GOAL
To demonstrate a highly selective solid-phase extraction (SPE) technique with Oasis® MAX µElution Plates for the extraction of testosterone from serum samples for LC-MS/MS analysis in clinical research.

BACKGROUND
To determine the pharmacokinetic, bioavailability, and safety of new formulations of testosterone gels in clinical research, accurate and precise quantification of testosterone at low physiological concentrations is required.¹ Historically, testosterone has been analysed by direct immunoassay methods. However, it has been reported that these immunoassay methods can suffer from a lack of specificity due to the cross reactivity of structurally similar steroid hormones i.e., dihydrotestosterone. This results in greater imprecision and inaccuracy, most notably at the lower concentrations of testosterone found in serum samples. LC-MS/MS provides a solution to this problem by providing a platform to discern between similar steroids through the use of chromatographic separation and selective MRM mass spectrometry. Additional selectivity can also be obtained through the use of sample preparation prior to analysis. The use of sample preparation can provide orthogonal selectivity to the LC-MS/MS dimension of the method. Potential interferences found in the MRM trace of the analyte of interest can be removed with selective sample preparation techniques such as mixed mode SPE.

Figure 1. Testosterone quantifier MRM chromatograms of extracted serum samples using an Oasis MAX µElution Plate, Oasis HLB µElution Plate, and protein precipitation. Samples were extracted in triplicate and the mean S/N value is shown. S/N is calculated on the raw data using peak to peak at ±2 SD.

The Oasis MAX µElution Plate enables optimized analytical sensitivity and selectivity for the analysis of testosterone in serum for clinical research.
THE SOLUTION

In this technology brief, an Oasis MAX µElution Plate was successfully employed for the selective extraction of testosterone from serum. This chemistry provides mixed-mode interaction which utilizes both hydrophobic and anion exchange functionality. The added selectivity of this sorbent is more proficient at removing interferences than conventional reversed-phase SPE.

Sample preparation and LC-MS/MS analysis

Serum samples were diluted with internal standard, ammonia, zinc sulphate, methanol, and water. Following centrifugation, sample supernatant was loaded onto the 96-well Oasis MAX µElution Plate (PN:186001829) following conditioning and equilibration. A high pH wash was performed with ammonia in 20% methanol and samples were eluted with methanol followed by water.

20 µL of each extracted sample was injected on an ACQUITY UPLC® I-Class/Xevo® TQD utilizing mobile phases consisting of 2 mM ammonium acetate and 0.1% formic acid in water and methanol. Separation was performed on an ACQUITY UPLC HSS C18 SB Column (PN:186004118). The MRM transitions used for this analysis were m/z 289.2 for the testosterone precursor ion and m/z 96.9 and 109.0 for the quantifier and qualifier product ions respectively. Testosterone-13C3 was used as the internal standard.

Results

The additional selectivity provided by the Oasis MAX chemistry compared to other extraction techniques, provides enhanced analytical sensitivity for ESI MS analysis of testosterone. This is observed in the extraction and quantification of testosterone in serum using optimized Oasis MAX µElution, Oasis HLB µElution, and protein precipitation extraction protocols. The largest S/N ratio observed for the quantifier ion of testosterone was obtained using the Oasis MAX µElution Plate for the sample preparation (Figure 1).

289.2 > 109.0

The Oasis MAX µElution Plate also removes an interference clearly present in the qualifier trace of the same serum samples, as can be seen in Figure 2. Over a calibration range of 0.17–51 nmol/L for testosterone the precision of the method was determined by extracting and quantifying three replicates of tri-level QC material on two occasions per day over five separate days (n = 30). The results of these experiments are seen in Table 1, where total precision and repeatability is ≤5.0% RSD.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total QC precision (RSD)</th>
<th>QC repeatability (RSD)</th>
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<tbody>
<tr>
<td>Testosterone</td>
<td>Low 5.0%</td>
<td>Mid 4.4% High 4.3%</td>
</tr>
<tr>
<td></td>
<td>Low 3.1%</td>
<td>Mid 3.0% High 1.4%</td>
</tr>
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Table 1. Total precision and repeatability for the analysis of testosterone in serum at low (0.51 nmol/L), mid (3.4 nmol/L), and high (34 nmol/L) concentration points.
SUMMARY

A LC-MS/MS method for the analysis of testosterone for clinical research has been developed. Sample preparation was performed using an Oasis MAX 96-well µElution Plate for the extraction of testosterone from serum. The benefits of this method include:

- Highly selective sample preparation, leading to a nearly 3 fold improvement in S/N relative to other evaluated techniques.
- Removal of interferences observed at low physiological concentrations of testosterone, which enables analytically sensitive analysis of testosterone in serum (LLOQ 0.17 nmol/L).
- Excellent reproducibility across the calibration range (≤5.0% RSD).

References