Optimisation and validation of ultrahigh performance liquid chromatography method for quantification of 25-hydroxyvitamin d in maternal plasma

ABSTRACT

This study aimed to optimise and validate a simple and efficient sample preparation and extraction method for the quantification of 25-hydroxyvitamin D (25OHD) in the maternal plasma sample. Different sample preparation methods and precipitation reagentto sample ratio used by previous studies were compared. An ultrahigh performance liquid chromatography method was developed and validated for simultaneous quantification of 25OHD2 and 25OHD3. The chromatographic separation was achieved using the COSMOCORE 2.6Cholester Column and methanol and 0.1% formic acid (79:21, %v/v) as mobile phase at a flow rate of 0.3 mL/min and diode array detection at 264 nm. The results demonstrated that a recovery of approximately 100% could be achieved by extracting samples using 1 mL of hexane, vortex for 10s and a total number of three extraction steps. The precipitation reagentto-sample ratio of 2.8 was optimum for the quantification of 25OHD2 and 25OHD3 in a pooled maternal sample. The value obtained for validation parameters meets the criteria of the Recommendations and Acceptance Criteria for Bioanalytical Method Validation by the Food and Drug Administration. The results showed that this method could be applied for routine quantification of 25OHD, particularly in the maternal plasma sample.

Keyword: 25-hydroxyvitamin D; Maternal; Ultrahigh performance liquid chromatography; Extraction