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Confident quantification of steroids: analysis in human plasma or serum by liquid chromatography-tandem mass spectrometry for clinical research

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Keywords

Steroids, offline sample preparation, plasma, serum, TSQ Quantis, mass spectrometry

Application benefits

- Simple offline sample preparation by protein precipitation
- Eight steroids in a single quantitative method

Goal

Development and implementation of a robust, reliable, sensitive analytical method for quantification of eight steroids in human plasma or serum using Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer.

Introduction

The endocrine system controls signaling pathways that direct critical physiological processes such as development, reproduction, and metabolism. A major group of signaling molecules of the endocrine system is the steroid hormone family. Steroid hormones are defined as endogenous hormones synthesized from a cholesterol backbone and subtle changes in steroid structure can lead to drastic differences in physiological function. The four classes of steroid hormones (estrogens, androgens, progestogens, and corticosteroids) are often grouped together, however, most steroid hormones are regulated at different concentrations by different species. Quantifying and analyzing only one class of steroids often lead to misleading information, and hence, there is a strong need for a comprehensive steroid hormone profile that include analysis and quantitation. While several technologies exist for quantitation of analytes in biological matrices, owing to its ease-of-use, selectivity, and specificity, liquid chromatography (LC) coupled to triple quadrupole mass spectrometers (MS/MS) has gained widespread popularity.

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In this report, a robust, reliable LC-MS/MS method for clinical research is developed for the quantification of eight steroids in human plasma or serum. The analytical method reported in here includes 11- and 21-deoxycortisol, 17- and 21-hydroxyprogesterone, androstenedione, cortisol, dehydroepiandrosterone sulphate (DHEAS), and testosterone.

Experimental

Target analytes

The analytes, corresponding internal standards, and concentration ranges covered by the calibrators used are reported in Table 1.

Sample preparation

Reagents included seven calibrators (including blank) and three controls from RECIPE Chemicals + Instruments GmbH (Munich, Germany), as well as six isotopically labeled internal standards for the quantification. Samples of 50 µL of plasma or serum were protein precipitated using 75 µL of precipitating solution containing the internal standards. Precipitated samples were vortex-mixed and centrifuged, and the supernatant was transferred to a clean plate or vial.

Liquid chromatography

The LC separation was achieved using mobile phases and analytical column provided by RECIPE. Details of the analytical method are reported in Table 2. Total runtime was 7.0 minutes.

Table 1. Concentration ranges covered by calibrators

Analyte	Internal Standard	Concentration Range (ng/mL)
Androstenedione	d7-Androstenedione	0.117–12.7
Cortisol	d4-Cortisol	2.75–325
DHEAS	d5-DHEAS	43.9–4851
11-Deoxycortisol	d5-11-Deoxycortisol	0.100–12.5
21-Deoxycortisol	d5-11-Deoxycortisol	0.117–14.7
17-Hydroxyprogesterone	d8-17-Hydroxyprogesterone	0.109–12.5
21-Hydroxyprogesterone	d8-17-Hydroxyprogesterone	0.196–24.4
Testosterone	d3-Testosterone	0.109–11.0

Table 2. LC method description

Gradient profile:	Time (min)	Flow rate (mL/min)	A (%)	B (%)
	0.00	0.5	73	27
	4.5	0.5	43	57
	4.51	0.5	0	100
	4.64	0.5	0	100
	4.75	0.8	0	100
	5.35	0.8	0	100
	5.36	0.8	73	27
	5.65	0.8	73	27
	5.66	0.5	73	27
	6.00	0.5	73	27
Injection volume:	40 µL			
Column temperature:	40 °C			

Mass spectrometry

Analytes and internal standards were detected by selected reaction monitoring (SRM) on a TSQ Quantis triple quadrupole mass spectrometer with heated electrospray ionization operated in positive mode. Extracted samples were injected onto a Thermo Scientific™ Transcend™ II TLX-2 system connected to TSQ Quantis mass spectrometer followed by detection using six isotopically labeled internal standards. A summary of the MS conditions is reported in Table 3. Two SRM transitions for each analyte were included in the acquisition method for quantification and confirmation, respectively.

Table 3. MS settings

Source type:	Heated electrospray ionization (HESI)
Vaporizer temperature:	350 °C
Capillary temperature:	325 °C
Spray voltage (positive mode):	3500 V
Sheath gas:	50 AU
Sweep gas:	1 AU
Auxiliary gas :	10 AU
Data acquisition mode:	Selected-reaction monitoring (SRM)
Collision gas pressure:	1.5 mTorr
Cycle time:	0.300 s
Q1 mass resolution (FWMH):	0.7
Q3 mass resolution (FWMH):	1.2

Method evaluation

Method performance was evaluated using the ClinMass® LC-MS/MS Complete Kit from RECIPE. The method performance was evaluated in terms of linearity of response within the calibration ranges, carryover, accuracy, and intra-assay precision for each analyte. Carryover was calculated in terms of percentage ratio between peak area of the highest calibrator and a blank sample injected just after it. Analytical accuracy was evaluated in terms of percentage bias between nominal and average back-calculated concentrations using quality control samples at three different levels provided by RECIPE (MS12083 batch #1226) prepared and analyzed in replicates of four. Intra-assay precision was evaluated on the same run (control samples at three levels, replicates of four) in terms of percentage coefficient of variation (%CV).

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 4.1 software.

Results and discussion

The method proved to be linear in the calibration ranges covered by the calibrators. It was not possible to detect the lowest calibrator for 17-hydroxyprogesterone but the method was linear in the range calibrator 2 through calibrator 6. Representative chromatograms for the lowest calibrator for testosterone, 21-deoxycortisol, and their internal standards are reported in Figure 1. Representative calibration curves for the same analytes are reported in Figure 2.

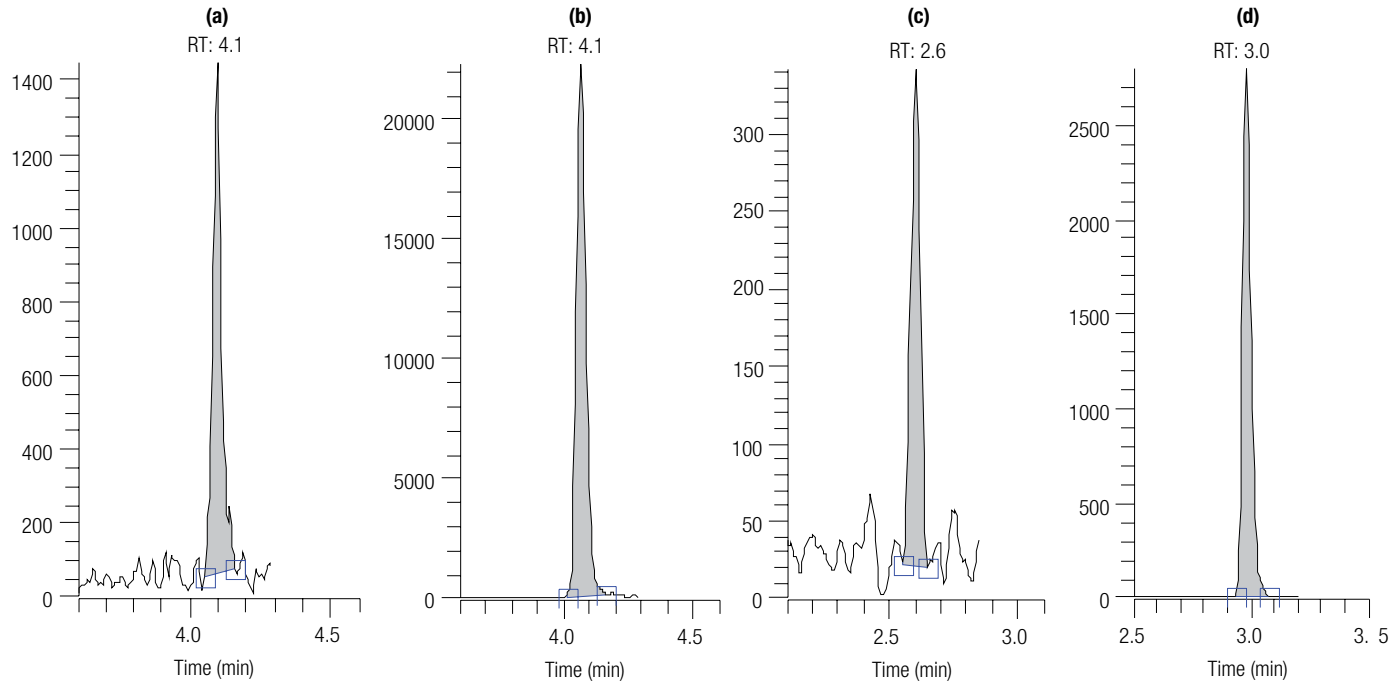

Figure 1. Representative chromatograms of the lowest calibrator for (a) testosterone, (b) d3-testosterone, (c) 21-deoxycortisol, and (d) d5-11-deoxycortisol

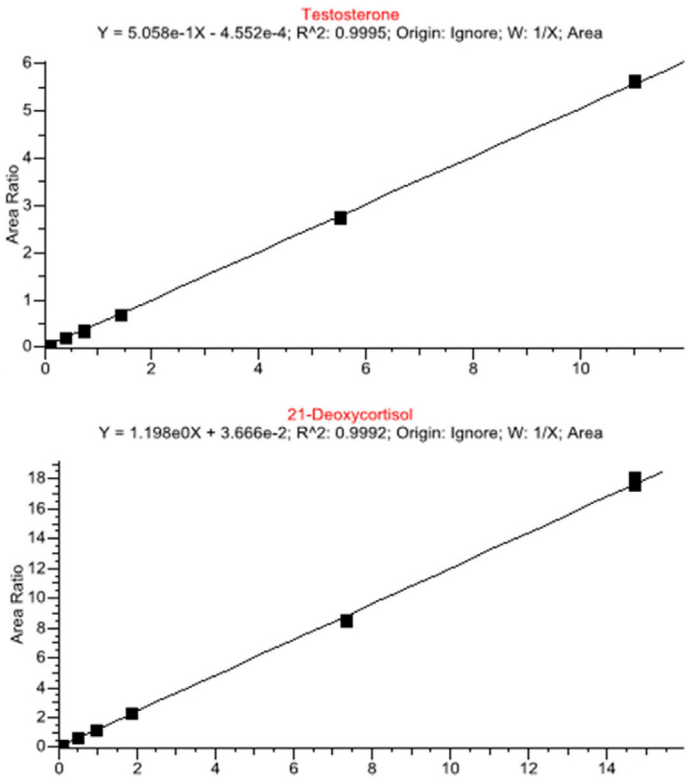

Figure 2. Representative calibration curves for (a) testosterone and (b) 21-deoxycortisol

The maximum registered carryover was 0.08% with the exception of androstenedione, which showed a value of 0.36%.

The data demonstrated outstanding accuracy of the method with the percentage bias between nominal and average back-calculated concentration for the control samples ranging between -4.4% and 5.3%. The %CV for intra-assay precision was always below 5.7% for all the analytes. Results for accuracy and intra-assay precision reported in Table 4.

Table 4 (part 1). Analytical accuracy and intra-assay precision results for control MS12083 batch #1226 for level I

Analyte	Level I			
	Nominal Concentration (ng/mL)	Average Calculated Concentration (ng/mL)	Bias (%)	CV (%)
Androstenedione	0.219	0.223	1.9	3.7
Cortisol	5.53	5.61	1.5	1.0
DHEAS	86.4	89.0	2.9	5.2
11-Deoxycortisol	0.225	0.232	3.0	2.1
21-Deoxycortisol	0.248	0.256	3.0	2.1
17-Hydroxyprogesterone	N/A	N/A	N/A	4.7
21-Hydroxyprogesterone	0.422	0.412	-2.5	4.4
Testosterone	0.198	0.196	-1.0	5.7

Table 4 (part 2). Analytical accuracy and intra-assay precision results for control MS12083 batch #1226 for level II

Analyte	Level II			
	Nominal Concentration (ng/mL)	Average Calculated Concentration (ng/mL)	Bias (%)	CV (%)
Androstenedione	0.604	0.604	0.0	2.5
Cortisol	15.4	16.0	3.7	0.9
DHEAS	235	241	2.3	4.0
11-Deoxycortisol	0.633	0.662	4.3	3.9
21-Deoxycortisol	0.692	0.699	1.0	3.9
17-Hydroxyprogesterone	0.599	0.574	-4.4	1.8
21-Hydroxyprogesterone	1.18	1.18	-0.4	5.0
Testosterone	0.540	0.546	1.1	1.6

Table 4 (part 3). Analytical accuracy and intra-assay precision results for control MS12083 batch #1226 for level III

Analyte	Level III			
	Nominal Concentration (ng/mL)	Average Calculated Concentration (ng/mL)	Bias (%)	CV (%)
Androstenedione	3.21	3.29	2.6	0.8
Cortisol	84.0	88.5	5.1	1.1
DHEAS	1292	1345	4.0	2.4
11-Deoxycortisol	3.54	3.70	4.3	1.1
21-Deoxycortisol	3.76	3.97	5.3	0.5
17-Hydroxyprogesterone	3.29	3.47	5.2	0.0
21-Hydroxyprogesterone	6.45	6.80	5.1	1.5
Testosterone	2.88	3.04	5.2	1.3

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Conclusions

A robust, reliable LC-MS/MS method for clinical research for the quantification of eight steroids in human plasma or serum was developed and implemented. The ClinMass LC-MS/MS Complete Kit from RECIPE ensured increased confidence in the result that was obtained. The method was analytically validated on a Transcend II system connected to a TSQ Quantis triple quadrupole mass spectrometer. The sample preparation procedure utilized in this method uses quick and simple offline protein precipitation with concomitant internal standard addition. The described method meets research laboratory requirements in terms of sensitivity, linearity of response, accuracy, and precision.

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