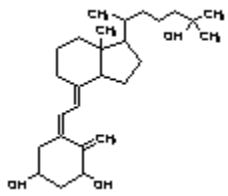
SPE Application Note for
Extraction and Separation of Vitamin D Metabolites from Plasma

This method was developed for the simultaneous extraction and fractionation of the vitamin D metabolites 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D from plasma or serum using a non-polar retention mechanism.

EXTRACTION PROCEDURE

ISOLUTE® SPE Column: MFC18 500 mg/3 mL Part # 240-0050-B

Pre-treatment:	To a 1 mL sample of serum or plasma, add 30 ul of tracer (1000 cpm) as internal standard. Mix thoroughly, and incubate for 15 mins. Add dropwize 1 mL of acetonitrile, mix, and centrifuge at 2000 g for 10 minutes at 20 C. To the supernatant, add 1 mL of phosphate buffer (0.4 M, pH 10.5), mix and centrifuge at 2000 g for 10 minutes at 20 C.
Solvation:	Solvate the column with hexane (2 mL), isopropanol (2 mL) and methanol (2 mL) sucessively.
Equilibration:	Rinse the column with water (2 mL).
Sample application:	Apply the sample supernatant at a flow rate of 1-2 mL/min.
Interference elution:	Rinse the column with water (5 mL) followed by 70:30 (v/v) methanol:water (5 mL). Dry the column by centrifugation at 2000 g for 10 mins at 20 C.
Analyte elution:	Elute 25-hydroxyvitamin D with 10:90 (v/v) dichloromethane:hexane (3 mL). Rinse the column with a further 2 mL of 10:90 (v/v) dichloromethane:hexane. Do not collect. Rinse the column with 1:99 (v/v) isopropanol:hexane (5 mL). Do not collect. Elute the 1,25-dihydroxyvitamin D with 3.5:96.5 (v/v) isopropanol:hexane (5 mL).
Structure	1,25-dihydroxyvitamin D is shown. 
Structural considerations	The analytes differ in polarity by one -OH group. This difference is utilized to fractionate the two metabolites.

Matrix considerations	The analytes are to be extracted from a polar, aqueous matrix.
Analytical method	Radioreceptor assay
Reagents	
General comments	1. This procedure uses the monofunctional C18 sorbent, which has significantly more secondary silanol interactions than standard trifunctional C18. In the initial extraction, the analytes are extracted from the polar matrix by non-polar C18 interactions. Fractionation of the vitamin D metabolites utilizes the polar silanol interactions, with the least polar metabolite eluted with a very non-polar solvent, and the more polar metabolite eluted with a more polar solvent.

ISOLUTE column part numbers represent the product configuration of choice for use with a vacuum sample processing station. For 96-well and alternative column configurations compatible with any SPE automation system, please contact Biotage.

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