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## 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 30ul of tracer (1000cpm) as internal standard. Mix thoroughly, and incubate for 15 mins. Add dropwise 1 ml of acetonitrile, mix, and centrifuge at 2000 g for 10 mins 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 mins at 20 C.

**Solvation:** Solvate the column with hexane (2 ml), isopropanol (2ml) and methanol (2 ml) successively.

**Equilibration:** Rinse the column with water (2 ml).

**Sample application:** Apply the sample supernatant at a flow rate of 1-2 ml / min.

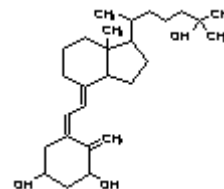
**Interference elution:** Rinse the column with water (5 ml) followed by 70:30 (v/v) methanol:water (5 ml). Dry the column by centrifugation at 2000 g for 10 mins at 20 C.

**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 utilised to fractionate the two metabolites.

#### Matrix considerations

The analytes are to be extracted from a polar, aqueous matrix.



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**Analytical method** Radioreceptor assay

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**Reagents**

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**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 utilises 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.

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ISOLUTE column part numbers represent the product configuration of choice for use with a vacuum sample processing station. For alternative configurations compatible with any SPE automation system, please consult your IST Distributor.

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