Ret Time	ID	DP	EP	CE	CXP
331.2	97	7.9	11-Deoxycorticosterone 1	60	10	26	12
331.2	109	7.9	11-Deoxycorticosterone 2	60	10	29	12
347.1	97	6.8	11-Deoxycortisol 1	60	10	30	12
347.1	109	6.8	11-Deoxycortisol 2	60	10	36	12
315.1	297.1	8.25	17-OH-Pregnenolone 1	50	10	20	10
315.1	159.1	8.25	17-OH-Pregnenolone 2	50	10	35	14
315.1	91	8.25	17-OH-Pregnenolone 6	50	10	70	10
331	97	8.15	17-OH-Progesterone 1	70	10	31	12
331	109	8.15	17-OH-Progesterone 2	70	10	38	12
347.1	121.1	6.18	21-Deoxycortisol 1	70	10	34	10
347.1	311	6.18	21-Deoxycortisol 3	70	10	23	10
291.1	255	8.5	5a-Dihydrotestosterone 1	55	10	22	22
291.1	159.1	8.5	5a-Dihydrotestosterone 2	55	10	31	14
287.1	109	7.68	Androstenedione 1	50	10	32	12
287.1	97	7.68	Androstenedione 2	50	10	28	12
347.1	121.1	6.6	Corticosterone 1	70	10	32	10
347.1	91	6.6	Corticosterone 2	70	10	81	10
363	121	5.2	Cortisol 1	50	10	30	6
363	97	5.2	Cortisol 5	50	10	32	10
271.2	253	8.2	DHEA 1	50	10	20	8
271.2	213	8.2	DHEA 2	50	10	23	12
271.2	91.1	5.8	DHEAS 2	70	10	65	10
271.2	253	5.8	DHEAS 5	70	10	20	14
299.1	281	8.9	Pregnenolone 8	70	10	21	14
299.1	159	8.9	Pregnenolone 11	70	10	30	8
299.1	159.1	7.92	Pregnenolone Sulfate 3	45	10	28	8
299.1	211.1	7.92	Pregnenolone Sulfate 4	45	10	27	12
315	109	8.65	Progesterone 1	50	10	32	12
315	97	8.65	Progesterone 2	50	10	28	12
289.1	109	8.05	Testosterone 1	60	10	32	12
289.1	97	8.05	Testosterone 2	60	10	30	12
271	145	7.97	Estradiol 1	-40	-10	-51	-16
271	143	7.97	Estradiol 2	-40	-10	-69	-16
359.2	189	4.4	Aldosterone 1	-55	-10	-27	-22
359.2	331.1	4.4	Aldosterone 2	-55	-10	-23	-20

RESULTS

The SCIEX 6500+ system enabled sensitive detection of the target analytes. Rapid polarity switching enabled the use of both +ESI and –ESI within the same run. Example chromatograms are shown in Figure 4, demonstrating the ability to detect 1 pg/mL Estradiol and Aldosterone in neat solvent.

Figure 4. Analysis of steroids in neat solvent at 1 pg/mL on the SCIEX 6500+ LC-MS/MS system. (a) Estradiol (b) Aldosterone

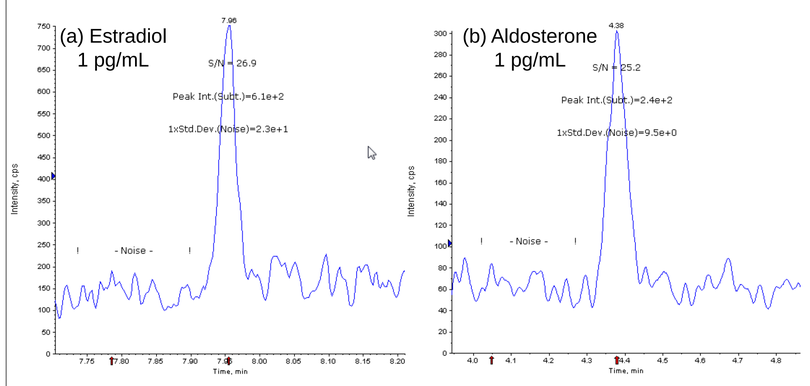
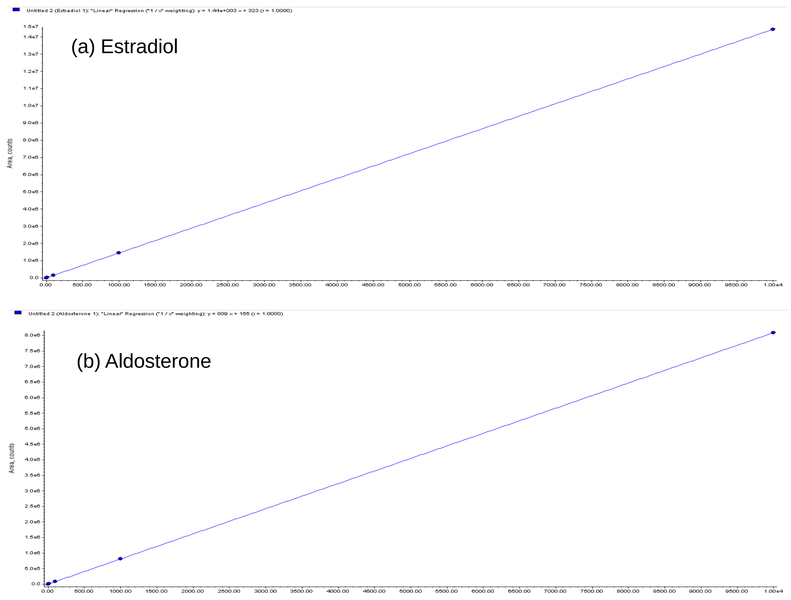
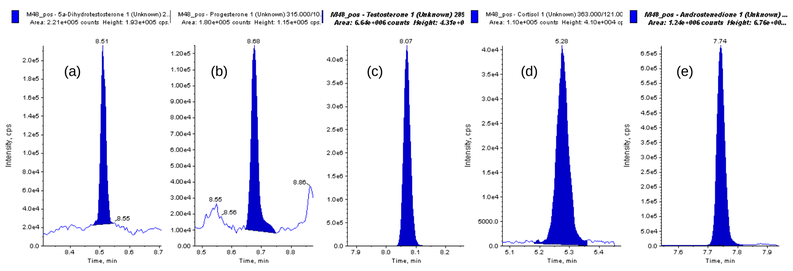


Figure 5. Linear calibration curves for steroids in neat solvent, from 1 pg/mL to 10 ng/mL. (a) Estradiol, (b) Aldosterone



To evaluate the multi-steroid method, several serum samples were analyzed. Shown below in Figure 6 are representative chromatograms for selected analytes detected and quantified in the serum samples. Our preliminary results demonstrate that the method is appropriate for quantifying steroids at the required levels.

Figure 4. Chromatograms showing several steroids that were detected and quantified in a male serum sample. (a) Dihydrotestosterone, (b) Progesterone, (c) Testosterone, (d) Cortisol, (e) Androstenedione.



CONCLUSIONS

We developed a sensitive method for the detection of a panel of steroids in serum. The method employs rapid polarity switching, enabling the analysis of steroids in both +ESI and –ESI modes within the same run. The use of the highly sensitive SCIEX Triple Quad 6500+ system enabled the measurement of analytes at concentrations <1 pg/mL, for selected steroids.

TRADEMARKS/LICENSING