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Published in:
Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences

DOI:
10.1016/j.jchromb.2023.123709

Journal of Chromatography B 1222 (2023) 123709

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Expeditious quantification of plasma tacrolimus with liquid chromatography tandem mass spectrometry in solid organ transplantation

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ARTICLE INFO

Keywords:
Tacrolimus
Transplant
LC-MS/MS
Bioanalysis
Alternative matrices

ABSTRACT

Traditionally, tacrolimus is assessed in whole blood samples, but this is suboptimal from the perspective that erythrocyte-bound tacrolimus is not a good representative of the active fraction. In this work, a straightforward and rapid method was developed for determination of plasma tacrolimus in solid organ transplant recipients, using liquid chromatography tandem mass spectrometry (LC-MS/MS) with heated electrospray ionisation. Sample preparation was performed through protein precipitation of 200 µl plasma with 500 µl stable isotopically labelled tacrolimus I.S. in methanol, where 20 µl was injected on the LC-MS/MS system. Separation was done using a chromatographic gradient on a C18 column (50 × 2.1 mm, 2.6 µm). The method was linear in the concentration range 0.05–5.00 µg/L, with within-run and between-run precision in the range 2–6 % and a run time of 1.5 min. Furthermore, the method was validated for selectivity, sensitivity, carry-over, accuracy and precision, process efficiency, recovery, matrix effect, and stability following EMA and FDA guidelines. Clinical validation was performed in 2333 samples from 1325 solid organ transplant recipients using tacrolimus (liver n = 312, kidney n = 1714, and lung n = 307), which had median plasma tacrolimus trough concentrations of 0.10 µg/L, 0.15 µg/L and 0.23 µg/L, respectively. This method is suitable for measurement of tacrolimus in plasma and will facilitate ongoing observational and prospective studies on the relationship of plasma tacrolimus concentrations with clinical outcomes.

1. Background

Tacrolimus is a macrolide lactone generally known for its use as immunosuppressant and largely applied to prevent graft rejection in solid organ transplant recipients. While a major part of the tacrolimus is bound to erythrocytes, its working mechanism is the inhibition of calcineurin in *T*-cells, that are located specifically in the plasma compartment [1,2]. Adverse effects to tacrolimus use include nephrotoxicity, neurotoxicity, diabetogenesis, and susceptibility for malignancies and infections [3]. Due to its small therapeutic window and high interindividual and intraindividual variability in its pharmacokinetics, tacrolimus is recommended for therapeutic drug monitoring (TDM) [2]. TDM is classically performed by means of whole blood tacrolimus levels, where different target ranges exist in matter of transplant type and time after transplantation.

The choice for using whole blood tacrolimus for TDM originates from the availability of the analytical methods in the 1990′s, when the drug was first approved. In that period, immunoassays (ELISA) were used for concentration measurements in both plasma and whole blood. While sensitivity of the ELISA was sufficient with lower limits of quantification of 0.1–0.5 µg/L, monoclonal antibodies showed cross-reactivity to tacrolimus metabolites with artificially higher measured drug concentrations [4,5]. Moreover, the analysis was time-consuming, since it took 24 h to produce results. The successively developed immunoassay (MEIA) was faster and could be completed within one hour, but was limited in specificity for plasma detection with a lower limit of

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https://doi.org/10.1016/j.jchromb.2023.123709
Received 31 January 2023; Received in revised form 24 March 2023; Accepted 3 April 2023
Available online 7 April 2023

quantification of 5 μg/L. Because of this technical limitation, whole blood became the most widely used matrix for tacrolimus TDM. However, the use of whole blood is currently debated by transplant professionals as this marker may not have a good correlation with transplant outcomes [6]. While a relationship was found between higher trough concentrations and toxicity [7,8], adverse events also occur in patients with blood concentrations within the therapeutic window. Therefore, other markers may be evaluated that give better reflections of effectivity and toxicity.

Tacrolimus as measured in plasma, in free unbound plasma fractions, or even in peripheral blood mononuclear cells or other specific cell selections may be good options to this end. Plasma contains both the protein-bound fraction and the free concentration, of which the prior is deemed constant [9,10]. Clinically, it would be more logical to measure tacrolimus in plasma or in the free fraction, because only free tacrolimus can cross cellular membranes, where it can either be pharmacologically active or cause adverse events [11]. Even more rational for its relevance would be the measurement of tacrolimus in its active compartment, being peripheral blood monoclonal cells or even *T*-cell selections, however, this may not give information on the risk for toxicity. [12–17]. Unfortunately, measuring free tacrolimus or *T*-cell concentrations require laborious techniques such as ultracentrifugation and gradient density centrifugation, which is hindering a high throughput of the samples and therefore cumbersome to implement in routine clinical practice. In contrast, it is relatively easy to measure plasma tacrolimus concentrations, due to the development of more sensitive LC-MS/MS equipment in recent years.

In this article, a newly developed and validated LC-MS/MS method is described for the quantification of tacrolimus in plasma with the use of an isotopically labelled internal standard. This method can be used for further investigation of the relationship between plasma tacrolimus concentrations and clinical outcomes.

2. Materials and methods

2.1. Chemicals and reagents

Tacrolimus was purchased from Cerilliant (Round Rock, TX, USA). Stable isotopically labelled (SIL) Tacrolimus [$^{13}C^{2}H_{4}$] was purchased from Alsachim (Illkirch-Graffenstaden, France). Analytical grade quality methanol was obtained from Biosolve (Valkenswaard, The Netherlands), and formic acid was obtained from Merck (Darmstadt, Germany). Ammonium formate was acquired from Thermo Fisher Scientific (Waltham, MA, USA). Ultrapure water was freshly prepared with a Milli-Q system (Millipore Corporation, Bedford, MA, USA). Pooled blank human plasma was made available per protocol of the University Medical Center Groningen (UMCG). For stability experiments in clinical samples, anonymous left-over material was used from routine care tacrolimus analyses. Plasma samples were used from participants of the TransplantLines Biobank and Cohort study (see section 2.6 'clinical validation' for further details).

2.2. Preparation of stocks, standards, and quality controls

All tacrolimus stock solutions had a concentration of 1000 mg/L in methanol. Working solutions of analytes of interest were prepared in various concentrations in methanol, and separate batches were prepared for calibration and quality control samples. All stock solutions and dilutions were stored at −20 °C. Calibration curves and quality control (QC) samples were freshly prepared in blank plasma. The calibration curve had concentrations of 0.050, 0.100, 0.250, 0.500, 1.00, 2.00, 4.00, and 5.00 μg/L. The separately prepared QC had concentrations of 0.050 μg/L (LLOQ), 0.100 μg/L (LOW), 2.00 μg/L (MED), and 4.00 μg/L (HIGH). Also, an over the curve (OTC) solution was prepared with a concentration of 10.0 μg/L, which was ten times diluted (DIL) in blank plasma before processing. In all preparations, the volume of working solution spiked to plasma was<5 % of the total volume. For the preparation of the internal standard (I.S.), stocks were diluted in methanol resulting in an I.S. solution with a concentration of 0.5 μg/L tacrolimus [$^{13}C^{2}H_{4}$].

2.3. Sample preparation

Samples were prepared by adding 500 μl of I.S. to an aliquot of 200 μl EDTA plasma in a 1.5 mL glass HPLC screw top vial (Fisher Scientific, Houston, Texas, USA). The mixture was vortexed for 1 min and subsequently placed in a −20 °C freezer for minimally 10 min for protein precipitation. After another minute of vortexing, the samples were centrifuged for 5 min at 10.000 *g*, after which 20 μl was automatically injected onto the LC-MS/MS system.

2.4. Equipment and conditions

Separation and detection were based on a previously validated method for immunosuppressants in whole blood [18]. The analysis was performed on a TSQ Quantiva LC-MS/MS, equipped with a Vanquish Horizon UPLC system with binary pump, autosampler and an Accucore C18 50 × 2.1 mm column with 2.6 μm solid-core particles. All instruments were from Thermo Fisher Scientific (Waltham, MA, USA).

Separation was performed using a gradient according to Table 1, with a constant flow of 1000 μl/min and a pressure upper limit of 1500 bar. The mobile phase consisted of ammonium formate buffer 0.02 M in ultrapure water (pH = 3.5) and methanol, where the concentration methanol was increased to elute the analyte of interest, with a maximum of 95 % methanol to flush the column. Preheater and column temperature was 60 °C. Total run time of the method was 1.5 min, with an injection-to-injection time of 2.3 min.

Detection was done in positive selected reaction monitoring (SRM) mode, with mass transitions shown in Table 2. Ionisation was acquired with heated-electrospray ionisation (H-ESI) with a positive static spray voltage of 4500 V. Arbitrary units for gasses were 50 for sheath gas, 25 for auxiliary gas and 0 for sweep gas. High-purity nitrogen was used for the source gas flows, while high-purity argon was used for the collision gas flows. Ion transfer tube temperature was 140 °C and the temperature at the vaporiser was 350 °C. Results were processed with Thermo Xcalibur QuanBrowser version 4.1.31.9 (Thermo Fisher Scientific, Waltham, MA, USA).

2.5. Analytical method validation

The method was validated according to the FDA's 'Guidance for Industry Bioanalytical Method Validation' and EMA's 'Guideline on bioanalytical method validation' [19,20]. Selectivity and sensitivity, carry-over, linearity, accuracy, and precision, process efficiency, recovery, matrix effect, and stability were assessed.

2.5.1. Selectivity, sensitivity, and carry-over

For selectivity, 6 individual blank plasma samples were analysed and

Table 1
Mobile phase gradient: A, Ammonium formate buffer 0.02 M (pH = 3.5) B, Methanol.

Time (min)	% Elution		Flow (μl/min)
	A	B	
0.00	70	30	1000
0.30	70	30	1000
0.31	27	73	1000
0.95	22	78	1000
0.96	5	95	1000
1.25	5	95	1000
1.26	70	30	1000
1.50	70	30	1000

Table 2
Mass spectrometer transitions.

Immunosuppressant	Retention time (min)	Collision energy (eV)	Mass transition (*m/z*)
Tacrolimus	0.99	20	821.5 > 768.4
Tacrolimus [$^{13}C^{2}H_4$]	0.88	20	826.6 > 773.5

compared with 6 samples with only I.S. and 6 samples with both I.S. and tacrolimus at LLOQ. The response should be under 5 % for the I.S. and < 20 % for the LLOQ. For sensitivity, the LLOQ should be at least 5 times the response of the blank sample. Carry-over was assessed during validation steps, where the response should be < 20 % in blank samples after the HIGH QC.

2.5.2. Linearity, accuracy, and precision

The 8-point calibration curve was generated over three separate days, where linearity was assessed on response. To decrease sample injections and turnaround time, accuracy and precision were also assessed with a 2-point calibration curve, consisting of the lowest and highest concentration of the linear range [21]. Accuracy and precision were then estimated in quintuplicate over 3 days. Bias and coefficient of variation (CV%) were calculated on peak height ratio to the I.S.. Regression, overall bias, and CV% were calculated using one-way ANOVA in an internally validated Excel worksheet. Bias and CV% should be under 20 % for the LLOQ and under 15 % for all other QC's.

2.5.3. Process efficiency, recovery, and matrix effects

Process efficiency, recovery and matrix effects were determined at LOW, MED, and HIGH in 6 lots of blank plasma, where ratios of tacrolimus to I.S. peak height were used. For process efficiency, spiked blank plasma was compared to spiked extraction fluid. For recovery, spiked blank plasma was compared to spiked plasma extract. For matrix effect, spiked plasma extract was compared to spiked extraction fluid. Following the EMA guidelines, also the I.S. normalised MF was calculated, and the CV% over the 6 batches is presented.

2.5.4. Stability

Stability of spiked plasma was assessed for 4 days at room temperature (20 °C), for 4 days in the refrigerator (4 °C), for 8 days at autoinjector temperature (10 °C), and after 3 freeze–thaw cycles. All samples were prepared in quintuplicate at LOW and HIGH concentrations, and % bias was calculated. All mean concentrations should be under 15 % bias as opposed to the nominal value.

Also, benchtop stability experiments were performed on 5 separate days in left-over tacrolimus-positive clinical samples, which were from routine tacrolimus analyses. Plasma was processed at baseline and after the EDTA whole blood was stored for 1, 2, 4, 5, 6, 8, 24, and 48 h at room temperature (20 °C). Samples were centrifuged on a temperature-controlled Mikro220R or Rotanta 460R centrifuge (Hettich, Tuttlingen, Germany) for 10 min at 20 °C at 1300 *g* and stored at −80 °C directly after being processed. Bias was calculated as the deviation from measurement t = 0. A mean bias of 20 % was deemed acceptable.

2.6. Clinical validation

The method has been used to measure tacrolimus in plasma samples from kidney, liver, and lung transplant recipients from the ongoing, prospective, TransplantLines Biobank and Cohort Study (NCT03272841) in the UMCG, The Netherlands. All participants have given written informed consent. The study has been approved by the local Institutional Review Board (METc 2014/077), adheres to the UMCG Biobank Regulation, and is in accordance with the WMA Declaration of Helsinki and Declaration of Istanbul [22]. EDTA plasma samples were collected before tacrolimus administration at several timepoints after transplantation. After collection, samples were centrifuged for 10 min at 1300 *g* and stored at −80 °C within 4 h after collection. Differences between the transplant groups were assessed with Kruskal Wallis test for numerical data and Chi-squared test for nominal data in SPSS statistics v.28 (IBM, Armonk, NY, USA). The relation of plasma tacrolimus trough concentrations with e.g., laboratory characteristics, adverse events, and clinical outcomes will be further assessed in future papers.

3. Results

3.1. Method validation

3.1.1. Selectivity and specificity

Representative ion chromatograms are shown in Fig. 1. No significant matrix interferences were observed at the expected retention times, and the RSD% of the 6 batches at LLOQ was 5.5 %. The intensity of the noise in the six blanks and blanks with I.S. was < 100, while the intensity of the peaks was > 500 for tacrolimus LLOQ and > 2000 for the internal standard. Carry-over was < 20 %.

3.1.2. Linearity, accuracy, and precision

The calibration curve was linear over the range 0.05–5.00 μg/L, showing typical regression with weighing 1/X and line y = 1.854 (SD: 0.015) + 0.006 (SD: 0.009), with a correlation coefficient of 0.999. All calibration standards were within the limits of their nominal value (<15 % for the LLOQ, <7.5 % for all other calibrators). The CV and Bias were < 10 % for all QC's, where within-day accuracy ranged from 1.6 % to 4.9 % and the between-day precision ranged from 2.4 % to 6.0 %, with an overall accuracy ranging from 2.9 % to 7.8 %. The overall bias ranged from −5.1 to 3.0 %. Accuracy and precision results per QC are shown in Table 3.

3.1.3. Process efficiency, recovery, and matrix effects

The results of the overall process efficiency, recovery, matrix effects with and without internal standards, and the I.S. normalised matrix factor precision for the method are shown in Table 3. The use of internal standard corrected for the present matrix effect, so that the matrix effect bias and imprecision were under 10 %.

3.1.4. Stability

Acceptable results were found in spiked plasma with a stability of 24 h at room temperature (20 °C), at 4 days in the refrigerator (4 °C), at 7 days at the autosampler (8 °C), and after three freeze–thaw cycles, as shown in Table 3. For the three latter parameters, these results were from the maximum time or number of cycles evaluated.

Variable results were found when routine clinical samples were processed after EDTA whole blood was stored for various periods at room temperature, as shown in Fig. 2. A total of 53 clinical samples were measured for tacrolimus in plasma at baseline on 5 separate days, with whole blood aliquots centrifuged at various timepoints on a temperature-controlled centrifuge set on 20 °C. A mean bias of 144 % and 198 % was found when whole blood aliquots were centrifuged at 24 and 48 h as compared to baseline. For whole blood aliquots that were centrifuged after 1, 2, 4 and 5 h, the mean bias was respectively −7.7 %, −12.6 %, −19.1 % and −4.5 %, where timepoints 6 and 8 h had a mean bias of > 20 %. A total of 24 clinical samples were measured in duplicate, where the mean CV% between the duplicates was 3.2 % (range 0.2–14 %).

3.2. Clinical validation

A total of 2333 samples were taken from 1325 tacrolimus-using liver, kidney, and lung transplant recipients, of which the characteristics at time of sample collection are presented in Table 4. Number of samples under the LLOQ was the highest among liver transplant recipients (N =

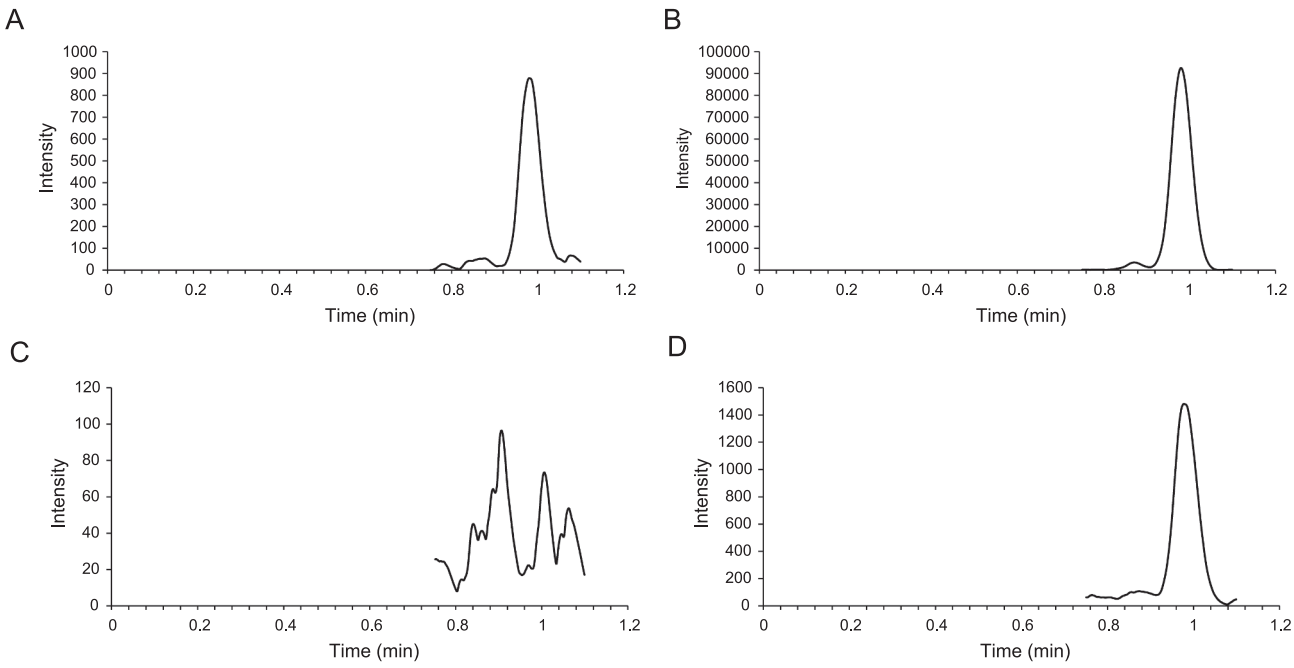

Fig. 1. Representative ion chromatograms of A) tacrolimus at LLOQ, B) tacrolimus at HIGH QC, C) blank sample, D) clinical sample of a tacrolimus user.

Table 3
Validation results of accuracy (bias) and precision (coefficient of variation, [CV %]), calculated with a 2-point calibration curve.

	Value at indicated quality control level				
	LLOQ	LOW	MED	HIGH	DIL
Nominal value (µg/L)	0.05	0.1	2.0	4.0	1.0
Accuracy and precision					
Within-day CV (%)	4.9	3.9	2.0	1.6	1.8
Between-day CV (%)	6.0	3.7	4.0	2.4	2.8
Overall CV (%)	7.8	5.4	4.5	2.9	3.4
Overall bias (%)	3.0	1.0	−5.1	−2.5	3.0
PEREME					
Process efficiency (%)		96	100	101	
Recovery (%)		104	107	106	
Matrix effect (%)		108	112	113	
I.S. normalised matrix effect (%)		93	94	94	
I.S. normalised matrix factor CV (%)		3.3	2.0	1.5	
Stability					
Room temperature 20 °C, 24 h (%)		−8.0		−8.7	
Refrigerator 4 °C, 4 days (%)		−5.7		−7.8	
Autosampler 8 °C, 8 days (%)		−1.0		3.4	
Freeze-thaw, 3 cycles (%)		−0.3		1.8	

54, 17 %), followed by kidney transplant recipients (N = 27, 2 %) and lung transplant recipients (N = 1, 0.3 %). Accordingly, the median [IQR] plasma tacrolimus concentration of liver transplant recipients was the lowest (0.10 [0.07 to 0.15] µg/L), followed by kidney (0.15 [0.10 to 0.21] µg/L) and lung (0.23 [0.17 to 0.34] µg/L) transplant recipients, as presented in Fig. 3. Similarly, median whole blood concentrations were lowest for the liver transplant recipients (3.8 [2.5 to 5.5] µg/L), followed by kidney (6.4 [5.0 to 7.8] µg/L) and liver (8.3 [7.0 to 9.8] µg/L). Data regarding whole blood tacrolimus concentrations were available at 2291 (98 %) sample collection moments. The ratio between plasma tacrolimus concentration and whole blood tacrolimus concentration was different for the type of organ transplant recipient ($p < 0.001$). Among liver transplant recipients, the median ratio was 0.026 [0.020 to 0.033], whereas the median ratio was 0.024 [0.018 to 0.031] among kidney transplant recipients and 0.029 [0.022 to 0.038] among lung transplant recipients. As shown in Fig. 4, no direct linear trend is visible over the entire whole blood range when plasma concentrations were plotted against whole blood.

4. Discussion

A simple and rapid LC-MS/MS method was developed for the quantification of tacrolimus in human plasma, with minimal sample preparation effort. Even so, this method showed good accuracy and precision, dilution integrity, process efficiency, recovery, matrix effect, and stability according to the FDA and EMA guidelines. Additional experiments were performed regarding benchtop stability. The calibration curve was linear in the range 0.05–5.00 µg/L. The injection-to-injection time was 2.3 min per sample and a gradient separated possible interfering peaks, where tacrolimus had a retention time of 0.99 min. As the sample preparation consists of only one additional step, it was swiftly applied for all 2333 samples.

For this method, a sensitive LC-MS/MS system was applied, and the injection volume was increased to 20 µl to reach a LLOQ of 0.05 µg/L. This increased injection volume had no influence on the chromatography and had no carry-over effect. The LLOQ was targeted lower in line with the expected concentrations of tacrolimus in plasma, which were previously reported to be on average 1.2–1.3 % of the whole blood values, but with high variability [23,24]. The whole blood trough concentrations of our population are targeted between 2 and 15 µg/L, with differences between transplant types. Indeed, a higher percentage of plasma measurements under LLOQ was found in liver transplant recipients, which have lower target trough concentrations. The whole blood samples taken at the same time as plasma samples under LLOQ had a median whole blood concentration of 2.6 µg/L. For overall tacrolimus whole blood concentrations of over 5.0 µg/L, the percentage samples under LLOQ was under 0.5 %.

In our population, tacrolimus plasma concentrations were variable with a median proportion of 2.4–2.9 % of the whole blood values. The highest plasma concentrations were seen in lung transplant recipients, which had significantly lower haematocrit values and higher whole blood concentrations than liver and kidney transplant recipients. Higher whole blood concentrations in general gave higher median plasma concentrations, with a trend towards a non-linear increase at tacrolimus whole blood concentrations of > 12 µg/L, which may suggest red blood cell saturation. In this research, only total (erythrocyte-unbound)

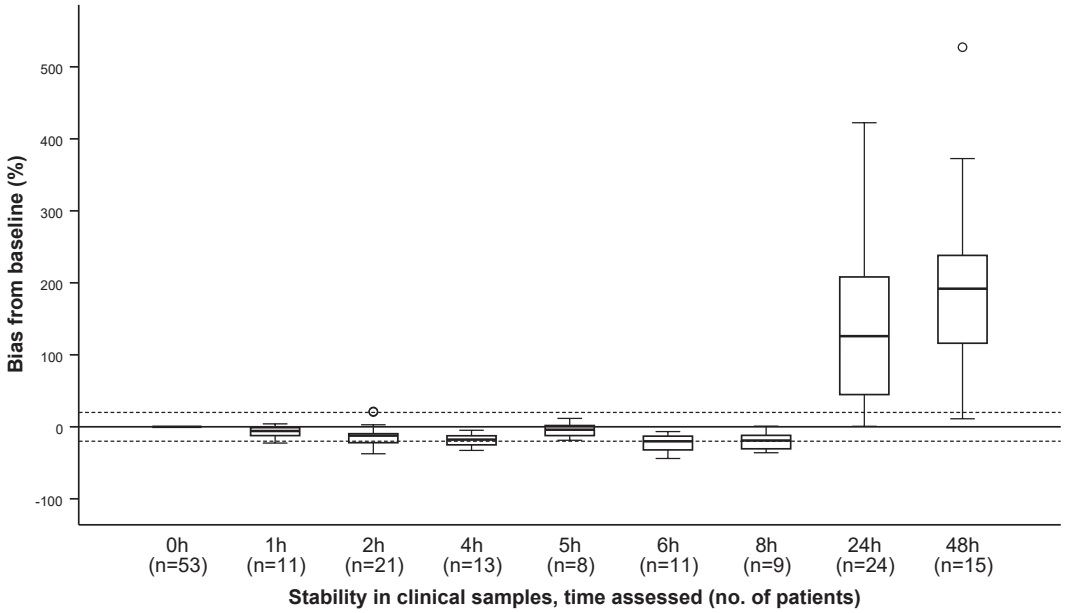

Fig. 2. Results from stability tests of plasma tacrolimus in left-over clinical samples, A total of 164 aliquots from 53 individual clinical samples were stored for various times at room temperature. *Change from baseline of the stability samples were normalised to baseline values. Dotted lines represent the 20% bias limit.*

Table 4
Patient characteristics at time of sample collection.

	Transplantation type[#]			
	Liver ($N_{samples}$ = 312)	**Kidney ($N_{samples}$ = 1714)**	**Lung ($N_{samples}$ = 307)**	**p**
Demographics				
Age, years	55 ± 14	55 ± 14	55 ± 13	0.98
Male sex, *n* (%)	186 (60)	1087 (63)	174 (57)	0.05
Time after transplantation				<0.001
± 3 months, *n* (%)	40 (13)	412 (24)	43 (14)	
± 6 months, *n* (%)	31 (10)	397 (23)	54 (18)	
± 12 months or longer, *n* (%)	241 (77)	905 (53)	210 (68)	
Laboratory measurements				
Haemoglobin, mmol/L	8.3 ± 1.3	8.0 ± 1.2	7.3 ± 1.0	<0.001
Haematocrit, L/L	0.40 ± 0.05	0.40 ± 0.05	0.37 ± 0.04	<0.001
eGFR, ml/min/1.73 m^2	71 ± 25	51 ± 17	54 ± 23	<0.001
Tacrolimus measurements				
Plasma tacrolimus				
Below detection level, *n* (%)	54 (17)	27 (2)	1 (0.3)	<0.001
Concentration above detection level, µg/L	0.10 [0.07 to 0.15]	0.15 [0.10 to 0.21]	0.23 [0.17 to 0.34]	<0.001
Whole blood tacrolimus, µg/L	3.8 [2.5 to 5.5]	6.4 [5.0 to 7.8]	8.3 [7.0 to 9.8]	<0.001
Ratio (plasma/whole blood)	0.026 [0.020 to 0.033]	0.024 [0.018 to 0.031]	0.029 [0.022 to 0.038]	<0.001

[#] Samples were taken from 253 liver, 855 kidney and 217 lung transplant recipients, respectively. Data regarding haematocrit, whole blood tacrolimus and ratio between plasma and whole blood tacrolimus were missing at 10, 42, and 121 sample collection moments. Data are reported as mean ± SD, n (%), or median [IQR] when appropriate.

plasma concentrations were assessed, while previous studies showed additional advancements in measuring free plasma concentrations [23–25]. Free drug concentrations are theoretically proportional to total plasma concentrations in steady state conditions, as higher free concentrations are also cleared faster when this clearance is non-saturable [11]. This was demonstrated in a previous study reporting a linear relationship between unbound fraction of tacrolimus in plasma and total plasma concentration in patient samples [9]. However, the high variation in plasma to whole blood ratios in this study implies that whole blood and plasma contain different sets of information and needs more research to be further understood.

While the benchtop stability of tacrolimus in plasma was agreeable, a substantial bias in plasma tacrolimus concentrations was observed in a benchtop stability experiment with clinical whole blood samples. This bias could be explained by time and temperature dependent erythrocyte binding and sample haemolysis, which was addressed in previous publications on this topic [11,23,26–28]. When temperature-dependent erythrocyte binding was studied in 48 samples, plasma concentrations were reported to be two to three times higher when stored at 37 °C in comparison to 20 °C [28]. However, warming of blood should be done with much care, as red blood cells are more prone to rupture at higher temperatures. As the majority (>90 %) of tacrolimus in the blood can be found in erythrocytes, in theory even slight haemolysis could falsely increase the plasma tacrolimus concentrations. The effect of haemolysis, which could in addition be the consequence of patient factors, mechanical damage during blood collection or improper sample handling, on plasma tacrolimus concentrations, will be focused on in future research. In addition, practical effect of time and temperature dependent binding should be further investigated. Until then, the time until centrifugation should be kept as short as possible, preferably within one hour, but with a maximum of five hours. Further storage of the plasma samples is allowed for up to four days at 4 °C. The current observation underlines the necessity to pay attention to differences between stability in spiked samples and real-world samples, as to make sure the bioanalyses reflect concentrations in the patient at the time of sample collection. Additional stability experiments in whole blood should be done for bioanalyses performed in plasma or serum, which is supported by new FDA recommendations [29].

Plasma concentrations of tacrolimus are not used in daily practice TDM, mainly due to two reasons. First, handling and processing of

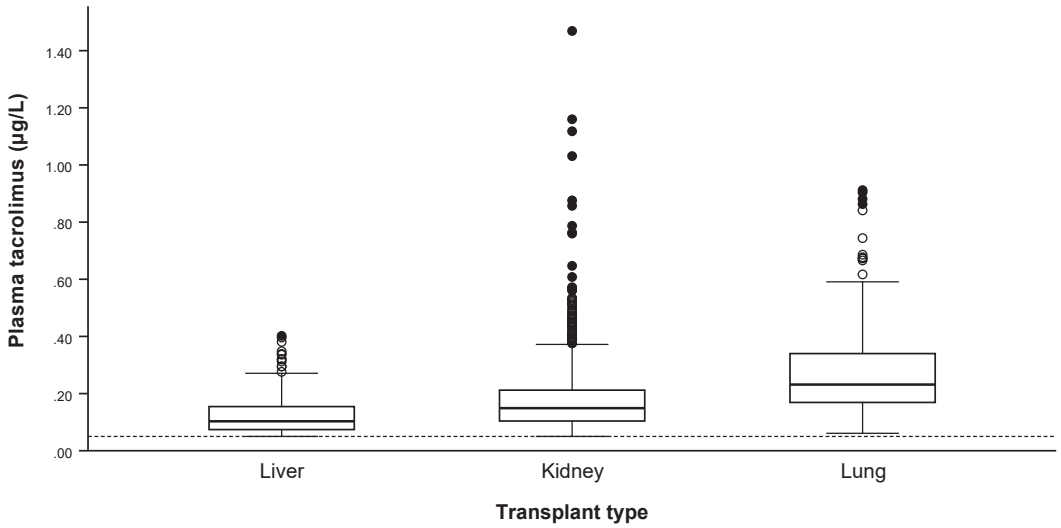

Fig. 3. Box plots of measured plasma tacrolimus concentrations per transplant type. The dotted line represents the LLOQ of the method (0.05 µg/L). *Median [IQR] plasma tacrolimus concentrations were 0.10 [0.07 to 0.15] µg/L for liver transplant recipients, 0.15 [0.10 to 0.21] µg/L for kidney transplant recipients and 0.23 [0.17 to 0.34] ug/L for lung transplant recipients.*

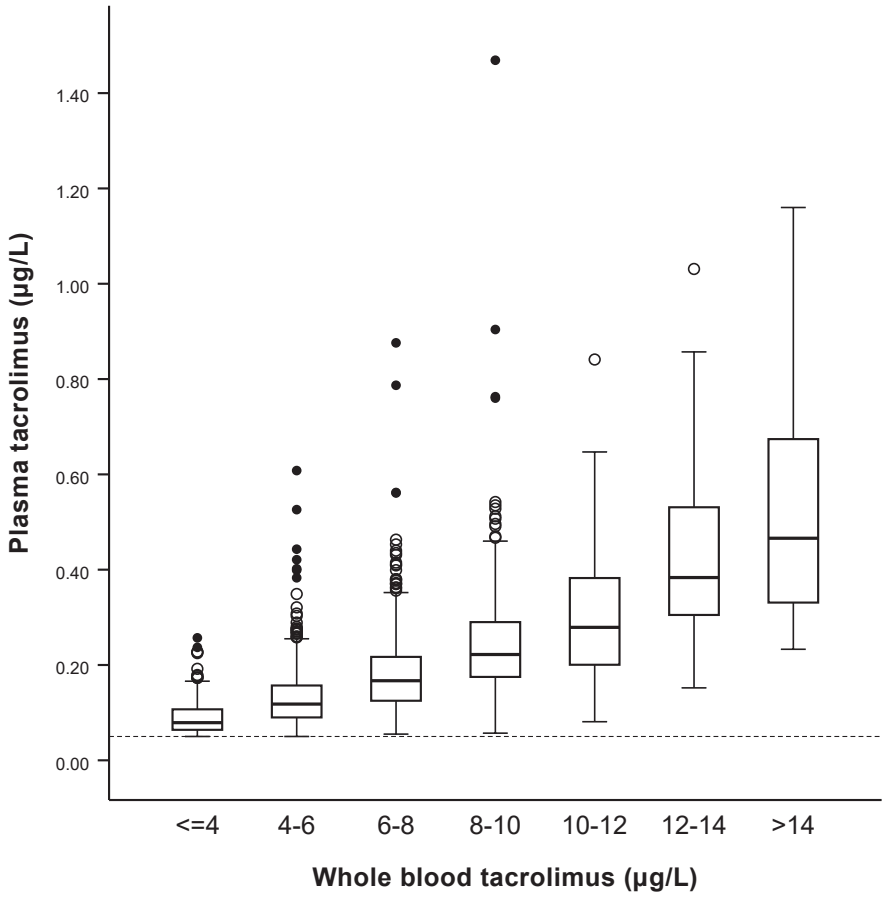

Fig. 4. Box plots of all measured plasma tacrolimus concentrations grouped per accompanied whole blood tacrolimus concentration. *The dotted line represents the LLOQ of the method (*0.05 µg/L*).*

plasma samples needs to be more strictly standardized as opposed to whole blood samples, as differences in temperature and haemolysis can result in substantial changes in plasma tacrolimus concentrations. Second, a relation between plasma concentrations and clinical outcomes or adverse events has not yet been established. Future publications will elucidate the pharmacokinetics of plasma tacrolimus concentrations and their relationship with adverse event occurrence, renal outcomes and graft function.

5. Conclusion

A simple and fast LC-MS/MS method was developed and validated to quantify tacrolimus in plasma, using stable isotopically labelled tacrolimus as an internal standard. This method can be applied for clinical studies to evaluate the relation between tacrolimus concentrations in plasma to adverse reactions and transplant outcomes.

CRediT authorship contribution statement

Tanja R Zijp: Validation, Data curation, Visualization, Writing – original draft. **Tim J Knobbe:** Data curation, Writing – original draft. **Kai van Hateren:** Methodology, Validation, Writing – review & editing. **Jan Roggeveld:** Formal analysis, Project administration. **Hans Blokzijl:** Resources, Writing – review & editing. **C Tji Gan:** Resources, Writing – review & editing. **Stephan JL Bakker:** Conceptualization, Supervision, Resources, Writing – review & editing. **Erwin M Jongedijk:** Methodology, Validation, Writing – review & editing. **TransplantLines Investigators:** Resources, Project administration, Funding acquisition. **Daan J Touw:** Conceptualization, Supervision, Writing – review & editing.

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.

Data availability

Public sharing of individual participant data was not included in the informed consent of the TransplantLines Biobank and cohort study, but data can be made available to interested researchers upon reasonable request by mailing to the data manager of the TransplantLines Biobank and Cohort study (datarequest.transplantlines@umcg.nl).

Acknowledgements

The authors thank all transplant recipients for their involvement. The TransplantLines Biobank and Cohort study was supported by a grant from Astellas BV (TransplantLines Biobank and Cohort study) and Chiesi Pharmaceuticals BV (PA-SP/PRJ-2020-9136), and co-financed by the Dutch Ministry of Economic Affairs and Climate Policy by means of the PPP-allowance made available by the Top Sector Life Sciences & Health to stimulate public-private partnerships. The funders had no role in the design of the study, or the writing of the manuscript.

Ethics approval

The study protocol of the TransplantLines Biobank and Cohort study has been approved by the local medical ethics review committee (METc 2014/077).

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