Rapid measurement of tacrolimus in whole blood by paper spray-tandem mass spectrometry (PS-MS/MS)

Run-Zhang Shi [a], El Taher M. El Gierari [b], Nicholas E. Manicke [c,*], James D. Faix [a]

[a] Stanford University School of Medicine, Palo Alto, CA, United States

[b] Stanford Hospital & Clinics, Palo Alto, CA, United States

[c] Department of Chemistry and Chemical Biology, Indiana University-Purdue University Indianapolis, Indianapolis, IN, United States

* Corresponding author at: Department of Chemistry and Chemical Biology, Indiana University-Purdue University Indianapolis, 402N Blackford, LD 326, Indianapolis, IN46202, United States.

phone: +1 317 274 6819

E-mail address: nmanicke@iupui.edu (N.E. Manicke)

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1. Introduction

Tacrolimus (FK506, Prograf®), a calcineurin inhibitor, is widely prescribed as an immunosuppressive drug for solid organ transplant recipients. Dose adjustment based on therapeutic drug monitoring (TDM) leads to better clinical outcome, especially in pediatric transplant recipients (1, 2)]. Most clinical laboratories use either immunoassay or liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure tacrolimus.

Automated immunoassays provide quick turnaround time but lack standardization across platforms, and may suffer from cross reactivity with tacrolimus metabolites (3). When clearance is impaired, leading to buildup of drug metabolites, such cross-reactivity can result in a significant over-estimation of the tacrolimus concentration. Additionally, the cost-per-test for immunoassay is higher than for mass spectrometry (MS) assays (4, 5). Many of the tacrolimus immunoassays still require manual sample pretreatment before automated analysis by the instrument, while only a selective few may include on-board hemolysis of whole blood specimens to be truly automated.

LC-MS/MS methods have superior analytical specificity and lower reagent cost, especially if one considers the ability to multiplex several different immunosuppressive drugs. However, they usually require more specimen pretreatment, such as protein precipitation and/or extraction, and are therefore almost always performed in batch mode to improve efficiency. This results in longer turnaround times, which may delay adjustment of dose especially during the early phase of prophylaxis of allograft rejection. In addition, maintaining LC-MS/MS test methods in clinical laboratories

require considerable amount of expertise and resource, which are more often spent on liquid chromatography system up keeping and troubleshooting to minimize system downtime.

Paper spray (PS) is a direct technique that generates gas phase analyte ions directly from dried blood spots (DBS) or other fluids without the need for complex sample pretreatment and chromatography (6, 7). Sample extraction and ionization are all performed in automated fashion by the PS ion source directly from a paper substrate stored within a single-use cartridge (Figure 1). There is no punching, no offline extraction, and total analysis time (excluding time required to dry the sample) is only a few minutes. Compared to conventional LC-MS/MS methods, this new approach has the additional advantages of significant reduction of solvent/reagent waste and elimination of carry-over.

Briefly, paper spray-tandem mass spectrometry (PS-MS/MS) is performed by depositing whole blood and internal standard mixture onto a paper substrate and allowing it to dry. An appropriate solvent is applied to the rear of the paper so that it flows through the dried blood spot sample by capillary action. As the solvent wicks through the sample, soluble components are extracted into the solvent, leaving behind the bulk of proteins and lipids [6]. A high voltage (3-5 kV) is applied to the moist paper, and an electrospray is induced at the sharp tip of the triangular shaped paper in the cartridge, which is held stationary in front of the inlet to the MS. As solvent evaporates, gas phase ions of the analyte molecules are generated which can then be detected by MS. PS-MS/MS has been successfully used for quantitative analysis of xenobiotics (8-10) and endogenous compounds (11) from whole blood.

We evaluated the feasibility of this novel approach for tacrolimus TDM in our clinical diagnostic laboratory. Tacrolimus was the drug of choice for the study due to the high clinical demand for TDM as well as the highly desirable fast turnaround time. This is the first application of this technology in a clinical diagnostic laboratory, and the first reported cross-validation of a paper spray assay against validated FDA approved immunoassays and LC-MS/MS assays.

2. Materials and Methods

2.1. Preparation of reagents, standards, and QCs

FK506 monohydrate (MW 822; ≥98% purity) was purchased from Sigma-Aldrich (St. Louis, MO). Tacrolimus (MW 804, certified 1 mg/ml in acetonitrile; 99.4% purity) was purchased from Cerilliant (Round Rock, TX). Stable isotope labeled internal standard (SIL-IS) of [^{13}C, $^{2}H_2$]-FK506 (MW 807; 98% isotopic purity) was purchased from Toronto Research Chemicals (Toronto, Canada). Liquichek whole blood immunosuppressant levels 1, 2, 3 and 4 (Bio-Rad, Hercules, CA) and Tac Control levels 1, 2 and 3 (More Diagnostics; Los Osos, CA) were used as quality controls (QCs). HPLC grade methanol was purchased from VWR (Radnor, PA). Chloroform was purchased from EMD Millipore (Billerica, MA). Sodium acetate anhydrous (NaOAc) was purchased from Sigma-Aldrich. All other drugs and chemicals used were of highest purity commercially available. FK506 monohydrate powder was carefully weighed and dissolved in methanol to obtain a 1 mg/ml stock solution. The stock was further diluted in methanol to obtain working stock solutions. Calibration standard solutions of tacrolimus at various concentrations were obtained by spiking pure FK506 solutions into

FK506-free whole blood, keeping the organic solvent content at less than 5%. A separate tacrolimus stock solution from Cerilliant was used to confirm concentrations of tacrolimus stocks made in-house. Tacrolimus and its SIL-IS solutions were checked for impurity.

2.2. Sample pretreatment

A 200 µl aliquot of patient whole blood collected in K_2EDTA collection tube, calibration standards (at 0, 0.5, 3.0, 10.0, and 30.0 ng/ml) prepared in drug free pooled whole blood, or QC material was transferred to labeled sample vials and mixed well with 50 µl of SIL-IS solution ([^{13}C, $^{2}H_2$]-FK506 at 30 ng/ml). An aliquot (10 µl) of each of the sample mixtures was then carefully spotted onto the middle of dry porous triangular shaped paper (Whatman 31ET-Chr) contained in disposable cartridges (Prosolia, Indianapolis, IN) following manufacturer's instructions. Drying of the blood spots was accelerated by loading up to 40 cartridges into the cartridge holder and placing it in an oven at 40°C for 20 min, or by placing up to 8 cartridges in a cartridge dryer provided by Prosolia which blows warm (~37°C) air over the blood spots for 20 min. Cartridges could otherwise be left at ambient temperature for about 90 minutes until they are completely dry. Result comparison was performed between replicate blood spotted cartridges being dried under different conditions and at different length of time, such as room temperature, drying by the cartridge dryer, and drying in oven at 40°C. Comparison study was performed by using different blood volume of 200 µl, 100 µl and 50 µl, with proportionally reduced SIL-IS solution volume of 50 µl, 25 µl, and 12.5 µl, respectively.

2.3. Ion source and MS/MS optimization

Sample analysis was performed using an automated paper spray ion source (Prosolia) interfaced to a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA). The paper spray ion source serves the combined functions of an auto-sampler and ionization source, in automatically loading the cartridges in the instrument, delivering the solvent, positioning the cartridge in front of the MS inlet, and ejecting the spent cartridge after analysis. The paper spray ion source is latched on to the front of the MS using a mechanism similar to the commercial ion source housing but is specifically designed and made for the make and model of the MS. The ion source was programed to deliver the extraction/spray solvent (40% Methanol and 60% Chloroform, 0.1% NaOAc; 170 µl) to the cartridges at an optimal rate to extract and elute analytes to the tip of the paper by capillary action. Sodium acetate was included to 1) increase the stability of the spray ionization by increasing solvent conductivity and 2) facilitate the generation of sodium adducts of tacrolimus and its SIL-IS. After the solvent application, the automated ion source moved the cartridge in line with the MS inlet, with the sharp tip of the paper positioned about 4 mm away from the inlet. An ionization voltage of +3.4 kV was applied, initiating production of analyte ions from the tip of the paper which were drawn directly into the MS inlet. More information about experimental and fundamental details can be found in the literature (8, 9, 12). MS conditions were optimized using continuous infusion of tacrolimus and SIL-IS solutions into the commercial H-ESI source by a syringe pump, and SRMs for both compounds selected accordingly (Table 1). XCalibur software (Thermo Scientific) was used to control the MS and to process data.

For the SIL-IS, [13C,$^{2}H_{2}$]-FK506 was listed as the major component, but due to impurities in the compound, the +4 m/z peak at m/z 830 was nearly as intense. This peak at m/z 830 was used during analysis to reduce overlap between the SIL-IS and the naturally occurring isotope peaks of the unlabeled tacrolimus.

2.4. Data collection and analysis

Data was collected for 60 seconds by toggling the spray voltage on and off, resulting in a total of 50 scans for each SRM channel. The average time required per sample analysis was 3 minutes, which included extraction and data collection. The more intense of the two SRMs (m/z 826 → 616 for tacrolimus and m/z 830 → 620 for the IS) was used for quantitation, while the other transition was used for confirmation. The area under the curve (AUC) for the tacrolimus quantifier ion during the entire 60 second analysis was determined and normalized by that of the SIL-IS quantifier ion. No background subtraction was performed. A calibration curve was constructed by linear regression (without weighting) from calibration standards analyzed within the same batch, and used for calculation of all patient sample concentrations as well as QCs for that batch. Tacrolimus results were confirmed when the quantifier and qualifier SRM ions were averaged over the entire scan time and ratio of the two was within mean ±25%.

2.5. Method validation

PS-MS/MS assay was evaluated for intra-day (n=10) and inter-day (n=12) imprecision using QC materials at multiple levels of tacrolimus (Bio-Rad and More

Diagnostics). Imprecision was further evaluated by repeatedly analyzing patient samples (n=95) with known tacrolimus concentration (0.0-29.0 ng/ml) everyday over 3-5 days in order to generate a precision profile. Assay linearity was evaluated by serial dilution of the highest calibration standard in drug free whole blood (8 levels). Limit of detection (LOD) was defined as three times the standard deviation of the reading obtained from drug free blood divided by the slope of the calibration curve ($3* s_b/m$) (13). The lower limit of quantitation (LLOQ) was determined by 1) determining interday test imprecision in patient samples with varying tacrolimus concentrations and 2) calculating the LLOQ using a statistical approach ($10*s_b/m$)(13). The LLOQ was set at a concentration where the imprecision decreased to less than 20% and was also greater than $10*s_b/m$. Assay accuracy was determined by the closeness of quantitative results to their predetermined concentrations measured in samples spiked with pure tacrolimus. Accuracy was further evaluated by comparing PS-MS/MS results to those obtained by validated FDA approved immunoassays (Architect, Abbott Diagnostics, Abbott Park, IL; Dimension RXL, Siemens, Malvern, PA), an LC-MS/MS assay validated in-house, and a validated LC-MS/MS method performed at a reference laboratory (Mayo Clinic Laboratories, Rochester, MN). Robustness of the method was evaluated by testing the effects of hemolysis, lipemia and icterus. Specificity was evaluated by spiking patient samples of known tacrolimus concentration at low and medium therapeutic ranges, with high concentrations of each of 25 pure steroid, vitamin, diuretic, and immunosuppressive compounds (f/c: 200-500 ng/ml). Selected sample cartridges were dried and analyzed a second time to determine the feasibility of repeat analysis without re-spotting sample. Study was also carried out to compare results from different batches

using a common single calibration, versus results from multiple batches using calibration included in each batch.

Note on terminology

The term chronogram is used here to indicate ion signal as a function of time. Use of the more commonly used term “chromatogram” is discouraged because no chromatography (i.e. separation of analytes) is being performed.

3. Results

3.1. Optimization

An extraction/spray solvent composition of 40% methanol, 60% chloroform containing 0.1% NaOAc was found to be optimal to generate tacrolimus signal with maximum intensity and duration. Reconstituted tacrolimus and its SIL-IS solutions were checked for impurity, and no contaminant compound or unlabeled tacrolimus was found in SIL-IS solutions. Continuous syringe pump infusion experiments revealed abundant sodium adducts of tacrolimus (m/z 826.5) in full mass scan. Ammonium adducts (NH_4^+) could also be generated at similar intensities by spiking ammonium acetate into the solvent rather than sodium acetate. The sodium adduct of tacrolimus was deemed to give more selective fragment ions during MS/MS, and was therefore chosen over the ammonium adduct for this assay. The two most intense product ions and the optimal ion source and MS instrument settings were obtained and used for SRM analysis (Table 1). Cartridges spotted with pure tacrolimus and SIL-IS solutions were dried and used to

confirm the settings and SRM selections. Different drying conditions tested had no impact to tacrolimus results. Sample mixture preparation using smaller blood aliquots of 100 µl and 50 µl gave equivalent test results, and 200 µl was selected for the method. In case of insufficient blood samples submitted, especially by pediatric and anemic transplant patient, a lower sample volume may be used.

3.2. Interference study

During paper spray analysis, the chemicals extracted from the DBS are introduced directly to the mass spectrometer without prior separation or extraction. Because of this lack of pretreatment, the effect of hemolysis, lipemia, and icterus on analyte ion intensity was evaluated in comparison to that of pure compound solutions (in methanol) and of regular whole blood samples without visible hemolysis, lipemia, or icterus. There was no systematic difference in the AUC or the shape of the chronogram between these groups. Variability in the shape of the chronograms was observed, but it was not consistently associated with particular samples. The similarity of the SIL-IS chronogram from the same sample essentially normalized such variability (Figure 2), providing reliable quantitative results. Experience from analysis of hundreds of patient samples revealed that up to 10% samples required reanalysis due to failure of the initial sample cartridge to generate adequate signal intensity and/or failure of the preset ion ratio criterion. Up to 90% of repeat analysis was successful, however, and was able to meet the ion ratio criteria and produce quantitative results. On rare occasions, a sample may need to be repeated a third time, usually after making dilution, to generate successful ionization and quantitative result.

3.3. Precision, linearity, and accuracy

The inter-day precision (n=10) for the analysis of Bio-Rad QC materials was 12%, 14%, 14%, and 12% CV at tacrolimus concentrations of 4, 9, 16, and 25 ng/ml, respectively. Analysis of a different set of QC materials (More Diagnostics) showed inter-day precision (n=12) to be 13%, 8%, and 5%, at tacrolimus concentration of 4.5, 10.5, and 24.5 ng/ml, respectively. Bio-Rad control samples appeared to result in higher imprecision than the other QC samples, possibly due to difference in sample matrix, where the two manufacturers may include a number of different proprietary additives. The assay was linear within the analytical measurement range of 1.5-30 ng/ml as determined by serial dilution of the highest calibration standard with drug free whole blood, as was also demonstrated by the typical calibration curve (Figure 3). The assay accuracy was evaluated by quantifying standards prepared in drug free whole blood spiked with certified of tacrolimus standard solution. The accuracy of the assay was within 95-105%. Accuracy was also evaluated by comparing the concentrations determined by PS-MS/MS to those determined by two immunoassays and two LC-MS/MS assays. Linear regression analysis (y = mx +b) was used for the assessment with the following slope (m) and y-intercept (with 95% confidence limit): (1) to Siemens Dimension RXL (Figure 4A): m = 0.87-1.01, b = -0.61-0.79; difference of mean = 0.4 ng/ml. (2) to Abbott Diagnostics Architect (Figure 4B): m = 0.98 -1.06, b = -0.22-0.71; difference of mean = 0.3 ng/ml. (3) to LC-MS/MS at a reference lab (Figure 4C): m = 0.83-0.96, b = -0.20-1.04; difference of mean = 0.5 ng/ml. (4) to LC-MS/MS method developed in-house (Figure 4D): m = 0.89-1.0, b = -0.82-0.28; difference of mean = 0.7

ng/ml. Finally, recent external proficiency samples from CAP (College of American Pathologists) were analyzed and results were at the LC-MS/MS peer group mean (data not shown).

3.4. Limits of detection and quantitation.

The limit of detection was determined by repeat analysis of whole blood samples without tacrolimus to obtain the mean and standard deviation for the blank signal (N = 25 over 17 different days). The LOD, calculated using $3*s_b/m$, was 0.2 ng/mL. The LLOQ was determined by plotting precision as a function of concentration in patient samples (Figure 5) and determining the concentration at which the %CV was less than 20%. Additionally, the LLOQ was calculated from the formula $10*s_b/m$, which gave a concentration of 0.7 ng/mL. The LLOQ was conservatively set at 1.5 ng/mL using these two criteria. This LLOQ was further confirmed by determining test imprecision at various tacrolimus concentration of spiked drug free whole blood sample.

3.5. Ion suppression and recovery

Matrix effects were evaluated by comparing the absolute signal of the SIL-IS (calculated as the area under the curve for the quantifier ion) in methanol versus pooled whole blood. Calibration standards in methanol and drug free whole blood were prepared and analyzed on three separate days. The absolute signal of the SIL-IS for the calibrators prepared in methanol, averaged across all three days, was 1.3E5 compared to 3.6E4 when prepared in whole blood. This is a relative difference of 3.5 fold, with a 95% confidence interval of 2.7 to 4.5. The higher SIL-IS signal in methanol

compared to whole blood likely arises from a combination of lower analyte recovery from the dried blood matrix and ion suppression.

Difference in matrix effect among patient samples was also evaluated. Thirty three random patient blood samples were analyzed on three consecutive days. The average absolute signal of the SIL-IS obtained for each patient sample was compared to the average signal obtained for all of the samples. None of the patient samples were significantly different from the pooled mean at the 95% confidence level, indicating that no significant differential ion suppression/recovery effects existed in this group.

3.6. Specificity and reanalysis of cartridges

No detectable difference was noted in samples with low and medium tacrolimus concentrations after **s**piking high concentrations of each of 25 pure steroid, vitamin, diuretic, and immunosuppressive drugs up to final concentrations of 500 ng/ml. Due to the need to reanalyze samples, we examined the option of reanalyzing cartridges containing patient blood spots. The reanalysis was carried out after drying the cartridges (n=40) a second time following the first analysis. Thirty repeat analyses using the same cartridge produced comparable results, while 10 (25%) repeat analyses had spurious results, leading to the conclusion that using a new cartridge for repeat analysis was necessary.

Discussion and Conclusion

We evaluated PS-MS/MS for TDM of tacrolimus in a clinical diagnostic laboratory. The method was tested with real patient samples, and was shown to be

simple and rapid. The method uses simple sample pretreatment and completely eliminated chromatography, which simplified the analysis of tacrolimus by MS while retaining the superb sensitivity and specificity. To maintain high precision and accuracy, the method used a SIL-IS to better control the variability arising during the analysis, particularly during extraction of the drug from the DBS and also during ion generation from the paper tip.

The instrumental analysis time for PS-MS/MS was comparable to many typical tacrolimus LC-MS/MS assays. PS-MS/MS had some other advantages over more traditional HPLC-MS/MS methods for tacrolimus quantitation, however. First, the sample preparation steps are simpler because blood cell lysis, protein precipitation, and centrifugation steps can be omitted. The simplification of the sample preparation improved turnaround times and made the method more amenable to random access analysis. Second, because PS-MS/MS required less bench work and simplified the analytical measurement process and instrumentation by removing the HPLC system, there is a potential for reductions in labor and expertise requirements to maintain and perform an MS based assays. Third, solvent consumption was lower, and practically no solvent waste (<1 mL per day) was generated. Fourth, aside from the solvent no components of the system require regular replacement, unlike the analytical column in an HPLC based assay. Finally, there is no potential for carry-over because the ionization, extraction, and fluid path are contained within a disposable cartridge.

Because extraction and ionization occur simultaneously in PS and cannot be separated, we evaluated matrix effects as a whole (including both ion suppression and recovery) by comparing the absolute signal of the SIL-IS in calibrators prepared in

methanol and drug free whole blood. Signal for calibrators prepared in methanol was about 3.5 times higher than in whole blood calibrators. This difference likely arises from a combination of ion suppression and poorer recovery from the dried blood matrix compared to paper alone. It should be noted that the presence of matrix effects does not prevent accurate quantitation. Indeed, many clinical assays are well-known to exhibit matrix effects. In the PS-MS/MS assay developed here, matrix matched whole blood calibrators and a stable isotope labeled internal standard were used to account for variation arising during extraction and ionization, and resulted in a much simplified MS method with satisfactory accuracy and precision. Moreover, comparison of 33 different patient samples showed no significant difference in internal standard response among the patient samples.

Tacrolimus is normally detected by MS as the ammonium adduct. For the PS-MS/MS assay developed here, we chose to detect tacrolimus as sodium adduct instead for several reasons. First, the three most abundant fragment ions obtained for the $[M+Na]^+$ ion were about 2 to 3 times more intense than the $[M+NH_4]^+$ ion. Second, the fragmentation for the $[M+Na]^+$ ion was deemed to be more selective because the most intense fragment ions, which were m/z 616, 415, and 445, arose from relatively large neutral losses, whereas the two most intense fragment ions for the $[M+NH_4]^+$ ion arose from neutral losses of only 34 and 53 amu. Larger neutral losses, particularly those that result in fragment ions in the middle of the mass range, tend to be more selective. This consideration becomes even more important in PS-MS/MS due to the elimination of chromatography. Finally, a number of tacrolimus LC-MS/MS assays using tacrolimus sodium adduct were in published reports (14, 15).

The whole blood volume required per sample analysis was as little as 50 µl. The total sample volume required for the assay was set by the need to mix an accurate volume of the blood with the IS solution. In this report, 200 µl of blood sample was used, but this could be reduced to 50 µl or less, provided the blood volume was measured accurately. Reduction of the blood draw volume would be ideal for pediatric organ transplant patients, in which poor circulation and anemia are common. Another feature of PS-MS/MS is that the assay is performed on DBS. Because of the relative ease of storage and transportation of DBS (16), the method may be amenable to off-site blood collection in clinics with a simple finger prick collection. Further development to the blood collection cartridge and method validation would be required prior to taking this step.

The novel approach of PS-MS/MS is suitable for therapeutic monitoring of tacrolimus. The PS-MS/MS method reported here for tacrolimus had excellent LLOQ and accuracy, and was found to be well suited for a clinical lab with limited expertise in the maintenance and trouble shooting of LC-MS/MS. PS-MS/MS cannot replace LC-MS/MS for all applications, particularly where closely related isomers must be distinguished (e.g. testosterone and DHEA or morphine and norcodeine). This study demonstrates that PS-MS/MS is a viable alternative method for tacrolimus measurement, having lower ongoing costs than immunoassays and being more easily implemented in some clinical labs than LC-MS/MS because it eliminates chromatography and associated user training and trouble shooting, generates very little solvent waste (around 1 ml/day), and requires much less sample preparation.

Acknowledgements

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References

1. Wallemacq P, Armstrong VW, Brunet M, Haufroid V, Holt DW, Johnston A, et al. Opportunities to optimize tacrolimus therapy in solid organ transplantation: Report of the european consensus conference. Ther Drug Monit 2009;31:139-52.
2. Delaloye JR, Kassir N, Lapeyraque AL, Alvarez F, Lallier M, Beaunoyer M, et al. Limited sampling strategies for monitoring tacrolimus in pediatric liver transplant recipients. Ther Drug Monit 2011;33:380-6.
3. Architect tacrolimus [package insert]. Abbott Laboratories, 2009.
4. Hetu PO, Robitaille R, Vinet B. Successful and cost-efficient replacement of immunoassays by tandem mass spectrometry for the quantification of immunosuppressants in the clinical laboratory. J Chromatogr B 2012;883:95-101.
5. Poquette MA, Lensmeyer GL, Doran TC. Effective use of liquid chromatography-mass spectrometry (lc/ms) in the routine clinical laboratory for monitoring sirolimus, tacrolimus, and cyclosporine. Ther Drug Monit 2005;27:144-50.
6. Liu JJ, Wang H, Manicke NE, Lin JM, Cooks RG, Ouyang Z. Development, characterization, and application of paper spray ionization. Anal Chem 2010;82:2463-71.
7. Wang H, Liu JJ, Cooks RG, Ouyang Z. Paper spray for direct analysis of complex mixtures using mass spectrometry. Angew Chem-Int Edit 2010;49:877-80.
8. Espy RD, Manicke NE, Ouyang Z, Cooks RG. Rapid analysis of whole blood by paper spray mass spectrometry for point-of-care therapeutic drug monitoring. Analyst 2012;137:2344-9.
9. Manicke NE, Abu-Rabie P, Spooner N, Ouyang Z, Cooks RG. Quantitative analysis of therapeutic drugs in dried blood spot samples by paper spray mass spectrometry: An avenue to therapeutic drug monitoring. J Am Soc Mass Spectrom 2011;22:1501-7.
10. Manicke NE, Yang QA, Wang H, Oradu S, Ouyang Z, Cooks RG. Assessment of paper spray ionization for quantitation of pharmaceuticals in blood spots. Int J Mass Spectrom 2011;300:123-9.
11. Yang Q, Manicke NE, Wang H, Petucci C, Cooks RG, Ouyang Z. Direct and quantitative analysis of underivatized acylcarnitines in serum and whole blood using paper spray mass spectrometry. Anal Bioanal Chem 2012;404:1389-97.
12. Yang Q, Wang H, Maas JD, Chappell WJ, Manicke NE, Cooks RG, Ouyang Z. Paper spray ionization devices for direct, biomedical analysis using mass spectrometry. Int J Mass Spectrom 2012;312:201-7.
13. Long GL, Winefordner JD. Limit of detection a closer look at the iupac definition. Analytical Chemistry 1983;55:712A-24A.
14. Hetu PO, Robitaille R, Vinet B. Successful and cost-efficient replacement of immunoassays by tandem mass spectrometry for the quantification of immunosuppressants in the clinical laboratory. J Chromatogr B Analyt Technol Biomed Life Sci 2012;883-884:95-101.
15. Poquette MA, Lensmeyer GL, Doran TC. Effective use of liquid chromatography-mass spectrometry (lc/ms) in the routine clinical laboratory for monitoring sirolimus, tacrolimus, and cyclosporine. Ther Drug Monit 2005;27:144-50.
16. Cheung CY, van der Heijden J, Hoogtanders K, Christiaans M, Liu YL, Chan YH, et al. Dried blood spot measurement: Application in tacrolimus monitoring using limited sampling strategy and abbreviated auc estimation. Transpl Int 2008;21:140-5.

Table 1. TSQ Vantage MS/MS and SRM Parameters

Name	Paren	Produc	Width	Time (s)	CE	Q1 PW	Q3 PW	S-Lens
FK506	826.5	443.1	0.01	0.1	44	0.30	0.70	182
$[M + Na]^+$	826.5	616.3	0.01	0.1	32	0.30	0.70	182
FK506-$^{13}C,^{2}H_2$	830.5	447.2	0.01	0.1	44	0.30	0.70	182
$[M + Na]^+$	830.5	620.3	0.01	0.1	32	0.30	0.70	182

Parent: parent ion; Product: product ion; CE: collision energy; PW: peak width

Figure Captions

Figure 1. Schematic demonstrating the workflow for a PaperSpray analysis.

Figure 2. Rows 1-3: Representative PS-MS/MS data for three patient samples; Left column shows the extracted ion chronograms for the sodium adducts of tacrolimus (m/z 826 → 616, solid line) and the SIL tacrolimus (830 → 620, dashed line). Right column shows the stick SRM spectra for the quantifier ion (m/z 826 → 616) and qualifier ion (m/z 826→ 443) to show the ion ratio for the three patient samples

Figure 3. PS-MS/MS calibration curve.

Figure 4. Method comparison with two immunoassays (A and B) assays and two HPLC-MS/MS assays.

Figure 5. PS-MS/MS precision profile for 95 patient showing interday precision as a function of concentration

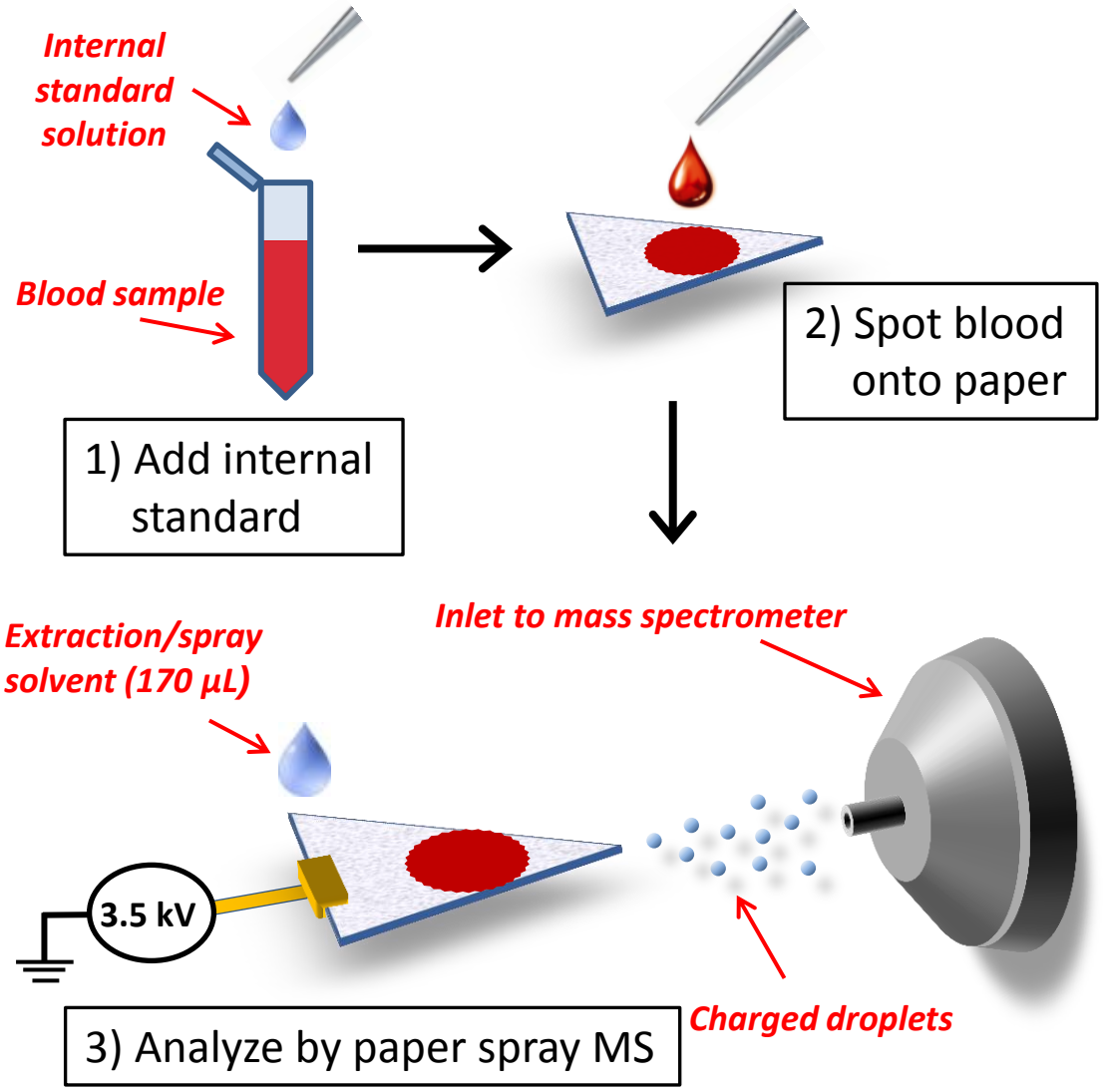

Internal standard solution
Blood sample
1) Add internal standard
2) Spot blood onto paper
Extraction/spray solvent (170 µL)
Inlet to mass spectrometer
3.5 kV
Charged droplets
3) Analyze by paper spray MS

Figure 1

Figure 2

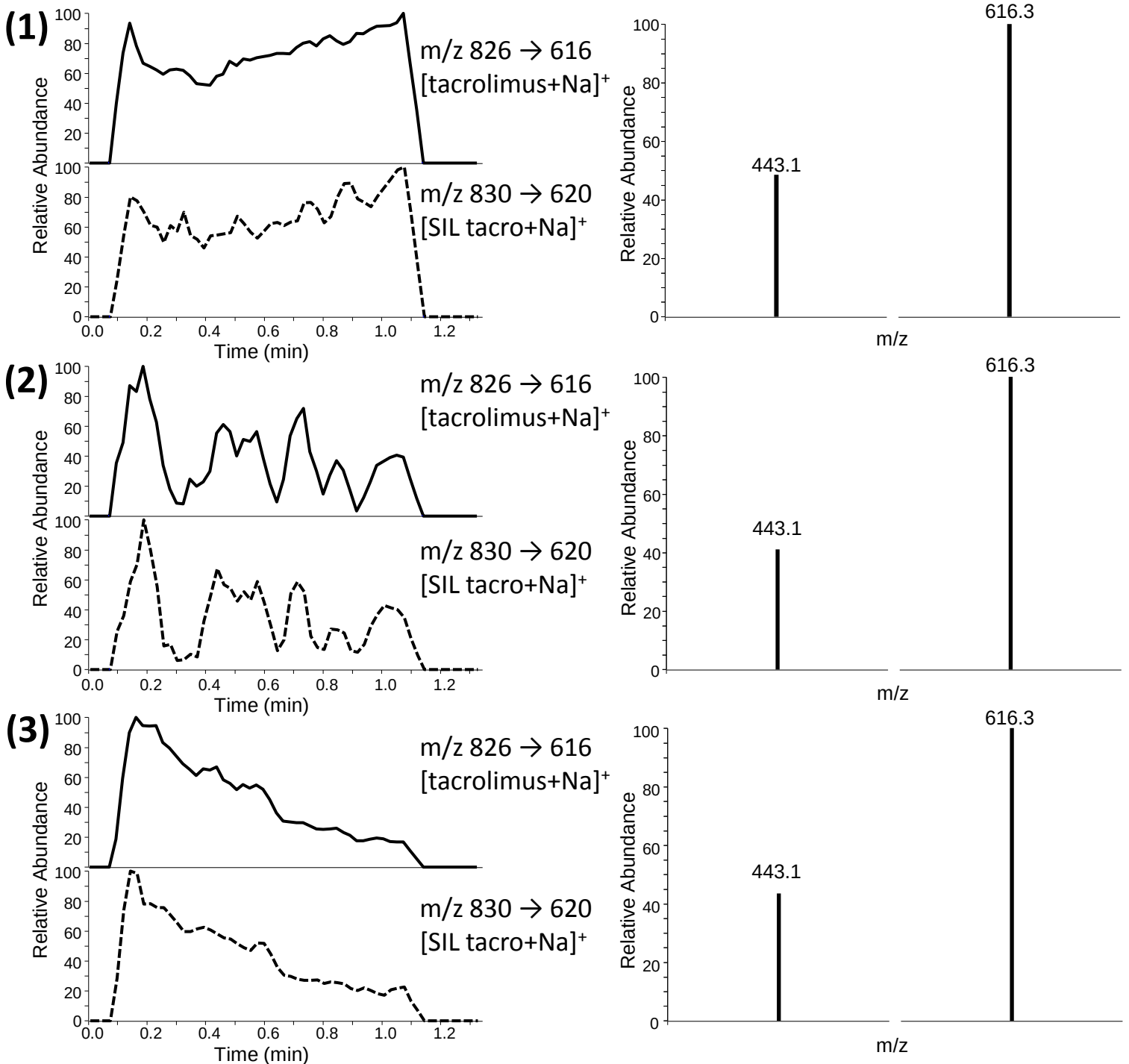

(1)
m/z 826 → 616
[tacrolimus+Na]+
m/z 830 → 620
[SIL tacro+Na]+
Relative Abundance
Time (min)
443.1
616.3
m/z
(2)
m/z 826 → 616
[tacrolimus+Na]+
m/z 830 → 620
[SIL tacro+Na]+
Relative Abundance
Time (min)
443.1
616.3
m/z
(3)
m/z 826 → 616
[tacrolimus+Na]+
m/z 830 → 620
[SIL tacro+Na]+
Relative Abundance
Time (min)
443.1
616.3
m/z

Figure 3

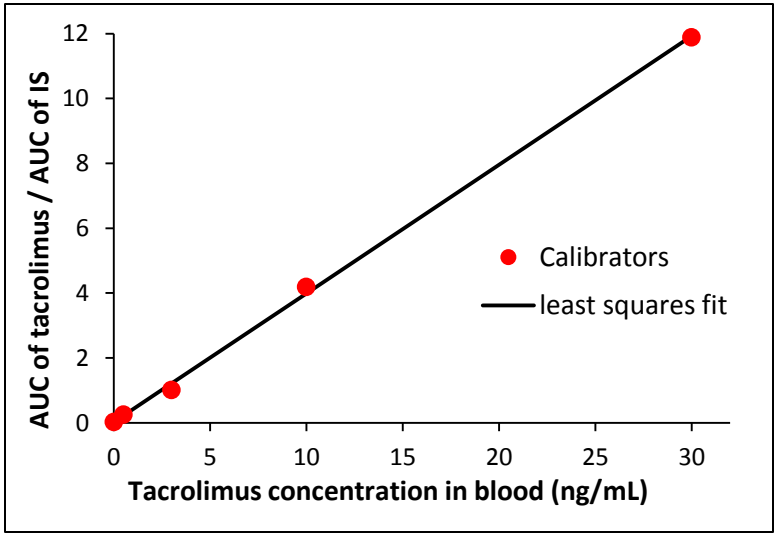
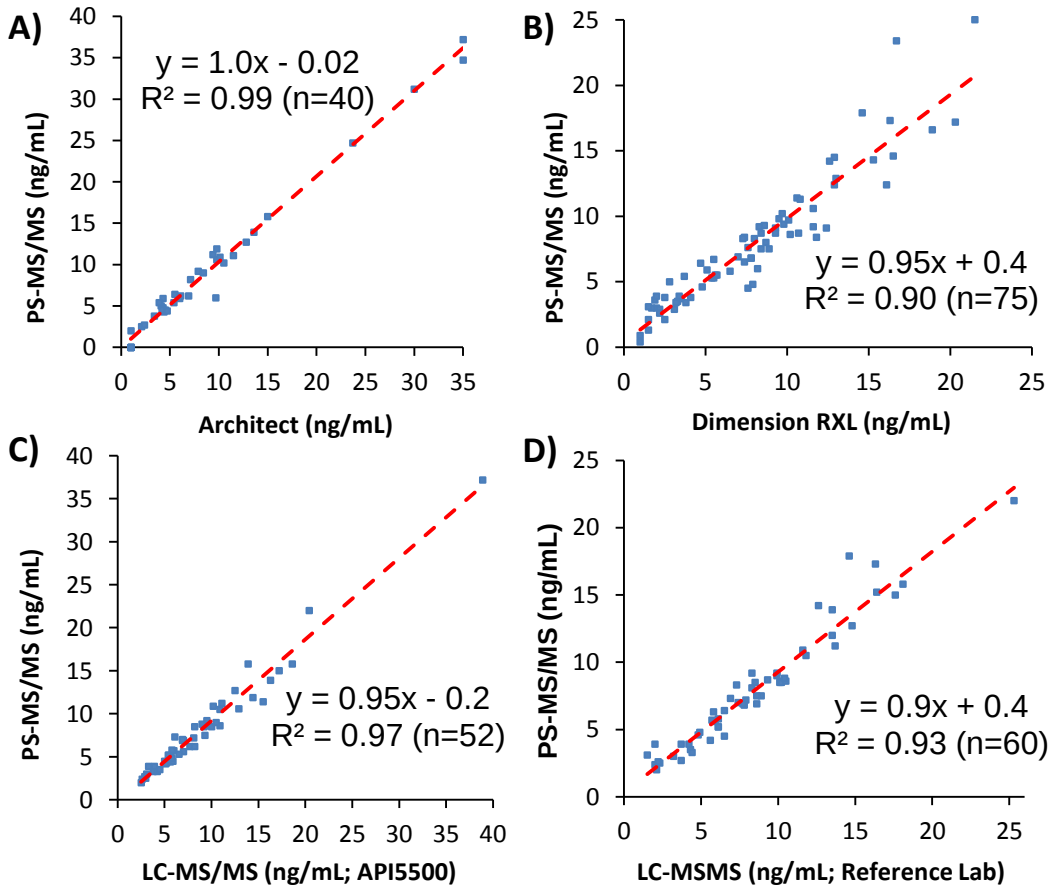
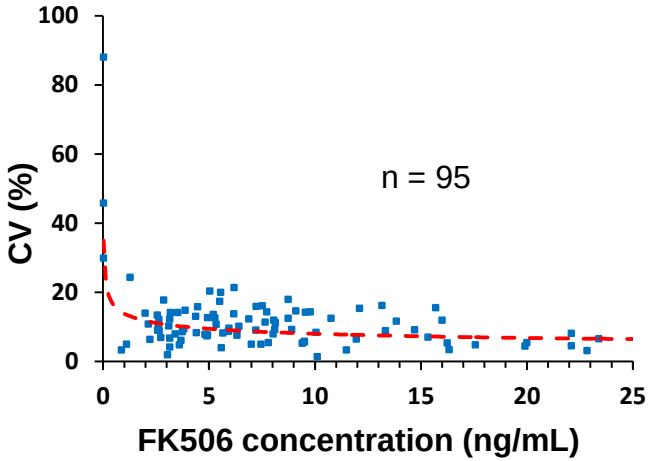

AUC of tacrolimus / AUC of IS
12
10
8
6
4
2
0
0
5
10
15
20
25
30
Tacrolimus concentration in blood (ng/mL)
Calibrators
least squares fit

Figure 4


A)
y = 1.0x - 0.02
R² = 0.99 (n=40)
PS-MS/MS (ng/mL)
Architect (ng/mL)
B)
y = 0.95x + 0.4
R² = 0.90 (n=75)
PS-MS/MS (ng/mL)
Dimension RXL (ng/mL)
C)
y = 0.95x - 0.2
R² = 0.97 (n=52)
PS-MS/MS (ng/mL)
LC-MS/MS (ng/mL; API5500)
D)
y = 0.9x + 0.4
R² = 0.93 (n=60)
PS-MS/MS (ng/mL)
LC-MSMS (ng/mL; Reference Lab)


100
80
60
40
20
0
CV (%)
n = 95
0
5
10
15
20
25
FK506 concentration (ng/mL)