### Overview

**Purpose:** Comprehension of the full text was necessary for the quantification of Vitamin D.

**Methods:** Employed LC/MS/MS techniques using Thermo Scientific Velos Pro mass spectrometer. Analysis performed using Thermo Scientific LC-MS 2.6.1 Quantitation software.

**Results:** Challenges in Vitamin D Analysis were noted due to the complexity of the Vitamin D system. The use of MS2 and MS3 approaches was imperative for accurate quantification. The correlation plots showed good linearity (R² > 0.98) across the concentration range for both MS2 and MS3 methods. The correlation of Vitamin D levels between MS2 and MS3 methods was excellent (Y = 0.098 + 0.0818*X, R² = 0.9988).

**Conclusion:** The use of both MS2 and MS3 methods provided accurate quantification with excellent linearity. The collaborative data showed comparable results between MS2 and MS3 methods, validating the methods' reliability.

### Sample Preparation

1. 250 μL each sample was treated with 200 μL of 0.1 M NaOH, vortexed and left at 4°C for 1 hour. 
2. 100 μL of internal standard solution was added and vortexed.
3. The mixture was centrifuged at 1500 g for 5 minutes.
4. The supernatant was used for the subsequent analysis.

### Liquid Chromatography

An Agilent 1260 Infinity HPLC system was employed with a reversed-phase C18 column (5 μm, 4.6 mm × 150 mm) and a mobile phase of acetonitrile-water (0.1% formic acid solution). The flow rate was set at 0.5 mL/min.

### Mass Spectrometry

An Agilent 6410 Triple Quadrupole mass spectrometer was used in positive electrospray ionization mode. The MS conditions were as follows:
- Ion source temperature: 350°C
- ne spray voltage: 4500 V
- Capillary voltage: 3500 V
- Dry gas: nitrogen (1.5 L/min)
- Dry gas temperature: 300°C
- Dry gas flow: 10 L/min

### Conclusion

In this study, we used a full scan MS3 approach for data acquisition. This choice was made because the MS2 approach requires larger ionization of the molecule, which is often not possible. The use of MS3 allows for a more accurate quantification of the Vitamin D levels. The collaborative data showed comparable results between MS2 and MS3 methods, validating the methods' reliability.

### Acknowledgements

We would like to thank Kara Lynch and Shannon Kastner from the University of California - San Francisco for supplying fresh plasma sample sets. These methods are For Research use Only. Not for use in Diagnostic Procedures. Any non-commercial use of copyrighted materials will require permission from the copyright owner. The data presented herein is protected by intellectual property rights of others.

### Table: Sample Preparation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment</th>
<th>Temperature</th>
<th>Concentration (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>200 μL NaOH</td>
<td>4°C</td>
<td>100 μL Internal Standard</td>
</tr>
</tbody>
</table>

### Table: Mass Spectrometry

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion Source</td>
<td>Temperature: 350°C</td>
</tr>
<tr>
<td>Spray Voltage</td>
<td>4500 V</td>
</tr>
<tr>
<td>Capillary Voltage</td>
<td>3500 V</td>
</tr>
<tr>
<td>Dry Gas</td>
<td>Nitrogen (1.5 L/min)</td>
</tr>
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<td>Dry Gas Flow</td>
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</tr>
</tbody>
</table>

### Graphs

- Figure 1: Chromatograms for 25-OH Vitamin D2, 25-OH Vitamin D3, and Full Scan MS3 Base Peak Extracted Ion Chromatogram for 25-OH Vitamin D3.
- Figure 2: Peak Top Full Scan MS2 Spectra for 25-OH Vitamin D2 (top), Internal Standard (middle) and 25-OH Vitamin D2 (bottom) for Donor 46.
- Figure 3: Peak Top Full Scan MS3 Spectra for 25-OH Vitamin D2 (top), Internal Standard (middle) and 25-OH Vitamin D2 (bottom) for Donor 46.
- Figure 4: Full Scan MS3 Total Ion Chromatogram for 25-OH Vitamin D2 (top), Internal Standard (middle) and 25-OH Vitamin D2 (bottom) for Donor 46.