



25-OH VITAMIN D3 AND 25-OH VITAMIN D2 IN PLASMA BY LC/MS

REF LC19110

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.



INSTRUCTIONS FOR USE



For *in vitro* diagnostic use



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INTENDED USE

In vitro diagnostic medical device for the detection and monitoring of plasma Vitamin D2 and D3 levels as an aid in determination of the state of hypovitaminosis D. Manual method for professional use.

KIT CONTENT FOR 100 TESTS

CONTENT	DESCRIPTION	PACKAGING	WARNING
Reagent A	Deproteinization Solution	4 x 5 ml	Store at -25°C  -15°C after addition of the Reagent B
Reagent B	Internal Standards Solution	1 x 200 μl	Stable 6 months at -25°C  -15°C
Multiparametric Calibrator in plasma		7 x 1 x 1 ml	(packed separately – Code LC19016 - see data sheet)
Reagent M1	Mobile Phase M1	1 x 500 ml	Store at $+2^{\circ}\text{C}$  $+8^{\circ}\text{C}$
Reagent M2	Mobile Phase M2	1 x 500 ml	Store at $+2^{\circ}\text{C}$  $+8^{\circ}\text{C}$

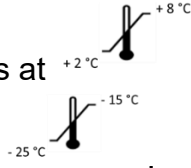
ACCESSORIES AND CONSUMABLES AVAILABLE SEPARATELY

CODE	DESCRIPTION	PACKAGING
LC19016	Calibrator for 25-OH-Vitamin D3 and 25-OH Vitamin D2 in plasma by LC/MS	7 x 2 x 1 ml
LC19019	Control for 25-OH-Vitamin D3 and 25-OH Vitamin D2 in plasma by LC/MS	3 x 5 x 1 ml
SK19110	Starter kit for 25-OH-Vitamin D3 and 25-OH Vitamin D2 in plasma by LC/MS	4 x 1 ml
LC19011	25-OH-Vitamin D3 and 25-OH Vitamin D2 in plasma by LC/MS – Mobile Phase M1	4 x 500 ml
LC19021	25-OH-Vitamin D3 and 25-OH Vitamin D2 in plasma by LC/MS – Mobile Phase M2	4 x 500 ml
LC19130	Deuterated Internal Standards Solution in plasma	1 x 200 µl
ZRE9309A52	RAPTOR BIPHENYL Analytical Column (50 x 2,1 mm. 2,7 µm)	1 Pc
Z51843550	Clear glass vials with reduced volume goblet form from 15 µl to 1,5 ml	1 x 100 Pcs
Z51820717	Caps for clear glass vials with reduced volume goblet form from 15 µl to 1,5 ml	1 x 100 Pcs
L-30-210	Eppendorf Test tubes by 1,5 ml with caps	1 x 1000 Pcs

INSTRUMENTS

DESCRIPTION	WARNING
LC-MS triple quadrupole instrument medium-high level (S/N almost 10.000/1 for 1 pg of injected Reserpine) Analysis in binary gradient – Work modality MRM, APCI positive Divert valve at 6 ways Binary Pump	Required
Operational Computer	Required
Autosampler	Required

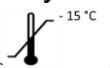
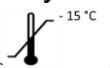
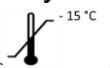
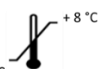
MATRIX - COLLECTION AND STORAGE PROCEDURE

MATRIX	PROCEDURE
Plasma	Dispense 3 ml of whole blood into a tube with EDTA as an anticoagulant. Centrifuge at 4000 rpm for 5 minutes. Separate plasma. Stable for 4 weeks at $+2^{\circ}\text{C}$ or 4 months at -25°C 

REFERENCE VALUES

Vitamines	Normal Range (ng/ml)
25-OH Vitamin D2	20 - 120
25-OH Vitamin D3	20 - 120

ANALYTICAL PROCEDURE

STEP	DESCRIPTION																				
1	Dispense 50 µl of Reagent B in each bottle containing 5 ml of Reagent A according to use. Stable 3 months at  . In Eppendorf tubes, dispense in sequence: <table><tr><td></td><td>Calibrator (µl)</td><td>Sample (µl)</td><td>Controls (µl)</td></tr><tr><td>Calibrator</td><td>100</td><td></td><td></td></tr><tr><td>Sample</td><td></td><td>100</td><td></td></tr><tr><td>Controls</td><td></td><td></td><td>100</td></tr><tr><td>Reagent A (taken directly from  and used cold)</td><td>200</td><td>200</td><td>200</td></tr></table> Close the vials with caps - Vortex for 30 sec.		Calibrator (µl)	Sample (µl)	Controls (µl)	Calibrator	100			Sample		100		Controls			100	Reagent A (taken directly from  and used cold)	200	200	200
	Calibrator (µl)	Sample (µl)	Controls (µl)																		
Calibrator	100																				
Sample		100																			
Controls			100																		
Reagent A (taken directly from  and used cold)	200	200	200																		
2	Put tubes at  for 10 minutes.																				
3	Centrifuge at 15.000 rpm for 15 minutes.																				
4	Take the supernatant and transfer into vial with insert																				
INJECTION	Inject 20 µl of the solution into the LC system																				

GRADIENT

Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	Flow (µl/min)
0.2	60	40	400
0.3	25	75	400
2.5	20	80	400
2.6	0	100	400
3.5	0	100	400
3.51	60	40	400
4.5	60	40	400

PARAMETERS

Parameters set on APCI 4500

CURTAIN GAS (CUR)	35
COLLISION GAS (CAD)	8
ION SPRAY VOLTAGE (IS)	5.500 V
TEMPERATURE (TEM)	470°C
ION SOURCE GAS 1 (GS1)	50
ION SOURCE GAS 2 (GS2)	/

FRAGMENTATION OF ANALYTES AND DEUTERATED INTERNAL STANDARD (IS)

ANALYTES	RT (min)	FRAGMENTATION		DEUTERATED I.S. USED FOR CALIBRATION	RT (min)	FRAGMENTATION	
		PARENT ION (Q1)	FRAGMENT ION (Q3)			PARENT ION (Q1)	FRAGMENT ION (Q3)
25-OH Vitamin D2	2.5	395.0	209.0	25-OH Vitamin D2-d3	2.5	398.0	230.0
		395.0	269.0			398.0	212.0
25-OH Vitamin D3	2.3	383.3	257.2-211.2	25-OH Vitamin D3-d6	2.3	389.3	263.2

TECHNICAL FEATURES - PART I

ABBREVIATION	MEANING
C LLOQ	Concentration at low limit of quantification
C1	Low concentration
C2	Medium concentration
Max C	The highest concentration

ANALYTES	CONCENTRATIONS USED TO CALCULATE REPRODUCIBILITY AND ACCURACY (ng/ml)			
	C LLOQ	C1	C2	MAX C
25-OH Vitamin D2	2	7,5	30	75
25-OH Vitamin D3	2	7,5	30	75

ANALYTES	ACCURACY INTRA SERIE (RELATIVE ERROR %)			
	CLLOQ (%)	C1 (%)	C2 (%)	MAX C (%)
25-OH Vitamin D2	9,84	4,50	4,29	5,15
25-OH Vitamin D3	13,14	6,37	4,03	3,67

ANALYTES	ACCURACY INTER SERIE (RELATIVE ERROR %)			
	CLLOQ (%)	C1 (%)	C2 (%)	MAX C (%)
25-OH Vitamin D2	10,94	6,04	5,33	5,17
25-OH Vitamin D3	17,74	7,61	6,46	6,21

TECHNICAL FEATURES - PART II

ANALYTES	REPRODUCIBILITY INTRA SERIE (COEFFICIENT OF VARIATION %)			
	CLLOQ (%)	C1 (%)	C2 (%)	MAX C (%)
25-OH Vitamin D2	5,33	4,78	5,65	1,63
25-OH Vitamin D3	2,99	1,73	3,13	3,29

ANALYTES	REPRODUCIBILITY INTER SERIE (COEFFICIENT OF VARIATION %)			
	CLLOQ (%)	C1 (%)	C2 (%)	MAX C (%)
25-OH Vitamin D2	10,79	6,13	7,22	5,72
25-OH Vitamin D3	8,89	6,61	4,52	6,44

TECHNICAL FEATURES - PART III

ANALYTES	LLOD (ng/ml)	LINEARITY ng/ml	COEFFICIENT OF CORRELATION R2 + STD DEV
25-OH Vitamin D2	0,6	2 – 1.000	0,9977 ± 0,0016
25-OH Vitamin D3	0,6	2 – 1.000	0,9982 ± 0,0005

ANALYTES	RECOVERY (%)	MATRIX EFFECT (%)	CARRY OVER (%)	LOTS VARIABILITY (%)
25-OH Vitamin D2	108,0	110,7	2,43	3,58
25-OH Vitamin D3	91,8	111,8	12,05	9,23

REFERENCE CHROMATOGRAMS

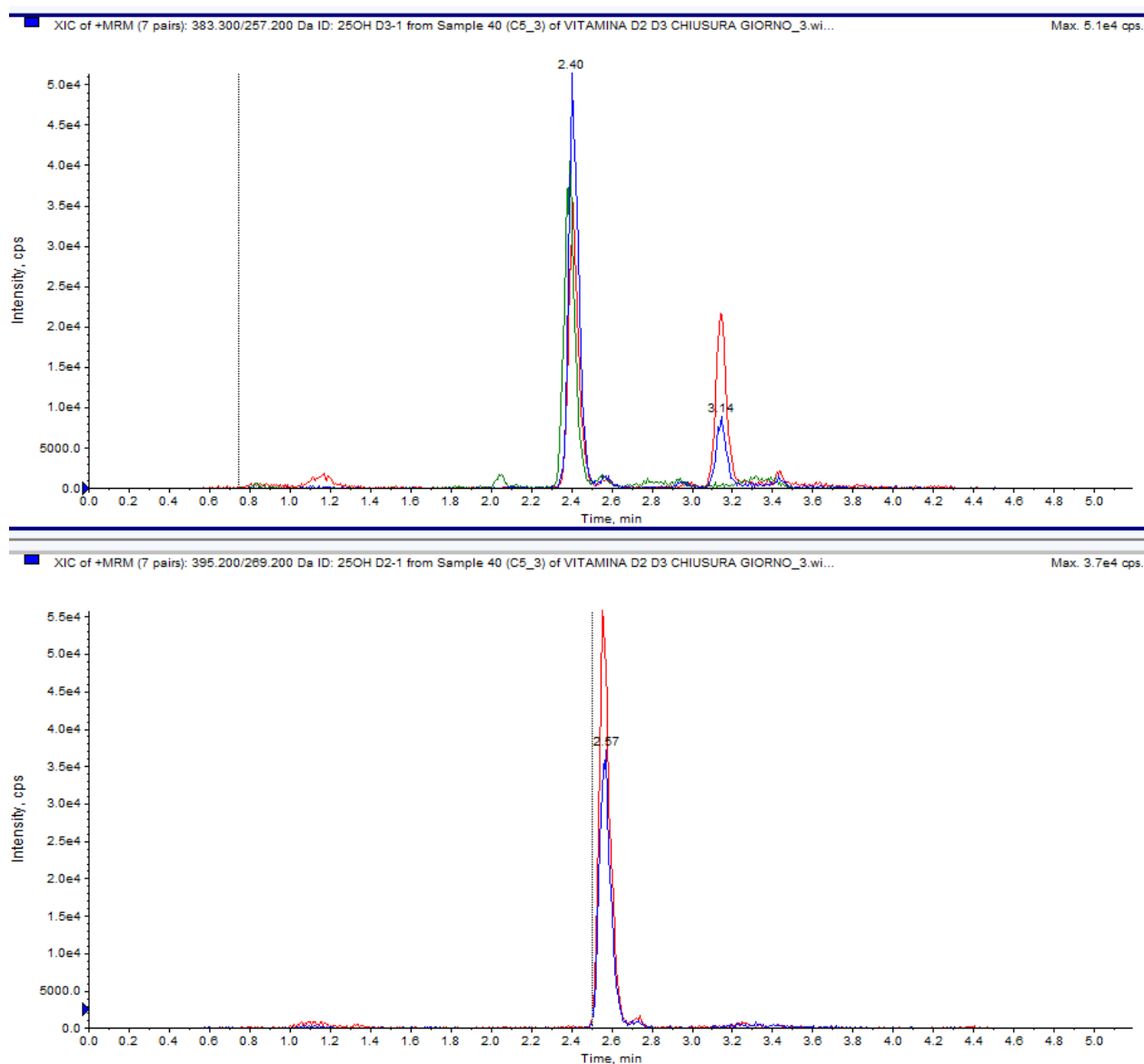


Fig.1 Calibrator level 5 for Vitamins 25 OH D3/D2. At the top there are traces related to vitamin 25 OH D3 and the corresponding internal standard, at the bottom the traces related to vitamin 25 OH D2.

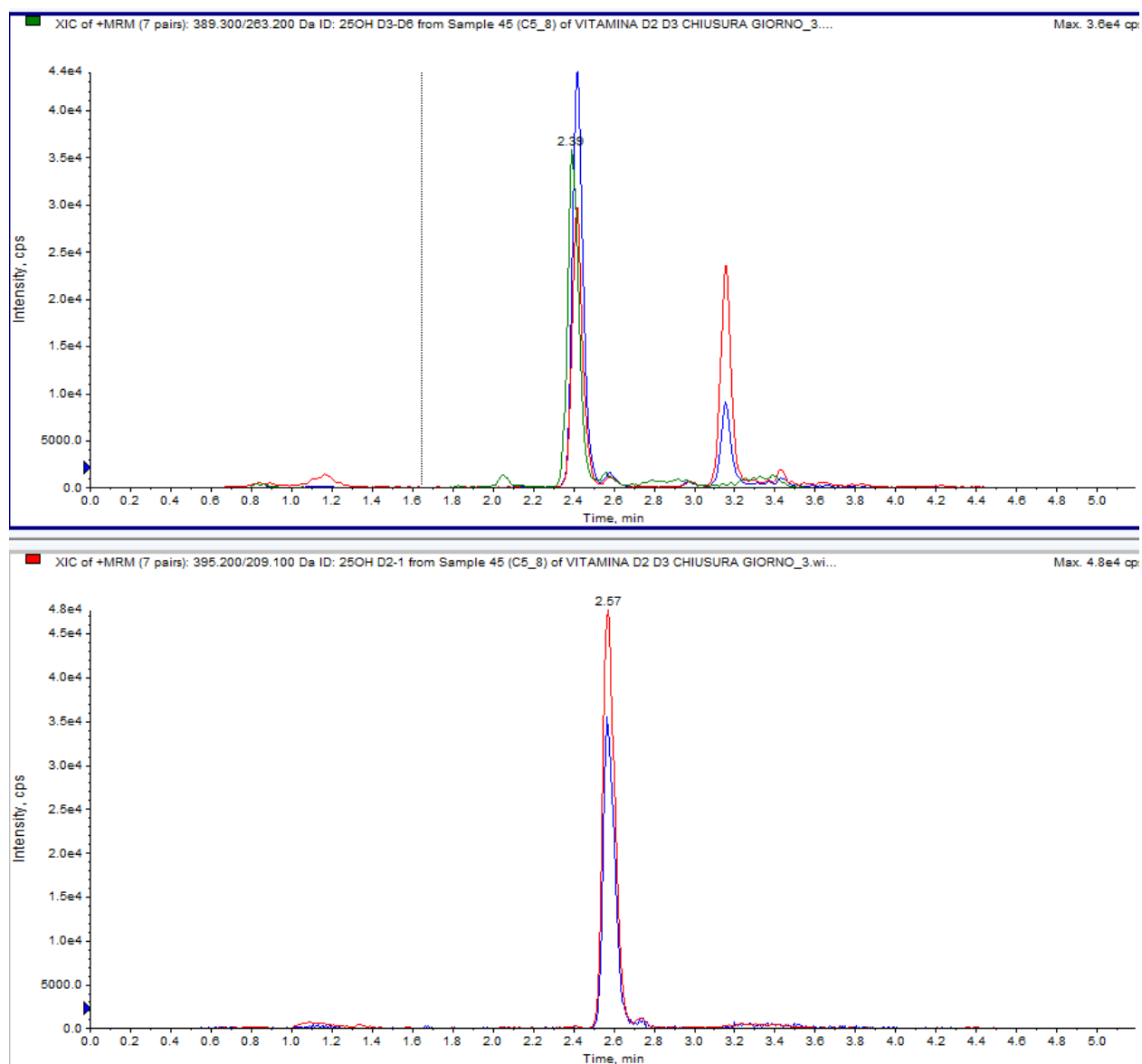


Fig. 2 Control level 2 for Vitamins 25 OH D3/D2. At the top there are traces related to vitamin 25 OH D3 and the corresponding internal standard, at the bottom the traces related to vitamin 25 OH D2.

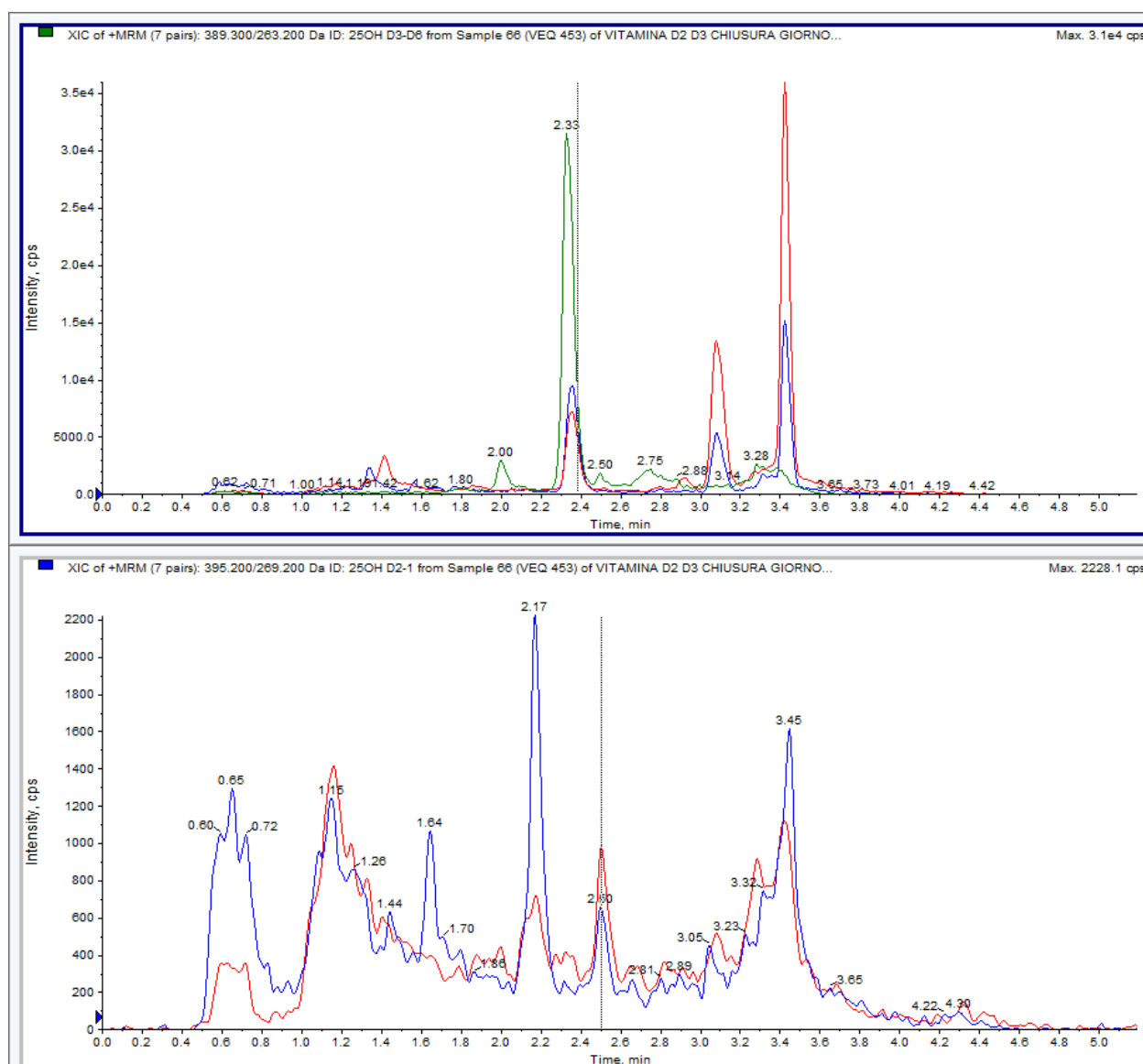


Fig. 3 Real sample treated for dosage of vitamins 25 OH D3 e D2. At the top there are traces related to vitamin 25 OH D3 and the corresponding internal standard, at the bottom the traces related to vitamin 25 OH D2.

GENERAL WARNINGS

1. STORAGE AND SHELF LIFE

All the components are ready-to-use. They are stable for 3 years at $+2^{\circ}\text{C}$  $+8^{\circ}\text{C}$ except from Reagent A (Deproteinization Solution) and B (Internal Standard Solution) that must be stored at -15°C  -25°C .

After opening the shelf life of our internal standards is 6 months if stored at -15°C  -25°C .

2. NEW COLUMN TREATMENT

Install the new analytical column (RAPTOR BIPHENYL (50 x 2,1 mm - 2,7 μm) thermostated at 60°C . Disconnect the detector and flux a solution of Acetonitrile and set flow at 0,3 ml/minute for 30 minutes. **Don't recycle the washing solutions.**

3. COLUMN CONDITIONING

Conditionate the new analytical column RAPTOR BIPHENYL (50 x 2,1 mm - 2,7 μm) with Mobile Phases M1:M2 (80:20) set flow at 400 μl / minute for 10 minutes. Finally inject the H_2O HPLC grade and verify the quality of the HPLC run.

It is not possible to make analysis at recycling phase. If room temperature is $> 20^{\circ}\text{C}$ store the

Mobile Phase at $+2^{\circ}\text{C}$  from an analytical session to an another.

4. COLUMN CLEANING

Disconnect the detector. Wash the column with a solution of Mobile Phase M2 at flow of 400 μl /minutes for 10 minutes. Store the column in a solution of Mobile Phase M2.

5. AUTOSAMPLER SYRINGE WASHING

Wash the syringe with Methanol before use.

ABBREVIATIONS

ABBREVIATIONS	MEANING
e.g. 7 x 2 x 1 ml	N° of levels x N° of vials x volume
e.g packaging of the starter kit 3 x 1 ml	N° of vials x volume Note: each vial contains one molecule (analyte or labelled IS)
25-OH Vitamin D2	25-Hydroxy Vitamin D2
25-OH Vitamin D3	25-Hydroxy Vitamin D3
IS	Internal Standard
pc/pcs	Piece/pieces
RT	Retention Time
STD DEV	Standard Deviation

BIBLIOGRAPHY

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5. Kornelia Galior *et al.* 10 years of 25-hydroxyvitamin-D testing by LC-MS/MS-trends in vitamin D deficiency and sufficiency Bone Reports Volume 8, June 2018, Pages 268-273
6. Jiri Adamec *et al.* Development and optimization of an LC-MS/MS-based method for simultaneous quantification of vitamin D2, vitamin D3, 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 J. Sep. Sci. 2011, 34, 11–20

REFERENCE STANDARDS

Quality Assurance System compliant with regulations:

UNI CEI EN ISO 13485:2016,

UNI EN ISO 9001:2015

Any modification made to the Medical Device unless authorized by Eureka S.r.l. - Lab Division makes this declaration invalid.

We declare that the devices comply with the following **Technical Standards**:

UNI CEI EN ISO 14971:2012

UNI CEI EN ISO 15223-1:2017

UNI EN ISO 18113-2:2012

We declare that the device is sold in a non-sterile package.

IVD SYMBOLS

NUMBER	SYMBOL	DESCRIPTION
1		Manufacturer
2		Product Model Number / Designation or Part Number
3		Caution, consult accompanying documents
4		Product Lot Number
5		Use By (Expiry)
6		Consult Operating Instructions
7		Date of manufacture
8		Temperature limitation
9		For <i>in vitro</i> diagnostic use
10		European Union conformity
11		Packaging