



Increased Sensitivity of 25-Hydroxyvitamin D₃ Detection Using a Strata™-X PRO SPE Plate

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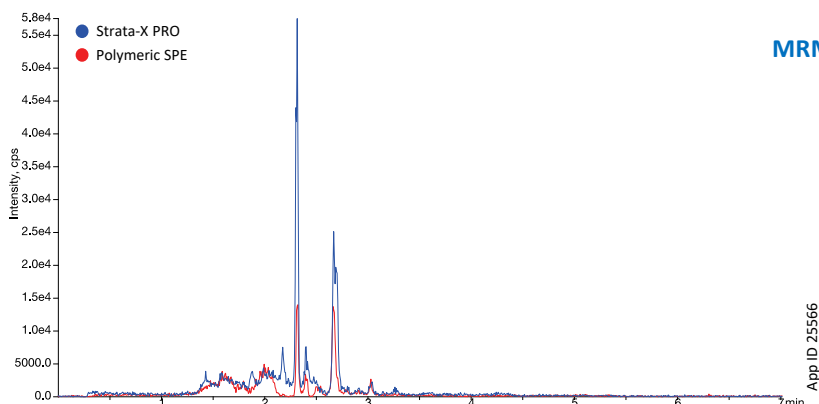
Overview

Matrix interferences, such as phospholipids, can cause challenges when measuring serum levels of 25-Hydroxyvitamin D₃ by LC-MS/MS. In this application note, we describe a method optimization approach using a Strata-X PRO solid phase extraction (SPE) plate (Part No.: [8B-S536-TAK](#)) and a Kinetex 2.6 μm XB-C18 LC column. By removing the phospholipids from serum samples, there is a reduction in ion suppression yielding higher sensitivity with lower limits of detection, when compared to polymeric SPE.

Sample Preparation

Step	Polymeric SPE	Strata-X PRO
Condition:	1 wash 600 μL Methanol	Not Required
Equilibrate:	1 wash 600 μL Water	Not Required
Load:	600 μL serum / 0.1 % Formic Acid in Water (1:1, v/v)	600 μL serum / 0.1 % Formic Acid in Water (1:1, v/v)
Wash:	1 wash 600 μL 30 % Methanol in Water	1 wash 600 μL 30 % Methanol in Water
Elute:	1 wash 600 μL 0.1 % Formic Acid in Acetonitrile / Methanol (90:10, v/v)	1 wash 600 μL 0.1 % Formic Acid in Acetonitrile / Methanol (90:10, v/v)

Figure 1. Comparison of Sensitivity Between Polymeric SPE and Strata-X PRO for 25-Hydroxyvitamin D₃



LC Conditions

Column: Kinetex™ 2.6 μm XB-C18

Dimensions: 50 x 2.1 mm

Part No.: [00B-4496-AN](#)

Guard: SecurityGuard™ ULTRA

Guard Part No.: [AJ0-8782](#)

Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Methanol

Gradient:	Time (min)	%B
	0	60
	1	100
	4	100
	4.01	60
	7.01	60

Flow Rate: 0.45 mL/min

Injection Volume: 20 μL

Temperature: 25 °C

Instrument: Agilent®

Detection: MS/MS

Detector: SCIEX® 4500 Triple Quad™ (APCI)

MS/MS Conditions

Ion Spray Voltage: 5500 V

Polarity: Positive

Scan Type: MRM

CUR: 10

GS1: 25

NC: 3

EP: 10

CXP: 10

Temperature: 400 °C

MRM Transitions

Analyte	Q1 (m/z)	Q3 (m/z)	CE
25-Hydroxyvitamin D ₃	383.4	257.2	23.0
25-Hydroxyvitamin D ₃	383.4	159.1	36.0



Need a different column size or sample preparation format?

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