

# Extraction of Vitamin D Metabolites From Human Plasma Using ISOLUTE<sup>®</sup> SLE+

## Introduction

This application note describes the extraction of Vitamin D metabolites from human plasma using ISOLUTE SLE+.

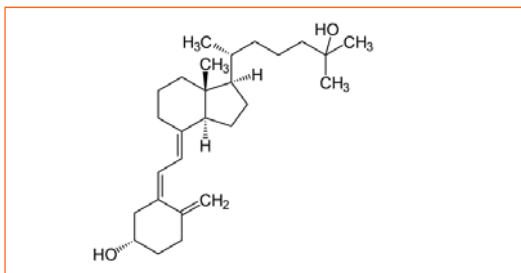


Figure 1. Structure of 25-OH Vitamin D<sub>2</sub>

This method describes the use of ISOLUTE SLE+ plates for the extraction of Vitamin D metabolites 25-OH Vitamin D<sub>2</sub> and 25-OH Vitamin D<sub>3</sub>. Incorporated into the procedure is an integral protein binding disruption step which maximizes analyte recovery and eliminates the need for any offline protein precipitation.

ISOLUTE SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation time.

## Analytes

25-OH Vitamin D<sub>2</sub> and 25-OH Vitamin D<sub>3</sub>.

## ISOLUTE SLE+ Procedure

- ISOLUTE SLE + Configuration:** ISOLUTE SLE+ 200 Supported Liquid Extraction Plate, part number 820-0200-P01
- Sample pre-treatment:** To a mixture of 50 µL HPLC grade water and 50 µL isopropanol (propan-2-ol) add 100 µL of sample (plasma) and mix for 10 seconds.
- Sample loading:** Load pre-treated plasma (200 µL total volume) onto the ISOLUTE SLE+ plate, leave the samples to absorb for 5 minutes under gravity and then apply a pulse of vacuum for 2-5 seconds if not fully absorbed onto sorbent.
- Analyte elution:** Apply 500 µL of heptane, wait 5 minutes to allow the solvent to soak, apply a short pulse of vacuum if solvent not fully absorbed. Apply a second 500 µL of heptane, allow to soak for 5 minutes and then apply another short pulse of vacuum.
- Post extraction:** Evaporate eluate to dryness without heat and reconstitute in 0.1% formic acid in HPLC grade water/ 0.1% formic acid in methanol (20:80 v/v, 100 µL). Cap samples and vortex gently for 60 seconds.

## Additional information

Analytes are particularly sensitive to light. Amber glassware is recommended where possible.

## HPLC Conditions

**Instrument:** Acquity UPLC fitted with a 20  $\mu$ L Loop.  
**Column:** Acquity UPLC BEH C18 column (1.7 $\mu$ , 50 x 2.1 mm id).  
**Mobile Phase:** A = 0.1% formic acid (aq), B = 0.1% formic acid/MeOH at a flow rate of 0.4 mL/min.

**Gradient:**

Time	% A	% B
0	20	80
2	0	100
3.1	0	100
3.15	20	80
4.6	20	80

**Injection Volume:** 15  $\mu$ L (partial loop with overfill).

**Sample Temperature:** 10 °C.

**Column Temperature:** 40 °C.

## Mass Spectrometry Conditions

**Instrument:** Quattro Premier XE triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. *Table 1.* shows the positive ions acquired in the multiple reaction monitoring (MRM) mode.

**Desolvation Temperature:** 450 °C

**Ion Source Temperature:** 150 °C

Scan Function	Analyte	MRM Transition	Cone Voltage (V)	Collision Energy (eV)
1	25-OH Vitamin D <sub>2</sub>	395.0 > 119.0	20	27
2	25-OH Vitamin D <sub>3</sub>	401.4 > 383.4	20	9

**Table 1.** MRM transitions for Vitamin D metabolites

## Results

All results show recoveries above 85% with RSDs below 10%, Figure 2. shows typical analyte recoveries, Figure 3. shows an example mass chromatogram. LOQ values are 25 ng/mL for 25-OH Vitamin D<sub>2</sub> and D<sub>3</sub>, high recoveries are also possible for the other Vitamin D metabolites.

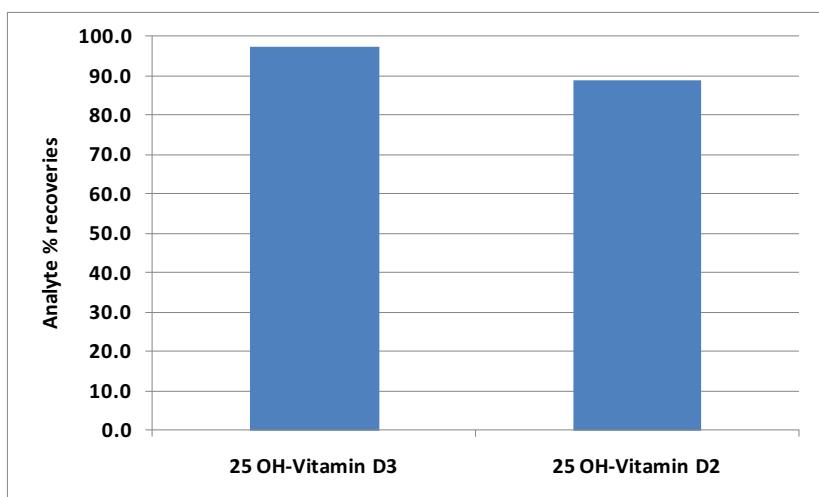


Figure 2. Typical analyte % recoveries (n = 7)

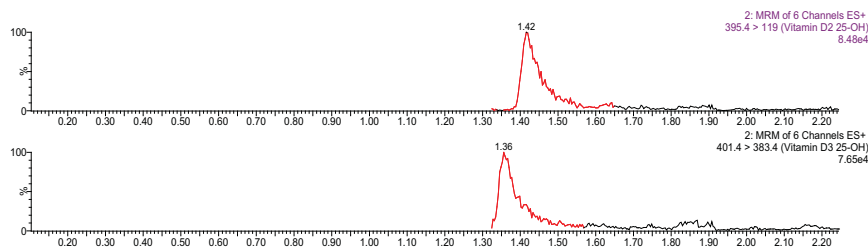


Figure 3. Mass chromatogram showing Vitamin D<sub>2</sub> 25-OH, Vitamin D<sub>3</sub> and 25-OH at 100 ng/mL.

## References

This application note is based on the poster 'Vitamin D and Metabolites: Evaluation of Supported Liquid Extraction (SLE) prior to LC-MS/MS Analysis.', L Williams et al, presented at MSACL, San Diego, CA, 5-9 February, 2011.

## Ordering information

Part number	Description	Quantity
820-0200-P01	ISOLUTE SLE+ 200 Supported Liquid Extraction	1

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