

# REVOLUTION

## in Vitamin D2-D3 Analysis



## World's First Walk Away UHPLC Vitamin D2-D3 Analyzer

Quantitative & Precise & Accurate Results in 3 Mins

## About Zivak



### UNIQUE SOLUTION FROM ONE COMPANY

Zivak Technologies is an international special company providing ready to use LC-MS/MS and HPLC analysis kits in the clinical diagnostic field. The Research&Development company also supplies its own fully automated sample preparation and injection systems which enables laboratories around the globe to make efficient use of their chromatography instruments as well as their employees in a fast, accurate and cost efficient way.

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## Which Diseases Are Associated With Vitamin D Deficiency?

- Osteoporosis, Osteopenia, Osteoarthritis
- 17 Varieties Of Cancer (Including Breast, Prostate And Colon)
- Heart Disease
- High Blood Pressure
- Obesity
- Metabolic Syndrome And Diabetes
- Autoimmune Diseases
- Gout Disease
- Parkinson's Disease
- Depression And Seasonal Affective Disorder
- Alzheimer's Disease
- Periodontal Diseases



## Which Symptoms Are Associated With Vitamin D Toxicity?

Hypervitaminosis D is a state of Vitamin D toxicity. Symptoms of vitamin D poisoning Include:

- Dehydration
- Vomiting
- Decreased Appetite
- Irritability
- Constipation
- Fatigue
- Muscle Weakness

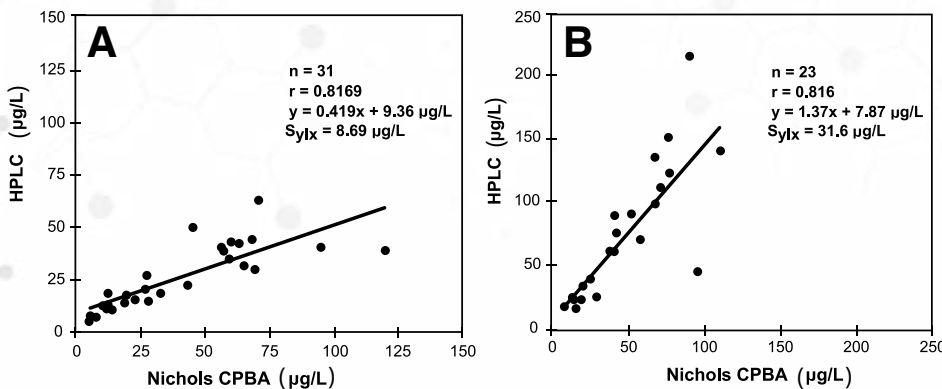


## Why We Analyze 25-OH Vitamin D2 and D3 Separately?

Circulating 25-Hydroxyvitamin D (25-OH-D) is widely recognized as the best indicator of vitamin D status. In blood circulation the two major vitamin d metabolites are 25-Hydroxyvitamin D3 (25-OH-D3) and 25-Hydroxyvitamin D2 (25-OH-D2). 25-OH-D3 is mainly derived from vitamin D3 produced by sunlight in the skin, while 25-OH-D2 is derived from plants in the diet. There are currently two main types of methods used routinely for measuring 25-OH-D: methods based on chromatographic separation followed by non-immunological direct detection and competitive immunoassays.

Most immunoassays depend on an antibody that can detect both 25-OH-D2 and 25-OH-D3 together, but the proportion of 25-OH-D2 detection is variable. Two non-immunological methods for direct detection are currently available: high pressure liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (lc-ms/ms). Chromatographic methods are able to measure 25-OH-D2 and 25-OH-D3 independently with excellent accuracy and sensitivity.

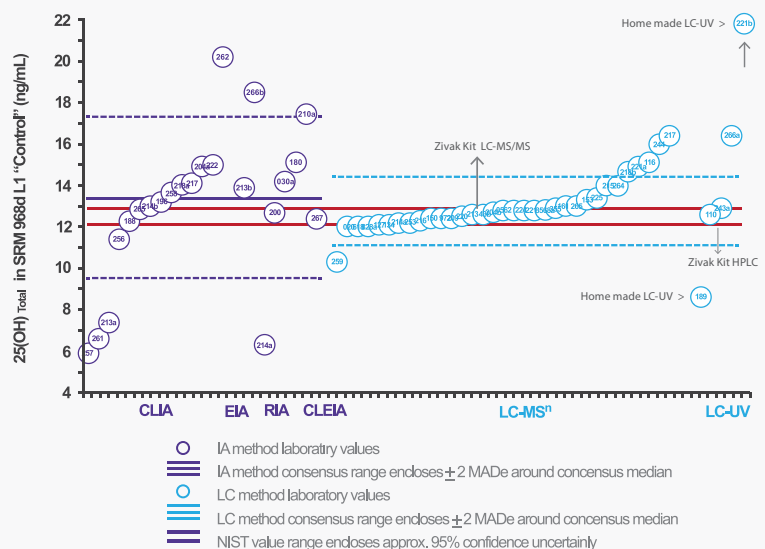
On the other hand, there is many scientific studies and papers showing inaccuracy measuring total vitamin D with commercial immunoassays without separating and measuring vitamin D2 and vitamin D3 metabolites individually. It is recommended that any 25-OH-D assay in clinical laboratories should measure both 25-OH-D2 and 25-OH-D3 separately in order to report a total 25-OH-D value. The evaluated immunoassays measure total 25-OH-D (both 25-OH-D3 and 25-OH-D2), but the cross-reaction with 25-OH-D2 and 25-OH-D3 differs from 52 to 100% in different methods.



The scientific papers showing that variability among current commercial immunoassays measuring Total Vitamin D bring into question the benefit these assays for patients.

Bland–Altman difference plot for the proposed HPLC method vs the Nichols Advantage CPBA. Comparisons of the assays with patient samples containing predominantly 25(OH)D3 (A) or predominantly 25(OH)D2 (B).  
Clinical Chemistry 52:6 1120–1126 (2006)

Participant results for 25(OH)D Total in SRM 968d Level1 (Control) as determined by immunoassay (CLIA, EIA, RIA and CLEIA) and LC (LC-MSn and LC-UV) methods.



- IA method laboratory values
- ▬ IA method consensus range encloses ±2 MADE around consensus median
- LC method laboratory values
- ▬ LC method consensus range encloses ±2 MADE around consensus median
- ▬ NIST value range encloses approx. 95% confidence uncertainty

## What VD-200 Offering Vitamin D2 and D3 Analysis?

### WALK AWAY SYSTEM

HPLC and LC-MS/MS methods are accepted as Gold Method for separate analysis of 25-OH metabolites of Vitamin D. Usage of these chromatographic methods are being increased significantly on clinical laboratories recent years. The major disadvantage is the time-consuming and laborious extraction procedure that limits the number of samples that can be processed per day.

Many of these laboratories cannot use their chromatography systems effectively due to these systems required qualified staff, complex sample preparation steps, high kit prices/ operation costs and no availability of walk away systems.

VD-200 enables to analyze Vitamin D2/D3 samples fully automated – without any human intervention- from primer sample tube. With VD-200, the low throughput in chromatography analysis will not be a problem for the laboratories any more.

### COST EFFECTIVE

Due to large number of Vitamin D2/D3 samples, many laboratories cannot run all other LC-MS/MS specific analysis on their LC-MS/MS systems effectively.

VD-200 is especially designed the for the laboratories to provide them a cost- effective solution with a fully automated system and make customer's expensive LC-MS/MS systems free for specific tests which have to be run on LC-MS/MS system.



The evaluated immunoassays measure total 25-OH-D (both 25-OH-D2 and 25-OH-D3). But the cross-reaction with 25-OH-D2 and 25-OH-D3 differs from 52 to 100% in different methods. Chromatographic gold methods are which able to measure 25-OH-D2 and 25-OH-D3 independetly with excellent accuracy and sensitivity.

#### Literature:

1. Risteli J, Winter WE, Kleerekoper M, Risteli L. Bone and mineral metabolism. In: Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. Editors: Burtis CA, Ashwood ER, Bruns DE, 5th Edition, Elsevier, St. Louis, MO, USA 2012: pp. 1733- 801.
2. Lai JK, Lucas RM, Clements MS, Harrison SL, Banks E. Assessing vitamin d status: pitfalls for the unwary. Mol nutr food res 2010;54(8):1062-71. Review.
3. Vogeser M. Quantification of circulating 25-hydroxyvitamin D by liquid chromatography-tandem mass spectrometry. J steroid biochem mol biol 2010;121(3-5):565-73.
4. De La Hunty A, Wallace AM, Gibson S, Viljakainen H, Lamberg-Allardt C, Ashwell M. Uk food standards agency workshop consensus report: the choice of method for measuring 25-hydroxyvitamin D to estimate vitamin d status for the uk national diet and nutrition survey. Br J Nutr 2010;104(4):612-9.
5. Binkley N, Krueger DC, Morgan S, Wiebe D. Current status of clinical 25-hydroxyvitamin D measurement: an assessment of between-laboratory.

## What is the Revolution?

### VD-200 High Sensitivity with Special D-Detector.

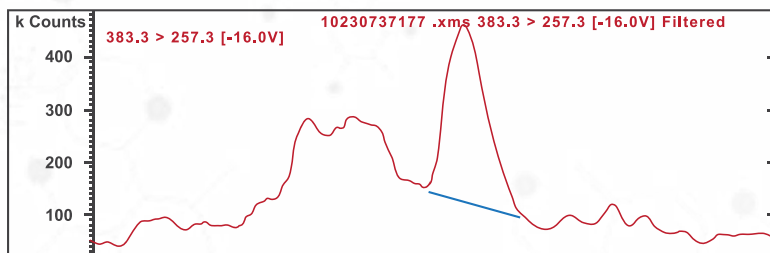
Zivak Technologies offers higher sensitivity than entry level LC- MS/MS by new design Special D detector based on single wavelength UV. The limit of detection is 0.3 ng/mL quantitation for 25-OH vitamin D2 and D3 while the limit of quantitation is 0.9 ng/mL . The quantitation limit is lower than most of entry level LC-MS/MS systems which has quantitation limits variable between 1.5 – 5 ng/mL.

#### LC-MS/MS Vit D3

C: 5,3 ng/mL

Run Time: 1,8 min

Calculated LOQ: 3,5 ng/mL

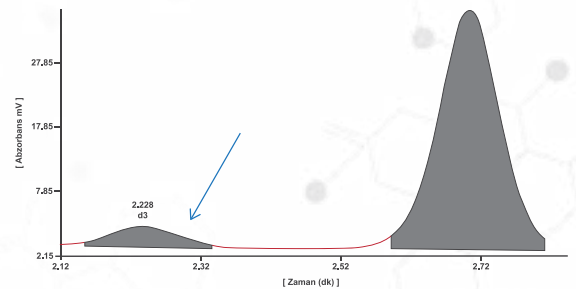


#### Zivak Vit D3 VD-200 UHPLC Special D Detector

C: 5,3 ng/mL

Run Time: 3 min.

Calculated LOQ: 0,9 ng/mL



VD-200 is especially designed the for the laboratories to provide them a cost-effective solution with a fully automated system and make customer's expensive LC-MS/MS systems free for spesific tests which have to be run on LC-MS/MS system.

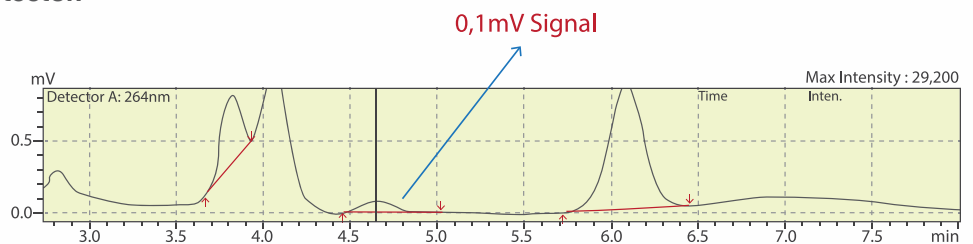


Zivak Special D detector has minimum 10 times better sensitivity than any other commercially available variable wavelength UHPLC-UV detector.

#### Commercial HPLC-UV Detector

C: 10,1 ng/mL

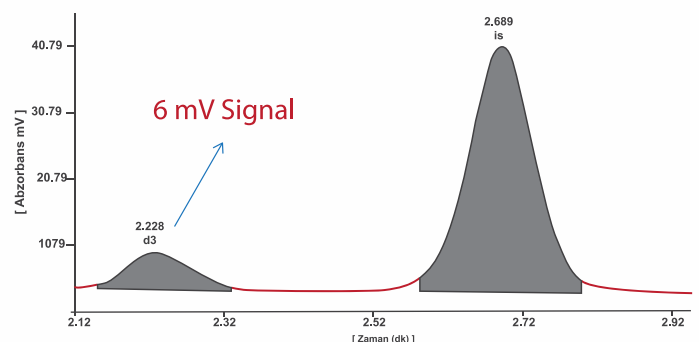
Run Time: 8 min



#### Zivak Vit D3 VD-200 UHPL Special Detector

C: 10,1 ng/mL

Run Time: 3 min



## Direct Bidirectional LIS Data Transfer From Automated Barcode Reading To Result

The VD-200 was especially designed for routine Vitamin D2/D3 testing clinical labs. VD-200 enables to analyze Vitamin D2/D3 samples fully automated – without any human intervention- from primer sample tube after the technician place the samples on the sample tray.

The Zivak VD-200 system automatically generates sample sequence by reading barcode from primary serum tube which barcoded by Hospital LIS system. **PLACE YOUR SAMPLES AS YOU GET** without align the barcodes.

Due to rotational barcode reading which followed by automated sample preparation, the system is independent from placing order or position of barcode of the samples in sample tray. Also this system fulfills the requirements for the positive sample identification of CE-IVD regulations.



Status	Active	Sample Name
		32991935144
		10270347956
		10260890845
		10270347563
		10270347569

Zivak VD-200 Walk-away UHPLC Vitamin D2-D3 Analyzer is a Plug-and-Play device and can be serviced by HPLC user.



The sequence list directly generated from the barcodes of the samples. There is no possible sample mismatch from the transfer of the samples from primary serum tube to standart chromatography vials or well plates. Therefore, there is no possibility to mismatch of the sample identity and results which directly transferred to the LIS system of the laboratory by the VD-200 software.

## Automated Sampling & Preparation From Serum Tube With Liquid Level Sensing

All sample preparation steps for 25-OH Vitamin D2-D3 analysis made by the unique modules of VD-200 Walk Away UHPLC Analyzer. The volume of serum samples is not an issue for VD-200 system since Liquid Level Sensing capability from closed lid serum tube. Zivak VD-200 systems gives quantitative, precise and accurate Vitamin D2 and D3 results in 3 minutes. This TEST TIME includes all steps from primary serum tube to results. The processes listed below can be done by robotic arms with these unique modules;

- ⊗ Barcode reading from serum tube.
- ⊗ Sample transfer from serum tube.
- ⊗ Reagent adding.
- ⊗ Vortex mixing.
- ⊗ Contrifuge.
- ⊗ Injection and chromatographic run.



System is not required any human intervention during all steps. By the way, 240 accurate results will be on the desk in 12 hours fully automated.

## 240 Test Capacity / 12 Hours

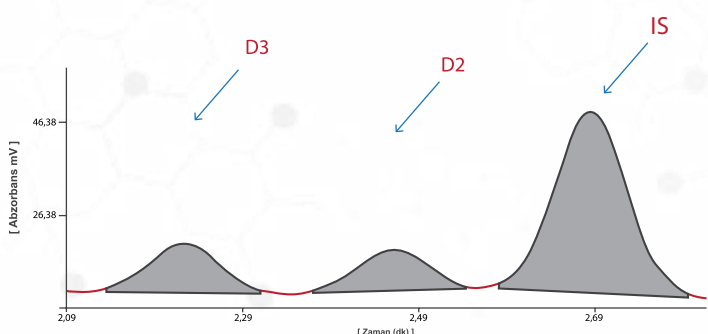
Standart HPLC-UV methods for Vitamin D2-D3 analysis are generally takes 6-8 minutes. This 6-8 minutes run time which does not include sample transfer from serum tube, reagent adding, protein precipitation, vortex mixing and centrifuge steps. Using HPLC-UV methods are difficult for medical laboratories and hospitals which has over 50 samples per day. For this reason, automated and high-throughput chromatography methods is highly appreciated.

Zivak VD-200 systems gives quantitative, precise and accurate Vitamin D2 and D3 results in 3 minutes. This **TEST TIME** includes all sample preparation steps from primary serum tube to results such as barcode reading, sample transfer from serum tube, reagent adding, vortex mixing, centrifuge and injection from the upper phase and analysis time. **With VD-200 walk away analyzer 240 accurate results will be on the desk full automated.**

**This state-of-art design gives robust, reliable and low carry-over results during longer column life.**

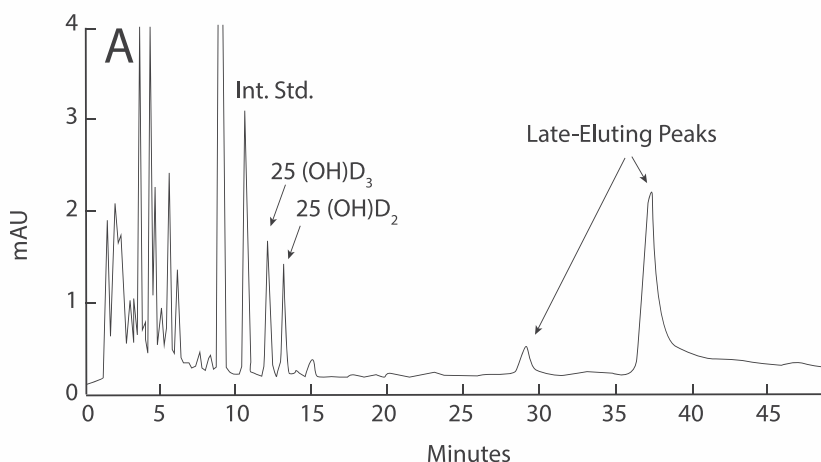
### Lower Calibration Chromatogram from Zivak VD-200 UHPLC Vitamin D2-D3 Analyzer with Special D-detector

Excellent D2-D3 Separation in 3 minutes.



Analysis Date	18 April 2016 - 14:58:15		
Barcode	22391937964		
Compound Name	Retention Time(min)	Area (ug/L)	Result
25-OH Vitamin D3	2,330	5,110	21,102
25-OH Vitamin D2	2,610	2,170	23,065
IS	2,818	9,856	---
<b>Total Vitamin D</b>			<b>44,167</b>

With the excellent resolution around 120 bar pressure and online SPE module, the VD-200 system can eliminate late peaks in 3 minutes test time. For the non-automated standart HPLC-UV systems, the late peaks must be eliminated with expensive and laborious SPE procedures during sample preparation.



With the affordable and short-time payback investment on VD-200 Walkaway UHPLC Vitamin D2-D3 Analyzer, the expensive LC-MS/MS systems can be used for special analyses which must be run on LC-MS/MS systems.



## Fully Validated System With Ready to Use Vitamin D2 and D3 Analysis

This system fully validated with Zivak Vitamin D2/D3 UHPLC Analysis Kit which includes all necessary reagents, calibrator and controls. Due to total validation of the VD-200 system with the analysis kit, all validation parameters including variations from automation and analysis.

### VALIDATION TABLE

25-OH Vitamin D3			25-OH Vitamin D3		
LOD	(ng/mL)	0.3	LOD	(ng/mL)	0.3
LOQ	(ng/mL)	0.9	LOQ	(ng/mL)	0.9
Accuracy	(%)	98.2	Accuracy	(%)	98.5
Intra-Assay Precision	(%CV)	2.1	Intra-Assay Precision	(%CV)	2.5
Inter-Assay Precision	(%CV)	3.2	Inter-Assay Precision	(%CV)	3.6
Linearity	(R <sup>2</sup> )	0.9995	Linearity	(R <sup>2</sup> )	0.9990



With the excellent resolution around 120 bar pressure and online SPE module, the VD-200 system can eliminate late peaks in 3 minutes test time.

### Lower Kit, Maintenance And Capital Costs

The aim of Zivak Technologies is to give opportunity laboratories around the globe to make efficient use of their chromatography instruments as well as their employees in a fast, accurate and cost efficient way.

The immunoassay systems seems cheaper way of the analysis of vitamin D status. There is many scientific studies and papers showing inaccuracy measuring total vitamin d with commercial immunoassays without separating and measuring vitamin D2 and vitamin D3 metabolites individually. It is recommended that any 25-OH-D assay in clinical laboratories should measure both 25-OH-D2 and 25-OH-D3 separately in order to report a total 25-OH-D value. Zivak VD-200 walk-away UHPLC vitamin D2-D3 analyzer gives the chance customers to analyze 25-OH-D2 and 25-OH-D3 separately with the cost which around immunoassay systems.

Zivak VD-200 walk-away UHPLC vitamin D2-D3 analyzer has better sensitivity if compared entry-middle level of LC-MS/MS systems. But the maintenance cost of VD-200 system is significantly lower than any LC-MS/MS system.

As known, lc-ms/ms systems needs many environmental supplies e.g. Nitrogen generator, argon gas, turbomolecular pumps, rough vacuum pumps and ESI/APCI needles. These parts are has limited lifetime and has to be serviced by the experts.

Most of medical laboratories their LC-MS/MS systems occupied with vitamin D2-D3 analysis since their high number vitamin D2-D3 samples.



## KIT

Zivak ready to use 25-OH Vitamin D2-D3 UHPLC Analysis kit is used with VD-200 Walk away UHPLC Analyzer.

25-hydroxy metabolites of Vitamin D2 and D3 are extracted from human serum samples using an organic extraction solution after deproteinisation step. Separation was made with an isocratic elution on Zivak 25-OH Vitamin D HPLC column and measured by special D-detector which specially designed for 25-OH Vitamin D2-D3 UHPLC Analysis.

AutoMass Software automatically checks the results with low and high levels of the controls for the intra-day and inter-day precision.

## VALIDATION TABLE

ZV-4027-0200-10		25-Hydroxyvitamin D2-D3 Serum VD-200 UHPLC Analysis Kit	
Materials Supplied	ZV-4027-05S1-10	1 x 2 mL	Serum Calibrator Level 1, lyophilised
	ZV-4027-05K1-10	1 x 2 mL	Serum Control Level 1, lyophilised
	ZV-4027-05K2-10	1 x 2 mL	Serum Control Level 2, lyophilised
	ZV-4027-02R1-10	3 x 37 mL	Reagent 1, Contains aqueous buffer
	ZV-4027-02R2-10	2 x 40 mL	Reagent 2, Contains internal standard
	ZV-4027-02R3-10	1 x 12 mL	Reagent 3, Contains dilution buffer
	ZV-4027-02MP-10	1 x 0,75 L	Mobile Phase, Contains organic solvent
	ZV-4027-02MT-10	1 x 0,75 L	SPE buffer, Contains organic solvent
	ZV-4027-02WB-10	1 x 0,5 L	Washing Solution, Contains organic solvent
	ZV-4027-02KK-10_REV00	1 x 1 pc	User Guide
Materials Not Supplied with	ZV-4027-02S2-10	1 X 2 ML	Serum Calibrator Level 2, lyophilised (Optional)
	ZV-4027-02S3-10	1 X 2 ML	Serum Calibrator Level 3, lyophilised (Optional)
	ZV-4027-02TM-10	1 X 2 ML	Test Mix Solution (Optional)
	ZS-9003-0100-00	2X100 PCS	1.8 ml glass autosampler vial
	ZS-9004-0100-00	2X100 PCS	1.8 ml glass autosampler vial caps pre-slit
	ZV-4027-02C1-10	25-Hydroxyvitamin D2-D3 Serum VD-200 UHPLC Analytical Column	
	ZV-4027-02C2-10	25-Hydroxyvitamin D2-D3 Serum VD-200 UHPLC Trap Column Housing	
	ZV-4027-CSPE-10	25-Hydroxyvitamin D2-D3 Serum VD-200 UHPLC Trap Column Cartridge	

## TECHNICAL SPECIFICATIONS

### VD-200 Specifications

#### GENERAL

- ⊗ Power Requirements:
  - Operating Voltage: 220-230V AC
  - Operating Frequency: 50 – 60 Hz
  - Operating Current: 3A Max
- ⊗ Dimensions(W x D x H): 1020mm x 825mm x 676mm
- ⊗ Working Temperature: 10 – 40 °C
- ⊗ Storage Temperature: -20 – 70 °C
- ⊗ Humidity: %30 – 80 RH
- ⊗ Sound Level: < 60dB @1m

#### SAMPLING & INJECTION

- ⊗ Sample Capacity: 108 (13 mm Diameter Serum Tube - 5 mL volume)
- ⊗ Loop Volume: 100 uL
- ⊗ Injection cycle time: 3 minutes
- ⊗ Syringe volume: 250 uL for injection, 10 mL for reagents
- ⊗ Wash Solvent: External wash solvent bottle.
- ⊗ Wetted parts in flow path: Metal tubing stainless steel number, PTFE, teflon, glass
- ⊗ Liquid Detection: cLLD (Capacitive Liquid Level Detection)
- ⊗ Switching Time of Valves: Less than 1 second
- ⊗ Column Oven: Constant Temperature (35°C)
- ⊗ Valves : 2 x 6-port Injection & Switching valves

#### PUMPS

- ⊗ 2 x UHPLC Pumps
- ⊗ Flow Rates: 0.001 – 5.0 mL/min
- ⊗ Pressure: 18000 psi Maximum
- ⊗ Pressure Accuracy: ± 2% of full scale pressure
- ⊗ Pressure Zero Offset: ± 2psi
- ⊗ Flow Accuracy: ± 2% for a flow rate of 0.20 mL/min and above, with 80:20 Water/IPA @ 1000psi
- ⊗ Flow Precision: 0.5% RSD
- ⊗ Working Temperature: 10 – 30 °C
- ⊗ Humidity: %20 – 90 RH

#### DETECTOR

- ⊗ Special D-detector with 0.9 ng/mL LOQ for Vitamin D3 & D2
- ⊗ Cell Length: 70 mm
- ⊗ Working Temperature: 20 – 30 °C
- ⊗ Fixed Wavelength: 264 nm

#### ANALYTICAL PERFORMANCE

- ⊗ Injection Mode: Full-Loop
- ⊗ Injection Reproducibility: Full loop injection < %0.3RSD
- ⊗ Carry Over: - Less than %0.05 while standard wash  
- Less than %0.01 while extra wash

#### PROGRAMMING

- ⊗ User Interface: Automass Chromatography Software V2 for VD-200
- ⊗ Injection Mode: Full-Loop
- ⊗ Injection volume: 100 uL
- ⊗ Wash: Needle wash
  - Wash inside and outside of the needle
  - Wash between injections
  - Wash between vials

#### COMMUNICATION

- ⊗ CIB to PC communication with CAT5 ethernet cable

**ZIVAK**<sup>®</sup>  
TECHNOLOGIES

FUTURE OF ANALYSIS

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