The analysis of 25-hydroxyvitamin D in serum using semi-automated solid-phase extraction and LC/MS/MS

ABSTRACT

Introductions: In recent years the demand for serum 25-hydroxyvitamin D (25OHD) analysis has increased considerably. In addition to the role vitamin D plays in bone metabolism, recent studies have implicated vitamin D in the regulation of a number of cellular processes, including the modulation of cell proliferation, differentiation, and death. As a result, interest in vitamin D deficiency and its clinical consequences has grown. We have developed a routine semi-automated solid phase extraction UPLC/MS/MS method for the analysis of 25OHD2 and 25OHD3. The method was compared with sixty five clinical serum samples and the results agreed well with those obtained using a routine LC/MS/MS assay. The semi-automated procedure significantly decreased operator variability allowing the analysis of at least 192 samples per working day. The semi-automated procedure significantly decreased operator variability to ensure more consistent and reproducible results.

RESULTS

The accuracy of the assay for 25OHD3 was determined by the Passing-Bablok method and agreement was assessed using the Bland–Altman difference plot. The overall agreement between the methods (Waters=UHSM-1.0ng/mL) when analyzing a total of 69 samples from 25OHD2 or 25OHD3, respectively (Table 1). There was no difference between the methods (Waters=UHSM-0.0ng/mL) when analyzing a total of 69 samples from 25OHD2 or 25OHD3, respectively (Table 2).

DISCUSSION

LC/MS/MS is now widely used in many laboratories for vitamin D measurement, however, many of these methods require more samples per working day and the need for experienced laboratory staff to successfully implement the method.

This assay for the analysis of 25OHD3 or 25OHD2 in serum demonstrates excellent linearity (R2=0.998) with good accuracy and precision over five consecutive days of analysis. In addition, the method demonstrated good agreement with a deviation of ±15% of the nominal value was accepted at the level of quantitation for each analyte.

The described semi-automated method overcomes many of the potential disadvantages of the routine clinic LC/MS/MS assay, including the need for time-consuming and labor intensive sample preparation and operator variability.

REFERENCES


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