

Sensitive quantitation of testosterone in human serum

In this technical note, the combination of a rapid sample preparation procedure consisting of a liquid-liquid extraction with a robust and sensitive LC-MS/MS method using the SCIEX QTRAP 6500+ enabled low-pg/mL detection of testosterone extracted from human serum. The method demonstrated excellent precision and accuracy at the lowest calibrator [0.5 pg/mL]. Excellent linearity was observed in the calibration series [1-1000 pg/mL], with a r^2 value of 0.9954. Low-level sensitivity was achieved at the lowest calibrator [0.5 pg/mL] with a signal-to-noise ratio [S/N] of 5:1. In addition, the method showed excellent precision [8.0%] and accuracy [97.7%] at the lowest calibrator [0.5 pg/mL], demonstrating the quantitative performance of the assay.

Key benefits of testosterone analysis from human serum using the QTRAP 6500+ system

- **Low-pg/mL level sensitivity and excellent quantitative performance:** Sensitive quantitation of testosterone was performed with excellent precision [8.0%] and accuracy [97.7%] at the lowest calibrator level [0.5 pg/mL]
- **Rapid sample preparation:** Testosterone was extracted from human serum samples using a liquid-liquid extraction [LLE], evaporation, and reconstitution sample preparation
- **Excellent linearity:** Calibration curve for the testosterone showed an r^2 value of 0.9954 across the calibration range [1-1000 pg/mL]

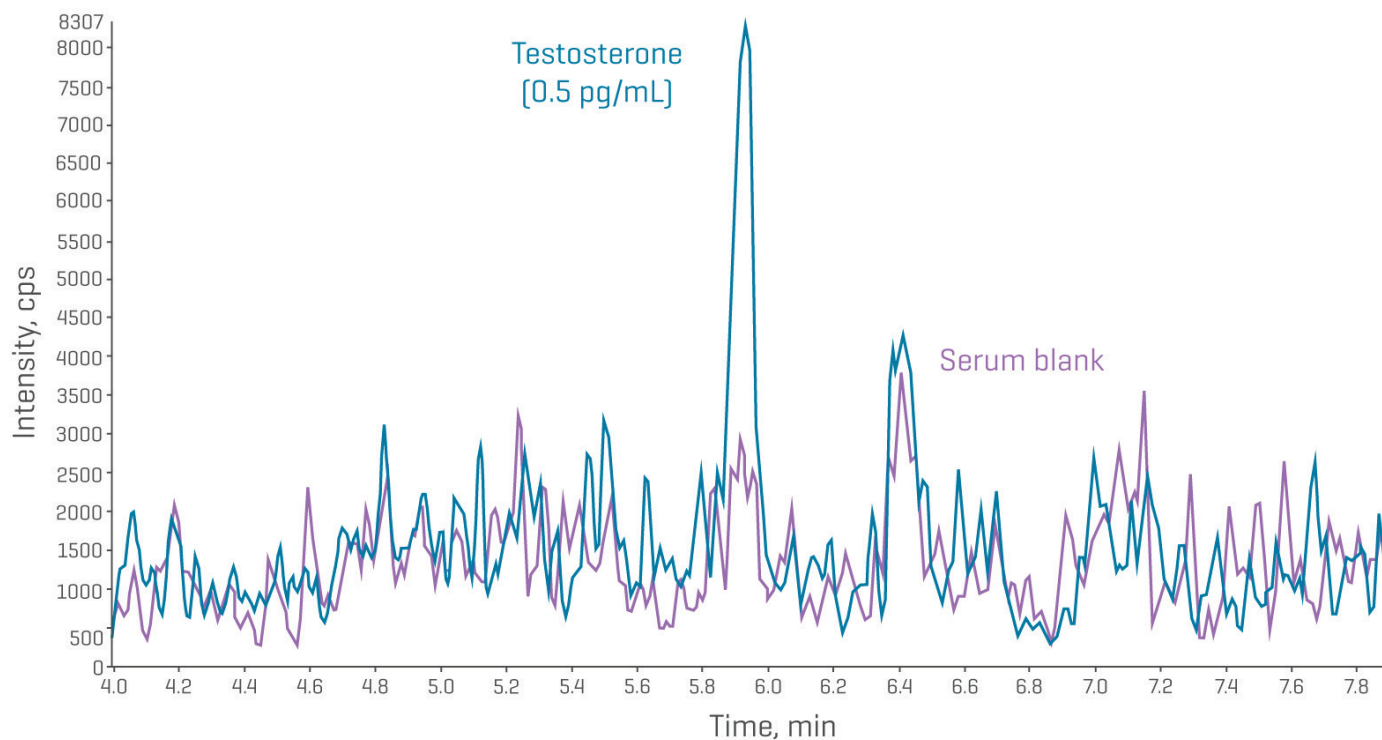


Figure 1. Analytical sensitivity of a 0.5 pg/mL testosterone standard extracted from serum matrix. Analysis of a 0.5 pg/mL testosterone standard in matrix shows a S/N of 5:1 based on a peak-to-peak algorithm.

Introduction

Testosterone, a steroid hormone primarily associated with male physiology, also plays important roles in female health, influencing mood, bone density, and reproductive function.^{1,2} In women, testosterone is produced in smaller quantities by the ovaries and adrenal glands, circulating at much lower concentrations than in men. Accurate quantitation of testosterone in female and male biological samples is essential in clinical research, particularly for studies investigating hormonal imbalances, endocrine disorders, or the effects of therapeutic interventions.

Methods

Sample preparation: Testosterone was extracted from human serum using a liquid-liquid extraction (LLE), evaporation, and reconstitution sample preparation procedure.

Liquid chromatography: Chromatographic separation was achieved using a [Phenomenex Kinetex C8 column](#) (50 x 2.1 mm, 2.6 μ m, 00B-4497-AN). Mobile phase A was ammonium fluoride in water and mobile phase B was ammonium fluoride in methanol. The total run time was 12 minutes.

Mass spectrometry: Data was collected using a [QTRAP 6500+ system](#) with an IonDrive Turbo V ion source and operated in electrospray ionization (ESI) positive mode. The Scheduled MRM algorithm was used in [SCIEX OS software](#) [version 3.1.6] to collect the appropriate number of data points for quantifiable data. Compound-dependent parameters were optimized by infusion.

Data processing: Data processing was performed using [SCIEX OS software](#) [version 3.1.6] to collect 10-12 data points for quantifiable data. Peak integration was achieved using the MQ4 algorithm. Quantitative analysis was conducted in the Analytics module of SCIEX OS, where calibration curves, concentration calculations, and assay precision statistics were automatically generated.

Results and discussion

Figure 1 shows testosterone in a control human serum sample at final concentrations of 0.5-1000 pg/mL. The extracted ion chromatograms showed a signal-to-noise (S/N) ratio of 5:1 at the lowest matrix calibrator concentration [0.5 pg/mL], as calculated using a peak-to-peak algorithm.

The quantitative performance of the method was investigated by injecting a series of 3 calibrator samples spiked at each concentration between 0.5-1000 pg/mL. Linearity and precision were assessed across the calibration range. **Figure 2** shows the calibration curve for testosterone. The plot shows an excellent linear response across the calibration series, with an r^2 value of 0.9954.

The accuracy and precision values were calculated from 3 replicate injections of the lowest matrix calibrator analyzed. The accuracy was 97.7% and precision [%CV] was 8.0%.

Conclusions

A fast and sensitive LC-MS/MS method for the detection of testosterone in human serum samples was developed. The method demonstrated:

- Fast sample preparation which consisted of liquid-liquid extraction
- Excellent linear responses across the calibration series, with an r^2 value of 0.9954
- Good sensitivity resulting in S/N of 5:1 at the lowest calibrator level [0.5 pg/mL]
- High quantitative performance of the method, resulting in excellent precision [8.0%] and accuracy [97.7%] at the lowest calibrator level [0.5 pg/mL]

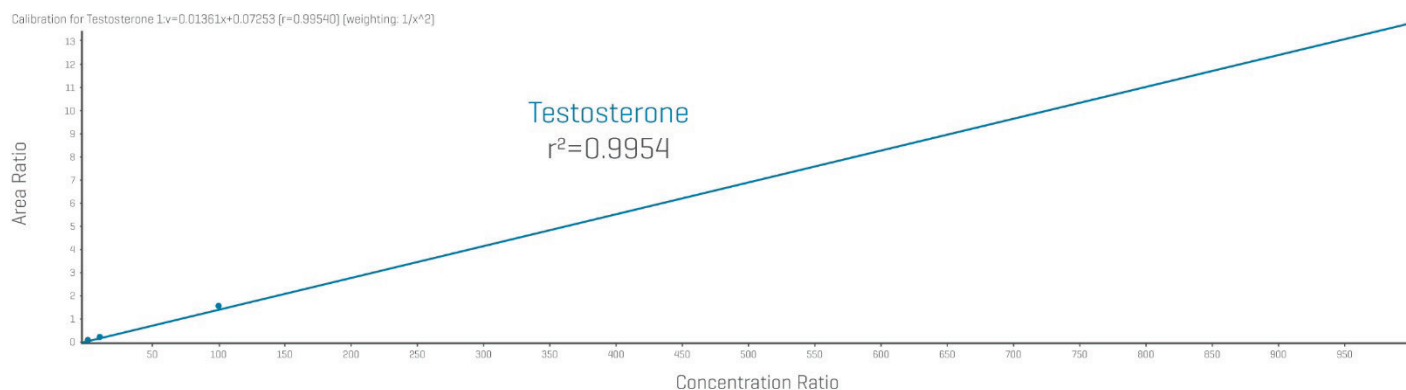


Figure 2. Linear calibration curve for testosterone extracted from serum matrix using the primary MRM transition. The calibration curve using the matrix calibrators was analyzed across the concentration range [0.5-1000 pg/mL]. The curve was generated using linear regression and $1/x^2$ weighting, resulting in a r^2 value of 0.9954.

References

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2. Holst JP, Soldin OP, Guo T, Soldin SJ: Steroid hormones: relevance and measurement in the clinical laboratory. *Clin. Lab Med.* **2004**, *24*, 105-118. DOI: [10.1016/j.cll.2004.01.004](https://doi.org/10.1016/j.cll.2004.01.004)

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