Evaluation of Total Vitamin D (D$_2$ / D$_3$) on a fully automated Liquid-Chromatography Mass-Spectrometer

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Introduction
Serum vitamin D analysis continues to be performed by immunoassay methods in many clinical laboratories, despite national/international guidelines recommending analysis by liquid chromatography-mass spectrometry (LC-MS/MS). Wide scale adoption of LC-MS/MS in routine clinical laboratories is restricted by the technical expertise required to set up and run assays and the cost of the necessary equipment. The Thermo Scientific™ Cascadion™ SM Clinical Analyser (Analyser) is the world’s first fully automated LC-MS/MS system; encompassing sample preparation, liquid chromatography and mass spectrometry within a single system. The Cascadion™ SM 25-Hydroxy Vitamin D Assay is for the determination of total 25-hydroxy vitamin D (25-OH Vit D) in human serum and plasma through the quantitative measurement of 25-Hydroxy Vitamin D$_3$ and 25-Hydroxy Vitamin D$_2$. It is commercially available and is CE-IVD compliant.

Aim
To carry out an independent evaluation of the Cascadion Vitamin D assay and its contributing D$_2$ and D$_3$ components.

Method
- Vitamin D$_2$ / D$_3$ validation
  - Stripped serum was analysed 20 times to assess the limit of blank.
  - Pooled serum was run ten times within a batch and on five consecutive days to assess imprecision.
  - A high sample was diluted 1:2, 1:4, 1:8 and 1:16 to assess linearity.
  - A high calibrator was run twice followed by two PBS samples and repeated 10 times to assess carryover.
  - Three samples were spiked with internal quality control to assess recovery.
  - External quality assurance (EQA) samples and reference material were assessed to analyse accuracy.
  - Internal quality control (IQC) samples from 6 independent suppliers were analysed to assess suitability.

Results
- The Vitamin D$_3$ component has an analytical range of 8.49 – 329.47 nmol/L. The D$_2$ component analytical range is 8.24 – 319.83 nmol/L
- D$_2$ and D$_3$ components showed no response observed for the limit of blank.
- Linearity was acceptable (r$^2$ >0.99).
- No carryover was observed.
- Recovery was acceptable (90%-110%).
- Imprecision was good; C.V.s across the analytical range for D$_3$ and D$_2$ were all <5.38%.
- No matrix effects were observed using third party controls.
- The relative bias observed was acceptable using NIST, DEQAS and RIb samples.

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