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Quantification of immunosuppressants in human whole blood by liquid chromatography-tandem mass spectrometry for clinical research

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Keywords

Immunosuppressants, cyclosporin A, tacrolimus, sirolimus, everolimus, human blood, mass spectrometry

Application benefits

- Quantification of four very important immunosuppressants in a single run
- Robust method with minimal offline sample preparation: simple protein precipitation followed by online SPE

Goal

Implementation of an analytical method for the quantification of cyclosporin A, tacrolimus, sirolimus, and everolimus in human blood on a Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer.

Introduction

A robust analytical method for the quantification of cyclosporin A, everolimus, sirolimus, and tacrolimus in human whole blood is reported. This method was developed using LC-MS for clinical research. The method involves a simple protein precipitation step followed by online solid phase extraction (SPE) using a Thermo Scientific™ Transcend™ II TLX-1 system. A Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer with heated electrospray ionization was used for detection by selected reaction monitoring (SRM) using isotopically labeled internal standards for each analyte. Method performance was evaluated using the MS1100 ClinMass® LC-MS/MS Complete Kit for Immunosuppressants in Whole Blood, advanced – on-line Analysis from RECIPE, to obtain limits of quantification, linearity ranges, accuracy, and intra- and inter-assay precision for each analyte.

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Experimental

Target analytes

- Cyclosporin A
- d_{12}-Cyclosporin A (internal standard)
- Everolimus
- $^{13}C_2d_4$-Everolimus (internal standard)
- Sirolimus
- $^{13}Cd_3$-Sirolimus (internal standard)
- Tacrolimus
- $^{13}Cd_2$-Tacrolimus (internal standard)

Sample preparation

Reagents included calibrators and controls from RECIPE at seven (including blank) and three different levels, respectively, covering the concentration ranges reported in Table 1. Each analyte was quantified using a corresponding isotopically labeled internal standard.

Sample cleanup was performed by a simple preliminary protein precipitation with internal standard addition followed by online SPE on a Transcend II TLX-1 system.

Table 1. Concentration ranges covered by calibrators.

Analyte	Concentration Range (ng/mL)
Cyclosporin A	25.8–1243
Everolimus	1.45–49.4
Sirolimus	1.62–52.9
Tacrolimus	1.37–45.1

Liquid chromatography

The LC separation was achieved using mobile phases, an SPE cartridge, and an analytical column provided by RECIPE; details of the analytical method are reported in Figure 1. Total runtime was 2 minutes. A schematic representation of the LC configuration is reported in Figure 2.

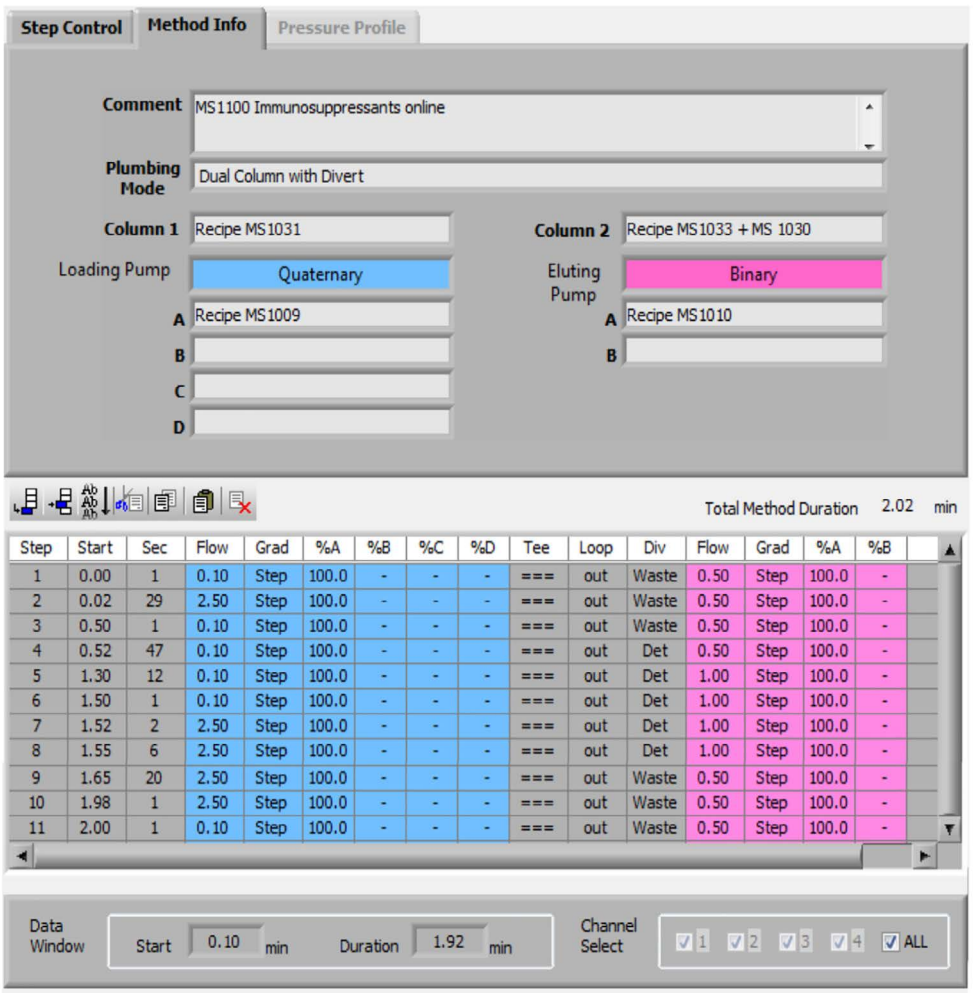

Figure 1. LC method description.

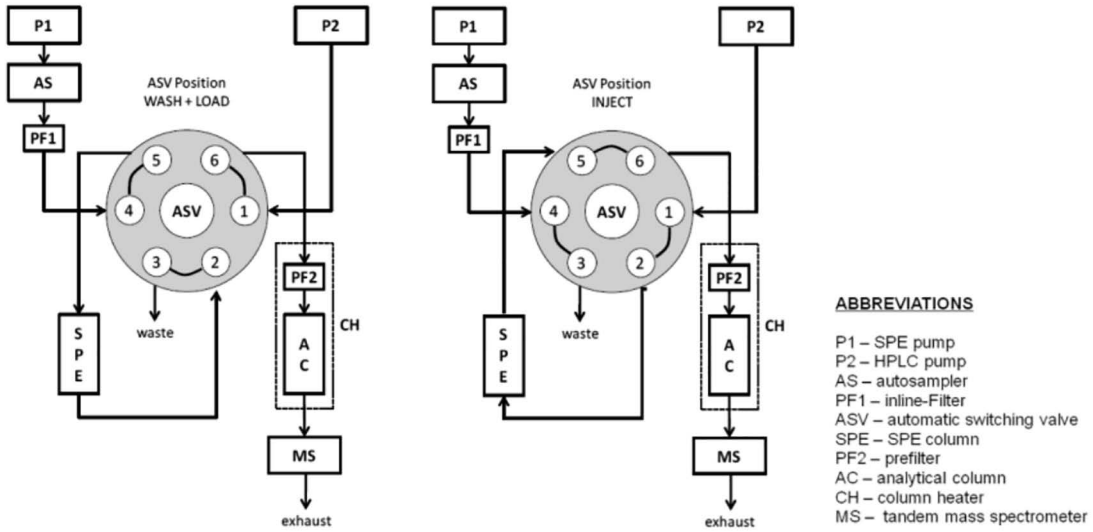

Figure 2. Schematic representation of the Transcend II TLX-1 system configuration used for online SPE.

Mass spectrometry

Analytes and internal standards were detected by SRM on a TSQ Endura triple quadrupole mass spectrometer with heated electrospray ionization operated in positive mode. Two SRM transitions for each analyte were included in the acquisition method for quantification and confirmation, respectively.

Method evaluation

The method performance was evaluated by obtaining limits of quantification, linearity ranges, accuracy, and intra- and inter-assay precision for each analyte. Analytical accuracy was evaluated in terms of trueness of measurement using the Proficiency Test Samples #601-22 and #601-62 from INSTAND e.V. prepared and analyzed on five different days in single runs each day. Intra-assay precision was evaluated in terms of percentage coefficient of variation (%CV) using the controls at three different levels in replicates of eight (n=8) prepared and analyzed in one batch. Inter-assay precision was evaluated on the same controls in replicates of three (n=3) prepared and analyzed on five different days.

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software version 3.3.

Results and discussion

The method proved to be linear not only in the calibration range covered by the calibrators but also in a wider range obtained by diluting the lowest calibrator up to 10-fold. The lower limits of quantification (LLOQ) were 14.0 ng/mL for cyclosporin A, 0.85 ng/mL for everolimus, 1.87 ng/mL for sirolimus, and 0.31 ng/mL for tacrolimus, with correlation factors (R^2) always above 0.99. Representative chromatograms for the lowest calibrator are reported in Figure 3. Representative calibration curves for cyclosporin A and everolimus are reported in Figure 4.

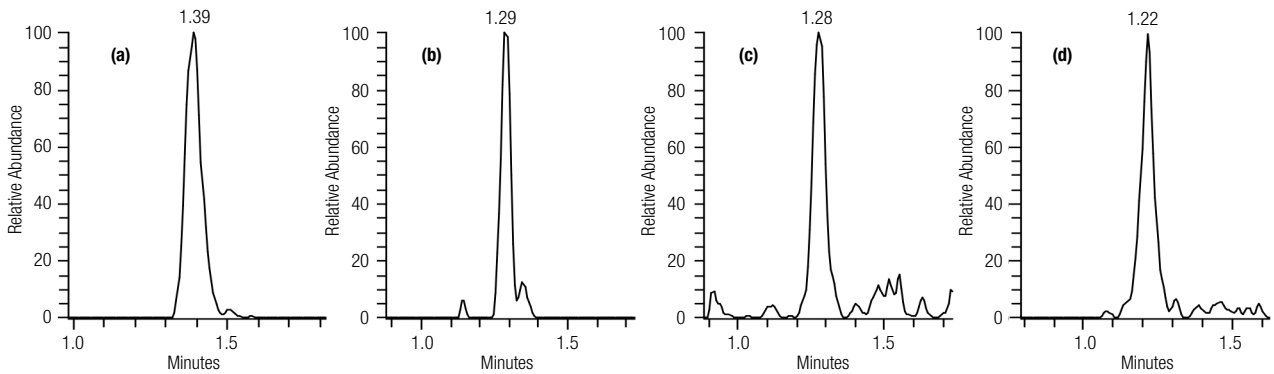

Figure 3. Representative chromatograms for the lowest calibrator for (a) cyclosporin A (25.8 ng/mL), (b) everolimus (1.45 ng/mL), (c) sirolimus (1.62 ng/mL) and (d) tacrolimus (1.37 ng/mL).

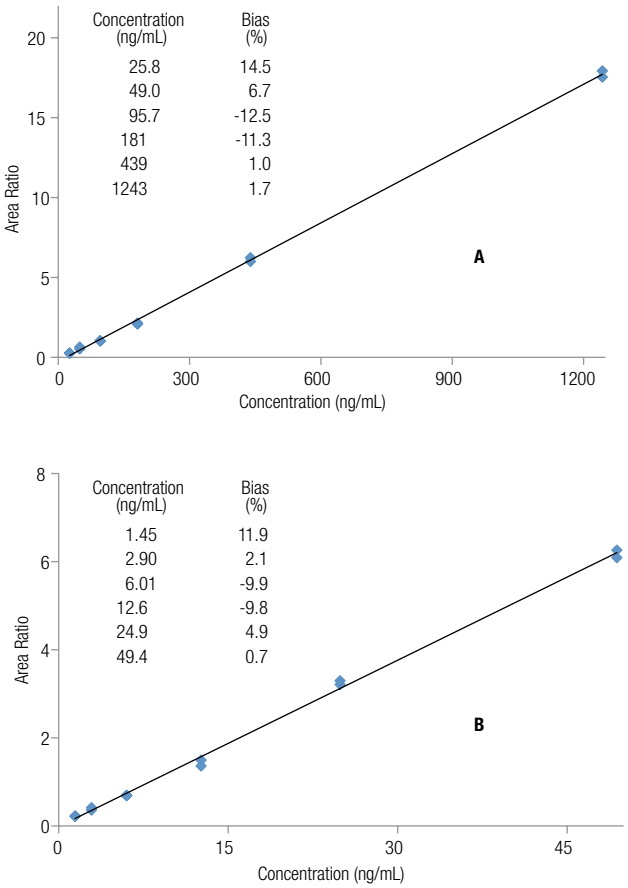

Figure 4. Representative calibration curves for (a) cyclosporin A and (b) everolimus.

The data showed remarkable accuracy with the percentage bias between nominal and average back-calculated concentration for these control samples always being between -21.5% and 15.1%. Results are reported in Table 2.

The %CV for intra-assay precision was always below 9.9% for all the analytes at all levels (Table 3). The maximum %CV for inter-assay precision including all the analytes was 12.9% (Table 4).

Table 2. Analytical accuracy results (n=5).

Analyte	Control	Nominal Concentration (ng/mL)	Measured Concentration (ng/mL)	CV (%)	Bias (%)
Cyclosporin A	601 (03/2016) 22	72.9	63.0	13.3	-13.5
	601 (10/2015) 62	256	201	15.8	-21.5
Everolimus	601 (03/2016) 22	0.19	N/A	N/A	N/A
	601 (10/2015) 62	8.55	7.36	13.5	-13.9
Sirolimus	601 (03/2016) 22	3.64	4.19	19.2	15.1
	601 (10/2015) 62	0.16	N/A	N/A	N/A
Tacrolimus	601 (03/2016) 22	3.83	3.69	9.6	-3.5
	601 (10/2015) 62	7.85	7.03	12.6	-10.5

Table 3. Intra-assay precision results (n=8).

Analyte	MS8830 #519		MS8831 #519		MS8832 #519	
	Average Concentration (ng/mL)	CV (%)	Average Concentration (ng/mL)	CV (%)	Average Concentration (ng/mL)	CV (%)
Cyclosporin A	54.8	4.05	111	4.57	210	1.51
Everolimus	3.75	9.93	12.7	9.92	20.1	6.95
Sirolimus	3.54	7.90	14.1	3.83	23.4	5.62
Tacrolimus	3.62	6.21	7.63	6.44	14.9	6.29

Table 4. Inter-assay precision results (n=15).

Analyte	MS8830 #519		MS8831 #519		MS8832 #519	
	Average Concentration (ng/mL)	CV (%)	Average Concentration (ng/mL)	CV (%)	Average Concentration (ng/mL)	CV (%)
Cyclosporin A	52.9	6.65	105	9.80	200	8.05
Everolimus	3.79	11.20	11.8	11.90	19	8.15
Sirolimus	4.32	12.90	12.9	12.60	21.5	11.10
Tacrolimus	3.57	9.73	7.47	11.90	14.6	7.47

Conclusions

This study details development of a liquid chromatography-tandem mass spectrometry method for clinical research for the quantification of cyclosporin A, everolimus, sirolimus, and tacrolimus in human whole blood. An MS1100 ClinMass LC-MS/MS Complete Kit for Immunosuppressants in Whole Blood, advanced – on-line Analysis from RECIPE was implemented and analytically validated on a Transcend II TLX-1 system coupled to a TSQ Endura triple quadrupole mass spectrometer. The method involves minimal sample preparation and meets research laboratory requirements for sensitivity, linearity of response, accuracy, and precision.

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