

CONFIDENCE IN YOUR CALIBRATORS: ASSESSMENT OF MASSTRAK™ ENDOCRINE CALIBRATOR AND CONTROL SETS

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INTRODUCTION

The routine analysis of steroid hormones is critical in understanding the function of metabolic pathways that impact sexual characteristics, inflammation and blood pressure. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) is fast becoming a sought-after technique in steroid analysis due to the advantages it provides over traditional ligand-binding techniques. These benefits include improvements in analytical sensitivity and selectivity, and the capability of multi-analyte quantitative detection in a single run. However, many LC-MS/MS methods lack harmonization or standardization. The Waters™ MassTrak™ Endocrine Steroid Calibrator and Quality Control (QC) Sets (IVD) contain metrologically traceable materials, aiding laboratories in their compliance to ISO 15189, and provide confidence in the accuracy and harmonization of results when using validated LC-MS/MS methods. The performance of the MassTrak Endocrine Steroid Calibrator and QC Sets (IVD) was evaluated using an in-house developed LC-MS/MS method.

THE MASSTRAK SOLUTION

MassTrak Endocrine Steroid Calibrator and Quality Control Sets

- The MassTrak Endocrine Steroid Calibrator and Quality Control Sets* contain progesterone, 11-deoxycorticosterone, corticosterone, 17-OHP, 21-deoxycortisol, 11-deoxycortisol, cortisol, DHEA, androstenedione, testosterone, DHT and DHEAS in lyophilized serum.



Figure 1. The MassTrak Endocrine Calibrator and Quality Control Sets

Features

- The MassTrak Endocrine Steroid Calibrator and QC Sets are pre-weighed, ready to use (following reconstitution) materials that provide a number of benefits, including:
 - Metrological Traceability for compliance with ISO 15189
 - Lot-to-lot consistency for improvements in longitudinal performance of steroid hormone LC-MS/MS methods
 - CE IVD* label
- The provision of these materials also provides significant savings in time and resource, as shown in Table 1.

Calibrator Preparation Steps	MassTrak Endocrine Steroid Calibrator Set
Source Certified Reference Material	✓
Source Matrix	✓
Gravimetric Preparation of Standards	✓
Preparation of Calibrators	✓
Stability Evaluation	✓
Calibrator Accuracy Evaluation	✓
Lot-to-Lot Evaluation	✓
Measurement of Uncertainty	✓

Table 1. The MassTrak Endocrine Steroid Calibrator Set improves laboratory efficiency, saving time and resource compared to the preparation of in-house calibrators

***The MassTrak Endocrine Steroid Calibrator and Quality Control Sets are not available for sale in all countries. For information on availability please contact your local representative**

METHODS

Materials

- The MassTrak Endocrine Steroid Calibrator and Quality Control Sets were used for calibrator and QC materials.
- Total precision was determined by extracting and quantifying five replicates of tri-level QC material on one occasion per day over five consecutive days (n=25). Repeatability was determined by analyzing five replicates at each QC level.
- Analytical method bias for testosterone, androstenedione, 17-OHP, DHEAS, and cortisol was determined using EQA samples obtained from UK NEQAS (Birmingham, UK).

Methods

- 125µL serum samples were pre-treated with internal standard, methanol and water. Samples were mixed and centrifuged.
- A small aliquot of supernatant was transferred to the collection plate and the remaining sample supernatant was transferred to an Oasis™ MAX µElution Plate, washed with 1% formic acid in 10% acetonitrile followed by 1% ammonia in 10% acetonitrile. Analytes were eluted with 60%_(aq) acetonitrile into the collection plate containing supernatant. 50mM Ammonium bicarbonate pH 7.4 was then added, which enhances DHEAS chromatographic peak shape.
- Using a Waters ACQUITY UPLC™ I-Class System, samples were injected onto a 2.1 x 100mm Waters CORTECS™ C₈ Column with an in-line filter, using a methanol/water/0.1mM ammonium fluoride gradient and analyzed with a Waters Xevo™ TQ-S micro detector in positive/negative ESI, using Multiple Reaction Monitoring.
- The scan conditions for the steroids were grouped into four time windows based on elution order to optimize acquisition conditions.
- The analysis time per sample was approximately 7.0 minutes injection to injection.

RESULTS

Chromatography, Analytical Sensitivity and Linearity

- Baseline resolution of the 12 steroid hormones, including isomeric and structurally similar steroids, was achieved using the CORTECS C₈ column.
- The analytical sensitivity of the developed method for the lowest calibrator is shown in Figure 2, with S/N (PtP) > 10 for the 12 steroid hormones.
- Linearity of the calibration ranges was demonstrated with mean r² values for the calibration lines >0.994 across the 12 steroid hormones.

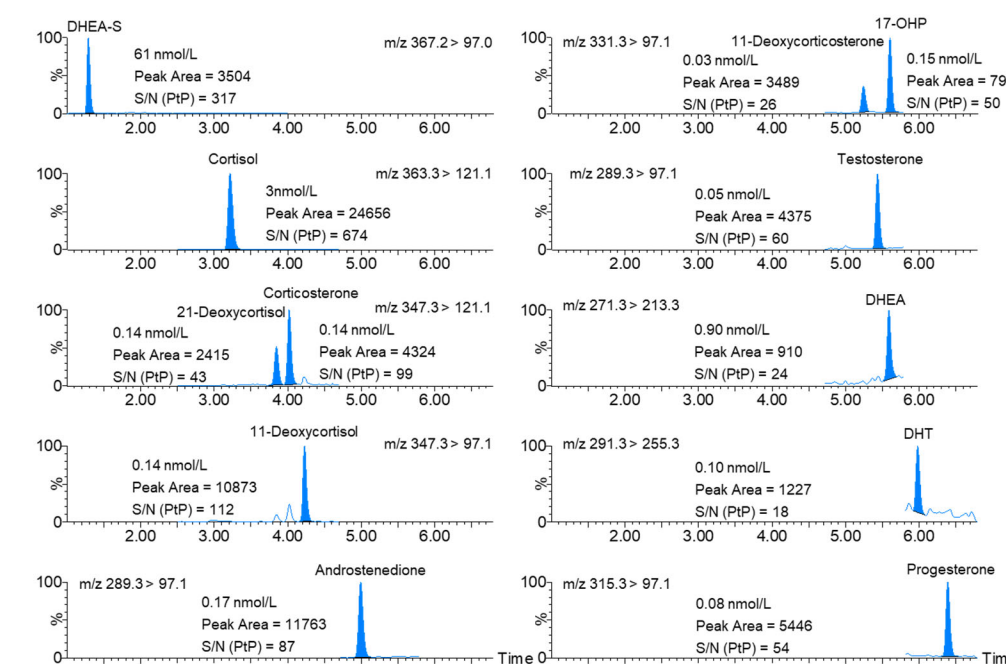


Figure 2. Analytical sensitivity of the 12 steroid hormones in the lowest calibrator found in the MassTrak Endocrine Steroid Calibrator Set

Total Precision and Repeatability

- Total precision and repeatability of the MassTrak Endocrine Steroid Quality Control Sets using the developed LC-MS/MS method were ≤7.7% CV over five analytical runs (Figure 3).

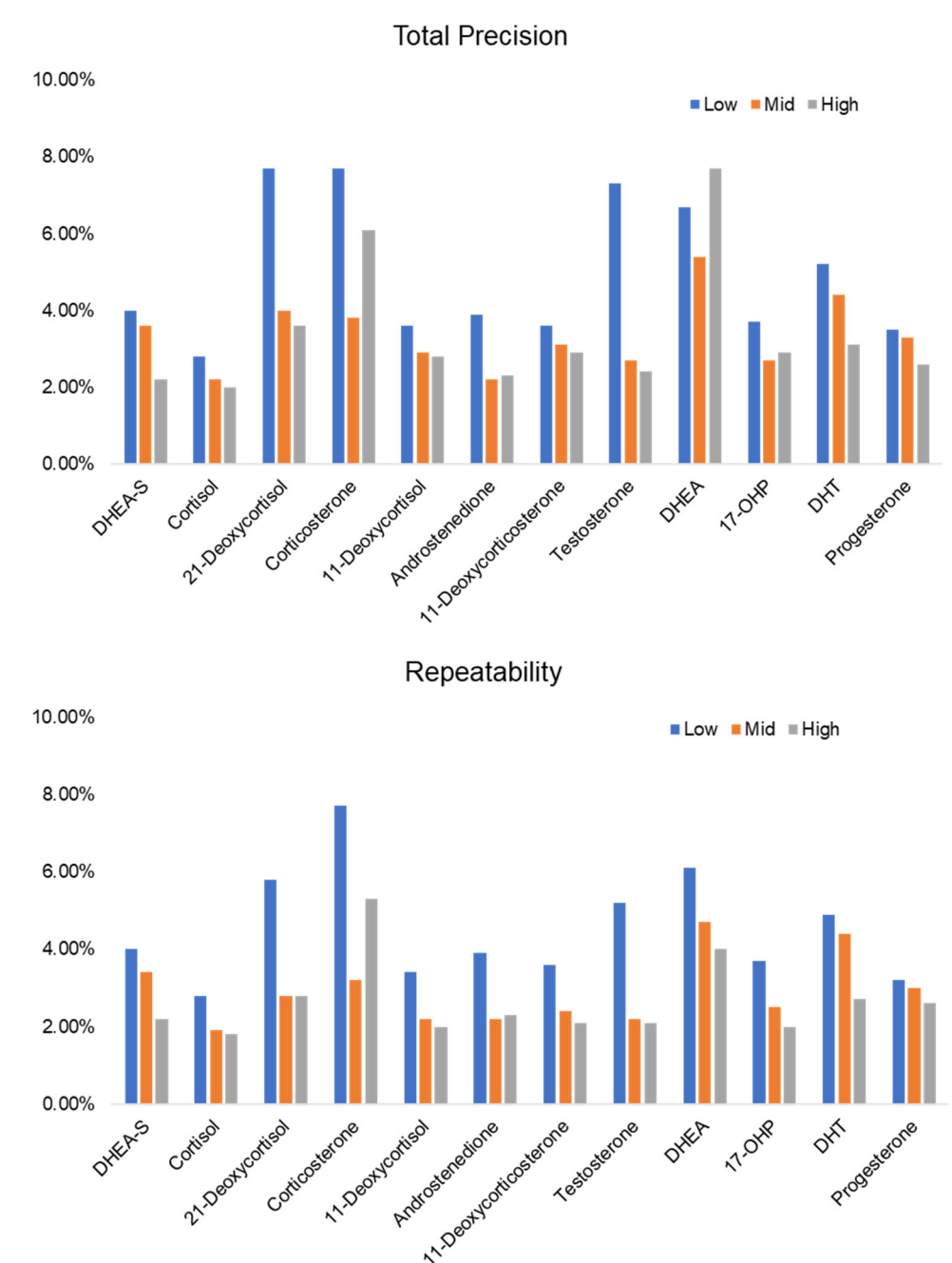


Figure 3. Total precision and repeatability of the 12 steroid hormones in the MassTrak Endocrine Steroid Quality Control Set

QC Accuracy and EQA Agreement

- QC accuracy for the steroids across three concentrations ranged from 91.0 – 112.4%.
- Mean method bias for External Quality Assessment (EQA) samples was within ±7.8% for testosterone, androstenedione, DHEAS, cortisol, 17-OHP and progesterone.

CONCLUSION

- MassTrak Endocrine Steroid Calibrator and QC Sets (IVD) contain metrologically traceable calibrators, aiding laboratories in their compliance to ISO 15189
- Significant savings in time and resource can be made by using pre-made calibrator and QC materials
- MassTrak Endocrine Steroid Calibrators are reproducible and accurate across manufacturing lots and cover the relevant reference ranges for each steroid hormone
- Excellent reproducibility and accuracy of the MassTrak Endocrine Steroid Quality Control Set and EQA samples were demonstrated with an in-house LC-MS/MS method

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