Long Abstract

Rapid Quantitative Analysis of 25-OH Vitamin D_2 and D_3 in Patient Serum using a Novel Weak Anion Exchange Disposable Tip Extraction (DPX) and LC-MS/MS Detection

Gary Woodward[1], William Brewer[2], Martin Roch[1], Rachel Webster[1]

[1]Clinical Biochemistry, University Hospitals Birmingham NHS Foundation Trust, Queen Elizabeth Hospital Birmingham, Birmingham, UK. [2]Department of Chemistry and Biochemistry, University of South Carolina, Columbia, South Carolina, US

Introduction. In the last decade clinical laboratories have been confronted with a renewed interest in vitamin D analysis. The increased demand and workload in hospital laboratories requires quicker and more efficient analysis methods. Thus, clinical methods now need to be both analytically sound and higher-throughput. As such, various extraction methods have been adopted based largely on liquid-liquid or solid-phase extractions with LC-MS/MS detection or immunoassay. We present a novel high-throughput method for the analysis of vitamin D_2 and D_3 using weak anion exchange disposable pipette extraction (DPX-WAX) validated in routine patient serum samples against conventional liquid-liquid (L/L) extraction. DPX-WAX incorporates loosely contained sorbent material within a pipette tip, which is mixed with the sample solution upon aspiration and expulsion (Fig. 1).

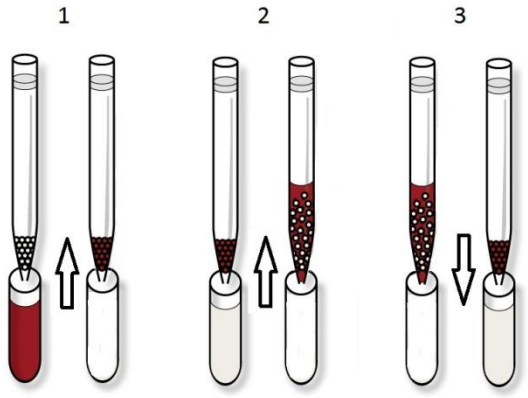

Figure 1. DPX-WAX cleanup method that permits protein crash in the pipette tip without centrifugation. Step 1 is aspirating the serum sample; step 2 is aspirating the acetonitrile and mixing with the serum sample and the sorbent; and step 3 is dispensing the clean sample solution into the vial or well for injection.

Methods. Patient plasma samples (n=32) were prepared using L/L extractions as described previously by Maunsell *et. al.* (2005) and DPX-WAX extractions were performed by aspirating 100 µL of plasma sample followed by 150 µL of internal standard solution ($[^2H_6]$25-OH D_3 in acetonitrile) as described in Fig. 1. The clean extract was analysed using a Waters 2795 HPLC with a Kinetix C18 column (5µm, 50 x 2.10 mm) coupled to a Waters Quattro Premier XE MS/MS detection (MRM 413>355 for D_2 and 401>159 for D_3). Statistical analysis was performed using Analyse-it for Microsoft Excel.

Results. DPX-WAX extraction of patient serum allowed for quantitative LC-MS/MS separation of vitamin D_2 and D_3. Using both patient samples and spiked steroid stripped serum preparations, limits of quantification were shown to be 10 and 5 µM (defined as a s/n >10 and CV <10%) for vitamin D_2 and D_3, respectively, with a mean recovery of 103% at concentrations greater than 5 µM. Passing-Bablok analysis of standard calibration material (Chromsystems, Germany) dilutions and spiked steroid stripped serum demonstrated linearity with a coefficients of determination (R^2) of 98% and 99% with a Y value of 0.95 and 1.00 for vitamin D_2 and D_3, respectively, across a concentration range of 2.7 – 89.5 µM. The precision observed following DPX-WAX sample preparation gave a mean CV of 13.6 and 7.6 % for vitamin D_2 and D_3, respectively. When comparing DPX-WAX with L/L extraction, Passing–Bablok analysis demonstrated a proportional bias of 1.09% and 1.12%, with an R^2 value of 0.98 and 0.99 for vitamin D_2 and D_3, respectively. The Bland–Altman bias plot confirmed that the results were comparable between the extraction techniques.

Conclusions. These results indicate that DPX with weak anion exchange sorbent is effective for vitamin D analysis in a routine clinical setting. Furthermore, the DPX-WAX method allowed for rapid sample clean-up (1-2 min per sample) that is amenable to automated platforms.

References

Maunsell Z *et al*. Routine Isotope Dilution Liquid Chromatography-Tandem Mass Spectrometry Assay for Simultaneous Measurement of the 25-Hydroxy Metabolites of Vitamins D2 and D3. *Clin Chem*, 2005; 51:9 1683-1690.