

Method for the Extraction of Vitamin D from Plasma Using ISOLUTE® C18 and RapidTrace® Workstation

Introduction

This application note describes for the extraction of Vitamin D from plasma using ISOLUTE and RapidTrace Workstation.

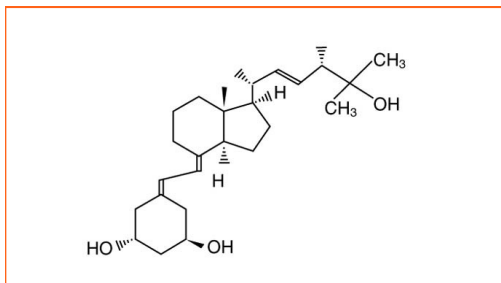


Figure 1. Structure of Vitamin D

Vitamin D (Figure 1) is the collective term for a group of fat soluble pro-hormones which exist as two major forms, D₂ (ergocalciferol) and D₃ (cholecalciferol). Vitamin D is biologically inert within the body; however, two subsequent hydroxylation reactions provide the main active metabolites; 25-hydroxy-vitamin D and 1 α , 25-dihydroxy-vitamin D. Humans receive vitamin D either through diet or exposure to sunlight (UVB). Deficiency can result in various disorders including osteoporosis, liver and kidney problems to increased risk of various cancers and multiple sclerosis, making this compound extremely clinically relevant.

Columns

ISOLUTE C18 (EC) (3mL 200mg part number 221-0020-B) is a non-polar silica based sorbent used to extract acidic, base and neutral compounds through non-polar interactions. The residual silanols on the silica surface are subsequently end capped (EC) to minimize secondary silanol interactions.

'The methods offered within this application are meant to represent a starting point and guide for method development and some amendment may be necessary.'

Analytes

25-hydroxy-vitamin D and 1 α , 25-dihydroxy-vitamin D.

The SPE steps were configured schematically using the RapidTrace software as follows:

RapidTrace procedure

	Step	Source	Destination	Volume (mL)	Flow (mL/min)
1	Condition	Methanol	Organic Waste	2.0	15.00
2	Condition	Water	Aqueous Waste	2.0	15.00
3	Load	Sample	Organic Waste	1.0	1.00
4	Rinse	Water	Aqueous Waste	2.0	15.00
5	Rinse	Methanol : Water	Organic Waste	2.0	15.00
6	Purge-Cannula	Methanol	Cannula Waste	2.0	30.00
7	Collect	Methanol	Fraction 1	1.0	0.50
8	Purge-Cannula	Methanol	Cannula Waste	2.0	30.00
9	Purge-Cannula	Water	Cannula Waste	2.0	30.00

Biotage recommends TurboVap Workstations post elution to blow down your samples.

Reagent lines

Line 1: Water

Line 2: Methanol

Line 5: Methanol : water (v/v, 50:50)

Ordering information

Part number	Description	Quantity
221-0020-B	ISOLUTE C18 (EC) 3mL 200mg	50
C50000	RapidTrace 3 mL Workstation	1
C50974	Tube Rack (13 x 100 mm Sample Tubes & 12 x 75 Fraction Tubes)	1
C40707	Test Tubes-13 x 100 mm, Uncap	1000
C44651	Test Tubes-12 x 75 mm, Uncap	1000
C52006	RapidTrace Start-Up Kit	1
C52689	RapidTrace Notebook Controller	1

RapidTrace Overview



The RapidTrace has been designed to eliminate sample preparation bottlenecks and it is a modular, highly scalable, automated platform designed for high throughput. Units are available to accommodate 1 mL, 3 mL and 6 mL SPE cartridges. Up to 10 modular units can be connected together and controlled through a simple, easy-to-use software package. The systems are widely used within analytical industry and are ideal both for industrial settings and for efficient SPE method development.

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