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

Overview

A high throughput method for the quantification of vitamin D using protein sample preparation and high resolution, accurate mass (ARMS) quantitation with an Exactive Plus orbitrap mass spectrometer for clinical research use.

Methods

Several precipitated sera in injectors were evaluated using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Chromatographic separation was performed using a TurboFlow XL C18P (0.5x50 mm) column. The Orbitrap MS was calibrated for calibration and analysis using an Exactive Plus Orbitrap HILIC kit.

Results

The limits of quantitation (LLOQ) for 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 were determined to be 2 ng/mL, based on analysis of a standard curve with a correlation coefficient of 0.99 for both analytes using the Exactive Plus Orbitrap MS. A lock mass was added in order to maintain mass accuracy during the batch runs.

Introduction

25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 are commonly tested by clinical researchers. In this study, total cholesterol was coupled with triple quadrupole mass spectrometry. Ions at 395.32965-395.33203 F: FTMS + p APCI corona Full lock ms were used to analyze vitamin D3. The calculated concentration (ng/mL) was 0.0081235 to 0.0082813 ng/mL. The lock mass and lock ion were determined to be present in human serum.

Methods

Standard solutions of 25-hydroxyvitamin D2, 25-hydroxyvitamin D3, and deuterated 25-hydroxyvitamin D3, quantified against Cerilliant, Inc. were analyzed. The concentrations were 0.01, 0.1, 1, 10, 100 ng/mL. Standards were then analyzed for 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3, and derivatized standards were used to analyze D6-25-hydroxyvitamin D3. The lock mass was added in order to maintain mass accuracy during the batch runs.

Results

The quality of the data was determined by evaluating the correlation coefficient obtained using 1/X weighted linear regression analysis of the standard curves. The correlation coefficient obtained using 1/X weighted linear regression analysis of the standard curves were greater than 0.99 for both analytes (Figure 1). A relative standard deviation (RSD) test was performed in order to determine the variability of analysis of the standards curve with a relative standard deviation of 5 percent. The variability of analysis of the standards curve with a relative standard deviation of 5 percent varied between 3.6 percent and 5 percent.

Discussion

The LLOQ was determined to be 2 ng/mL, based on analysis of a standard curve with a correlation coefficient of 0.99 for both analytes using the Exactive Plus Orbitrap MS. The correlation coefficients obtained using 1/X weighted linear regression analysis of the standard curves were greater than 0.99 for both analytes (Figure 1). A relative standard deviation (RSD) test was performed in order to determine the variability of analysis of the standards curve with a relative standard deviation of 5 percent. The variability of analysis of the standards curve with a relative standard deviation of 5 percent varied between 3.6 percent and 5 percent.

Conclusions

• Reliable detection of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 in serum by high-resolution mass spectrometry. The system can be used to quantitate both analytes with high accuracy and precision.

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