Quantitative analysis of 9 steroid hormones in human serum

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This technical note demonstrates the accurate quantitation of 9 steroid hormones in human serum using a solid-phase extraction (SPE) procedure with analysis using the SCIEX QTRAP 4500 system. Low-nmol/L level sensitivity was achieved with excellent precision (ranging from 0.7 to 8.0%) and accuracy (ranging from 92.0 to 111%) at the lowest calibrator, demonstrating the quantitative performance of the assay. The signal-to-noise ratios (S/N) were 29:1 for corticosterone, 446:1 for cortisol, 55:1 for androstenedione, 35:1 for progesterone, 26:1 for 11-deoxycortisol, 16:1 for 21-deoxycortisol, 41:1 for 17-OH progesterone, 145:1 for testosterone and 1599:1 for DHEAS at the lowest calibrator measured. In addition, excellent linearity was observed across clinically relevant concentrations, with r^2 values above 0.99 across the calibration ranges for all the analytes.

Key benefits of steroid hormone analysis from human serum using the QTRAP 4500 system

- **Efficient sample preparation:** Steroid hormones were extracted from human serum samples using an efficient solid-phase extraction (SPE) procedure, requiring 100 µL of human serum
- **Excellent quantitative performance:** Sensitive quantitation of steroid hormones was performed with excellent precision and accuracy (4.6% and 111% for corticosterone at 0.2 nmol/L, 3.9% and 104% for cortisol at 8.3 nmol/L, 2.4% and 108% for androstenedione at 0.5 nmol/L, 0.7% and 110% for progesterone at 0.4 nmol/L, 8.0% and 107% for 11-deoxycortisol at 0.4 nmol/L, 1.2% and 92.0% for 21-deoxycortisol at 0.3 nmol/L, 7.4% and 98.6% for 17-OH progesterone at 0.3 nmol/L, 7.6% and 105% for testosterone at 0.9 nmol/L and 0.9% and 107% for DHEAS at 0.2 nmol/L)
- **Excellent linearity:** Calibration curves for the 9 steroid hormones showed r^2 values above 0.99 across the calibration ranges for all the analytes

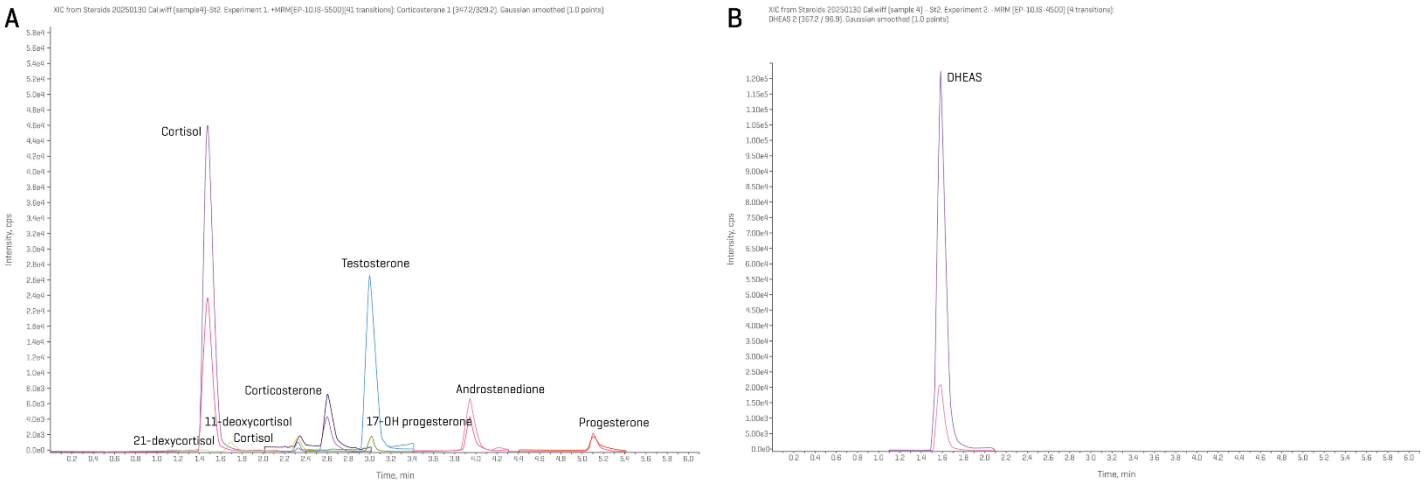

Figure 1: Chromatograms of the 9 steroid hormones at the lowest calibrator level extracted from serum matrix and analyzed using positive and negative polarity switching. Steroids analyzed in A) positive and B) negative mode using 50 ms polarity switching between positive and negative ESI modes are displayed from a single injection, where two MRM transitions were monitored per compound with > 10 points across the peaks for all compounds.

Introduction

Steroid hormones play vital roles in maintaining metabolic processes, immune regulation, and muscle integrity. Because these compounds exhibit extensive structural diversity and include many synthetic derivatives, precise determination of their concentrations in biological matrices is fundamental. Accurate analysis of steroid hormone levels enables a deeper understanding of hormonal balance and metabolic health, serving as a cornerstone for both clinical and biomedical research.

Methods

Sample preparation: Sample preparation was performed using Diagnotix's steroid panel basic' reagent set [https://www.diagnotix.com/nl/lcms-uhplc/Steroids] according to the manufacturer's specifications. 100 µL calibrators in serum matrix were used to perform the SPE procedure. This reagent set is only available in certain EU countries.

Liquid chromatography conditions: Chromatographic separation was achieved using a Phenomenex Kinetex Biphenyl column (150 x 2.1 mm, 2.6 µm, 00F-4622-AN). Mobile phases A and B from the reagent set were used. The total run time was 10 minutes at a flow rate of 800 µL/min. The injection volume was 20 µL. The LC gradient program is presented in **Table 1.**

Table 1: Chromatographic gradient for the analysis of steroid hormones in human serum.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	30	70
6.0	7	93
6.1	0	100
8.0	0	100
8.01	30	70
10.0	30	70

Mass spectrometry conditions: Mass spectrometry analysis was performed using the SCIEX Triple Quad 4500 system, operating in both positive and negative electrospray mode using rapid (50 ms) polarity switching between positive and negative ESI modes. Source and gas conditions are presented in **Table 2**. Compound-dependent parameters were optimized by infusion.

Table 2: Source and gas parameters for the analysis of steroid hormones in human serum using the QTRAP 4500 system.

Parameter	Value
Polarity	Positive and negative
Ion source gas 1	40 psi
Ion source gas 2	60 psi
Curtain gas	35 psi
Source temperature	550°C
Ion spray voltage	5500 V and -4500 V
CAD gas	9

Data processing: Data processing was performed using SCIEX OS software (version 3.1.6). Peak integration was achieved using the MQ4 algorithm. Quantitative analysis was conducted in the Analytics module of SCIEX OS, where calibration curves, concentration calculations, assay precision, and accuracy statistics were automatically generated.

Results and discussion

Figure 1 shows the chromatographic separation of the panel of 9 steroid hormones at the lowest calibrator level. The 10 min gradient, in combination with the column selection and mobile phase composition, resulted in separation of the panel of steroid hormones. The extracted ion chromatograms (XICs) showed (S/N) values of 29:1 for corticosterone, 446:1 for cortisol, 55:1 for androstenedione, 35:1 for progesterone, 26:1 for 11-deoxycortisol, 16:1 for 21-deoxycortisol, 41:1 for 17-OH progesterone, 145:1 for testosterone and 1599:1 for DHEAS at the lowest matrix calibrator measured, calculated using the peak-to-peak algorithm in SCIEX OS.

The quantitative performance of the method was investigated by injecting a series of calibrator samples spiked at the various concentration levels and injected in triplicate. Linearity, precision and accuracy were assessed across the calibration ranges for each of the 9 analytes. **Figure 2** shows the representative XICs for A) corticosterone and B) 11-deoxycortisol across their respective concentration ranges (0.2-44.2 nmol/L for corticosterone and 0.4-45.7 nmol/L for 11-deoxycortisol). **Figure 3** shows the calibration curves for the 9 steroid hormones over the analytes' respective calibration ranges. The plots show excellent linear responses across the calibration series, with r^2 values greater than 0.99 for analytes.

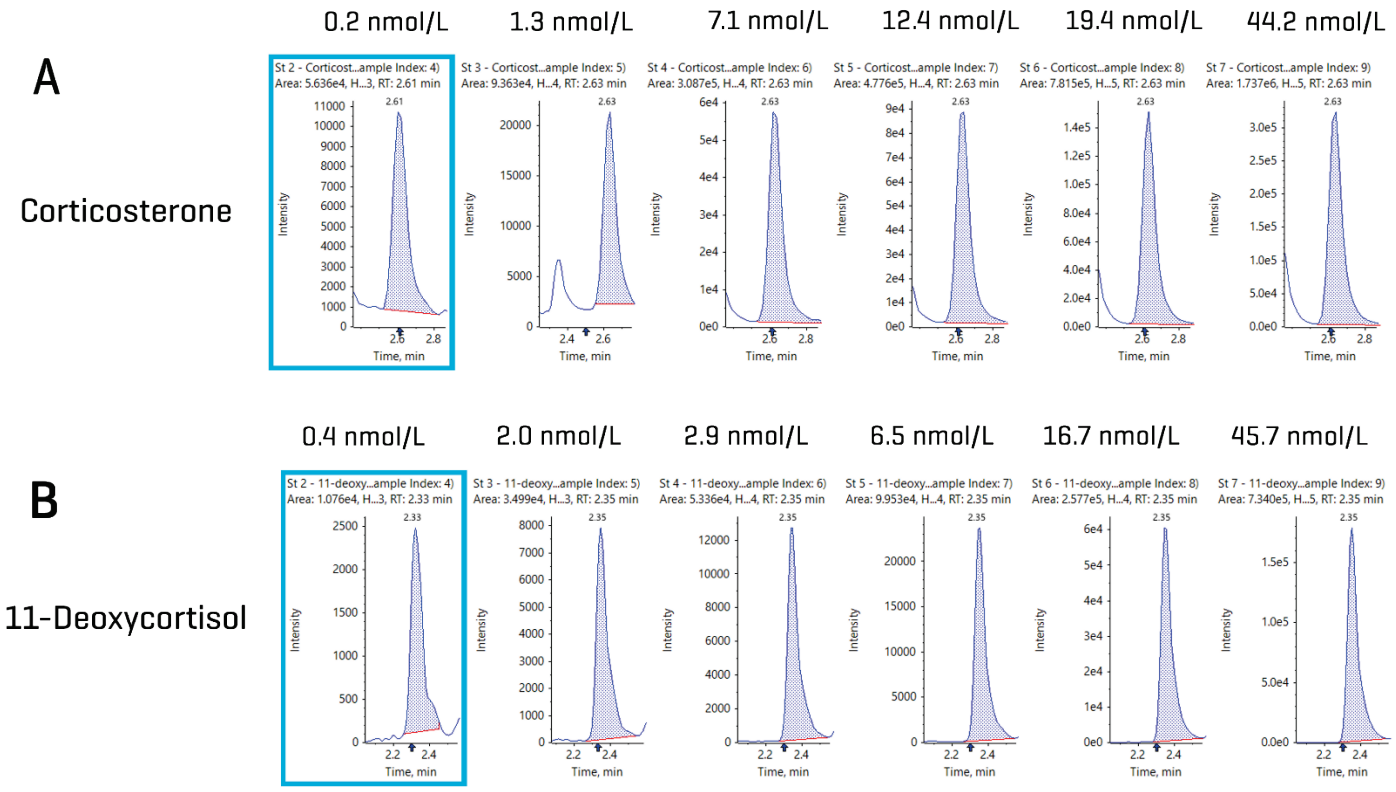

Figure 2: Extracted ion chromatograms for corticosterone and 11-deoxycortisol. The XICs show the signal for A) corticosterone and B) 11-deoxycortisol across their respective concentration ranges (0.2-44.2 nmol/L for corticosterone and 0.4-47.5 nmol/L for 11-deoxycortisol).

The precision and accuracy values were calculated from 3 replicates of the lowest matrix calibrators analyzed. The precision (%CV) and % accuracy were 4.6% and 111% for corticosterone at 0.2 nmol/L, 3.9% and 104% for cortisol at 8.3 nmol/L, 2.4% and 108% for androstenedione at 0.5 nmol/L, 0.7% and 110% for progesterone at 0.4 nmol/L, 8.0% and 107% for 11-deoxycortisol at 0.4 nmol/L, 1.2% and 92.0% for 21-deoxycortisol at 0.3 nmol/L, 7.4% and 98.6% for 17-OH progesterone at 0.3 nmol/L, 7.6% and 105% for testosterone at 0.9 nmol/L and 0.9% and 107% for DHEAS at 0.2 nmol/L.

Conclusions

A fast and accurate LC-MS/MS method for the detection of 9 steroid hormones extracted from human serum samples was developed using the SCIEX QTRAP 4500 system. The method demonstrated:

- Fast sample preparation, which consisted of a simple protein deproteination, requiring 100 µL of human serum
- Excellent sensitivity at the lowest calibrator level, resulting in signal-to-noise ratios (S/N) of 29:1 for corticosterone, 446:1 for cortisol, 55:1 for androstenedione, 35:1 for progesterone, 26:1 for 11-deoxycortisol, 16:1 for 21-deoxycortisol, 41:1 for 17-OH progesterone, 145:1 for testosterone and 1599:1 for DHEAS
- Excellent linear responses across the calibration series consisting of 6 calibrators, with r^2 values greater than 0.99 for analytes
- High quantitation performance of the method, resulting in excellent precision from 0.7 to 8.0% and accuracy from 92.0 to 111% across the steroid hormones

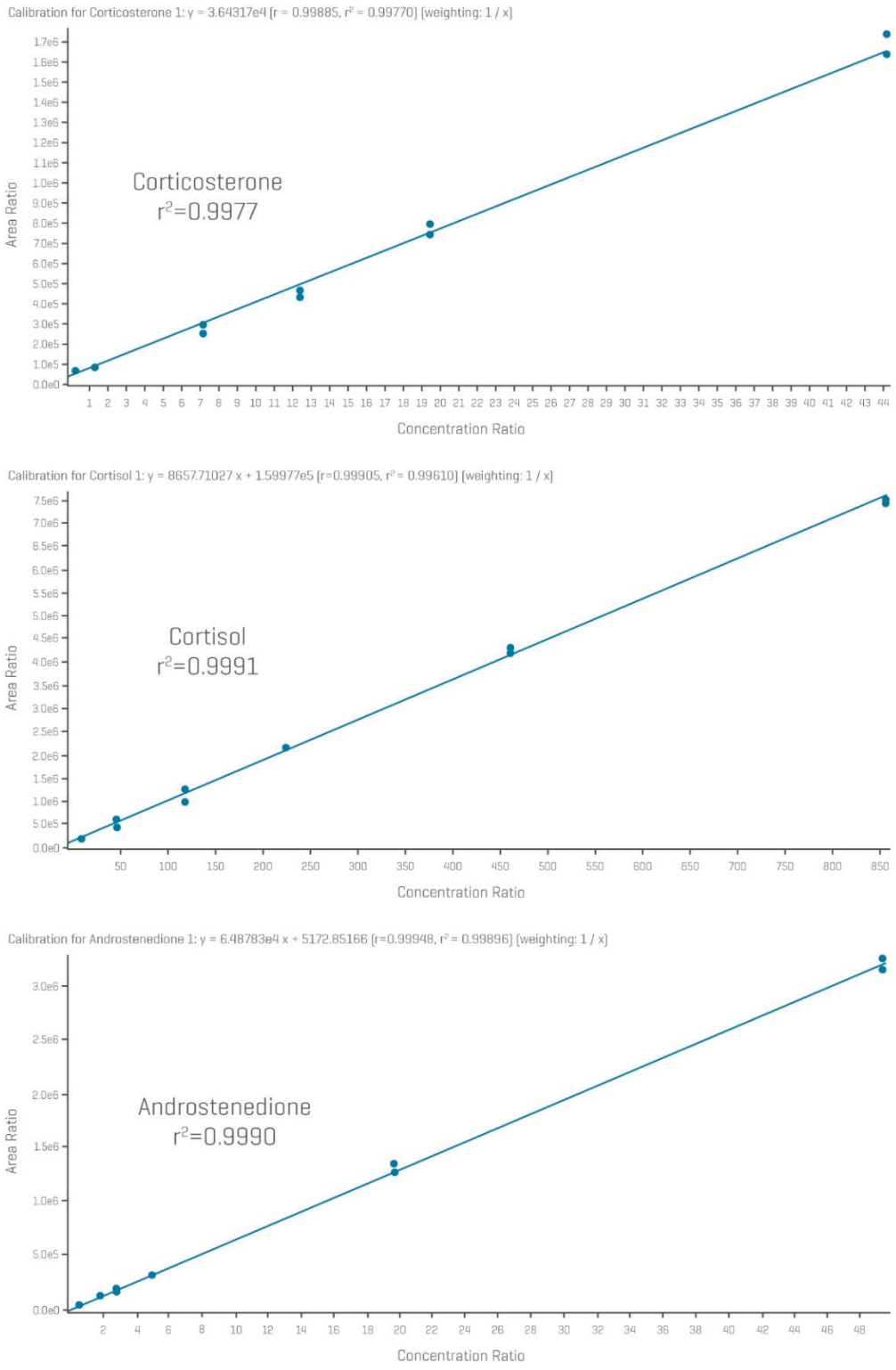

Figure 3. Linear calibration curves for the 9 steroid hormones extracted from serum matrix and analyzed using positive and negative polarity switching. The calibration curves were run in triplicate across the measured ranges shown in Table 1. The curves were generated using a linear regression and 1/x weighting, resulting in r^2 values >0.99 for all the compounds extracted from serum matrix.

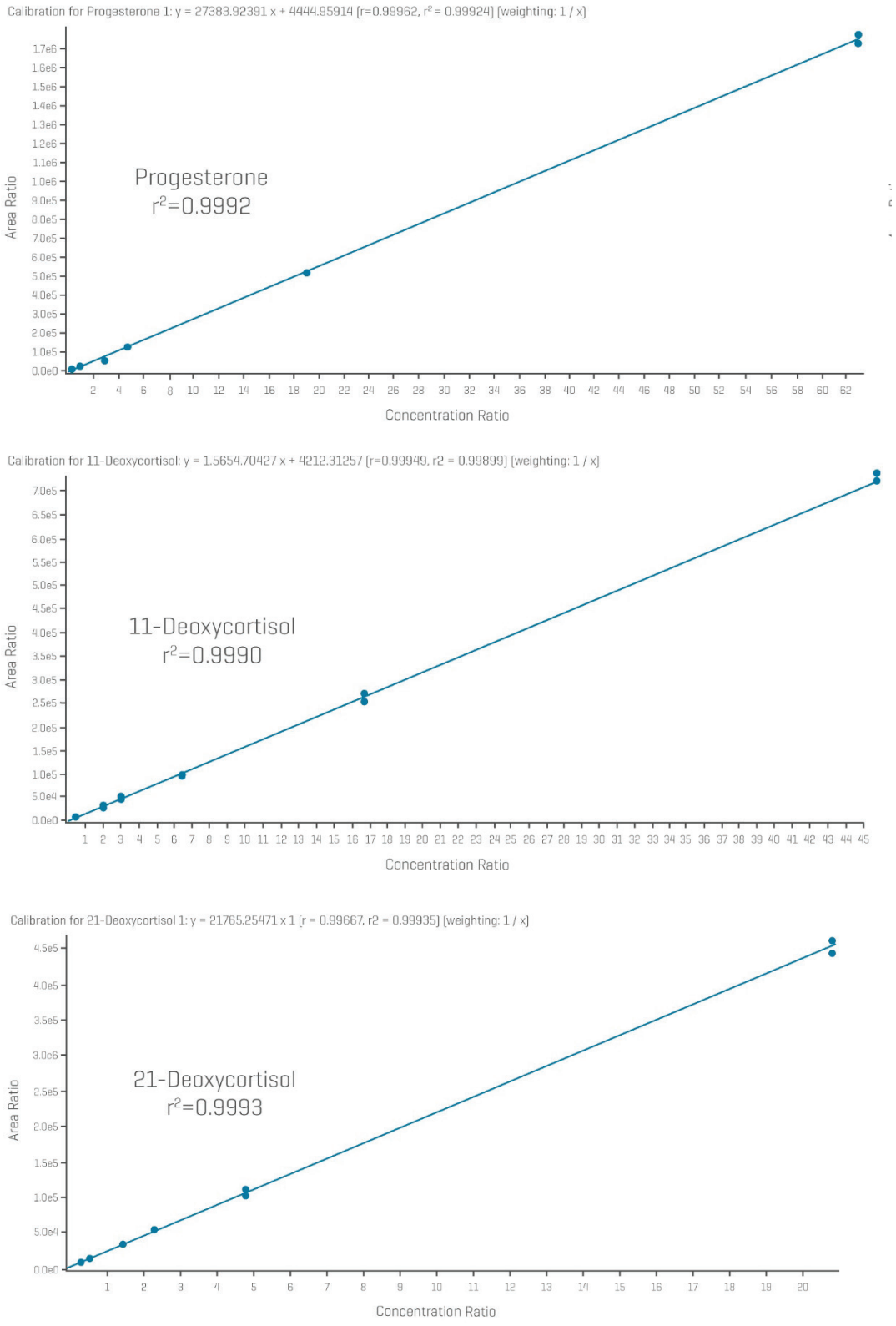

Figure 3 (continued). Linear calibration curves for the 9 steroid hormones extracted from serum matrix and analyzed using positive and negative polarity switching.

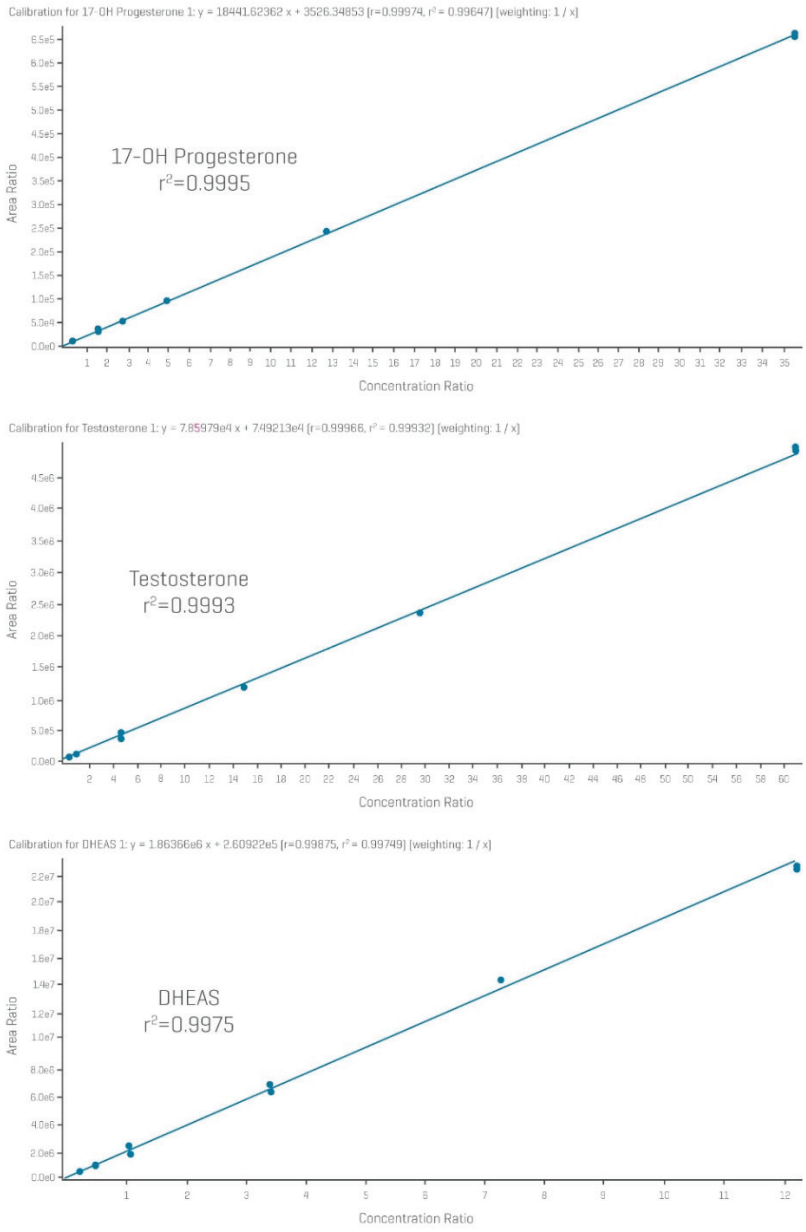

Figure 3 (continued). Linear calibration curves for the 9 steroid hormones extracted from serum matrix and analyzed using positive and negative polarity switching.

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