ENHANCEMENT OF SAMPLE TRACKING, THROUGHPUT AND ECOLOGIC FOOTPRINT OF AN IMMUNOSUPPRESSANT LC-MS WORKFLOW VIA FLUENT® AUTOMATION.

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BACKGROUND.

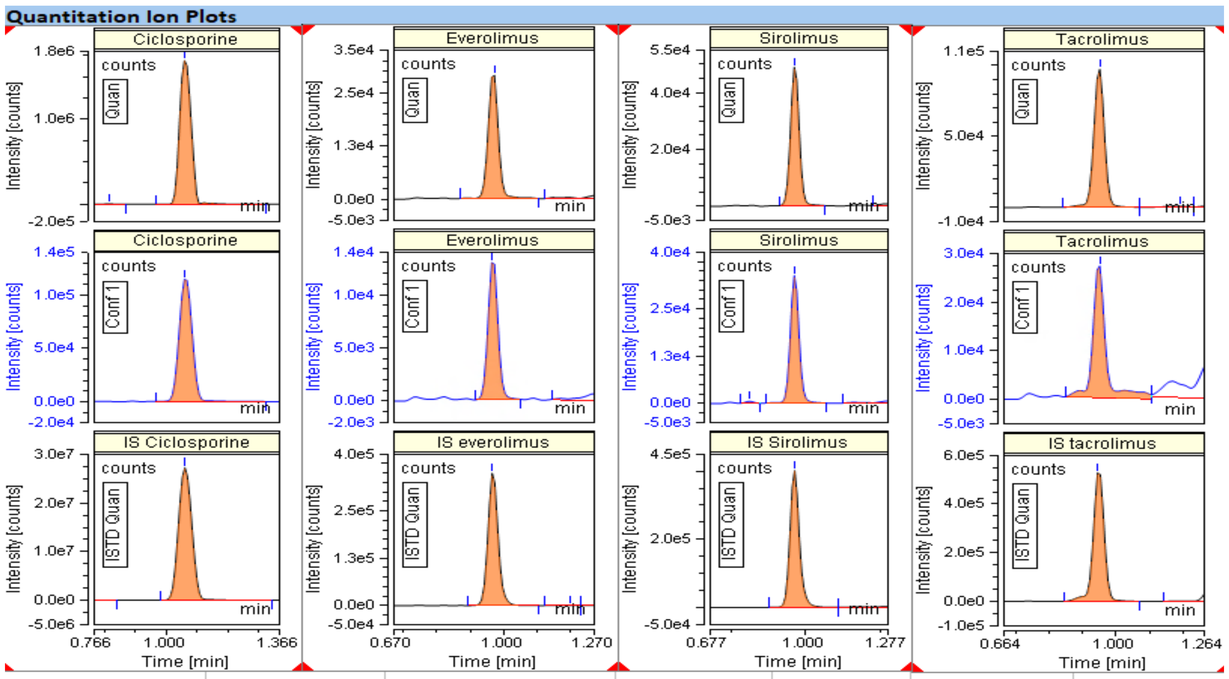
Immunosuppressants are critical agents used to modulate the immune system, primarily to prevent organ transplant rejection and to manage autoimmune diseases. Precise quantification of immunosuppressant drugs is essential for optimizing therapeutic efficacy and minimizing toxicity, as these agents often have narrow therapeutic windows and significant inter-individual variability in pharmacokinetics. Accurate measurement of immunosuppressant levels typically requires analysis of whole blood samples, which present unique challenges due to the complexity and variability of the matrix[1]. Automated sample preparation and analysis workflows have become increasingly important[2], enabling high-throughput, standardized, and reproducible processing of whole blood specimens. These advances help ensure reliable monitoring of immunosuppressant concentrations in research settings.

OBJECTIVE.

This study aimed to evaluate the sample preparation and analysis of four commonly used immunosuppressants, namely Tacrolimus, Everolimus, Sirolimus and Cyclosporine A, by automation compared to manual sample preparation. The focus was on evaluating improvements in sample tracking, processing efficiency, and resource consumption compared to conventional methods, and determining the suitability of the automated workflow for routine laboratory applications in immunosuppressant investigation.

METHODS.

Sample preparation followed the Tecan LC-MS Kit Ref # 30261557 / 30261558 (For Research use only. Not for use in diagnostic procedures. Distributed by Tecan, IBL International GmbH). Hereby, whole blood samples were being homogenized as well as extracted with a zinc sulphate and a deproteinization solution containing isotopically labeled internal standards of each of the analytes. Six calibrators and quality control samples at three concentration levels, within the expected physiological range, were reconstituted from a lyophilized whole blood matrix and treated as samples thereafter. Subsequently, the supernatant of these extracts was injected into an LC-MS/MS system consisting of a Thermo Scientific Vanquish UHPLC and a TSQ Quantis mass spectrometer operated by Chromeleon CDS. For the automated procedure, a Tecan Fluent® 780 liquid handler equipped with a robotic gripper arm, as well as an 8-channel pipetting arm with piercing tips, was utilized. The liquid handler was furthermore equipped with a 4-slot plate centrifuge, four orbital shakers and two tube rotators with integrated barcode scanners. Total durations of manual as well as automated sample preparation were taken. Furthermore, the analytical performance of both procedures and their respective environmental impact was evaluated. Precision was determined by 30-fold work-ups of all three QC levels, while contamination experiments were done by blank workups and injections, which followed the work-up and injections of the highest calibrator.



Component Type	Component Name	Retention Time min	Area counts*min	Amount	Peak Modified?
Analyte	Ciclosporine	1.06	71668	104.55	No
ISTD	IS Ciclosporine	1.06	1345637	1.00	No
Analyte	Everolimus	0.97	1026	3.83	No
ISTD	IS everolimus	0.96	12186	1.00	No
Analyte	Sirolimus	0.96	16390	5.46	No
ISTD	IS Sirolimus	0.96	14580	1.00	No
Analyte	Tacrolimus	0.95	3527	5.51	No
ISTD	IS tacrolimus	0.95	20008	1.00	No

Figure 1. Chromatograms displaying peak shapes of the MRM transitions of all target analytes, including their respective quantifier, qualifier, and internal standard for calibrator 3.

Disclaimer: The combined use of reagents, process script and instrument has to be validated individually on site by each laboratory.

RESULTS.

Time Requirements and Key Differences

In a five-plate scenario, the automated sample preparation workflow required approximately 42 minutes per plate, while the manual workflow took 67 minutes per wellplate. Both workflows included steps such as sample scanning, reagent and sample distribution, centrifugation, supernatant transfer, and plate transfers. However, the manual workflow required additional time for manual tube handling (19 minutes for de-capping and re-capping), a step that was omitted in the automated workflow due to the piercing capability of the sample tubes and pipette tips. This key difference contributed most to the overall time savings and increased efficiency observed with automation.

Table 1: Precision of Cyclosporine A, Everolimus, Sirolimus, and Tacrolimus tested in a 30-fold determination.

Precision	CV% QC Level I	CV% QC Level II	CV% QC Level III
Cyclosporine A	3.3	3.6	3.5
Everolimus	5	4	4.4
Sirolimus	6.1	4.8	4.3
Tacrolimus	4.2	3.4	3.6

Calibration and Controls

Calibration curves for both the manual and automated workflows were linear, with R^2 values greater than 0.99 (see Table 2 and Figure 2), and all controls were within their target ranges.

Precision and Cross-Contamination

The coefficients of variation for all analytes were below 10% (see Table 1), and no signs of cross-contamination were detected (<0.3%).

Linearity Range

Linearity was confirmed up to at least 2500 µg/l for Cyclosporine A, 200 µg/l for Everolimus, 200 µg/l for Sirolimus, and 100 µg/l for Tacrolimus (see Table 2).

Table 2: Linearity of Cyclosporine A, Everolimus, Sirolimus, and Tacrolimus.

Linearity	Linear up to at least	R^2
Cyclosporine A	> 2500µg/l	0.9975
Everolimus	> 200µg/l	0.9961
Sirolimus	> 200µg/l	0.9921
Tacrolimus	> 100µg/l	0.9982

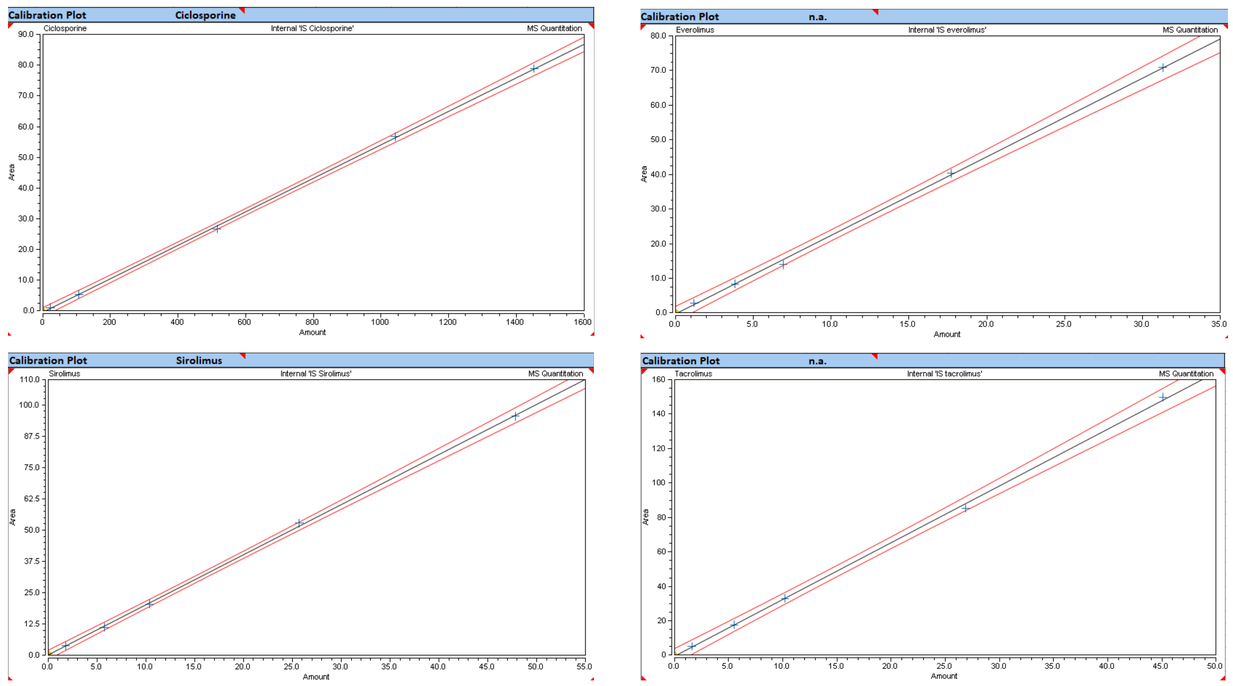


Figure 2: Calibration curves of Cyclosporine A, Everolimus, Sirolimus and Tacrolimus.

CONCLUSION.

Automation proved to be a robust and valuable enhancement of immunosuppressant LC-MS workflows. Sample prep time and, most importantly, staff occupation has been shown to be reduced significantly compared to a manual workflow. While pipetting steps themselves did not necessarily show significant differences in duration, especially the automated tube rotation in combination with cap piercing and automated barcode scanning had an immense impact on the overall workflow. With its analytical performance and its low environmental impact, the automated workflow should be the preferred fit for a sustainable and state-of-the-art laboratory environment.

1. Thomas M Annesley, Larry Clayton, Simple Extraction Protocol for Analysis of Immunosuppressant Drugs in Whole Blood, Clinical Chemistry, Volume 50, Issue 10, 1 October 2004, Pages 1845–1848, https://doi.org/10.1373/clinchem.2004.037416

2. More, D., Khan, N., Tekade, R. K., & Sengupta, P. (2024). An Update on Current Trend in Sample Preparation Automation in Bioanalysis: strategies, Challenges and Future Direction. Critical Reviews in Analytical Chemistry, 1–25. https://doi.org/10.1080/10408347.2024.2362707