APPLICATION NOTE

Therapeutic Drug Monitoring of Immunosuppressants with *MassTox*® Series A

Introduction

Therapeutic drug monitoring of immunosuppressants is indispensable for therapy following organ transplantation. Cyclosporin A, everolimus, sirolimus and tacrolimus are mainly prescribed for avoiding organ rejection. Laboratories working with *MassTox*® TDM Series A can perform therapeutic drug monitoring of more than 200 drugs with one column and an identical sample preparation. So far, it excluded the analysis of immunosuppressants. Here, we describe that *MassTox*® Series A can also be used for the analysis of cyclosporin A, everolimus, sirolimus and tacrolimus.

Material and Methods

Sample Preparation

The sample preparation was performed in line with the *MassTox*® Immunosuppressants protocol (Chromsystems). In brief: 50 µl of sample (EDTA-whole blood), reconstituted 6PLUS1® calibrator (order no 28039/XL) or *MassCheck*® control (order no 0081-0085, 0089) was pipetted into a 1.5 ml reaction vial. 25 µl of the reconstituted Internal Standard Mix (order no. 93946/RUO) and 100 µl Extraction Buffer (order no 93005/RUO) were added and mixed briefly. 250 µl of Precipitation Reagent (order no. 93003/RUO) was added, vortexed for 1 min and incubated for 2 min at ambient temperature, then centrifuged for 5 min at 15000 g. The supernatant was diluted with Dilution Buffer 1 (92007/RUO; ratio 1:1).

LC-MS/MS

10 – 50 µl were injected at a temperature for the autosampler of 8 °C – 15 °C. Substances were separated on MasterColumn® A (order. no 92110) with a column temperature of 70 °C. Flow rate, position of selection valve and binary gradient can be found in table 1. Detection was performed with electrospray ionisation in positive ion mode with a Sciex 4500 mass spectrometer. Used multiple reaction monitoring (MRM) transitions are found in table 2.

Time	Mobile Phase A (order no. 92001/RUO)	Mobile Phase B (order no. 92002/RUO)	Flow rate	MS/waste
0.00 min – 0.20 min	100 %	0 %	1.2 ml/min	Waste
0.21 min – 1.20 min	15 %	85 %	1.2 ml/min	
1.21 min – 1.80 min	15 %	85 %	0.7 ml/min	MS/MS
1.81 min – 2.90 min	0 %	100 %	0.7 ml/min	
2.91 min – 3.30 min	100 %	0 %	0.7 ml/min	
3.31 min – 3.50 min	100 %	0 %	1.2 ml/min	Waste

Table 1: Binary gradient profile

Quantification

A full calibration of the analysis system for each series of measurements has been performed with 6PLUS1® Multilevel Calibrator Set (order no. 28039/XL). Calibration curves were constructed by calculating the analyte to internal standard (ISTD) peak area ratio on the y axis against calibrator concentrations on the x axis. Then a calibration curve was plotted for all analytes using linear regression and 1/x weighting.

Analyte	Corresponding ISTD MRM	MRM 1	MRM 2	MRM 3
Cyclosporin A	Cyclosporin A-d12 (1231.9 → 1214.8)	1219.8 → 1202.8	1219.8 → 1184.8	1219.8 → 675.6
Everolimus	Everolimus-d4 (979.6 → 912.5)	975.6 → 908.5	975.6 → 926.5	-
Sirolimus	Sirolimus-d3 (934.6 → 864.5)	931.6 → 864.5	931.6 → 882.5	-
Tacrolimus	Tacrolimus-13Cd2 (824.5 → 771.6)	821.5 → 768.6	821.5 → 786.6	-

Table 2: MRMs and corresponding ISTD

Results

The analysis of the four immunosuppressants was performed with an analysis time of 3.5 min (see chromatogram in fig. 1). Coefficients of variation were below 10% [Inter-assay (CV = 2.1 % - 8.6 %) and intra-assay (CV = 1.9 % - 8.3 %)] for all clinically relevant concentration ranges (see tables 3.1 + 3.2). The lower limit and upper limits of quantification were determined and cover published therapeutic ranges for all four analytes (table 4).

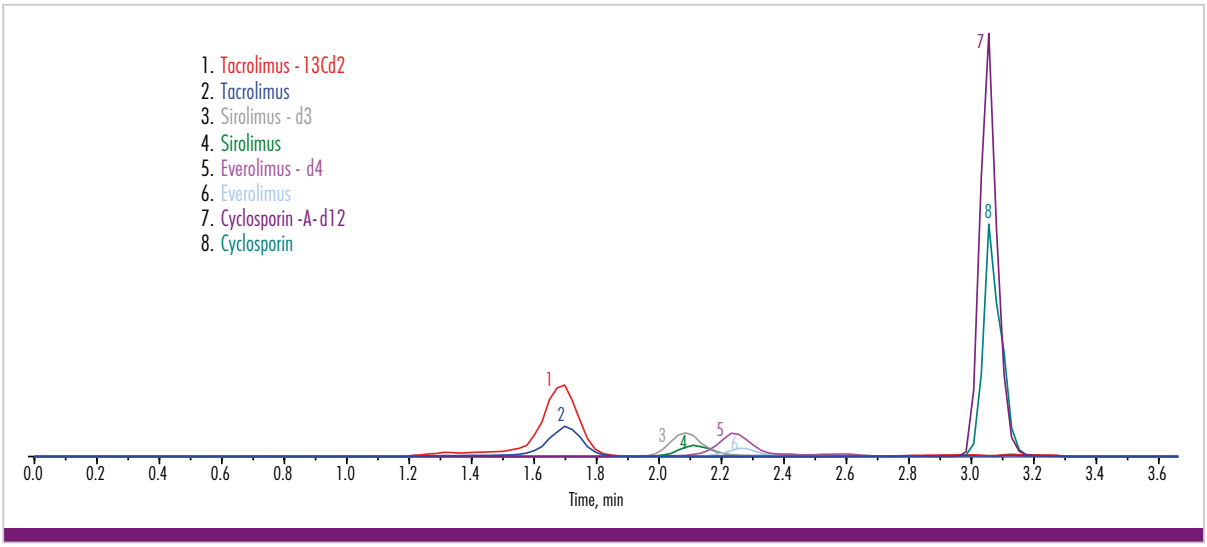

Fig. 1: Chromatogram for the four immunosuppressants and its internal standards.

	Level I, order no. 0082			Level II, order no. 0083		
		Intra-assay	Inter-assay		Intra-assay	Inter-assay
Analyte	Conc [µg/l]	CV [%]	CV [%]	Conc [µg/l]	CV [%]	CV [%]
Cyclosporin A	47.9	6.5	7.5	246.8	4.9	5.4
Everolimus	2.63	4.5	4.6	4.72	8.3	8.5
Sirolimus	2.92	4.0	4.4	9.82	3.1	4.3
Tacrolimus	2.75	4.0	4.9	7.28	4.5	4.5

Table 3.1: Intra- and inter-assay data

	Level III, order no. 0084			Level IV, order no. 0085		
		Intra-assay	Inter-assay		Intra-assay	Inter-assay
Analyte	Conc [µg/l]	CV [%]	CV [%]	Conc [µg/l]	CV [%]	CV [%]
Cyclosporin A	490.2	4.6	6.7	1196.5	3.3	8.6
Everolimus	9.12	2.6	3.1	31.13	3.1	3.6
Sirolimus	19.39	1.9	2.1	38.67	2.9	3.8
Tacrolimus	15.37	4.2	4.4	32.86	3.7	4.0

Table 3.2: Intra- and inter-assay data

Conclusions

We demonstrate in this application note that the immunosuppressants cyclosporin A, everolimus, sirolimus and tacrolimus in whole blood can be analysed by using a hybrid of the two Chromsystems kits: ***MassTox***® Series A and the ***MassTox***® **ONE***MINUTE* Immunosuppressants assay. The sample preparation is performed in line with the immunosupressants assay and with a dilution using a reagent of Series A. The sample is then applied to the analytical system of ***MassTox***® Series A without modification to the chromatographic setup.

While this approach is not CE-IVD compliant, customers can use this assay on a research-use basis, enabling the analysis of more than 200 drugs plus immunosuppressants in the laboratory while also eliminating the need for changes to the chromatographic setup.

[µg/l]	LLOQ	ULOQ
Cyclosporin A	5.68	2000
Everolimus	1.02	100
Sirolimus	0.50	100
Tacrolimus	0.56	100

Table 4: Upper and lower limit of quantification

Ordering Information

MassTox® Series A	
Product	**Order No.**
Mobile Phase 1	92001/RUO
Mobile Phase 2	92002/RUO
Rinsing Solution	92009/RUO
MassTox® TDM Master Column Series A	92110
Dilution Buffer 1	92007/RUO

MassTox® Immunosuppressants	
Product	**Order No.**
6PLUS1® Multilevel Whole Blood Calibrator Set	28039/XL
MassCheck® Immunosuppressants Whole Blood Controls Level I-IV, Blank	0081-0085, 0089
Internal Standard Set	93946/RUO
Precipitation Reagent	93003/RUO
Extraction Buffer	93005/RUO
Reaction vials, transparent	33006
Tuning Mix	93915/RUO

RUO: For research use only and not for not for diagnostic purposes.

Chromsystems Instruments & Chemicals GmbH · Am Haag 12 · 82166 Gräfelfing · Germany
Phone: +49 89 18930-0 · mailbox@chromsystems.com · www.chromsystems.com

Management system certified according to: ISO 9001, ISO 13485 (including MDSAP)