Robust and sensitive quantitation of 18 steroids in human serum using Thermo Scientific TSQ Certis Triple Quadrupole mass spectrometer

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Abstract

Purpose: To develop a robust and sensitive LC–MS/MS method for the simultaneous quantification of 18 endogenous steroids in human serum using Thermo Scientific™ TSQ Certis™ mass spectrometer.

Methods: Serum steroids were extracted and separated using a 9-min LC method. Calibration curves spanning wide concentration ranges were generated with using isotope dilution method. MS parameters were optimized, and performance was evaluated using commercial calibrators and QC samples.

Results: The method showed excellent sensitivity and linearity across broad dynamic ranges. Accuracy was within 30% of QC targets, with %RSD typically <14%. Robustness was confirmed over 311 injections, with consistent MS response (%RSD <17%) and stable performance.

Introduction

Accurate quantification of endogenous steroids in human serum is essential for biomonitoring physiological functions such as hormone regulation, reproduction, and immune response in clinical research laboratories. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) is the gold standard for steroid analysis due to its superior sensitivity, selectivity, and multiplexing capability compared to immunoassays. However, steroid measurements remain challenging because of low endogenous concentrations and complex serum matrices. Here, we describe a robust and sensitive LC–MS/MS method for the simultaneous quantification of 18 steroids in human serum using the Thermo Scientific TSQ Certis triple quadrupole mass spectrometer (Figure 1). The method leverages advanced ion source design and fast acquisition to deliver enhanced sensitivity, stability and throughput suitable for high-volume laboratory workflows.

Materials and methods

Sample preparation

Steroid standards were serially diluted in methanol to generate calibration working solutions. Calibration working solution of 10 µL was added to 10 µL of IS solution and 160 µL of 0.05% BSA in phosphate-buffered saline (PBS, w/v). Calibration samples were precipitated $ZnSO_4$ and ice-cold acetonitrile and reconstituted in 100 µL of 50% water/methanol for LC–MS analysis. A 5 µL was injected for LC–MS analysis.

Liquid chromatograph – mass spectrometry

Samples were analyzed on a Thermo Scientific™ Vanquish™ Horizon UHPLC system coupled to the TSQ Certis MS operated in selected reaction monitoring (SRM) mode. The mobile phase composition, analytical column details, LC gradient, and overlaid extracted ion chromatograms (EICs) are shown in Figure 3.

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software (v 5.2).

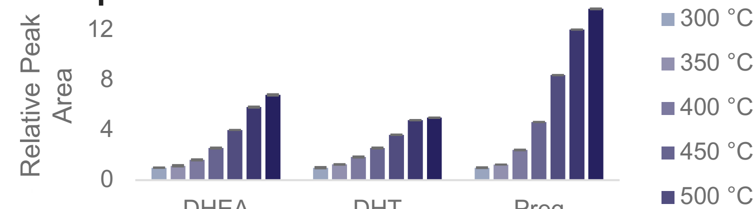
Results

MS parameters optimization

The Thermo Scientific™ OptaMax™ Plus HESI source on the TSQ Certis MS provided stable spray performance over a broad vaporizer temperature range. Figure 2 shows the effect of vaporizer temperature (300 – 600 ° C, in 50 ° C increments) on the MS responses. Up to a two-fold increase in relative peak area was observed for most steroids, while DHT, DHEA, and Preg exhibited significant signal enhancements (6 to 14-fold). A vaporizer temperature of 525 ° C was selected as the optimal global parameter for subsequent experiments.

Figure 1. Vanquish Horizon UHPLC coupled to TSQ Certis MS.

Figure 2. HESI source vaporizer temperature optimization. Significant response increases were observed in DHT and water loss precursors of DHEA and Preg with increasing vaporizer temperatures.



Quantification of steroids

Calibration curves were generated using linear regression with a 1/x weighting factor. All calibration curves exhibited coefficients of determination (R²) greater than 0.99 across up to four orders of magnitude in concentration. Limits of quantification (LOQs) were defined as the lowest concentration that reached |% Diff|, % RSD (of the calculated amount), %CV (of the peak areas) and relative ion ratio < % 20 (N = 4) (Table 1). Estrone, DHT, and testosterone were selected as representative examples to demonstrate the calibration curves and corresponding EICs of quantifier and qualifier ions at their LOQ levels (Figure 4).

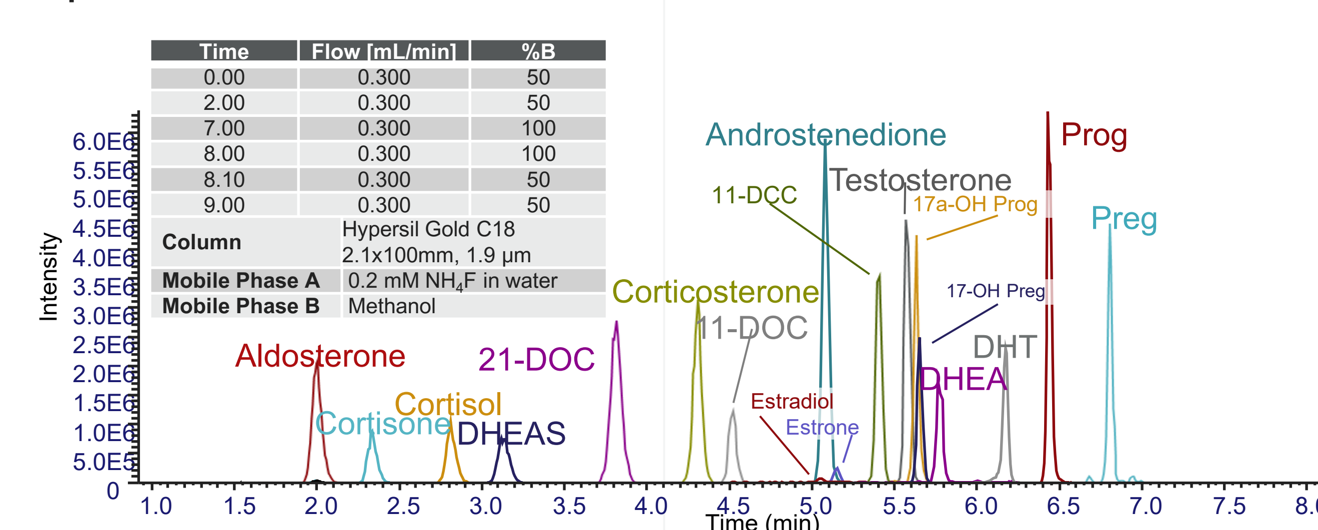
The developed method was then applied to the commercial serum steroid calibrator set (6PLUS1 Multilevel Serum Calibrator Set, Chromsystems Instruments & Chemicals GmbH, Germany), and quality control (QC) samples (Steroid Serum Control, UTAK Laboratories Inc, Valencia, CA). Calibration curves were externally constructed on peak areas.

All steroids demonstrated excellent linearity across their respective calibration ranges, with R² values exceeding 0.99. Expected concentrations and R² values for the calibrator set are summarized in Table 2. DHEA and estradiol were chosen as representative compounds to demonstrate the calibration curves and EICs of the quantifier ions in the STD 1 sample (Figure 5). Steroids in three QC samples, QC-L, QC-M, and QC-H, were quantified using calibration curves derived from the commercial calibrators. Measured steroid concentrations were within 30% of expected values, with %RSD values below 14% (Table 3).

Table 1. LOQ values of steroids (pg on column).

Compound name	LOQ (pg o.c.)	%RSD at LOQ	%CV at LOQ	Linear Range (pg o.c.)	R^2
Aldosterone	0.10	10.43	6.60	0.10 - 20	0.9994
Cortisone	0.10	8.21	6.77	0.10 - 200	0.9996
Cortisol	0.05	9.30	7.13	0.05 – 200	0.9998
DHEAS	0.10	11.70	5.13	0.10 - 200	0.9989
21-DOC	0.10	5.61	5.95	0.10 - 200	0.9991
Corticosterone	0.05	11.83	8.40	0.05 - 200	0.9997
11-DOC	0.02	11.67	8.59	0.02 - 20	0.9987
Androstenedione	0.02	10.38	8.14	0.02 - 20	0.9984
Estrone	0.20	4.20	3.24	0.20 – 400	0.9997
Estradiol	0.40	8.30	8.50	0.40 - 400	0.9994
11-DCC	0.02	5.89	4.49	0.02 - 200	0.9996
Testosterone	0.01	7.59	4.88	0.01 - 20	0.9984
17-OH-Prog	0.05	8.46	7.40	0.05 - 200	0.9998
DHEA	5.00	8.83	6.78	5.00 - 200	0.9940
17-OH-Preg	1.00	4.88	7.66	1.00 - 2000	0.9990
DHT	0.20	8.66	7.37	0.20 - 40	0.9981
Prog	0.05	7.07	6.01	0.05 - 20	0.9976
Preg	5.00	3.44	0.89	5.00 - 200	0.9976

Figure 3. The LC conditions, gradient, and representative EIC overlay of steroid separation in BSA matrix.



Time	Flow [mL/min]	%B
0.00	0.300	50
2.00	0.300	50
7.00	0.300	100
8.00	0.300	100
8.10	0.300	50
9.00	0.300	50
Column	Hypersil Gold C18 2.1x100mm, 1.9 µm	
Mobile Phase A	0.2 mM NH_4F in water	
Mobile Phase B	Methanol	

Figure 4. Calibration results of estrone, DHT, and testosterone in 0.05% BSA. (Top row) Quantifier (black trace) and qualifier (red trace) EIC overlay at the LOQ level, and (bottom row) their calibration curves and zoom-in to the lowest three calibration points. o.c. - on column.

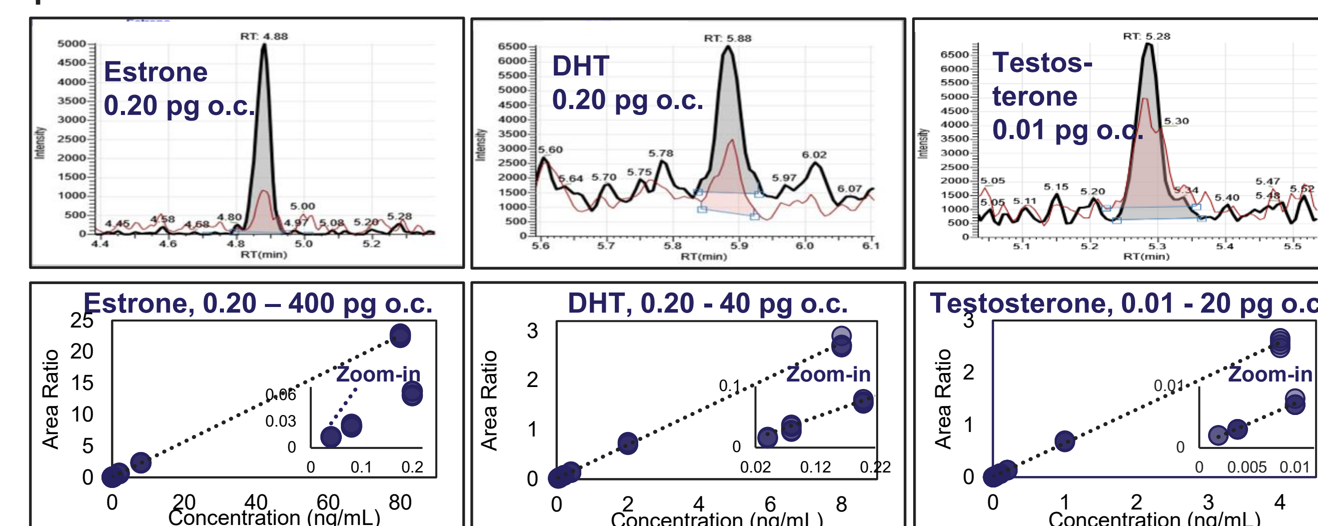


Table 2. Expected concentrations of steroids from 6PLUS1 Multilevel Serum Calibrator Set using external calibration (N = 3). .

Analyte	STD 1 (ng/mL)	STD 2 (ng/mL)	STD 3 (ng/mL)	STD 4 (ng/mL)	STD 5 (ng/mL)	STD 6 (ng/mL)	R^2
11-DCC	0.049	0.098	0.149	0.291	0.728	2.950	0.9998
17-OH Prog	0.089	0.484	0.978	1.930	3.930	22.500	0.9994
Androstenedione	0.200	0.409	0.820	1.470	4.830	14.800	0.9995
DHEA	0.886	4.940	9.460	14.100	27.900	55.900	0.9961
DHT	0.057	0.110	0.257	0.497	0.985	1.490	0.9945
Estradiol	0.039	0.101	0.261	0.516	1.540	5.240	0.9938
Progesterone	0.141	0.726	1.970	4.850	9.610	24.400	0.9992
Testosterone	0.055	0.252	0.984	2.880	5.760	11.600	0.9994

Figure 5. Calibration curves of DHEA and estradiol from 6PLUS1 Multilevel Serum Calibrator Set.

A. DHEA, SRM MS² *271.21 → 253* **B. Estradiol, SRM MS² *271.17 → 183***

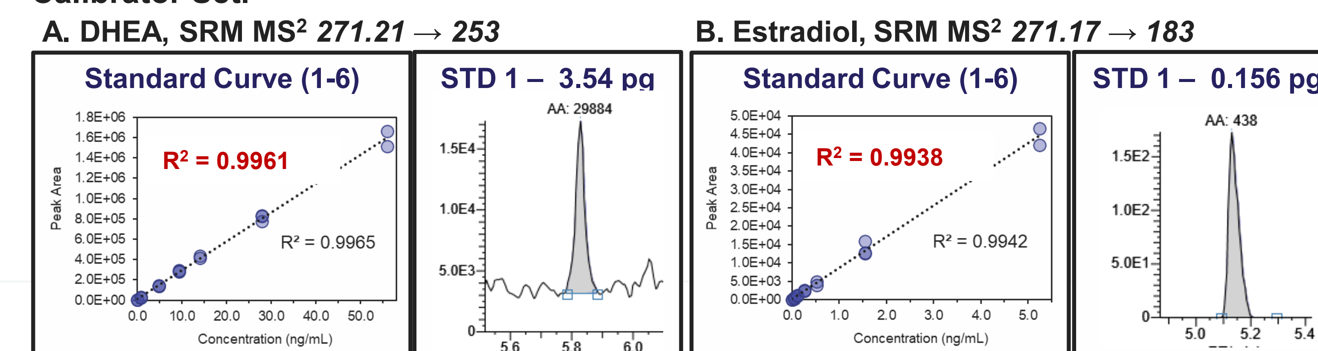
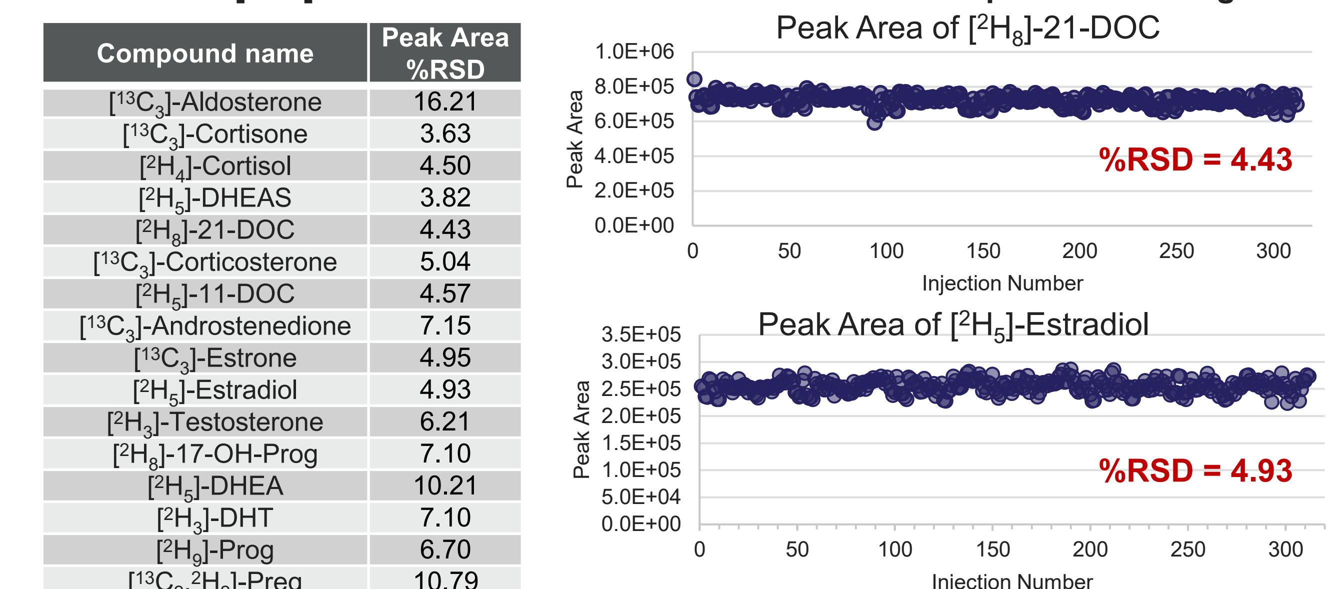


Figure 6. The robustness of the method was reflected via the %RSD of the IS peak areas over 311 injections of the steroid calibrators prepared using 0.05% BSA. [2H8]-21-DOC and [2H5]-Estradiol were selected to demonstrate the peak area changes.



Compound name	Peak Area %RSD
[$^{13}C_3$]-Aldosterone	16.21
[$^{13}C_3$]-Cortisone	3.63
[$^{2}H_4$]-Cortisol	4.50
[$^{2}H_5$]-DHEAS	3.82
[$^{2}H_8$]-21-DOC	4.43
[$^{13}C_3$]-Corticosterone	5.04
[$^{2}H_5$]-11-DOC	4.57
[$^{13}C_3$]-Androstenedione	7.15
[$^{13}C_3$]-Estrone	4.95
[$^{2}H_5$]-Estradiol	4.93
[$^{2}H_3$]-Testosterone	6.21
[$^{2}H_8$]-17-OH-Prog	7.10
[$^{2}H_5$]-DHEA	10.21
[$^{2}H_3$]-DHT	7.10
[$^{2}H_9$]-Prog	6.70
[$^{13}C_2$,$^{2}H_2$]-Preg	10.79

Results

Robustness

Excellent robustness of the method was demonstrated by the peak area changes of the IS in 311 injections of the calibration samples prepared using 0.05% BSA (Figure 6). For all steroids, the peak area %RSD values were below 17%, confirming the excellent quantitative stability and robustness of the system described.

Conclusions

A sensitive and robust LC–MS/MS method was developed for simultaneous quantification of 18 steroids in human serum using the Vanquish Horizon UHPLC and TSQ Certis MS with OptaMax Plus ion source. The method demonstrated excellent linearity, accuracy, and reproducibility across a wide dynamic range. Optimization of MS conditions, particularly vaporizer temperature, significantly improved sensitivity. Overall, the workflow delivers high sensitivity, selectivity, and long-term stability, providing a reliable solution for high-throughput steroid analysis in clinical and research settings.

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