Overview

A liquid chromatography method for the quantification of vitamin D using online sample preparation and high resolution accurate mass HR-MS quantification with an Exactive Plus mass spectrometer for clinical research was developed.

Methods

Spectrometer:
Matt Berube, Joe Dibussolo, Catherine Lafontaine, Yang Shi, Francois Espourteille

Mass Spectrometry

Mass Spectrometry

Vitamin D2 and 25-hydroxyvitamin D3 were quantified using an extraction method with a solid core HPLC column for separation and analysis using an Exactive Plus Orbitrap MS.

Results

The lower limit of quantitation (LLOQ) was determined to be 2 ng/mL for both analytes in BSA as indicated in Figure 3. Calibration curves and QCs were run in triplicate each day across four days. In addition, pooled patient serum samples were centrifuged and spiked with analytes for a final concentration of 20 ng/mL for 25-hydroxyvitamin D3 up to about 600 injections as indicated in Figure 7.

Sample Preparation

1. Add 100 µL of calibrants and controls into centrifuge tubes
2. Add 200 µL of precipitating reagent containing internal standard to each tube
3. Vortex each tube for 30 seconds
4. Centrifuge tubes at 5,000 RCF for 10 minutes
5. Aliquot supernatants into autosampler vials for analysis

In addition, pooled patient serum samples were centrifuged and spiked with analytes for a final concentration of 20 ng/mL for 25-hydroxyvitamin D3 up to about 600 injections as indicated in Figure 7.

Figure 1. Liquid Chromatography Method

FIGURE 1. Liquid Chromatography Method

FIGURE 2. Chromatograms at LLOQ of 2 ng/mL with 50 ng/mL internal standard

FIGURE 3. Chromatograms at LLOQ of 2 ng/mL with 50 ng/mL internal standard

FIGURE 4. Chromatograms of 40 ng/mL, 80 ng/mL and 120 ng/mL vitamin D3

FIGURE 5. QC Replicate injections. Three replicates of each QC level were run each day for four days and results compared.

Methods

High resolution accurate mass HR-MS quantification with an Exactive Plus mass spectrometer for clinical research was developed.

Figure 2. Chromatograms at LLOQ of 2 ng/mL with 50 ng/mL internal standard

FIGURE 3. Chromatograms at LLOQ of 2 ng/mL with 50 ng/mL internal standard

FIGURE 4. Chromatograms of 40 ng/mL, 80 ng/mL and 120 ng/mL vitamin D3

FIGURE 5. QC Replicate injections. Three replicates of each QC level were run each day for four days and results compared.

Conclusions

• Reliable detection of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 in serum by clinical researchers.
• Improved method robustness of approximately 600 serum injections between column changes.
• Ease of method set up compared to triple quadrupole MS methods.

Acknowledgements

We would like to thank the following for their assistance and collaboration:

Analysis of 25-Hydroxyvitamin D In Serum Using Automated On Line Sample Preparation Technique With High Resolution Orbitrap Mass Spectrometer

Matt Berube, Joe Dibussolo, Catherine Lafontaine, Yang Shi, Francois Espourteille
Thermo Fisher Scientific, Franklin, MA

Figure 2. Chromatograms at LLOQ of 2 ng/mL with 50 ng/mL internal standard

FIGURE 3. Chromatograms at LLOQ of 2 ng/mL with 50 ng/mL internal standard

FIGURE 4. Chromatograms of 40 ng/mL, 80 ng/mL and 120 ng/mL vitamin D3

FIGURE 5. QC Replicate injections. Three replicates of each QC level were run each day for four days and results compared.