

# Simultaneous on-line LC-MS/MS analysis of Everolimus, Tacrolimus, Sirolimus and Cyclosporin A in whole blood

Jos vd Elshout, Arjan Huyskens, Robert vd Wegen, Francisca de Jong & Jan Dankers

## Introduction

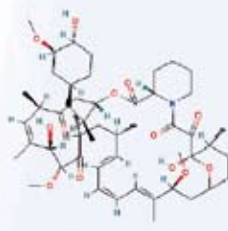
With the introduction of the novel immunosuppressive agents, new potentially more effective immunosuppressive regimens are undergoing clinical evaluation. In order to serve the pharmaceutical industry adequately for the analysis of these drugs for either Pharmacokinetic research or Therapeutic Drug Monitoring purposes, we have developed a combined method for a fast, sensitive and reliable analysis of Everolimus, Tacrolimus, Sirolimus and Cyclosporin A in whole blood.



Everolimus Mw 958



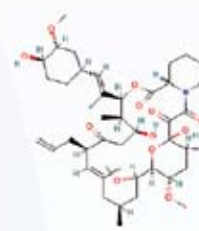
Tacrolimus Mw 804



Sirolimus Mw 914



Cyclosporin A Mw 1203



Ascomycin Mw 792

## Sample preparation

- 0.25 mL whole blood deproteinized with 1.5 ml 0.2 M ZnSO<sub>4</sub> / Methanol 30 /70
- Column: Zorbax Eclipse XDB-C8 150 X 4.6 mm
- Pre Column: Eclipse XDB-C8 12.5 X 4.6 mm
- Eluent: methanol + 0.1% Formic acid 20/80
- Injection volume: 25 µl

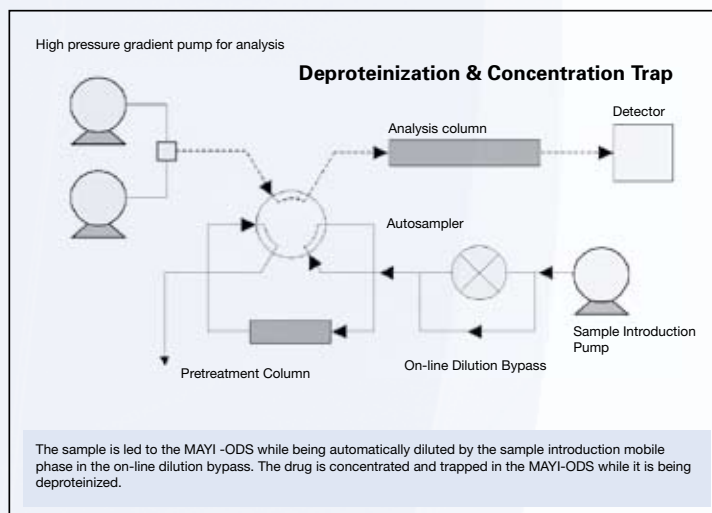
## LC-MS/MS conditions

API 4000, ion spray

HPLC: Shimadzu Co-Sense

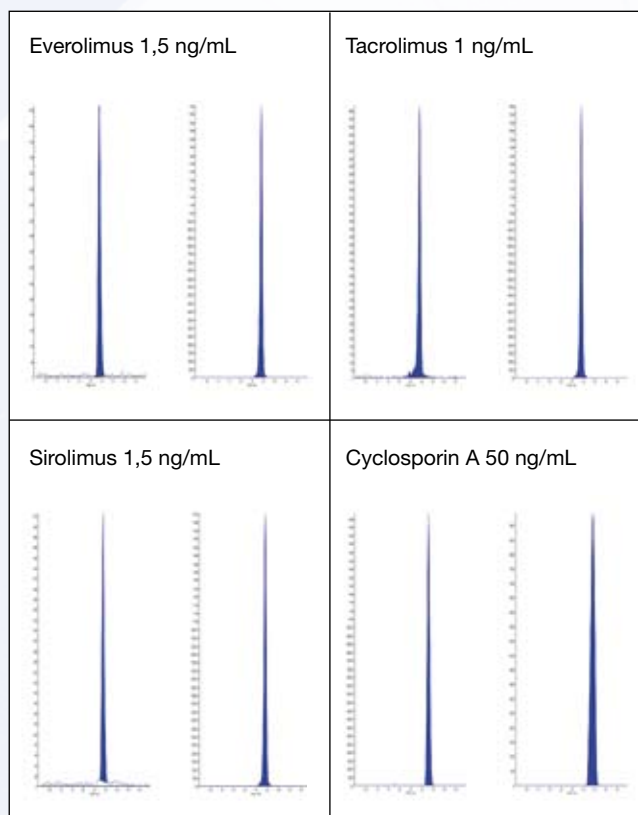
Everolimus	m/z 981 / 389
Tacrolimus	m/z 827 / 616
Sirolimus	m/z 937 / 409
Cyclosporin A	m/z 1225 / 1113
Ascomycin	m/z 815 / 604
Cyclosporin D	m/z 1239 / 1127

## On-line SPE



## Results

	Conc ng/mL	Precision (%) overall CV	Accuracy %
<b>Everolimus</b>			
Linear range	0.2 - 100		
LLOQ	0,2	11	97
QCL	1,5	6,7	105
QCM	5	4,8	110
QCH	40	4,3	109
<b>Tacrolimus</b>			
Linear range	0.2 - 100		
LLOQ	0,2	11	99
QCL	1	5,7	99
QCM	6	3,9	99
QCH	30	4,4	98
<b>Sirolimus</b>			
Linear range	0.2 - 100		
LLOQ	0,2	14	103
QCL	1,5	9,2	96
QCM	10	6,9	104
QCH	40	4,8	109
<b>Cyclosporin A</b>			
Linear range	5-1300		
LLOQ	5	5,6	103
QCL	50	6,4	111
QCM	240	3,7	110
QCH	800	3,1	87



## Conclusion

A sensitive method for the combined determination of Everolimus, Tacrolimus, Sirolimus and Cyclosporine in whole blood was validated successfully. This method proved to be robust for Therapeutic Drug Monitoring assay's in Clinical Trials.

## Literature

Analytico Medinet, project nr 4032051001: Method validation study for the simultaneous quantification of Everolimus, Sirolimus, Tacrolimus and Cyclosporin in EDTA human whole blood by LC-MS/MS.