Quantification of Corticosteroids and Androgens in Serum, Utilizing Waters MassTrak™ Steroid Serum Sets 2 and Sets 3 for Clinical Research

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Introduction

Steroid hormones encompass a large class of small molecules that play a central role in metabolic processes, such as the regulation of sexual characteristics, blood pressure, and inflammation. Enzymes that form part of the steroid biosynthetic pathway are pivotal in these metabolic processes, and their dysfunction can be examined through the correct measurement of steroid hormones in a clinical research setting. The availability of lyophilized calibrators and QCs reduces sample preparation time, aids in method harmonization, and assists with metrological traceability in accordance with ISO15189:2022, when used alongside an analytically selective chromatographic method.

Outlined is a quantitative clinical research method utilizing Waters MassTrak Steroid Serum Sets 2 and 3, Cals & QCs and Waters Oasis™ PRiME HLB µElution plate technology for the extraction of testosterone, androstenedione, 17-hydroxyprogesterone (17- OHP), dehydroepiandrosterone sulfate (DHEAS), Cortisol, 11-deoxycortisol and 21-deoxycortisol from human serum samples.

Sample Preparation
- Centrifuge all samples for 5mins at 3000 x g
- Transfer 100µL of each sample to an individual well on a 2mL 96 well plate.
- Add 25µL of MassTrak Steroid Internal Standard to each sample.

Extraction
- Add 200µL of LC-MS Grade Methanol to each well and mix.
- Add 550µL of LC-MS Grade Water to each well and mix.
- Centrifuge the 96 well plate at 4000 x g for 5mins.
- Load 600µL of supernatant onto the Oasis PRiME HLB 96 well µElution plate and slowly pull through on a low vacuum (100mbar).

Wash
- Wash with 150µL of 0.1% (v/v) ammonia in 35% (v/v) Methanol(aq)
- Wash with 150µL of 0.1% (v/v) formic acid in 35% (v/v) Methanol(aq)
- Elute with 30µL of 85:15 (v/v) Acetonitrile : Methanol into a 1mL collection plate.
- Add 70µL of LC-MS Grade Water and shake for 1min.
- Seal with 96 well Cap Mat Plate Seal.

Figure 1: Sample preparation workflow.

Chromatographic separation was performed on an ACQUITY™ UPLC™ I Class Plus (FL-I) System, using an ACQUITY UPLC HSS T3 Column, accompanied by a Xevo™ TQ-S micro, mass detector.

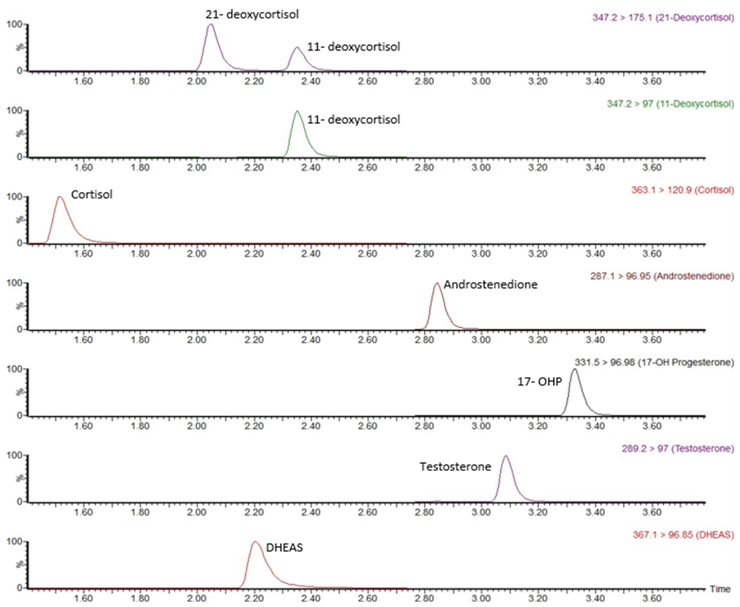

Figure 2: Chromatographic selectivity on the ACQUITY UPLC HSS T3 Column for the selection of Steroid Hormones

Figure 3: ACQUITY™ UPLC™ I Class Plus (FL-I) System, accompanied by a Xevo™ TQ-S micro mass detector.

An **accurate (±6%)** and **precise (±10%)** research method for the quantification of **steroid hormones** in serum.

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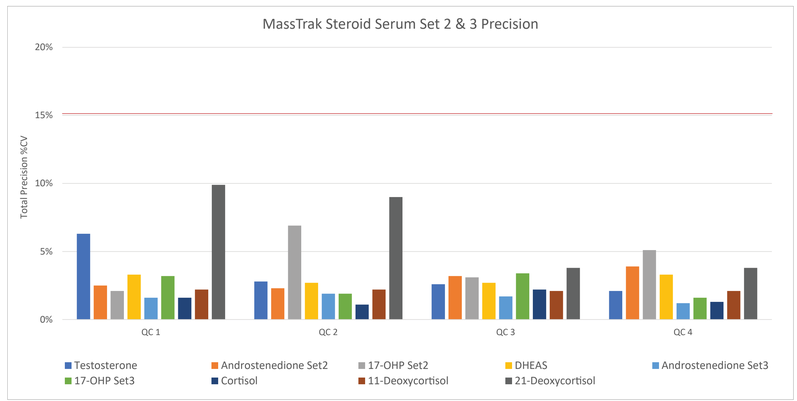
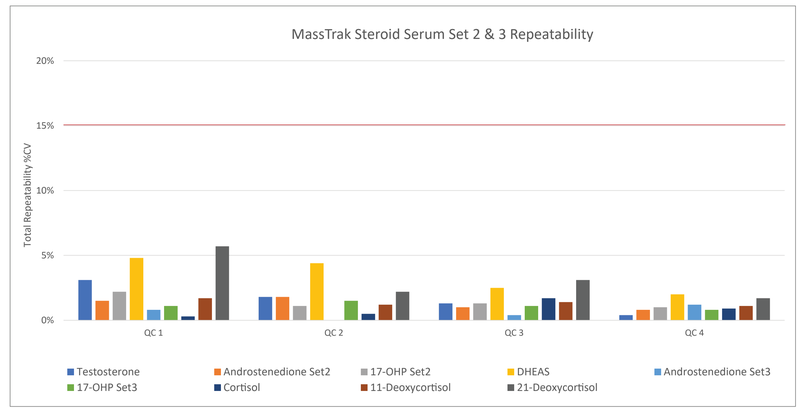
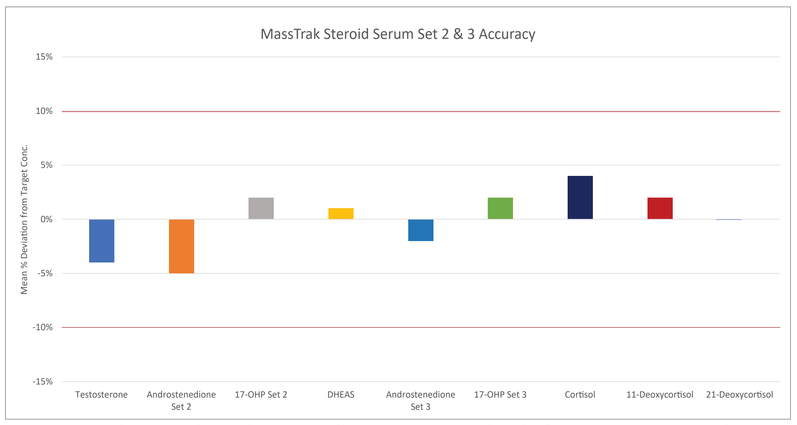
Figure 4: MassTrak Steroid Serum Calibrators and QCs



Graph 1: MassTrak Steroid Serum Set 2 & 3 Precision for each QC – two replicates over 5 days



Graph 2: MassTrak Steroid Serum Set 2 & 3 Repeatability for each QC – four replicates over 5 days



Graph 3: MassTrak Steroid Serum Set 2 & 3 Accuracy – Mean % deviation from target concentration at low, medium & high end of the calibration line

Key Takeaways

- Accuracy (±6%) and precision (±10%) have been confirmed through comparison to External Quality Assurance (EQA) schemes, in-house panels and QC material for all seven steroid hormones.
- Set 2 and Set 3 were analysed over a five-day period and were linear with a co-efficient of determination of (r2) > 0.999 for all analytes.
- Deming and Linear regression analysis were performed. No statistically significant bias was observed for each compound, with a mean method bias of ±1.3% for Set 2 and ±1.0% for Set 3.
- Analytical sensitivity using Signal: Noise (S/N) of the low calibrator (Calibrator 1) of each set, was >10:1 for each analyte across several analytical runs.

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