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Development and validation of an LC-MS/MS method for highly concentrated tacrolimus and cyclosporine samples prepared from pharmaceutical products to assess drug loss from feeding tubes

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ABSTRACT

Introduction: Tacrolimus and cyclosporine are common immunosuppressants utilized post-organ transplantation to manage allograft rejection. Both have narrow therapeutic indices and are frequently measured to support dose adjustments. Although nasogastric tubes are commonly used to provide nutritional support and serve as a route for immunosuppressant administration, they were never validated for such purposes.
Objective: To develop and validate a liquid chromatography – tandem mass spectrometry (LC-MS/MS) method for highly concentrated tacrolimus and cyclosporine samples prepared from pharmaceutical products to support the validation of feeding tube administration of these immunosuppressants.
Methods: The method involved stepwise dilutions with dimethyl sulfoxide before analysis using online sample preparation and LC-MS/MS. It was validated in a CLIA-certified clinical laboratory that measures immunosuppressants by LC-MS/MS and is designed to support clinical studies evaluating drug loss from feeding tubes.
Results: The method was linear between 6.8 μg/mL and 75 μg/mL for tacrolimus, and between 0.9 mg/mL and 10 mg/mL for cyclosporine, with $r^2 > 0.99$ and total precision <5 % at all QC levels. The method demonstrated good recovery using cyclosporine Certified Reference Material, tacrolimus European Pharmacopeia Reference Standard, and prepared pharmaceutical products. Minimal matrix effects were observed.
Conclusion: An analytical method was developed and validated for *in vitro* studies with simulated administration of tacrolimus or cyclosporine to assess loss during drug administration using feeding tubes.

1. Introduction

First described in 1921, nasogastric (NG) tubes have been extensively used for the last century in surgery, acute or chronic care, and management of nutrient intake in critically ill patients [1,2]. Inserted through the nose to pass through the pharyngeal cavity and reach the stomach, this procedure aids in stomach decompression and allows for the administration of nutrients or medications to individuals with poor voluntary oral intake [3]. The use of an NG tube for nutritional support is critical for post-operative recovery in individuals who have undergone major surgeries, such as organ transplantation. In one study, the incidence of bacterial infection was significantly reduced in liver transplant patients who have received NG feeding compared with the control group [4].

Tacrolimus and cyclosporine are immunosuppressants commonly utilized in post-organ transplantation to treat or reduce the incidence of allograft rejection [5]. Since their approval by the Food and Drug Administration (FDA), both immunosuppressants have been prescribed for numerous recipients after liver, intestinal, kidney, and heart transplantation, further demonstrating their clinical utility and success [6,7]. Both drugs act as calcineurin inhibitors that regulate T-cell activation [8].

Both tacrolimus and cyclosporine have narrow therapeutic indices, and CLIA-certified clinical laboratories in many major medical centers, including our institution, measure their concentrations in blood for therapeutic drug monitoring [9–12]. Although these

Abbreviations: AMR, analytical measurement range; DMSO, dimethyl sulfoxide; ISTD, internal standard; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MRM, Multiple Reaction Monitoring; NG tube, nasogastric tube; QC, quality control; SPLC, sample preparation and liquid chromatography.

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immunosuppressants can be administered both orally and intravenously, oral intake may not be feasible for patients experiencing oral aversion or difficulty swallowing, making NG tubes an alternative route for immunosuppressant administration [1,3,13,14]. However, additional primary studies are needed to support NG tube administration of tacrolimus or cyclosporine to address concerns regarding drug loss, which may occur due to adsorption (i.e., where the drug adheres to the inner surface of feeding tubes), absorption (i.e., where the drug dissolves into the material of feeding tubes), or both [15].

Here, we describe the development and validation of a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantitative analysis of highly concentrated tacrolimus and cyclosporine samples prepared from pharmaceutical products. This method is intended for *in vitro* studies with simulated administration of these immunosuppressants to assess drug loss from feeding tubes.

2. Materials and methods

1.1. Chemicals and materials

NEORAL® (cyclosporine) oral solution (100 mg/mL) was purchased from Novartis (Basel, Switzerland). PROGRAF® (tacrolimus) capsules (5 mg/capsule) were purchased from Sandoz Pharmaceutical Company (Basel, Switzerland). ORA-Plus® suspending vehicle was purchased from Perrigo Company (Dublin, Ireland). Simple syrup was purchased from Camber Pharmaceuticals (Piscataway, NJ). 6PLUS1® multilevel immunosuppressants calibrator set was purchased from Chromsystems (Munich, Germany). Dimethyl sulfoxide (DMSO), tacrolimus monohydrate (European Pharmacopoeia Reference Standard), and cyclosporine (Certified Reference Material) were purchased from Sigma-Aldrich (St. Louis, MO). Tacrolimus-^{13}C, D_2 was purchased from Toronto Research Chemicals (Ontario, Canada). Cyclosporine-D_{12} was purchased from Alsachim (Illkirch, France). Ammonium hydroxide, zinc sulfate heptahydrate, Optima™ acetone, LC-MS grade formic acid, methanol, and water were purchased from Fischer Scientific (Waltham, MA).

1.2. Diluted clinical pharmaceutical products

Tacrolimus suspensions were prepared by the Department of Pharmacy at Children's Hospital Los Angeles. Stock tacrolimus suspensions with a target concentration of 500 μg/mL were prepared by suspending contents from PROGRAF® (tacrolimus) capsules with a solution that contains equal volumes of ORA-Plus® suspending vehicle and simple syrup. Final tacrolimus suspensions for accuracy assessments were prepared by diluting the stock tacrolimus suspension with water to a target concentration of 45 μg/mL. Stock cyclosporine solutions with a target concentration of 5 mg/mL were prepared by diluting the NEORAL® (cyclosporine) oral solution with water. Final cyclosporine solutions for accuracy assessments were prepared by diluting the stock cyclosporine solution with water to a target concentration of 2.5 mg/mL.

1.3. In-house prepared calibrator set, quality control materials, and internal standard

A tacrolimus stock solution at 2 mg/mL was prepared by dissolving the tacrolimus European Pharmacopoeia Reference Standard in DMSO. A cyclosporine stock solution at 20 mg/mL was prepared by dissolving the cyclosporine Certified Reference Material in DMSO. Those stock solutions were combined and diluted with DMSO to prepare a calibrator stock solution that contains tacrolimus at 100 μg/mL and cyclosporine at 10 mg/mL. An in-house calibrator set that contains both tacrolimus and cyclosporine was prepared by diluting the calibrator stock solution with DMSO to six different concentrations. Concentrations of tacrolimus in the calibrator set were: 10, 20, 40, 50, 60, and 80 μg/mL; concentrations of cyclosporine in the calibrator set were: 1, 2, 4, 5, 6, and 8 mg/mL. Three quality control (QC) materials were prepared by diluting a separately prepared calibrator stock solution with DMSO to three different concentrations. Concentrations of tacrolimus in the QC materials were: 12.5, 37.5, and 75 μg/mL; concentrations of cyclosporine in the QC materials were: 1.25, 3.75, and 7.5 mg/mL.

The internal standard (ISTD) was a methanolic solution that contained tacrolimus-^{13}C, D_2 at 2.3 ng/mL, cyclosporine-D_{12} at 23 ng/mL, and zinc sulfate at 0.04 M.

1.4. Sample preparation

The in-house prepared calibrator set, QC materials, and highly concentrated tacrolimus and cyclosporine samples prepared from pharmaceutical products were prepared by stepwise dilution with DMSO in 1.5 mL microcentrifuge tubes. A 1:2,000 dilution was performed for the measurement of tacrolimus. A 1:20,000 dilution was performed for the measurement of cyclosporine. 50 μL of the final diluted specimens and the 6PLUS1® calibrators were vortexed with 200 μL of the ISTD solution at the highest speed on a multi-tube vortexer (Thermo Fisher Scientific, Waltham, MA) for 10 min. After centrifugation at 8000×*g* for 10 min, the supernatant was transferred to an autosampler for LC-MS/MS analysis. QC materials were prepared and measured on each day of sample preparation.

1.5. LC-MS/MS conditions

20 μL of each prepared sample were injected and analyzed using a Prelude sample preparation and liquid chromatography (SPLC) system (Thermo Fisher Scientific, Waltham, MA) coupled with a TSQ Quantiva tandem mass spectrometry system (Thermo Fisher Scientific, Waltham, MA) using the same method as described previously [16].

Briefly, chromatography was performed using a Cyclone-P Turbo-Flow column (0.5 x 50 mm) at 30C° for online extractions and a Accucore C8 column (2.6 mm, 30 x 3 mm) at 70C° for final separation. Mobile phase A consisted of 10 mM ammonium formate and 0.05 % formic acid in water; mobile phase B consisted of 10 mM ammonium formate and 0.05 % formic acid in methanol; mobile phase C consisted of acetonitrile, isopropanol, and acetone (45 %, 45 %, 10 %, vol/vol). The SPLC method can be found in Supplemental Table 2.

Quantification was performed using Multiple Reaction Monitoring (MRM) in positive ionization mode to monitor tacrolimus (*m/z* 821.5/768.5) and cyclosporine (*m/z* 1203.0/425.3), as well as tacrolimus-^{13}C, D_2 (*m/z* 824.4/771.0) and cyclosporine- D_{12} (*m/z* 1214.9/437.4) as internal standards.

Calibration curves were generated using TraceFinder software (Thermo Fisher Scientific, Waltham, MA) with 1/x weighting.

1.6. Validation procedures

The analytical measurement range (AMR) was determined by measuring six levels of AMR materials prepared by spiking various volumes of the tacrolimus stock solution and the cyclosporine stock solution into DMSO. All six levels of AMR materials were measured on the same day in duplicate.

Accuracy assessment was performed by 1) measuring QC and AMR materials, and 2) measuring final tacrolimus suspensions and final cyclosporine solutions prepared from pharmaceutical products and comparing measured concentrations with theoretical concentrations. Tacrolimus results were generated using the 6PLUS1® calibrator set. Cyclosporine results were generated by one of three approaches: 1) using the 6PLUS1® calibrator set; 2) using the in-house prepared calibrator set; or, 3) using the 6PLUS1® calibrator set corrected by a batch-specific correction factor that is calculated as below:

$$\textit{Correction factor} = \textit{average \% difference of measured and theoretical concentrations of the in} - \textit{house prepared QC materials (three levels)}$$

Precision studies were performed using the in-house prepared QC materials: results for tacrolimus were calculated using the 6PLUS1® calibrator set; results for cyclosporine were calculated using the 6PLUS1® calibrator set with the batch-specific correction factor. Repeatability (intra-day precision) was assessed by preparing and measuring each QC level (n = 20) on the same day. Reproducibility (inter-day precision) was assessed by preparing and measuring three levels of QC materials on 22 different days over three months. Carryover was assessed by alternating quadruplicate injections of prepared high and low QC materials for a total of 16 injections.

Matrix effects were assessed by matrix dilution studies and by post-column infusion studies. For the matrix dilution study, final tacrolimus suspensions (n = 2) and final cyclosporine solutions (n = 2) were diluted with DMSO to 100 %:0%, 80 %:20 %, 60 %:40 %, 40 %:60 %, and 20 %:80 % (final sample: DMSO, *v/v*) before sample preparation and measurement. ISTD responses, recoveries, and %CV were calculated. A post-column infusion study was performed using a protocol modified from [17], with tacrolimus-^{13}C, D_2 and cyclosporine-D_{12} infused and monitored as surrogates for tacrolimus and cyclosporine, since tacrolimus- and cyclosporine-free pharmaceutical products were not available. Post-column infusion was performed with the infusion of an ISTD mixture (100 ng/mL tacrolimus-^{13}C, D_2 and 1,000 ng/mL cyclosporine-D_{12} in methanol) at 3 μL/min via a T-joint after the column, with the ISTD signal intensities being monitored and compared during successive injections of (i) methanol, (ii) prepared final tacrolimus suspensions, and (iii) prepared final cyclosporine solutions using a surrogate ISTD solution without isotope-labeled ISTD.

3. Results

3.1. Chromatogram

Despite high DMSO concentration in samples prepared from highly concentrated pharmaceutical products, comparable chromatograms with the 6PLUS1® calibrator were obtained (Fig. 1). No interfering peak was observed in samples prepared from pharmaceutical products (Fig. 1).

3.2. Accuracy

Accuracy was assessed by measuring AMR materials and three levels of in-house prepared QC materials, and by comparing measured concentrations with theoretical concentrations. Measured with the 6PLUS1® calibrator set, AMR materials demonstrated acceptable recovery for tacrolimus with a mean recovery of 103 % and an over-recovery for cyclosporine with a mean recovery of 114 % (Supplemental Table 1). Acceptable recovery for tacrolimus and over-recovery for cyclosporine were also observed when measuring in-house prepared QC materials for the precision studies (Table 1): a

Table 1
Results from accuracy assessment by measuring QC materials from the inter-day precision study. Measurements of cyclosporine were performed using the 6PLUS1® calibrator Set, the in-house calibrator Set, and the 6PLUS1® calibrator Set with the batch-specific correction factor.

Tacrolimus (6PLUS1® Calibrator Set)

	Theoretical Conc. (μg/mL)	Mean Measured Conc. (μg/mL)	Mean Recovery (%)	SD Recovery (%)
QC1	12.5	13.3	106	4
QC2	37.5	39.2	105	4
QC3	75.0	76.6	102	3

Cyclosporine (6PLUS1® Calibrator Set)

	Theoretical Conc. (mg/mL)	Mean Measured Conc. (mg/mL)	Mean Recovery (%)	SD Recovery (%)
QC1	1.25	1.47	118	5
QC2	3.75	4.41	118	6
QC3	7.50	8.80	117	7

Cyclosporine (In-House Calibrator Set)

	Theoretical Conc. (mg/mL)	Mean Measured Conc. (mg/mL)	Mean Recovery (%)	SD Recovery (%)
QC1	1.25	1.27	102	4
QC2	3.75	3.76	100	3
QC3	7.50	7.47	100	2

Cyclosporine (6PLUS1® Calibrator Set with Correction Factor)

	Theoretical Conc. (mg/mL)	Mean Measured Conc. (mg/mL)	Mean Recovery (%)	SD Recovery (%)
QC1	1.25	1.21	97	3
QC2	3.75	3.63	97	3
QC3	7.50	7.25	97	3

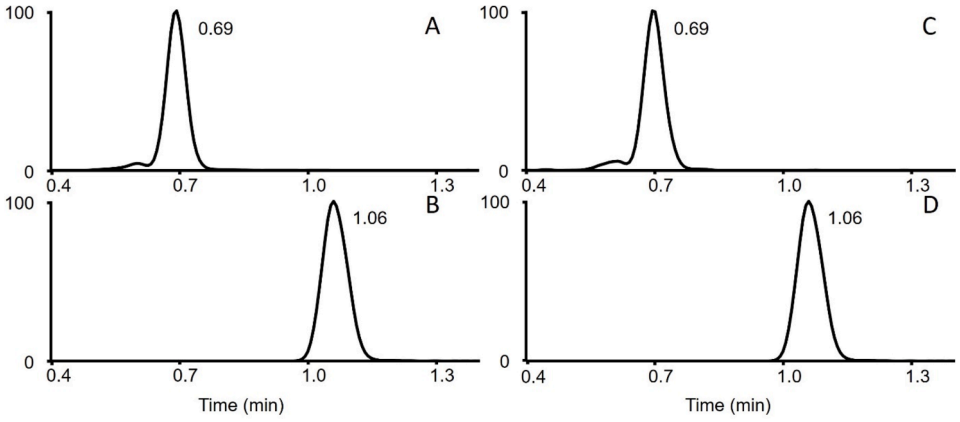

Fig. 1. Chromatograms from (A) the final tacrolimus suspension and (B) the final cyclosporine solutions prepared from pharmaceutical products, as well as the chromatograms of the lowest calibrator from the 6PLUS1® Calibrator Set for (C) tacrolimus and (D) cyclosporine.

mean recovery of 104 % was observed for tacrolimus, and a mean recovery of 118 % was observed for cyclosporine during the inter-day precision study. The mean recoveries for QC 1, 2, and 3 for cyclosporine were 118 %, 118 %, and 117 %, respectively (Table 1). The standard deviations for recoveries for the three levels of QC materials were similar (Table 1). The over-recovery for cyclosporine with the AMR materials and QC materials indicates a proportional bias when directly measured with the 6PLUS1® calibrator set.

To investigate the over-recovery issue of cyclosporine, an in-house calibrator set was prepared using cyclosporine Certified Reference Material. Results from the in-house prepared calibrator demonstrated an acceptable mean recovery of 100 % for QC materials (Table 1) and an acceptable mean recovery of 104 % for AMR materials (Supplemental Table 1). Given the proportional bias for cyclosporine when measured with the 6PLUS1® calibrator set, we proposed to correct the measured results with a batch-specific correction factor calculated from the measured results of all three levels of QC materials, allowing samples from pharmaceutical products to be batched together with clinical samples using the 6PLUS1® calibrator set. An acceptable mean recovery of 97 % from the QC materials and a mean recovery of 97 % from the AMR materials were observed using the correction factor (Table 1 and Supplemental Table 1).

Accuracy was also assessed by measuring pharmaceutical products (Table 2). Measurements of final tacrolimus suspensions (n = 3) yielded an acceptable mean recovery of 100 %. Final cyclosporine solutions were analyzed using the 6PLUS1® calibrator set, the in-house prepared calibrator set, and the 6PLUS1® calibrator set with the batch-specific correction factor, with mean recoveries of 119 %, 103 %, and 103 %, respectively (Table 2). Since the results from the 6PLUS1® calibrator set using a correction factor showed acceptable recovery, the remaining validation studies were continued using the 6PLUS1® calibrator set with the correcting factor.

3.3. Precision and carryover

In-house prepared QC materials were used to assess the precision of the method (Table 3). The intra-day coefficient of variations (CVs) ranged from 0.9 % to 1.5 % for tacrolimus and from 1.1 % to 2.4 % for cyclosporine (Table 3). The inter-day CVs ranged from 3.4 % to 4.1 % for tacrolimus and from 2.8 % to 3.3 % for cyclosporine (Table 3). No carryover effect was observed (data not shown).

3.4. Analytical measuring range

Six samples prepared with tacrolimus European Pharmacopoeia Reference Standard and cyclosporine Certified Reference Material at varying concentrations were analyzed with duplicate injections.

Table 3
Results from precision studies (inter-day and intra-day). Measurements of cyclosporine were performed using the 6PLUS1® calibrator set with the batch-specific correction factor.

Tacrolimus					
		Intra-day Precision (n = 20)		Inter-day Precision (n = 22)	
	Theoretical Conc. (μg/mL)	Mean (μg/mL)	CV (%)	Mean (μg/mL)	CV (%)
QC1	12.5	14.2	1.4	13.3	3.8
QC2	37.5	41.3	1.5	39.2	4.1
QC3	75.0	79.8	0.9	76.6	3.4
Cyclosporine					
		Intra-day Precision (n = 20)		Inter-day Precision (n = 22)	
	Theoretical Conc. (mg/mL)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)
QC1	1.25	1.25	2.4	1.21	3.3
QC2	3.75	3.65	1.4	3.63	2.8
QC3	7.50	7.27	1.1	7.25	2.9

Linearity was observed for both tacrolimus and cyclosporine, with R^2 values of 0.997 and 0.999, respectively (Fig. 2). The AMR for tacrolimus was determined to be 6.8 – 75 μg/mL using the 6PLUS1® calibrator set. The AMR for cyclosporine was determined to be 0.9 – 10 mg/mL using the 6PLUS1® calibrator set with the batch-specific correction factor.

3.5. Matrix effect

Matrix effects were assessed through matrix dilution studies and post-column infusion studies. The matrix dilution study involved diluting final tacrolimus suspensions (n = 2) and final cyclosporine solutions (n = 2) with increasing volumes of DMSO. Recoveries from tacrolimus ISTD ranged from 82 % to 111 %, while recoveries from cyclosporine ranged from 94 % to 104 % (Table 4).

Post-column infusion was conducted to evaluate ion suppression or enhancement from components of tacrolimus and cyclosporine pharmaceutical products [17,18]. No significant differences in signal intensity were identified at the expected retention time between the injection of methanol (blank) and the injection of prepared final tacrolimus suspensions prepared using a surrogate ISTD solution without isotope-labeled ISTD, indicating no significant ion suppression or enhancement from the formulation of the PROGRAF® (tacrolimus) capsules (Fig. 3A). Similarly, no significant ion suppression or enhancement was observed from the formulation of the NEORAL® (cyclosporine) oral solution (Fig. 3B).

Table 2
Results from accuracy assessment by measuring tacrolimus suspensions (n = 3) and cyclosporine solutions prepared from pharmaceutical products (n = 3). Measurements of cyclosporine were performed using the 6PLUS1® calibrator Set, the in-house calibrator Set, and the 6PLUS1® calibrator set with the batch-specific correction factor.

Tacrolimus		
Theoretical Conc. (μg/mL)	Measured Conc. (μg/mL)	Recovery (%)
45.45	43.53	96 %
45.45	46.26	102 %
45.45	46.41	102 %

Cyclosporine						
	6PLUS1® Calibrator Set		In-House Calibrator Set		6PLUS1® Calibrator Set with Correction Factor	
Theoretical Conc. (mg/mL)	Measured Conc. (mg/mL)	Recovery (%)	Measured Conc. (mg/mL)	Recovery (%)	Measured Conc. (mg/mL)	Recovery (%)
2.50	2.98	119 %	2.60	104 %	2.58	103 %
2.50	2.95	118 %	2.50	100 %	2.57	103 %
2.50	2.99	120 %	2.60	104 %	2.59	104 %

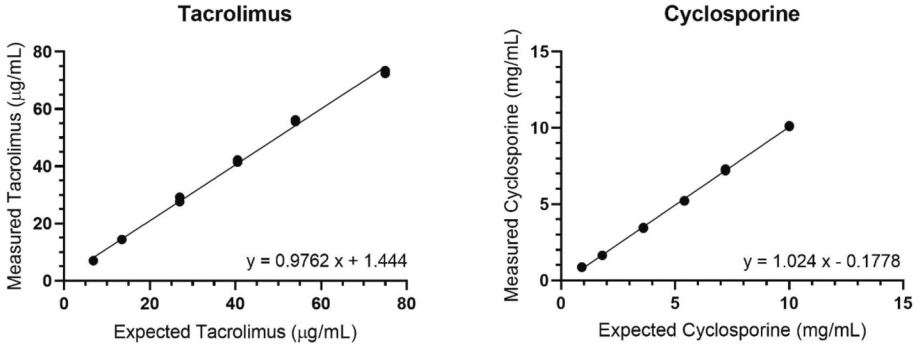

Fig. 2. Results from AMR assessment.

Table 4
Results from the matrix dilution study. Final tacrolimus suspensions (n = 2) and final cyclosporine solutions (n = 2) were diluted to five different ratios before sample preparation and measurements. ISTD responses were documented, with mean responses and %CV calculated from ISTD responses from all five matrix diluted samples, and recovery calculated from each matrix diluted sample.

Final Tacrolimus Suspensions

	Specimen 1		Specimen 2	
Final specimen: DMSO, *v/v*	ISTD Responses	Recovery (%)	ISTD Responses	Recovery (%)
100 %: 0 %	1,242,149	104 %	1,207,009	111 %
80 %: 20 %	1,168,084	98 %	1,174,578	108 %
60 %: 40 %	1,120,920	94 %	893,040	82 %
40 %: 60 %	1,320,862	110 %	1,190,021	110 %
20 %: 80 %	1,135,763	95 %	959,006	88 %
Mean	1,197,556		1,084,731	
%CV	7 %		14 %	

Final Cyclosporine Solutions

	Specimen 1		Specimen 2	
Final specimen: DMSO, *v/v*	ISTD Responses	Recovery (%)	ISTD Responses	Recovery (%)
100 %: 0 %	1,439,684	96 %	1,247,495	94 %
80 %: 20 %	1,550,269	103 %	1,344,707	101 %
60 %: 40 %	1,460,516	97 %	1,308,104	99 %
40 %: 60 %	1,545,127	103 %	1,341,778	101 %
20 %: 80 %	1,526,538	101 %	1,384,536	104 %
Mean	1,504,427		1,325,324	
%CV	3 %		4 %	

4. Discussion

Tacrolimus and cyclosporine are commonly used immunosuppressants for managing patients post- transplantation, and both have narrow therapeutic indices [11,12]. Although tacrolimus and cyclosporine have previously been administered via NG tubes, there is an unmet need to validate NG tubes for immunosuppressant administration with a method that measures them at much higher concentrations than their concentrations in the blood [3,15]. Paired with *in vitro* studies simulating the administration of these immunosuppressants, the method described herein could measure concentrations prior to and/or after dose administration through NG tubes to assess drug loss.

Additionally, this method leverages the existing workflow within the CLIA-certified laboratory at Children's Hospital Los Angeles that measures tacrolimus and cyclosporine in the blood. While tacrolimus demonstrated acceptable recoveries with the commercial 6PLUS1® calibrator set, there was a positive proportional bias for cyclosporine, verified with samples prepared from Certified Reference Material and cyclosporine solutions prepared from pharmaceutical products. Acceptable recoveries were obtained for cyclosporine using in-house prepared calibrators made from the cyclosporine Certified Reference Material, suggesting that the over-recovery was related to the use of the commercial 6PLUS1® calibrator set, although the cyclosporine from the 6PLUS1® calibrator set is traceable to Certified Reference Material, according to the package insert.

Since the 6PLUS1® calibrator set was not subjected to multiple dilutions with DMSO during sample preparation, one suspected source of the bias is the evaporation of DMSO during preparation for highly concentrated cyclosporine samples. However, DMSO was also used during sample preparation for highly concentrated tacrolimus samples, with no significant bias observed. Therefore, we believe the over-

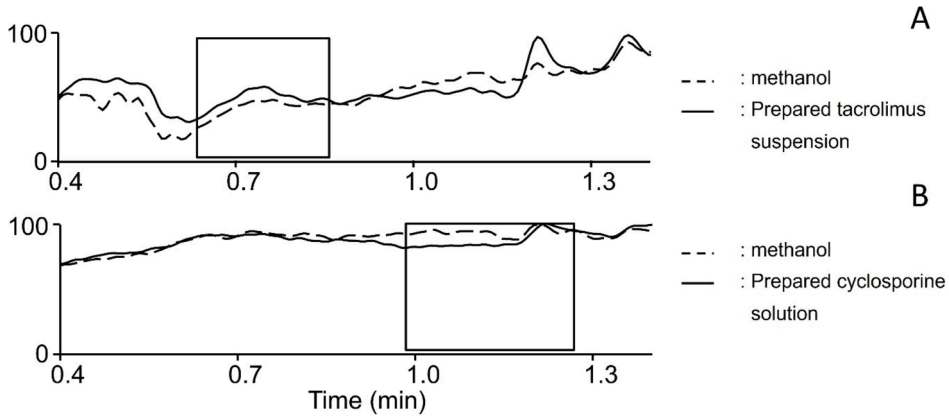

Fig. 3. Post-column infusion analysis with injected methanol (dashed lines) and final tacrolimus suspension (A) or prepared cyclosporine solution (B) prepared using a surrogate ISTD solution without isotope-labeled ISTD (solid lines). Isotope-labeled ISTD was infused and was used as a surrogate for each analyte. The highlighted regions represent where tacrolimus and cyclosporine are eluted.

recovery of cyclosporine cannot be attributed to the evaporation of DMSO. Another possibility for the over-recovery of cyclosporine is the presence of proprietary additives in the 6PLUS1® calibrator set, possibly used to extend shelf life, although minimal matrix effect was observed (Fig. 3 and Supplemental Table 1). This demonstrates the importance of using matrix-matched calibrators.

We performed a comprehensive study to understand the nature of the over-recovery. Since a proportional bias was demonstrated, we explored the possibility of using the 6PLUS1® calibrator set and correcting results with a batch-specific correction factor. Validation results demonstrated acceptable recoveries comparable to those obtained using the in-house prepared calibrator set: a mean recovery of 97 % was observed from the inter-day precision study using the correction factor, while a mean recovery of 100 % was recorded using the in-house prepared calibrator set.

Similarly, results using the correction factor were also acceptable when measuring the final cyclosporine solutions prepared from the NEORAL® (cyclosporine) oral solution. Consequently, we continued the validation studies using the 6PLUS1® calibrator set with a correcting factor. Although all three levels of QC materials were used to generate the batch-specific correction factor, theoretically, a proportional bias could be fully corrected using a correction factor calculated from only one level of QC material. However, since QC material with different tacrolimus and cyclosporine concentrations will be measured for each batch of sample analysis, we chose to calculate the correction factor using three levels of QC materials to account for the measuring range of the assay.

This method provides a solution for *in vitro* investigation of the loss of tacrolimus and cyclosporine when administered through NG tubes or other feeding tubes. Starting from PROGRAF® (tacrolimus) capsules, tacrolimus is initially prepared into a suspension of 500 μg/mL at the Department of Pharmacy in our hospital. In a simulated drug administration study, the suspension is further diluted before measurements. Based on discussions with collaborators who designed the study to evaluate drug loss from NG tubes, the AMR of our method (i.e., 6.8–75 μg/mL for tacrolimus) is appropriate. Likewise, although the cyclosporine concentration in the NEORAL® oral solution is 100 mg/mL, the AMR of our method (i.e., 0.9–10 mg/mL) is appropriate. If needed, our method can be modified to include fewer or more dilution steps to provide wider measurement ranges.

5. Conclusion

We have developed and validated a method for highly concentrated tacrolimus and cyclosporine samples prepared from pharmaceutical products in a CLIA-certified clinical laboratory. The method is designed to support *in vitro* studies with simulated administration of tacrolimus or cyclosporine to assess drug loss from feeding tubes and provide supporting evidence for immunosuppressant administration through feeding tubes for individuals with poor voluntary oral intake.

Ethics statement

This study did not use any human patient samples or specimens.

CRediT authorship contribution statement

Yi Xiao: Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Mari Ishak Gabra:** Writing – review & editing, Writing – original draft. **Edward Leung:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmsacl.2024.10.002.

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