



IMMUNOSUPPRESSANTS IN WHOLE BLOOD BY LC-MS/MS

REF LC75115

CYCLOSPORINE A, EVEROLIMUS, SIROLIMUS, TACROLIMUS

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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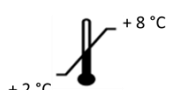
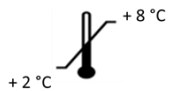
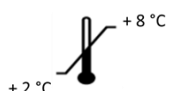
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INTENDED USE

In vitro diagnostic medical device for the detection and the quantitative analysis of Cyclosporine A, Everolimus, Sirolimus and Tacrolimus in whole blood as monitoring of immunosuppressive therapy. Manual method for professional use.

KIT CONTENT FOR 500 TESTS

CONTENT	DESCRIPTION	PACKAGING	WARNING
Reagent A	Deproteinization Solution	1 x 150 ml	
Reagent B	Internal Standard Solution	1 x 2.000 µl	
Reagent C	Lysing Solution	1 x 50 ml	
Multiparametric Calibrator in whole blood		7 x 6 x 1 ml	(Packed separately – Code LC75016 -see data sheet)
Reagent M1	Mobile Phase M1	5 x 500 ml	
Reagent M2	Mobile Phase M2	5 x 500 ml	

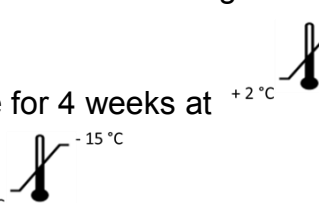
ACCESSORIES AND CONSUMABLES AVAILABLE SEPARATELY

CODE	DESCRIPTION	PACKAGING
LC75016	Whole blood Calibrator for Immunosuppressants	7 x 2 x 1 ml
LC75019	Whole blood Control for Immunosuppressants – Levels 1, 2 and 3	3 x 5 x 1 ml
SK75110	Starter kit for Immunosuppressants in whole blood	8 x 1 ml
LC75111	Mobile Phase M1	4 x 500 ml
LC75121	Mobile Phase M2	4 x 500 ml
LC75130	Labelled Internal Standard Mix Solution in whole blood	1 x 400 µl
L-30-210	Eppendorf Test tubes by 1,5 ml with caps	1 x 1000 Pcs
For HPLC system		
ZTF17126032130	ACCUCORE C 18 (30 x 2,1 mm - 2,6 µm) Analytical Column	1 Pc
For UHPLC system		
Z959757902	Analytical Column RRHD Eclipse Plus C18 (50 x 2,1 mm - 1,8 µm)	1 Pc
Mono-dimensional		
TF25403012101	Hypersil GOLD PFP Guard (10 x 2,1 mm - 3 µm) Prefilters - Specific for mono-dimensional modality	1 x 4 Pcs
TF84200	2mm DROP-IN HOLDER - Specific for mono-dimensional modality, una tantum	1 Pc
Bi-dimensional		
RE917950210	Ultra PFPP 10 x 4,0 mm (SPE on line - Specific for bi-dimensional modality)	1 x 3 Pcs
RE25021	Holder for 10mm Guard Trident Guard System - Specific for bi-dimensional modality, una tantum	1 Pc
S51843550	Clear Glass Vials with reduced volume (goblet form)	1 x 100 Pcs
S51820717	Caps for glass Vials with reduced volume (goblet form)	1 x 100 Pcs

INSTRUMENTS

DESCRIPTION	WARNING
LC/MS System with triple quadrupole (mode MRM) Divert Valve at 6 ways ESI (+) Interface Binary Pump Loop 50 µl	Required
Operational Computer	Required
Autosampler	Required

MATRIX - COLLECTION AND STORAGE PROCEDURE

MATRIX	PROCEDURE
Whole Blood	Test tube with anticoagulant EDTA. Stable for 4 weeks at $+2^{\circ}\text{C}$ or for 4 months at -25°C 

REFERENCE VALUES

Immunosuppressants	Therapeutic Range (ng/ml)
Cyclosporine A	10 – 2000
Everolimus	1 - 50
Sirolimus	1 - 50
Tacrolimus	1 - 50

ANALYTICAL PROCEDURE

STEP	DESCRIPTION																																								
1	Sample Preparation: Dispense 100 µl of Reagent B, 400 µl of Methanol and 500 µl of HPLC grade Water into each bottle of 10 ml. <u>Vortex for 5 sec.</u> N.B.: 20 µl of diluted sample standard is required, then prepare an appropriate volume of solution. Dispense in sequence into eppendorf tubes: <table><tr><td></td><td>Calibrator (µl)</td><td>Sample (µl)</td><td>Controls (µl)</td></tr><tr><td>Calibrator</td><td>50</td><td></td><td></td></tr><tr><td>Sample</td><td></td><td>50</td><td></td></tr><tr><td>Controls</td><td></td><td></td><td>50</td></tr><tr><td>Internal Standard (after dilution)</td><td>20</td><td>20</td><td>20</td></tr><tr><td></td><td colspan="3">Vortex for 10 sec.</td></tr><tr><td>Reagent C</td><td>100</td><td>100</td><td>100</td></tr><tr><td></td><td colspan="3">Vortex for 10 sec.</td></tr><tr><td>Reagent A</td><td>300</td><td>300</td><td>300</td></tr><tr><td></td><td colspan="3">Vortex for 10 sec.</td></tr></table> <u>Vortex for 20 sec.</u>		Calibrator (µl)	Sample (µl)	Controls (µl)	Calibrator	50			Sample		50		Controls			50	Internal Standard (after dilution)	20	20	20		Vortex for 10 sec.			Reagent C	100	100	100		Vortex for 10 sec.			Reagent A	300	300	300		Vortex for 10 sec.		
	Calibrator (µl)	Sample (µl)	Controls (µl)																																						
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2	Centrifuge at 14.000 rpm for 10 minutes. Take the supernatant and pour it into a glass vial.																																								
INJECTION	Inject 10 µl of the solution into the LC system																																								

GRADIENT

Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	Flow (µl/min)
0.00	100	0	500
0.50	100	0	500
SWITCHING VALVE			
0.51	100	0	600
1.50	5	95	600
3.00	5	95	600
SWITCHING VALVE			
3.01	100	0	500
3.50	100	0	500

PARAMETERS

Parameters set on triple quadrupole

	IMMUNOSUPPRESSANTS ESI POSITIVE (AGILENT 6460)		IMMUNOSUPPRESSANTS ESI POSITIVE (SCIEX 4500)
GAS TEMPERATURE	250°C	CURTAIN GAS (CUR)	25
GAS FLOW	5 L/min	COLLISION GAS (CAD)	10
NEBULIZER	60 psi	ION SPRAY VOLTAGE (IS)	5.500 V
SHEALTH GAS HEATER	300°C	TEMPERATURE (TEM)	550°C
SHEALTH GAS FLOW	12 L/min	ION SOURCE GAS 1 (GS1)	60
CAPILLARY	4.000 V	ION SOURCE GAS 2 (GS2)	60

FRAGMENTATION OF ANALYTES AND LABELLED INTERNAL STANDARDS (IS)

ANALYTES	RT (min)	FRAGMENTATION		LABELLED IS USED FOR CALIBRATION	RT (min)	FRAGMENTATION	
		PARENT ION (Q1)	FRAGMENT ION (Q3)			PARENT ION (Q1)	FRAGMENT ION (Q3)
Cyclosporine A	1.05	1.219,9	1.202,9	Cyclosporine A-D4	1.05	1.223,7	1.206,9
Everolimus	1.0	975,6	908,6	Everolimus ¹³ C2-D4	1.0	981,3	914,5
Sirolimus	1.0	931,6	864,5	Sirolimus ¹³ C-D3	1.0	935,4	864,6
Tacrolimus	1.0	821,5	768,5	Tacrolimus ¹³ C-D2	1.0	824	771

TECHNICAL FEATURES - PART I

ABBREVIATION	MEANING
C LLOQ	Concentration at low limit of quantification
C1	Low concentration
C2	Medium concentration
C3	High concentration
Max C	The highest concentration

ANALYTES	CONCENTRATIONS USED TO CALCULATE REPRODUCIBILITY AND ACCURACY (ng/ml)				
	C LLOQ	C1	C2	C3	MAX C
Cyclosporine A	0,05	25	60	300	500
Everolimus	0,05	1,25	2,5	12,5	25
Sirolimus	0,05	1,25	2,5	12,5	25
Tacrolimus	0,05	1,25	2,5	12,5	25

ANALYTES	ACCURACY INTRA SERIE (RELATIVE ERROR %)		ACCURACY INTER SERIE (RELATIVE ERROR %)	
	C1 (%)	C3 (%)	C1 (%)	C3 (%)
Cyclosporine A	3,4	10,1	12,0	13,9
Everolimus	6,6	6,0	8,2	6,8
Sirolimus	5,3	11,3	7,2	7,1
Tacrolimus	4,2	5,1	8,6	9,9

TECHNICAL FEATURES - PART II

ANALYTES	REPRODUCIBILITY INTRA SERIE (COEFFICIENT OF VARIATION %)			REPRODUCIBILITY INTER SERIE (COEFFICIENT OF VARIATION %)		
	C LLOQ %	C2 %	Max C %	C LLOQ %	C2 %	Max C %
Cyclosporine A	9,9	3,7	2,2	15,8	7,3	2,9
Everolimus	11,7	4,0	2,1	8,5	6,6	3,5
Sirolimus	9,7	8,1	1,9	8,4	7,0	5,6
Tacrolimus	1,6	4,6	4,4	16,9	7,1	3,7

TECHNICAL FEATURES - PART III

ANALYTES	LLOD (ng/ml)	LINEARITY (ng/ml)	COEFFICIENT OF CORRELATION R2 + STD DEV
Cyclosporine A	0,01	0,05 – 1.500	0,9941 ± 0,0060
Everolimus	0,01	0,05 - 100	0,9964 ± 0,0005
Sirolimus	0,01	0,05 - 100	0,9939 ± 0,0026
Tacrolimus	0,01	0,05 - 100	0,9951 ± 0,0026

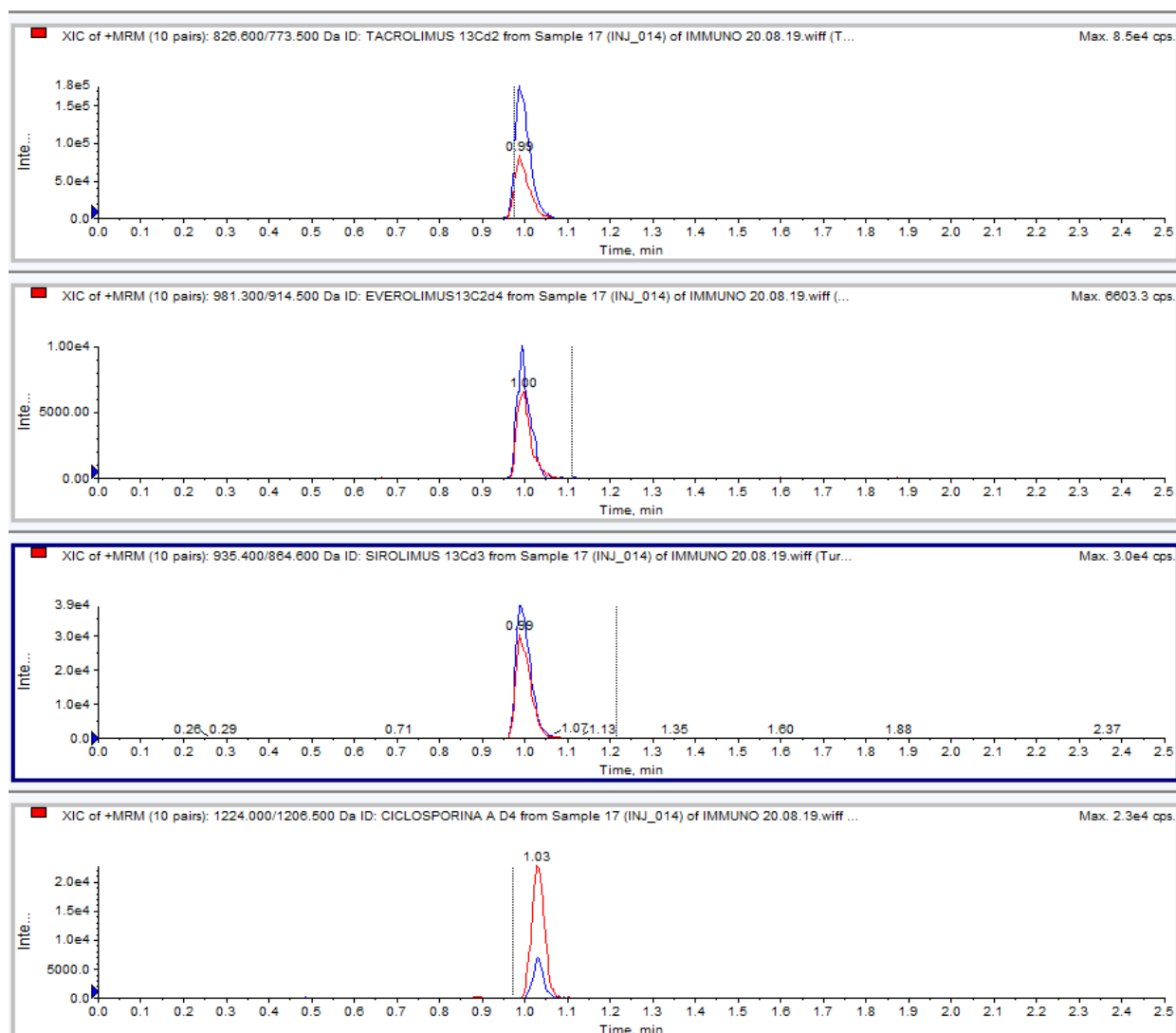
REFERENCE CHROMATOGRAMS

Fig.1 Calibrator level 5 for Immunosuppressants, from top to bottom, tacrolimus and its internal standard, everolimus and its internal standard, sirolimus and its internal standard, cyclosporine and its internal standard.

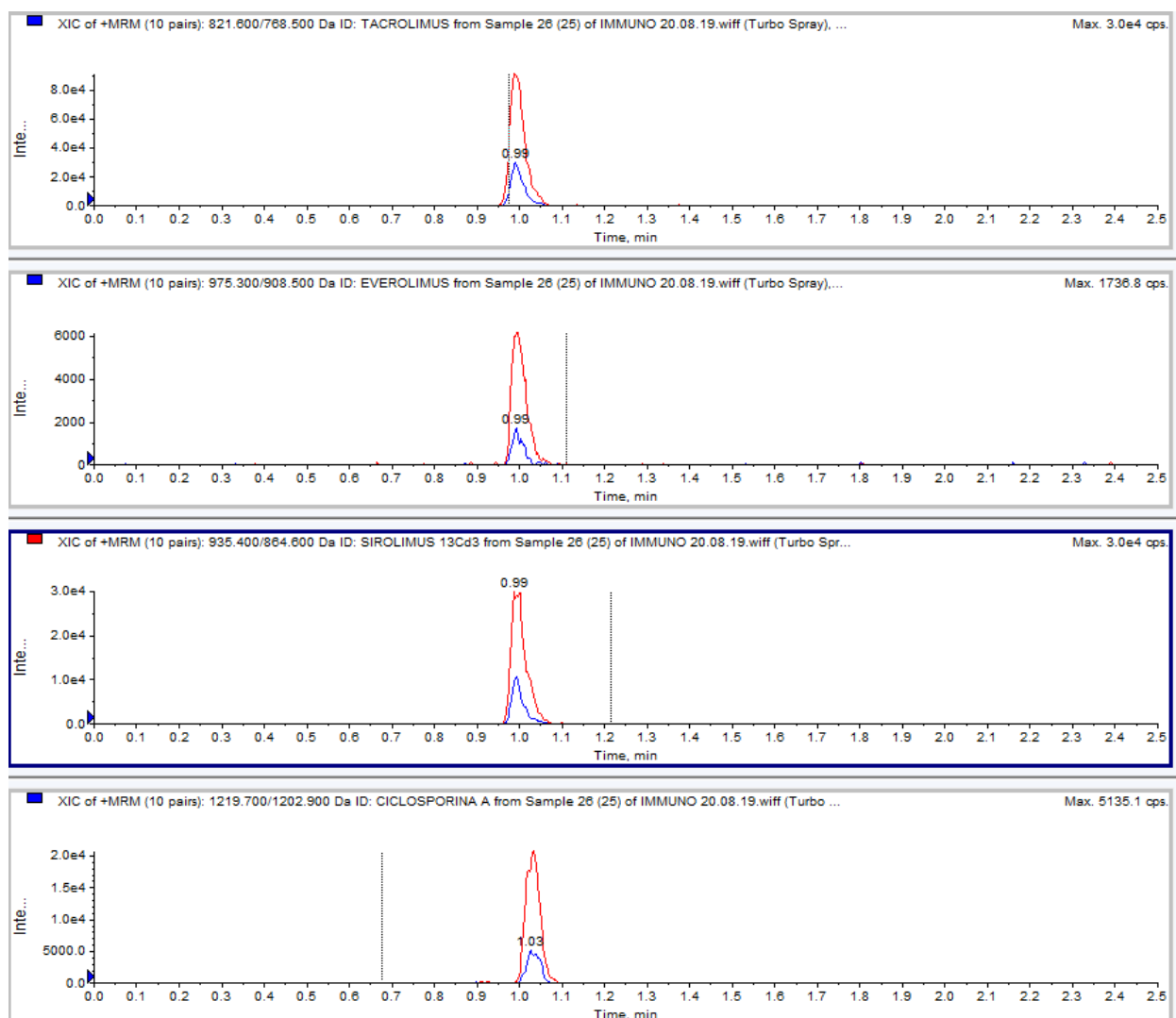


Fig.2 Control level 1 for Immunosuppressants, from top to bottom, tacrolimus and its internal standard, everolimus and its internal standard, sirolimus and its internal standard, cyclosporine and its internal standard.

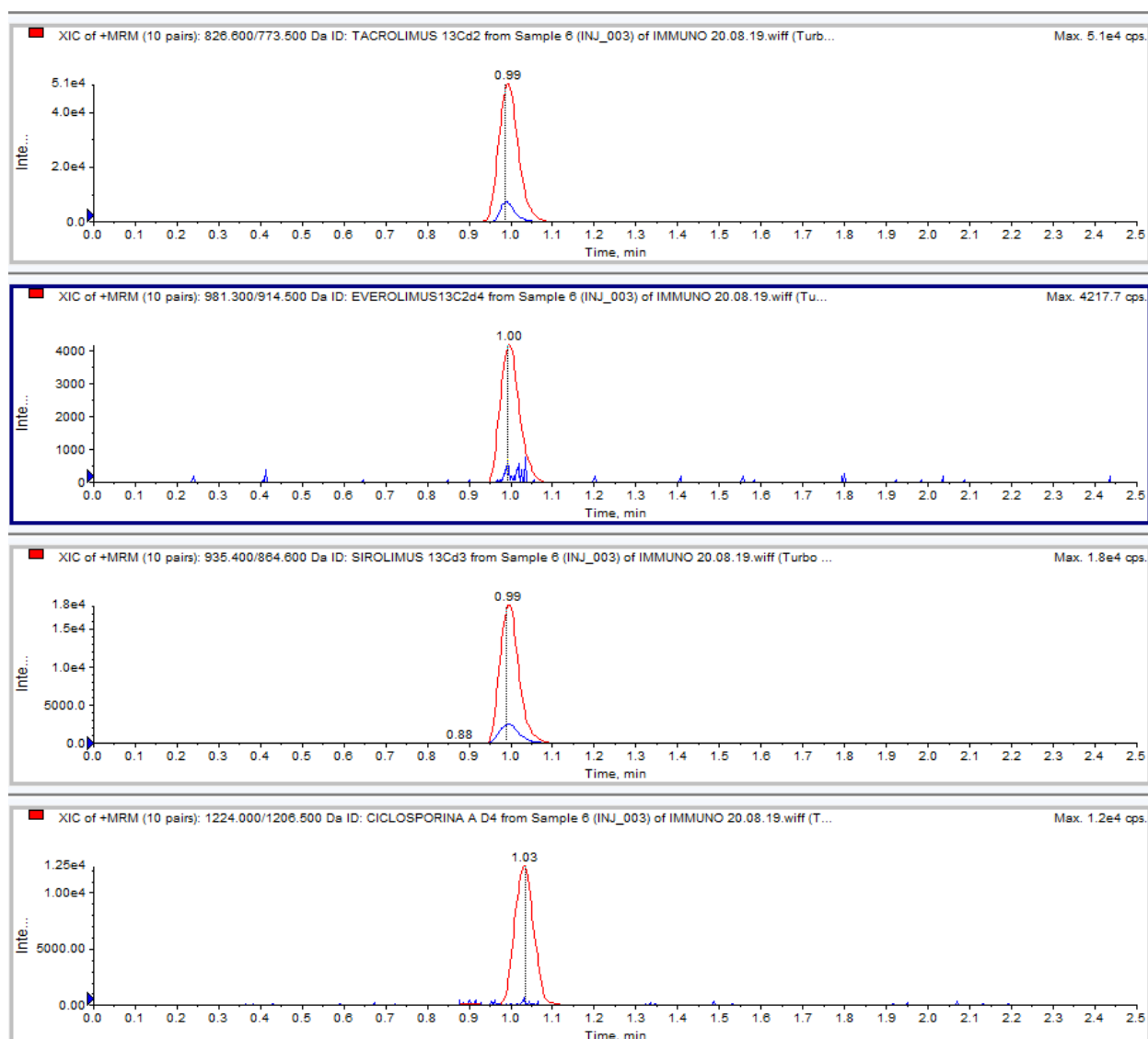


Fig.3 Treated sample for immunosuppressive assay (Tacrolimus 2 ng/mL, Sirolimus 1,7 ng/mL), from top to bottom, tacrolimus and its internal standard, everolimus and its internal standard, sirolimus and its internal standard, cyclosporine and its internal standard.

GENERAL WARNINGS

1. STORAGE AND SHELF LIFE

All the components are ready-to-use. They are stable for 3 years at  $+2^{\circ}\text{C}$ except from Reagent A (Deproteinization Solution) and Reagent B (Internal Standard Solution) that must be stored at  -25°C . After opening the shelf life of our internal standards is 6 months if stored at  -15°C .

2. NEW COLUMN TREATMENT

Install a new analytical column RRHD Eclipse Plus C18 (2,1 mm x 50 mm – 1,8 μm) thermostated at 60°C after the valve. Disconnect the detector and flux a solution of H_2O :Mobile Phase M2 (5 : 95 v/v) at the flow of 500 $\mu\text{l}/\text{min}$. **Don't recycle the washing solutions.**

3. COLUMN CONDITIONING

Condition the column with a solution of Mobile Phase M1:Mobile Phase M2 (5 : 95 v/v) set flow 500 $\mu\text{l}/\text{minute}$, with the valve in position B, for 10 minutes. Then, with the valve in the position A, flux with 100% of Mobile Phase M1 for 5 minutes. Then, make 4 injections of mobile phase before analytical session. **It's NOT possible to make analysis at recycling phase.**

If room temperature is $> 20^{\circ}\text{C}$ store the Mobile Phase at  $+2^{\circ}\text{C}$ between an analytical session and another.

ENRICHMENT COLUMN

Mono-dimensional

TF25403012101 Enrichment Column Hypersil GOLD PFP Guard 10 x 2.1 mm - 3 μm
TF84200 Drop in Holder 2 mm

Bi-dimensional

ZRE917950210 Enrichment Column Ultra PFPP 10 x 4.0 mm (SPE ON-LINE/Bi-dimensional Modality)
ZRE25021 Holder for 10 mm Guard Trident Guard System

Install enrichment column before valve and condition as analytical column.

4. COLUMN CLEANING

Disconnect the detector. Flux a solution of H_2O :Mobile Phase M2 (5 : 95 v/v) at the flow of 500 $\mu\text{l}/\text{min}$ for 15 minutes. Discharge. Store the column in the same solution.

5. AUTOSAMPLER SYRINGE WASHING

Wash the syringe and the needle with Methanol before use.

ABBREVIATIONS

ABBREVIATIONS	MEANING
e.g. 5 x 1 x 1 ml	N° of levels x N° of vials x volume
e.g packaging of the starter kit 27 x 1 ml	N° of vials x volume Note: each vial contains one molecule (analyte or labelled IS)
IS	Internal Standard
pc/pcs	Piece/pieces
RT	Retention Time
STD DEV	Standard Deviation

BIBLIOGRAPHY

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2. Katerina Sadilkova *et al.* Clinical validation and implementation of a multiplexed immunosuppressant assay in dried blood spots by LC–MS/MS Clinica Chimica Acta Volume 421, 5 June 2013, Pages 152-156
3. Zi-Shan Gong *et al.* A high-throughput LC-MS/MS method for the quantification of four immunosuppressants drugs in whole blood Clinica Chimica Acta Volume 498, November 2019, Pages 21-26
4. Remco A. Koster *et al.* Robust, High-Throughput LC-MS/MS Method for Therapeutic Drug Monitoring of Cyclosporine, Tacrolimus, Everolimus, and Sirolimus in Whole Blood Ther Drug Monit Volume 31, Number 1, February 2009
5. Michael Vogeser *et al.* Automated processing of whole blood samples for the determination of immunosuppressants by liquid chromatography tandem-mass spectrometr Clin Chem Lab Med 2006;44(9):1126–1130

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