iMethod™ Test

A Rapid iMethod™ Test for the Analysis of 25-Mono-Hydroxy-Vitamin D2 and 25-Mono-Hydroxy-Vitamin D3 in Human Plasma

The following description outlines the instrument requirements and expected results obtainable from the Spark Holland iMethod™ Test for the quantification of 25-Mono-Hydroxy-Vitamin D2 (MHVD2) and 25-Mono-Hydroxy-Vitamin D3 (MHVD3) in human plasma when using a Spark Holland Symbiosis PICO integrated online SPE and HPLC system with a mistral column oven and an Applied Biosystems/ MDS Analytical Technologies 4000 series (API 4000™ or 4000 QTRAP®) LC/MS/MS system.

Sample preparation is based upon the dilution of a human sample with protein disrupting buffer, followed by mixing and then injection with on-line sample clean up using a Spark HySphere C8EC SE sorbent SPE cartridge. More in depth sample preparation, and instrument parameter information is included as part of the standard operating procedure provided with the method. Solvents, standards and any supplies required for sample preparation are not.

The mobile phase consists of formic acid, water and methanol with separation on a Merck KGaA Chromolith SpeedROD RP-18e (endcapped), 50x4.6 mm HPLC column. An example chromatogram of the separation achieved is shown below in figure 1.

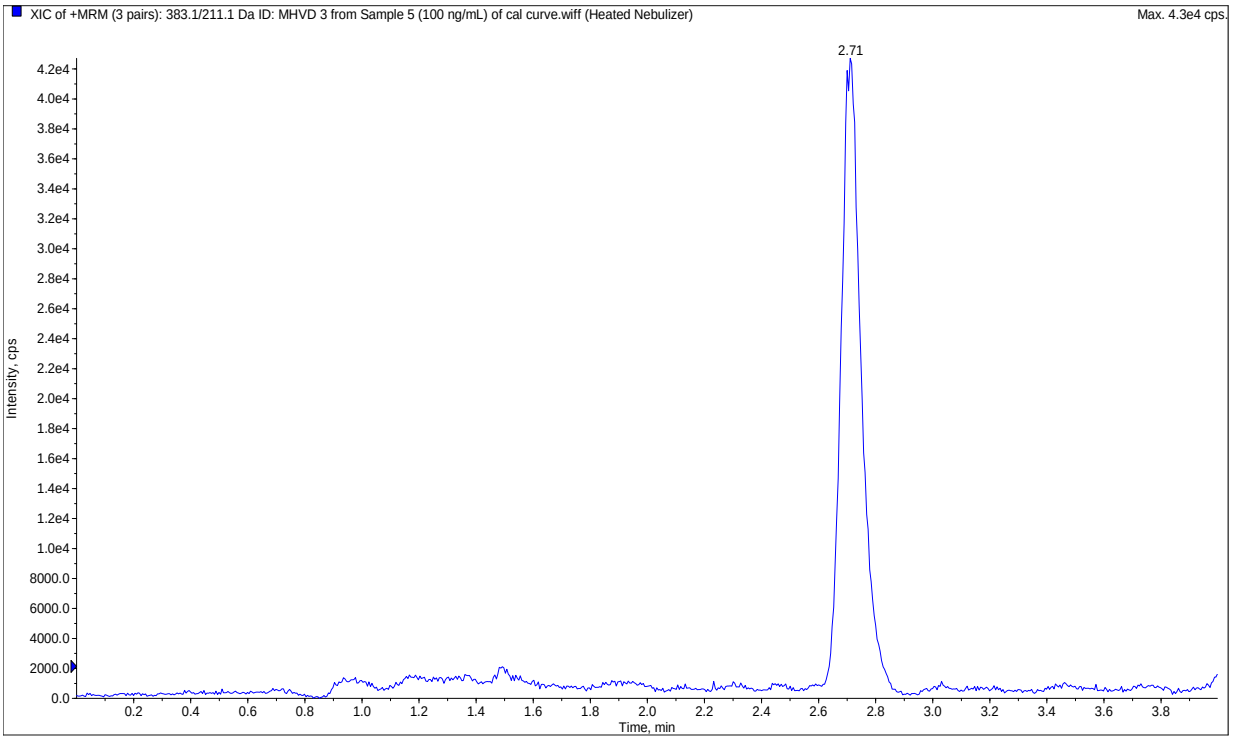

Figure 1:Chromatogram of a 100 ng/mL MHVD3 in plasma run on an API 4000™ LC/MS/MS System

Results

The following MHVD2 and MHVD3 calibration curves are representative of the performance obtained on the instrument using the method described here, and may not be representative of performance on any other instrument.

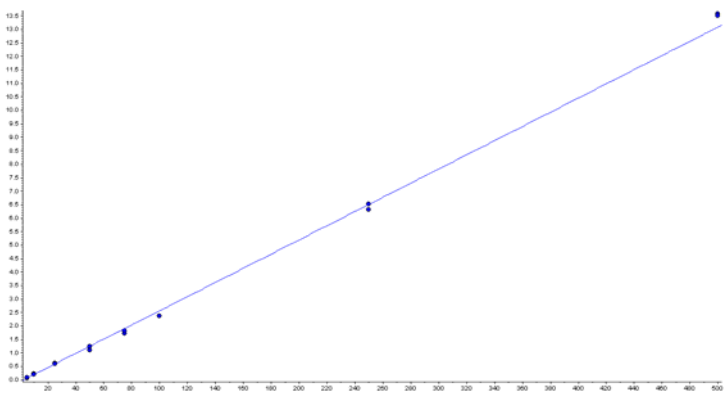

Figure 2: MHVD2 Cal Curve R=0.999 with 1/X weighting

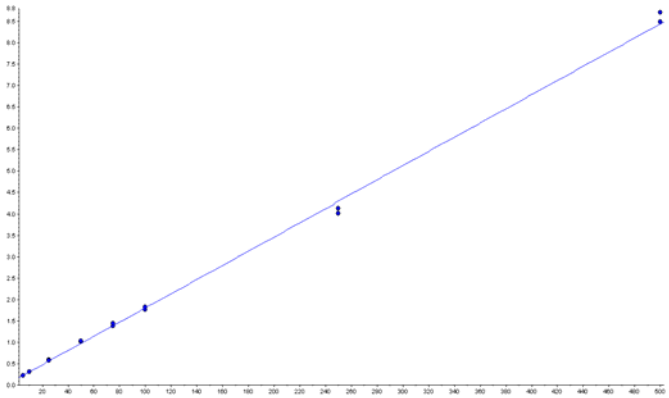

Figure 3: MHVD3 Cal Curve R=0.999 with 1/X weighting

The resulting Accuracies and Precisions (%CV) for the calibration standards and Quality Controls are shown in the tables below.

	MHVD3	MHVD3	MHVD2	MHVD2
Concentration (ng/mL)	%CV	Accuracy	%CV	Accuracy
5	3.35	92.70	6.06	107.67
10	3.23	100.08	2.41	108.35
25	1.76	104.53	1.95	102.79
50	1.40	105.14	8.05	93.54
75	3.18	101.77	3.01	93.04
100	2.91	99.27	0.05	92.53
250	2.07	94.61	2.39	98.56
500	1.78	101.91	0.28	103.52

Figure 4: Combined calibration curve data based upon 4 injections per level

Signal-to-noise

Concentration (ng/mL)	MHVD3 (100µL)	MHVD3 (250µL)	MHVD2 (100µL)	MHVD2 (250 µL)
1	–	4	–	4
5	–	10	–	16
10	8	16	18	40

Figure 5: Observed signal-to-noise (S/N) ratios for the MRM transitions of MHVD2 and MHVD3 for 100 µL and 250 µL injections using a Peak-to-Peak calculation.

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 Applied Biosystems/ MDS Analytical Technologies 4000 Series LC/MS/MS System
- Spark Holland Symbiosis PICO Online SPE HPLC system with a Mistral column oven
- Vitamin D calibrators and internal standards (Sigma Aldrich)
- LC/MS grade methanol, Water and Formic Acid
- A Merck KGaA Chromolith SpeedROD RP-18e (endcapped), 50x4.6 mm HPLC column
- Spark SPE cartridges filled with Applied Biosystems POROS® R1 sorbent
- Pipettes and standard laboratory glassware

Please note that the Phenomenex HPLC is required but not included with this iMethod™ Test.

Important Note

The purchase and use of certain of the 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. Applied Biosystems is not responsible for user's compliance with any statute or regulation, or for any permit or approval required for user to implement any iMethod™ procedure.

Legal Acknowledgements / Disclaimers

The iMethod™ Test described above has been designed by Applied Biosystems to provide the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. This method is provided for information purposes only. The performance of this method is not guaranteed due to many different potential variations, including instrument performance, tuning, and maintenance, chemical variability and procedures used, technical experience, sample matrices, and environmental conditions. It us up to 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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