

Cadenza CD-C18 HT

50 x 2 mm

Application

## High Throughput Reliable Quantitation of 25-hydroxyvitamin D in Serum by Offline Sample Preparation and a LC-MS/MS Instrument

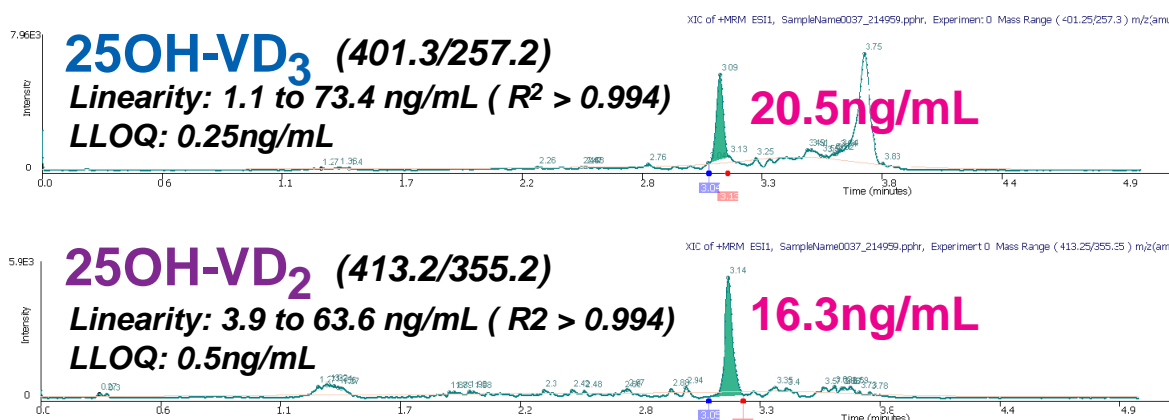
オフライン前処理による血清中の25-OHビタミンDのLC-MS/MS分析

### Sample Extraction

Sample preparation was carried out with the Orochem (Naperville, IL) PURITY Phospholipid Depletion Kit 96-well plate. The eluting step was performed with an Orochem Ezpress positive pressure manifold.

The extraction recovery rate on samples are about 60% for 25(OH) VD3 and 65% for 25(OH) VD2. Overall the extraction efficiency is about 50% for serum samples.

Step	Procedure
Load 1	300 µL of Vitamin D commercial precipitation reagent
Load 2	100 µL of serum sample, wait for 5 minutes,
Elution	apply a few pressure pulse until all solution passes through



Chromatograms of 25-Hydroxyvitamin D<sub>3</sub> and D<sub>2</sub> for a Recipe level I serum control

### LC Conditions

**Cadenza CD-C18 HT, 50 x 2 mm**

HPLC (Shimadzu UFLC)

A: 0.1% formic acid, 5mM AcONH<sub>4</sub> in H<sub>2</sub>O

B: 0.1% formic acid, 5mM AcONH<sub>4</sub> in MeOH

10%B (0-0.1min), 10-70%B (0.1-0.5min)

70-100%B(0.5-2.8min), 100%B(2.8-3.1min)

100-10%B(3.1-3.2min), 10%B(3.2-5min)

0.6mL/min, 32°C, 10 µL injection

### Mass Spectrometry

**IONICS 3Q 220 triple quadrupole MS**

MS Conditions

ESI Voltage (V)	5050
HSID Temp (°C)	175
Nebulizer Gas Setting	450
Drying Gas Setting	120
Source Temp (°C)	350

### Optimized MRM Parameters

Compound Name	Precursor (m/z)	Fragment (m/z)	CCL 2	CE
25(OH) VD <sub>3</sub>	401.3	257.2	-51	23
	401.3	383.2	-60	13
25(OH) VD <sub>2</sub>	413.3	355.2	-55	16
	413.3	395.2	-60	13

Ref) MSACL2014

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