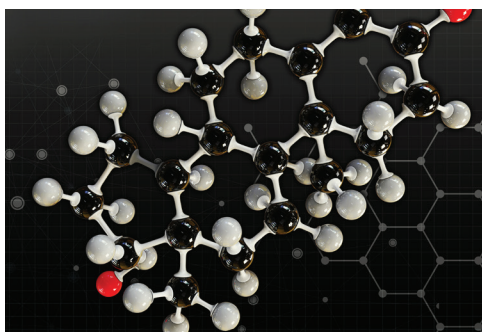


Analysis of Testosterone Using the Xevo TQ-XS for Clinical Research

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GOAL

To demonstrate the high analytical sensitivity and quantitative performance of the Xevo® TQ-XS for the analysis of serum testosterone for clinical research using 100 µL serum.

BACKGROUND

Testosterone is synthesized from androstenedione in the adrenal cortex, and is a key biomarker in many clinical research studies. Measurement of testosterone at low concentrations in serum by more traditional immunoassay methods are sometimes problematic due to cross-reactivity of structurally-related steroids (e.g. dihydrotestosterone), causing high imprecision and inaccuracy.

LC-MS/MS is an advanced analytical technique that provides high selectivity – differentiating between structurally-similar steroid hormones through selective sample preparation, chromatographic separation, and specific mass detection using Multiple Reaction Monitoring (MRM).

The Xevo TQ-XS (Figure 1) is a high-performance benchtop tandem quadrupole mass spectrometer expanding the scope of

The Xevo TQ-XS provides high analytical sensitivity for the analysis of testosterone from a small sample volume.



Figure 1. Waters® Xevo TQ-XS Mass Spectrometer.

ultimate analytical-sensitivity analysis. It features StepWave™ XS ion transfer optics delivering enhanced analytical sensitivity, robustness, and reliability. In addition, the new Xtended Dynamic Range (XDR™) Detector allows six orders of linear dynamic range. This ensures that methods are easily transferable onto this instrument, and that wide concentration ranges can be measured in a single analysis.

THE SOLUTION

The Xevo TQ-XS provides high analytical sensitivity for the analysis of testosterone from a small sample volume.

Sample preparation and LC-MS/MS analysis

Serum samples (100 µL) were precipitated with methanol and diluted with water prior to centrifugation. Sample supernatant was loaded directly onto the Oasis® PRiME HLB µElution Plate. Consecutive washes with 0.1% ammonia in 35% methanol and 0.1% formic acid in 35% methanol were performed. Samples were eluted with 85:15 (v/v) acetonitrile–methanol and diluted with water.

Samples were subsequently injected on an ACQUITY UPLC® I-Class System and Xevo TQ-XS Mass Spectrometer, utilizing a water/methanol/ ammonium acetate/formic acid gradient, and an ACQUITY UPLC HSS T3 Column with a VanGuard™ T3 pre-column.

MRM transitions monitored for testosterone were m/z 289.2 > 97.0 (quantifier) and 109.0 (qualifier). The internal standard testosterone-¹³C₃ had a MRM transition of m/z 292.2 > 100.0.

To convert conventional mass units (ng/mL) to SI units (nmol/L), multiply by 3.470.

Results

Using a 5 pg/mL solution of testosterone, triplicate injections were performed to demonstrate system analytical sensitivity for the analyte (Figure 2). The Xevo TQ-XS provides reproducible peak areas at this concentration (peak area <3% RSD, n=3).

The low calibration serum standard used for the LC-MS/MS method was assessed on the system over three separate days. This evaluation demonstrates reproducibility of a sample extracted and injected on the Xevo TQ-XS, on three separate occasions (Figure 3).

An accuracy evaluation using European Reference Material (ERM) was performed, demonstrating the excellent quantitative performance of the LC-MS/MS method on the Xevo TQ-XS. ERM DA345a and DA346a were extracted and analyzed, demonstrating method bias within ±3.3% (Figure 4).

Calibration lines (0.005–20 ng/mL) performed on three separate occasions were >0.997 (Figure 5). Total precision of the LC-MS/MS method was assessed over three separate days, with three replicates on each day at 0.02, 0.1, 1, and 14 ng/mL (n=18). Total precision and repeatability was ≤4.4% (Figure 6).

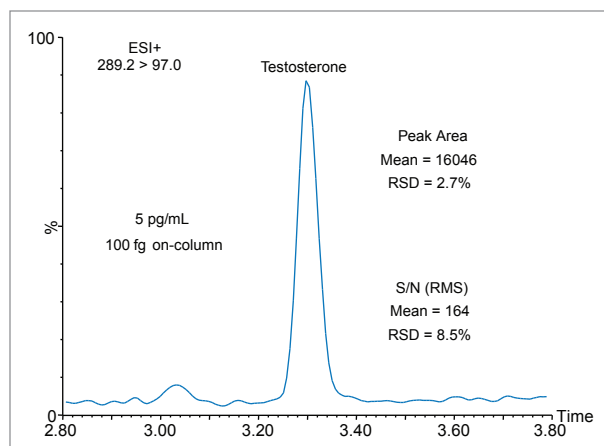


Figure 2. Injection of a 5 pg/mL solution (n=3, 100 fg on-column) of testosterone on the Xevo TQ-XS.

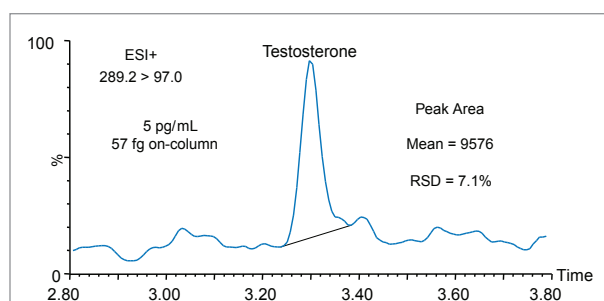


Figure 3. Analysis of the 5 pg/mL serum calibrator on the Xevo TQ-XS.

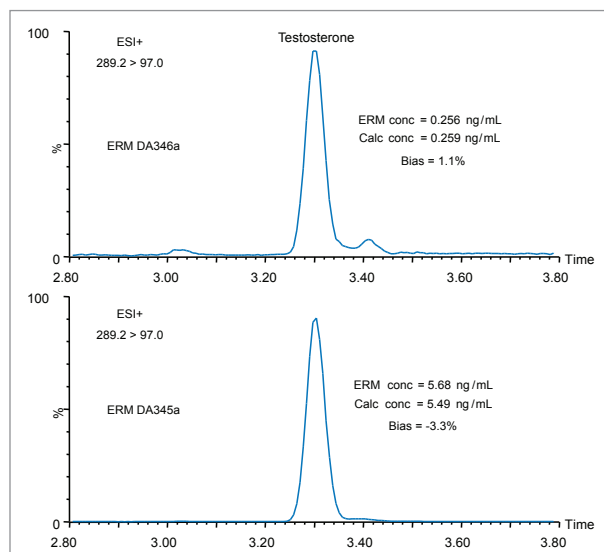
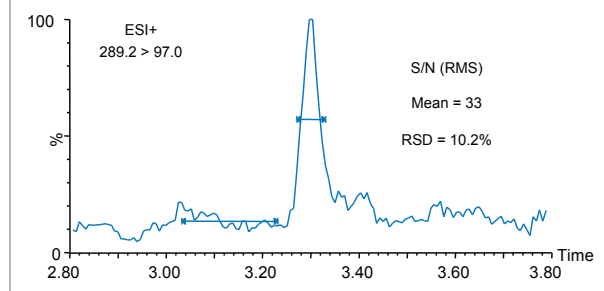


Figure 4. Injection of the ERM DA346a and ERM DA345a testosterone serum extracts with assigned values of 0.256 ng/mL (0.89 nmol/L) and 5.68 ng/mL (19.7 nmol/L), respectively.

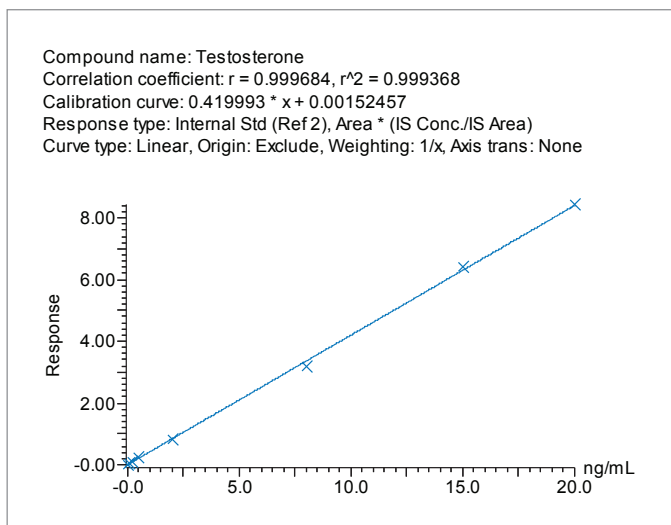


Figure 5. Calibration line for testosterone ranging from 0.005–20 ng/mL.

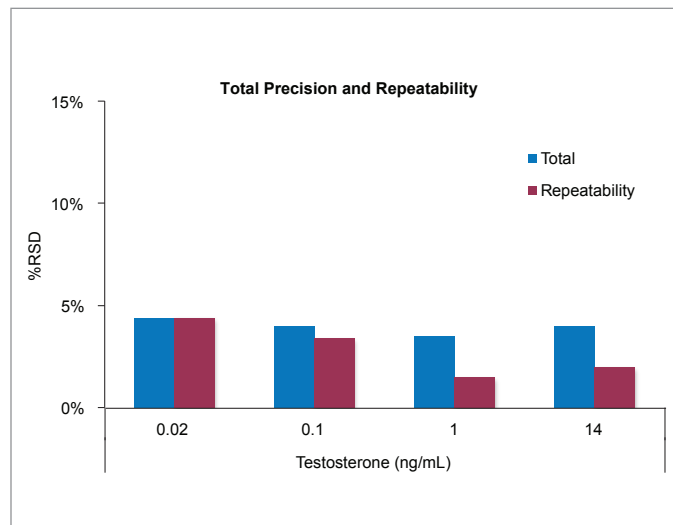


Figure 6. Total precision and repeatability on the Xevo TQ-XS ranging from 0.02–14 ng/mL.

SUMMARY

The Xevo TQ-XS has demonstrated excellent quantitative performance for the analysis of testosterone for clinical research, which includes:

- High analytical sensitivity testosterone in stripped serum at 5 pg/mL (0.017 nmol/L)
- Excellent analytical precision ($\leq 4.4\%$) for testosterone in stripped serum, ranging from 0.02–14 ng/mL
- Excellent agreement for ERM testosterone in serum, demonstrating method bias within $\pm 3.3\%$

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