

25-OH VITAMIN D₃ AND 25-OH VITAMIN D₂ IN PLASMA BY UV / VIS – FAST – CODE
Z19810

BIOCHEMISTRY

Quantification of 25-hydroxyvitamin D₃ (25-hydroxycholecalciferol) in serum is the best established indicator of vitamin D status. Vitamin D₃ (cholecalciferol) is absorbed in the diet and, given sufficient ultraviolet irradiation, nutritionally adequate amounts of vitamin D₃ are formed in the skin from its precursor, 7-dehydrocholesterol. In the liver, vitamin D₃ undergoes hydroxylation to 25-hydroxyvitamin D₃, which is further metabolized in the kidneys to form the active metabolites 1,25-hydroxyvitamin D₃ and subsequently 24,25-hydroxyvitamin D₃.

The prevalence of hypovitaminosis D has been recognized as substantial, even in regions with high sun exposure, contributing not only to osteoporosis but possibly to a loss of muscle strength in aging as well.

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CE

This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 003	Vitamin 25-OH-D3 and Vitamin 25-OH-D2 in plasma by UV - FAST	06/2024
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TECHNICAL FEATURES

Principle of the Method

25-OH Vitamin D₃ and **25-OH Vitamin D₂**, after deproteinization with a specific reagent, and after purification with Clean-up Columns are injected directly into isocratic HPLC system.

<u>Recovery :</u>	100%
<u>Sensitivity :</u>	1.5 ng/ml
<u>Linearity :</u>	2 - 500 ng/ml
<u>Normal Range :</u>	20 - 120 ng/ml

<u>CV%:</u>	25-OH-Vitamin D₃ 2,6% intra serie 3,4% inter serie
	25-OH-Vitamin D₂ 10,4%intra serie 10,7%inter serie

<u>Components of the kit :</u>	All the components are ready-to-use and stable 3 years at 2-8 °C except Calibrator (see data sheets).
Reagent A – Deproteinization Solution with Internal Standard, 1 x 90 ml	
Reagent B – Conditioning Solution N° 1, 1 x 130 ml	
Reagent C – Conditioning Solution N° 2, 1 x 120 ml	
Reagent D – Wash Solution, 1 x 70 ml	
Reagent E – Eluting Solution, 1 x 40 ml	
Reagent F – Test Solution, 1 x 1 ml	
Serum Calibrator lyophil. , 1 x 5 ml	<u>Code Z19116 (packed separately – see data sheets)</u>
Clean-up Columns , 100 pcs	
Reagent M – Mobile Phase, 3 x 500 ml	

<u>Minimum Instrumental equipment required:</u>	Isocratic HPLC System with loop of 25-50µl Spectrophotometric Detector UV/VIS $\lambda=265$ nm Chromatograms Recorder
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<u>Optional Equipment:</u>	Autosampler Sample Automatic Preparator Operational Computer
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<u>Blood Collection Procedure:</u>	Collect 5 ml of blood in a suitable test tube with EDTA as anticoagulant.
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<u>Pre-analytical Step :</u>	Plasma samples should be stored at –20 ° C for no longer than 3 months.
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WARNING: USE ONLY GLASS TUBES FOR EACH OPERATIVE STEP!
DON'T use absolutely plastic tubes!

PREANALYTICAL PROCEDURE

Pipette in a **glass tube** :

- 400 µl of Mobile Phase
- 100 µl of **Reagent F** - Test Solution

Vortex for 10 sec.

INJECTION :

- Inject 50 µl of this solution in the chromatographic system.

Verify that the Vitamin 25-OH- D₃ has retention time similar between 4,0 and 6,0 min. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

Important : Don't use this solution to calibrate!

ANALYTICAL PROCEDURE

STEP 1 : Calibrator, Controls and Samples Preparation:

Pipette in a **glass tube**:

	Calibrator	Sample	Controls
Calibrator	0,5 ml		
Sample		0,5 ml	
Control			0,5 ml
Reagent A – Deproteinization Sol.	0,75 ml	0,75 ml	0,75 ml

Warning: deproteinize directly on Vortex for at least 10 sec.

STEP 2 : Centrifuge at 10.000 rpm for 15 min.

STEP 3 : Place the supernatant (about 1,0 ml) in a clean **glass tube**.
Add 0,25 ml of H₂O HPLC grade.

Vortex for 10 sec.

STEP 4 : Clean-up Column Conditioning:

- 1,0 ml of **Reagent B** – Conditioning Sol. N° 1 and *discharge the percolated liquid*
- 1,0 ml of **Reagent C** – Conditioning Sol. N° 2 and *discharge the percolated liquid*

STEP 5 : Samples, Calibrator and Controls loading into Clean-up Columns:

- Dispense the supernatant (about 1,25 ml), previously prepared, into clean up columns.
(As Clean-up Columns is 1,0 ml, pipette 1 ml of supernatant each time).
- Discharge the percolated liquid, if needed a vacuum system could be used for percolation, at speed of 1 ml / min (drop by drop).

STEP 6 : Clean-up Column Wash:

- Pipette in Clean-up Columns 0,5 ml of **Reagent D – Wash Solution** and *discharge the percolated liquid*.

STEP 7 : Elution of 25-OH-D₃ :

- Pipette in Clean-up Columns 200 µl of **Reagent E – Eluting Sol.**
- Collect the eluate in a **glass tube**
- Add 25 µl of H₂O HPLC grade

Vortex for 10 sec.

N.B.: at this step, the sample is stable 7 days at 2-8 °C

INJECTION :

- Inject 25-50 µl of the eluate into the chromatographic system.

25-OH VITAMIN D₃ and 25-OH VITAMIN D₂ - FAST – Warnings

REAGENT F : TEST SOLUTION

25-OH VITAMIN D ₃	10.000 ng/ml
25-OH VITAMIN D ₂	10.000 ng/ml

SPECTROFOTOMETRIC DETECTOR PARAMETERS

λ	265 nm
GAIN	0,001 AUFS
INTEGRATION TIME	10 sec.

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL EC 4,6 x 50 mm, 2,7 μ m, the use of Javelin Prefilters (1 x 10 pcs.) cod. S90199511 is obligatory. Substitute the prefilter each 70 – 100 injections.

HPLC COLUMN CONDITIONING

Install a new analytical C₁₈ Reversed-Phase column (POROSHELL EC 4,6 x 50 mm, 2,7 μ m). Disconnect the detector and filter a solution of H₂O: Acetonitrile (20 : 80 v/v) set flow at 0,8 ml / min. **Don't recycle the washing solutions.** Filter the mobile phase with a vacuum system and a suitable filter of 0,22 μ m. Condition the column with the mobile phase at a flow of 0,8 ml / min. and discharge the first 30 ml. Condition further on the column for 30 min. also at recycling phase. Finally inject the Chemical Standard and verify the quality of the HPLC run.
It is NOT possible to make analysis at recycling phase. If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

At the end of the session of analysis to flush a solution of H₂O: acetonitrile (20: 80 v / v) for 30 min and discharge. The column is again ready for the next session of analysis. Store in a solution of H₂O : Acetonitrile (20 : 80 v/v).

INJECTION NEEDLE CLEANING

Wash with a solution of H₂O : Ethanol or Methanol or Acetonitrile (80 : 20 v/v).

HPLC PARAMETERS

LOOP	25-50 μ l
FLOW	0,8 ml/min.
PRESSURE	About 80 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z19116	Calibrator for 25-OH-Vitamine D ₃ and 25-OH-Vitamine D ₂	4 x 5 ml
Z19119	Control for 25-OH-Vitamine D ₃ and 25-OH-Vitamine D ₂ - Levels 1 and 2	2 x 5 x 5 ml
Z19811	Mobile Phase	4 x 500 ml
Z699975902	Poroshell EC (50 x 4,6mm –2,7 μ) Analytical Column	1 Pc
S90199511	Javelin Prefilters	1 x 10 Pcs
S51843550	Clear glass vials with reduced volume (globet form) from 1,5 ml to 15 μ l	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 μ l to 15 μ l and for amber vials	1 x 100 Pcs
Z1636/26	Pyrex tubes of 10 ml with SWL caps	1 x 40 Pcs

25-OH VITAMIN D₃ and 25-OH VITAMIN D₂
(Reference Chromatograms)

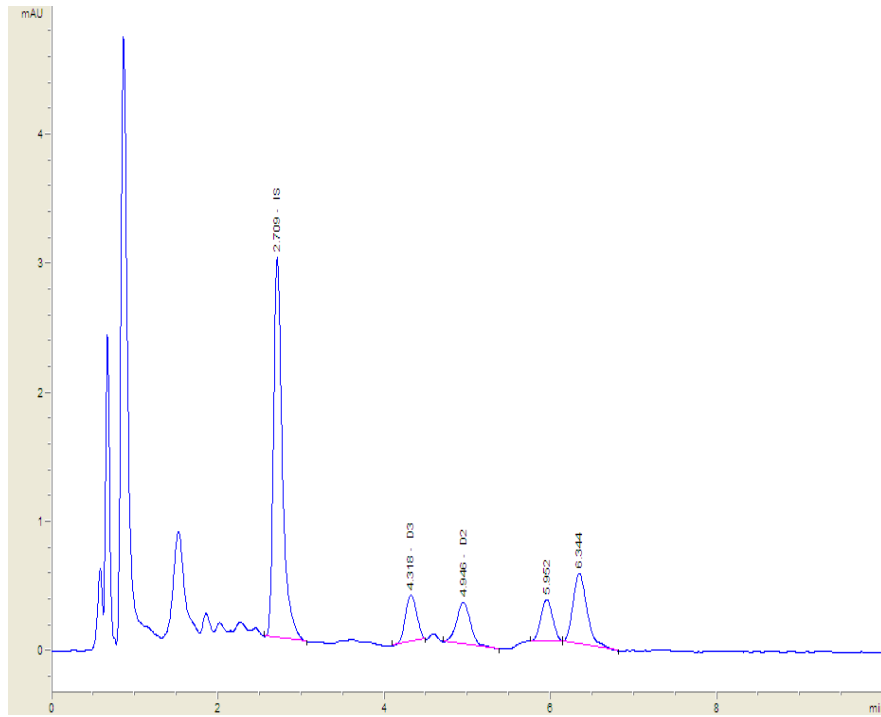


Fig. 1:	Plasma Calibrator
	R.T. 2.7 Internal Standard
	R.T. 4.3 25-OH D ₃ 26,0 ng/ml
	R.T. 4.9 25-OH D ₂ 29,6 ng/ml

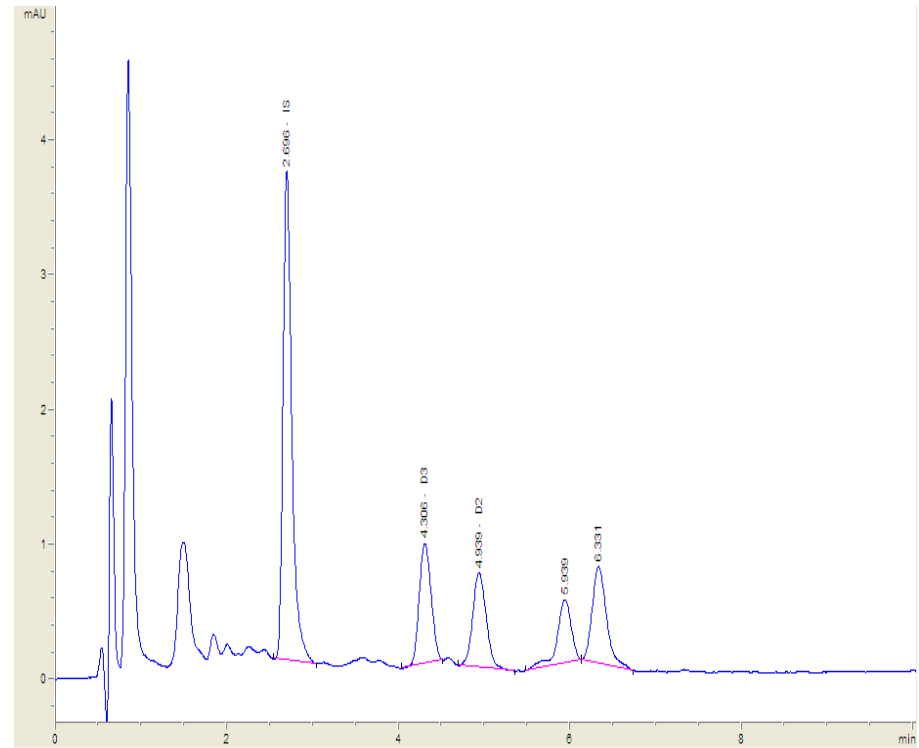


Fig. 2:	Plasma Control Level 2
	R.T. 2.7 Internal Standard
	R.T. 4.3 25-OH D ₃ 64,1 ng/ml
	R.T. 4.9 25-OH D ₂ 63,3 ng/ml