**Background**

Sample preparation is generally the bottleneck in a laboratory’s analytical workflow. With online sample cleanup, sample turnaround time, robustness, and data quality are significantly improved for the LC/MS/MS analysis of both vitamin D forms.

**The Approach**

A five minute method that features online sample cleanup provides a simple, accurate, and rapid analysis of 25-OH vitamin D<sub>2</sub> and D<sub>3</sub> in plasma and serum. Time consuming and labor intensive sample preparation is reduced when analytes are trapped on an Eclipse Plus C18 Guard Column, washed, and eluted onto a Poroshell 120 EC-C18 column for further chromatographic separation. Analysis with the combination of an Agilent 6430 Triple Quadrupole Mass Spectrometer coupled to an Agilent 1200 Infinity Series LC delivers the outstanding sensitivity and specificity required to confidently identify and quantify 25-OH vitamin D<sub>2</sub> and D<sub>3</sub> in difficult biological matrices.

With easy-to-use MassHunter software, a simple work flow can be established to rapidly quantify vitamin D. Linearity of calibration curves, as well as accuracy of quality control and sample data, can be quickly verified. Processed data can be viewed in a variety of customizable report formats or it can exported to a laboratory informatics system (LIMS) in .xml or .csv format.
Figure 2: Representative chromatogram demonstrating the rapid and sensitive LC/MS/MS analysis of 25-OH vitamin D$_2$ (blue) and D$_3$ (green).

Figure 3: Excellent linearity and sensitivity allows the accurate quantitation of 25-OH vitamin D$_2$ and D$_3$.

**Key Benefits**

- Rapid, high-throughput analysis of 25-OH vitamin D$_2$ and D$_3$
- Sensitive and accurate quantitation within five minutes
- Simple, straightforward online sample cleanup
- Quick results interpretation from advanced software tools
- A specific, cost-effective, and time-saving method

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