A Rapid iMethod™ Test for Testosterone

iMethod™ Test for Testosterone V.1.0 for Cliquid® Software

The following outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for research into the quantitation of Testosterone when using an AB SCIEX 3200 QTRAP® or API 3200™ LC/MS/MS System.

The method included is for the routine analysis of Testosterone in human serum. Calibrators are created by spiking charcoal filtered serum at known concentrations. Control samples at clinically relevant low and high concentrations are used to verify the calibration range. The method uses a deuterated Testosterone (D3) analog as an internal standard.

The sample preparation is a simple protein precipitation using methanol followed by injection onto a Phenomenex Luna 3.0 µm C18(2), 50 x 2 mm HPLC column, included with the method, connected to the APCI source of the mass spectrometer. The analytical run has a total LC run time of 3 minutes per sample.

Figure 1. Representative chromatograms from a 30 ng/mL sample of testosterone: a) quantifier ion, b) qualifier ion, and c) deuterated testosterone internal standard. The sample was run on a 3200 QTRAP® LC/MS/MS system with a Shimadzu Prominence LC system using a 3 minute method.
During the method evaluation, quantitation limits for each analyte were sufficient to allow the analytical method to be used for quantitation with a linear dynamic range of 1-400 ng/mL. Typical intra-assay precision (n=6) for the low QC concentration (1 ng/mL) gives a %CV value of 5, with an accuracy deviation of ±5% and a Signal to Noise (S/N) of 30. S/N is the peak height divided by the noise measured at 3 standard deviations of the noise.

<table>
<thead>
<tr>
<th>Analyte (Transition)</th>
<th>STD Level (ng/mL)</th>
<th>%CV</th>
<th>S/N</th>
<th>LOD (ng/mL) @ S/N of 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone-1 (289/97)</td>
<td>1</td>
<td>5</td>
<td>30</td>
<td>0.17</td>
</tr>
<tr>
<td>Testosterone-2 (289/109)</td>
<td>1</td>
<td>8</td>
<td>20</td>
<td>0.25</td>
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</table>

**Calibration**

The following calibration curves represent the linear dynamic range of 1-400 ng/ml for both quantifier and qualifier MRM transitions.

![Figure 2. Representative calibration curve for quantifier (289-97) MRM transition. Experiment was performed on a 3200 QTRAP® with a Shimadzu Prominence LC system in a 3 minute run. CV’s (n=6) for this experiment did not exceed 7% at the LLOQ (1 ng/mL). Accuracy deviation was within ±3% for all calibrators and controls.](image1)

![Figure 3. Representative calibration curve for qualifier (289/109) MRM transition performed on a 3200 QTRAP® LC/MS/MS system with a Shimadzu Prominence LC system in a 3 minute run. CV’s (n=6) for this experiment did not exceed 7% at the LLOQ (1 ng/mL). Accuracy deviation was within ±3% for all calibrators and controls.](image2)
Please note that the results presented above were obtained using a single instrument and single set of standards and samples. Prior to production use, the method should be fully validated with real samples, and the results here may not be typical for all instruments. Variations in LC column properties, chemicals, environment, instrument performance and sample preparation procedures will impact performance, thus these results should be considered as informative rather than representative.

System Requirements

In order to run this method as outlined above, the following equipment and reagents are required:

- An AB SCIEX 3200 series (3200 QTRAP® or API 3200™)
- A Shimazdu Prominence 20A HPLC system with reservoir tray and bottles, CBM-20A system controller, 100 µl mixer, 2 isocratic LC-20AD pumps, 3 channel degasser, SIL-20AC autosampler and column oven or an Agilent 1200 HPLC system with binary pump (no static mixer), well plate autosampler and thermostated column oven
- Charcoal filtered serum (www.goldenwestbio.com)
- Testosterone standard and deuterated internal standard (www.sigmaalrich.com)
- HPLC grade water, acetonitrile, methanol and formic acid
- 1.5 ml Eppendorf tubes
- A BetaBasic Javelin Guard Column 185 μm 20 x 2.1 mm (included)
- Phenomenex Luna 3.0 μm C18(2), 50 x 2 mm HPLC column (included)
- A centrifuge able to accommodate Eppendorf tubes and run at 14,000 rpm
- Pipettes and standard laboratory glassware

Ordering Information

<table>
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<tr>
<th>Product Name</th>
<th>Part Number</th>
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<tr>
<td>iMethod™ Test for Testosterone V.1.0 for Cliquid® Software</td>
<td>1037087</td>
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Important Note

While the information provided above outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for Testosterone, please note that the results obtained do require some experience with LC/MS/MS and sample preparation procedures. As such, web-based and onsite training are available to assist in the deployment of the iMethod™ Test and are recommended for inexperienced users. Please consult your local sales representative for more details.

The iMethod™ Test described above has been developed by AB SCIEX to provide all the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. The performance of this method will need to be verified in a given lab due to potential variations in instrument performance, maintenance, chemicals and procedures used, technical experience, sample matrices and environmental conditions. It is the responsibility of the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst® Software may be required to do so.

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