A rapid iMethod™ Test for Vitamin D analysis

*iMethod™ Test for Vitamin D Kit from Chromsystems for Cliquid® Software*

The following information outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for the quantitation of 25-hydroxyvitamin-D₃ and 25-hydroxyvitamin-D₂ when using an AB SCIEX API 3200™ or 3200 QTRAP® LC/MS/MS system in conjunction with Chromsystems MassChrom® Kit for the determination of 25-OH-Vitamin D₃ and 25-OH-Vitamin D₂. This method is also verified for use on the API 4000™ and 4000 QTRAP® LC/MS/MS systems.

The method included is for the routine analysis of 25-OH-Vitamin D₃ and D₂ in human serum and plasma samples. Calibration is performed using a lyophilized multilevel plasma calibrator set of known concentration. Lyophilized plasma control samples at clinically relevant low and high concentrations serve to establish the target analyte range. The method uses a deuterated 25-OH-Vitamin D₃ as an internal standard to correct for sample and instrument variability. Samples are analyzed using an atmospheric pressure chemical ionization (APCI) source for maximum sensitivity.

Figure 1: Example Chromatogram of overlaid MRM traces for the quantifier and qualifier MRM transitions for Chromsystems calibrator level I at 17.8 ng/mL or 44.4 nmol/L 25-OH-Vitamin D₃ and 19.5 ng/mL or 47.2 nmol/L 25-OH-Vitamin D₂, as performed on a 3200 QTRAP® system with a Shimadzu LC system in a 5-minute run.
Separation is performed using a Chromsystems analytical and trap column in conjunction with either a Shimadzu Prominence or Agilent 1200 HPLC system configured for on-line sample preparation according to the configuration included in the documentation with this method, for an overall LC run time of 5.0 minutes.

This method was evaluated using Chromsystems 3PLUS1® multilevel calibrators. Control sample responses were obtained at clinically relevant low (27.7 ng/mL or 69.1 nmol/L 25-OH-Vitamin D₃, 29.6 ng/mL or 71.7 nmol/L 25-OH-Vitamin D₂) and high (58.0 ng/mL or 145 nmol/L 25-OH-Vitamin D₃, 60.7 ng/mL or 147 nmol/L 25-OH-Vitamin D₂) concentrations. %CV and S/N values for the target analytes were obtained using 20 low level control replicates. The estimated limits of quantification (LOQ) for each analyte are more than sufficient to allow the analytical method to be used as either a screening or confirmation technique.

**Calibration**

The following calibration curves represent the linear dynamic range from 19.5 ng/mL to 76.2 ng/mL for OH-Vitamin D₂ and from 17.8 ng/mL to 74.1 ng/mL for OH-Vitamin D₃ for both the quantifier and qualifier transitions.

![Figure 2: Representative calibration curves for 25-OH-Vitamin D₃ and 25-OH-Vitamin D₂ included in the method are shown above. AB SCIEX recommends that calibration is performed using the “linear” regression mode and “1/x” weighting.](image)

The following table shows the observed signal-to-noise (S/N) ratios for the quantifier MRM transitions in a MassCheck® control level I sample, including the %CV estimates from 20 replicate samples.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>S/N*</th>
<th>%CV</th>
<th>Estimated LOQ (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-OH-Vitamin D₃</td>
<td>65</td>
<td>4.5</td>
<td>3.6</td>
</tr>
<tr>
<td>25-OH-Vitamin D₂</td>
<td>90</td>
<td>7.5</td>
<td>2.4</td>
</tr>
</tbody>
</table>

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 API 3200™, 3200 QTRAP® API 4000™ or 4000 QTRAP® LC/MS/MS system.
- 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 1100/1200 HPLC system with binary pump (no static mixer), well plate autosampler and thermostated column oven.
- A Chromsystems Analytical and Trap HPLC column
- 25 Hydroxy Vitamin D calibrators and controls (www.chromsystems.com)
- 25 Hydroxy Vitamin D$_3$-d$_6$ internal standards (www.medicalisotopes.com)
- HPLC grade Water, Acetonitrile, Methanol, Hexane and Formic Acid (www.sigmaaldrich.com)
- 1.5 mL disposable polypropylene microcentrifuge tubes
- 2 mL glass HPLC vials with 250 µL insert and PTFE-lined caps
- Microcentrifuge capable of 14000 rpm or 16000 g
- Pipettors (mechanical, variable volume) 0-200 µL and 0-1000 µL
- Disposable pipette tips
- Dry block heater equipped with microtube insert blocks and nitrogen gas sample concentrator

Please note that this method can also be run on other HPLC systems, given that they are supported for use by Cliquid® Software and the retention times are updated to reflect the configuration used.

Ordering Information

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>iMethod™ Test for Vitamin D Kit from Chromsystems</td>
<td>1039051</td>
</tr>
<tr>
<td>for Cliquid® Software</td>
<td></td>
</tr>
</tbody>
</table>

While the information provided above outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for Vitamin D, please note that the results obtained do require some experience with LC/MS/MS and sample preparation procedures. As such, web-based and on-site 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.

Important Note

The purchase and use of certain chemicals listed above may require the end user to possess any necessary licenses, permits or approvals, if such are required in accordance with local laws and regulations. It is the responsibility of the end user to purchase these chemicals from a licensed supplier, if required in accordance with local laws and regulations. The suppliers and part numbers listed below are for illustrative purposes only and may or may not meet the aforementioned local requirements. 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.

The suppliers identified in this document are provided for information purposes only and is not intended to be an exhaustive representation of all manufacturers or suppliers of the referenced product. AB SCIEX makes no warranties or representations as to the fitness or the continued fitness of a specific product by any of the manufacturers/suppliers referenced herein or the supplier. AB SCIEX assumes no responsibility or contingent liability, including indirect or consequential damages, for any use to which the purchaser may put the referenced suppliers’ products, or for any adverse circumstances arising therefrom.