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Successful and cost-efficient replacement of immunoassays by tandem mass spectrometry for the quantification of immunosuppressants in the clinical laboratory☆

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ABSTRACT

The purpose of this paper is to describe the implantation of mass spectrometry in replacement of immunoassays for the measurement of immunosuppressant drugs in the clinical setting, from scientific and financial perspectives. A straightforward, rapid, and economical method was developed for the simultaneous quantification of tacrolimus, sirolimus, and cyclosporine. Following a simple protein precipitation step, supernatants are injected on a small C_{18} guard cartridge and gradient elution of the immunosuppressants is performed in a total chromatographic run time of 2.25 min. Sodium adducts of the compounds and internal standards are quantified by electrospray tandem mass spectrometry. The method shows inter-assay impression of less than 10–15% for all compounds with good extraction efficiency (89–104%) and minimal matrix effects, except for sirolimus where ion suppression is more pronounced. The method correlates well with chemiluminescent microparticle immunoassays (on the Abbott Architect analyzer), although the immunoassay results are significantly higher than those obtained by HPLC–MS/MS. The transition from immunoassays to mass spectrometry was well received by the laboratory staff, and significant reductions in reagent costs have been realized (>$250,000 CAD per year). With these savings, the purchase and installation of two complete HPLC–MS/MS systems was completely financed in less than three years.

1. Introduction

Successful transplantation is at present the only curative treatment for end-stage organ failure and, in turn, the success of allograft transplantation depends on a good immunosuppressive therapy. Immunosuppressant drugs such as tacrolimus (FK-506, Prograf®, Advagraf®), cyclosporine (Sandimmune®, Neoral®), and sirolimus (rapamycin, Rapamune®) are used, in combination with corticosteroids, to prevent transplant rejection. Tacrolimus and cyclosporine are calcineurin inhibitors that block T-cell activation and proliferation by preventing interleukin-2 (IL-2) release by helper T-cells [1–3]. Sirolimus inhibits mTOR (mammalian target of rapamycin) and precludes IL-2 induced T-cell proliferation by preventing progression of the cell cycle from the G_1 to the S phase [1,2]. Optimal immunosuppressive therapy using these immunosuppressant drugs relies greatly on therapeutic drug monitoring, since these compounds have variable pharmacokinetic profiles and relatively narrow therapeutic indexes [4–8]. Dose adjustment using therapeutic drug monitoring ensures that sufficient immunosuppression is achieved and reduces the risk of experiencing toxic side-effects [9–11], including nephrotoxicity, neurotoxicity, diabetes mellitus, and hyperlipidemia [12–15]. Therapeutic drug monitoring of immunosuppressant drugs can be performed by immunoassays or by chromatographic methods.

In the beginning of 2008, two high pressure liquid chromatography systems coupled to tandem mass spectrometers (HPLC–MS/MS) were implanted in our laboratory for the simultaneous analysis of the immunosuppressant drugs tacrolimus, sirolimus, and cyclosporine. We had been using an EMIT method (enzyme multiplied immunoassay technique) to quantify tacrolimus and cyclosporine on a Viva analyzer from Dade Behring (now Siemens) since 2001, while sirolimus was quantified by an in-house HPLC-UV method. At that period, mass spectrometry was not well established in clinical laboratories and immunoassays were the method of choice for tacrolimus and cyclosporine measurements. Even though immunoassays for the quantification of immunosuppressant drugs usually require a manual sample pretreatment step (red blood cells lysis, centrifugation, and collection

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of the supernatant) before analysis on the automatic analyzer, the ease of execution and the rapid turnaround time of these assays still constitute a major advantage. On the other hand, immunoassays are very costly (>$10 per sample) and their specificity varies as antibodies can cross-react with drug metabolites [10,16–19].

In early 2005, we started to think about acquiring mass spectrometers because the supply contract for our tacrolimus and cyclosporine immunoassays was coming to an end. Mass spectrometry was now more common in clinical laboratories, notably in the pharmacology-toxicology area [20]. The advantage of mass spectrometry (and more so of tandem mass spectrometry) is that the great specificity and sensitivity of this technology allows for the simultaneous quantification of several compounds with similar chemical properties in a single assay, using only minimal sample pre-treatment steps and short chromatographic run times. Mass spectrometry was implanted in our laboratory for specificity (accuracy) issues, but also for financial reasons (*i.e.* to lower costs per sample for the analysis of immunosuppressants) and because of the added flexibility for future development that this technology permits.

Although the initial investment to buy mass spectrometers and the associated costs for the service contract was high, the very low costs of reagents and consumables per patient sample rapidly compensated the initial expense. In our laboratory, the savings generated by switching from immunoassays to mass spectrometry lead to a complete financing of two tandem mass spectrometer systems in less than three years. In the present paper, we will summarize the introduction of mass spectrometry for quantification of immunosuppressants in the clinical laboratory at our university hospital from the scientific, administrative, and financial perspectives.

2. Experimental

2.1. Chemicals

Tacrolimus, sirolimus, and cyclosporine (cyclosporine A) standards were purchased from LC Laboratories (Woburn, MA, USA). Cyclosporine D was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Ascomycin, sodium acetate, and zinc sulphate were purchased from Sigma–Aldrich (Saint-Louis, MO, USA). HPLC grade methanol was purchased from Fisher Scientific (Ottawa, Ontario, Canada). Quality control material (Lyphochek Whole Blood Immunosuppressant Control) was purchased from Bio-Rad Laboratories (Montréal, Québec, Canada).

2.2. Preparation of calibrators

Stock solutions of each immunosuppressant were prepared in methanol (0.1 mg/mL for tacrolimus and sirolimus, and 1 mg/mL for cyclosporine). A solution containing all three compounds was then prepared by dilution in 70% methanol to obtain a final concentration of 0.8 μg/mL for tacrolimus and sirolimus, and 20 μg/mL for cyclosporine. Calibrators were prepared in pooled human whole blood by adding dilutions of this mix of immunosuppressants (pool made from anonymous patient samples tested beforehand to verify absence of immunosuppressants). The calibrators were prepared fresh each week and were stored refrigerated.

2.3. Sample processing

Samples were prepared for analysis by transferring 50 μL of calibrator, control, or patient whole blood (using a positive displacement pipette) to a 1.5 mL microcentrifuge tube containing 30 μL of internal standard solution (83 ng/mL ascomycin and 833 ng/mL cyclosporine D in 70% methanol). Samples were vortex mixed briefly before adding 0.5 mL of methanol to precipitate the proteins. Samples were vortex mixed again, and after 10 min of mechanical stirring (1100 rpm in a Vortemp 56 shaker, Labnet Internationnal, Woodbridge, NJ, USA), the samples were centrifuged at 11,000 × *g* for 5 min. Aliquots of the supernatants (100 μL) were then transferred into HPLC vials containing 100 μL of 6 mM zinc sulphate.

2.4. LC–MS/MS analysis

Samples were injected (20 μL) on 1200 series HPLC systems (equipped with two binary pumps, a column heater and associated column-switching valve, absorbance detectors, and an automatic injector with needle washing capability) coupled to 6410 electrospray tandem mass spectrometers (Agilent Technologies, Montréal, Québec, Canada). Chromatography was performed on a SecurityGuard pre-column (C_{18}, 4 mm × 3 mm, 10 μm, cat. #AJ0-4287, Phenomenex, Torrance, CA) at a temperature of 50 °C. Mobile phase A was 100 μmol/L sodium acetate in water, and mobile phase B was 100 μmol/L sodium acetate in methanol. The initial chromatographic conditions were 60% B at a flow rate of 0.6 mL/min, and gradient elution of the immunosuppressants was performed by increasing to 100% B from 0.10 to 1.00 min and maintaining 100% B until 1.50 min. The pre-column was then washed and reconditioned as follows: 100% B at 1 mL/min from 1.51 to 1.90 min followed by 60% B at 1 mL/min from 1.91 to 2.25 min. Total chromatographic run time was 2.25 min with a programmed overlap sample injection (injector valve switched to bypass at 0.5 min). Solvent flow was diverted directly to waste instead of the mass spectrometer from 0 to 0.5 min and from 1.8 to 2.25 min. Detection of the sodium adducts of the different compounds was accomplished by tandem mass spectrometry in positive mode with a dwell time of 100 ms. Details of the mass spectrometer settings are listed in Table 1. Ascomycin was used as the internal standard for the quantification of both tacrolimus and sirolimus, while cyclosporine D was used as the internal standard for the quantification of cyclosporine.

2.5. Precision and accuracy

Intra-assay precision was determined by replicate analysis ($n = 7$) of three samples with different concentrations of immunosuppressants in a single run. Inter-assay precision was calculated from the Lyphochek Whole Blood Immunosuppressant quality control data accumulated from September 15th 2010 to April 29th 2011 using Unity Real Time QC Data Management software (Bio-Rad Laboratories).

Intra-assay accuracy was determined by comparing the measured concentration of immunosuppressants with the expected value in the lowest and highest calibrators ($n = 8$ in a single run). Inter-assay accuracy was calculated in the same manner, but over different runs ($n = 100$ different runs).

Table 1
Mass spectrometer parameters.

Compound	Fragments (*m*/*z*)	Fragmentor voltage	Collision energy
Tacrolimus	826.4 → 616.2	200 V	35 V
Sirolimus	936.6 → 409.3	360 V	60 V
Cyclosporine	1224.8 → 1112.0	300 V	75 V
Ascomycin	814.2 → 604.1	260 V	40 V
Cyclosporine D	1238.9 → 1126.4	300 V	75 V

Source parameters: gas temperature: 350 °C; gas flow: 10 L/min; nebulizer pressure: 40 psi; capillary voltage: 4000 V.

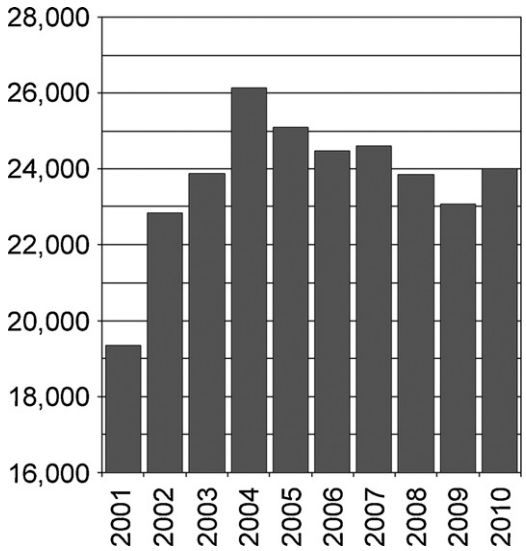

Fig. 1. Total number of tacrolimus, sirolimus, and cyclosporine measurements per year.

2.6. Evaluation of sample recovery and ion suppression

Extraction efficiency was calculated by spiking tacrolimus, sirolimus, and cyclosporine at final concentrations of 1.03 ng/mL for tacrolimus and sirolimus, and 25.86 ng/mL for cyclosporine in 5 extracts of blank human whole blood (10 μL of concentrated solution added to 200 μL of extracts). The MS/MS peak areas from these spiked extracts were compared with the peak areas obtained from processed samples of whole blood containing 24 ng/mL tacrolimus and sirolimus, and 600 ng/mL cyclosporine (which, after sample processing, is expected to give the same peak areas as the spiked samples).

The presence of ion suppression effects on the MS/MS response was evaluated by spiking equivalent amounts of immunosuppressants in extracts of blank whole blood ($n = 15$ different patient samples) or extracts of water ($n = 10$) and by comparing the peak areas from these two sets of samples.

2.7. Correlation with immunoassays

Immunosuppressant measurements in patient samples were performed both by LC–MS/MS and on an ARCHITECT ci16200 integrated chemistry/immunoassay system (Abbott Laboratories Limited, Diagnostics Division, Mississauga, ON, Canada) using chemiluminescent microparticule immunoassays (immunoassays were performed at Hôpital Maisonneuve-Rosemont). All samples were measured in duplicate with both methodologies ($n = 40$ for tacrolimus and sirolimus, and $n = 36$ for cyclosporine). The correlation between the methods was computed by weighted Deming regression analyses using the CBstat version 5.1.1 software (K. Linnet, purchased from the American Association for Clinical Chemistry online store, cat. #2475, www.aacc.org).

3. Results

In 2008, two LC–MS/MS systems were implanted in our laboratory for the quantification of tacrolimus, sirolimus, and cyclosporine. Because of the relatively high number of analyses performed annually at our hospital (Fig. 1), a high-throughput method was needed. For this reason, a simple protein precipitation was chosen to extract immunosuppressants, and a rapid chromatographic method was developed (using only a guard cartridge) for the simultaneous measurement of the three immunosuppressant drugs by tandem mass spectrometry. In the selected conditions, all immunosuppressants and corresponding internal standards were eluted from the cartridge in approximately 1.4 min, for a total chromatographic run time of 2.25 min. By programming an injection overlap, the throughput was increased to roughly 24 samples/h. The immunosuppressants were detected as their respective sodium adducts in multiple reaction monitoring mode, and the MS/MS response was shown to be linear from 2.5 to 24 ng/mL for tacrolimus and sirolimus, and from 62.5 to 600 ng/mL for cyclosporine (average correlation coefficient of 0.990, 0.979, and 0.991, respectively, $n = 100$ different assays). Samples that exceeded these ranges were re-assayed following dilution with blank whole blood. To avoid excessive re-assaying, cyclosporine 2 h post-dose samples were automatically diluted 1 in 3 prior to analysis. Overall, 0.66% of samples needed retesting after dilution (data not shown).

Table 2
Method precision.

Compound	Intra-assay precision			Inter-assay precision		
	Mean (ng/mL)	CV	n	Mean (ng/mL)	CV	n
Tacrolimus	3.6	6.5%	7	4.0	9.3%	364
	7.5	6.5%	7	9.6	7.7%	364
	12.1	2.3%	7	15.6	7.5%	364
Sirolimus	5.0	11.7%	7	5.5	14.1%	327
	9.3	12.5%	7	10.7	11.0%	333
	18.2	4.7%	7	22.3	10.1%	331
Cyclosporine	85.4	4.6%	7	85.2	7.8%	362
	165.7	5.1%	7	161.9	7.2%	362
	265.7	3.6%	7	283.7	6.9%	360

3.1. Precision and accuracy

The method showed good precision, with intra-assay and inter-assay imprecisions below 10% for tacrolimus and cyclosporine, and below 15% for sirolimus (Table 2). The method was also shown to be accurate by comparing the measured immunosuppressant concentrations with the expected values for the lowest and highest calibrators (Table 3). Our laboratory also participated in the monthly ASI International Proficiency Testing Scheme (Analytical Services International Ltd., www.bioanalytics.co.uk). Results for samples of the proficiency testing scheme were always within the acceptable ranges of the peer group mean. The average biases (±standard deviation) from the last 12 challenges were $-0.5 \pm 9.2\%$, $-1.5 \pm 16.5\%$, and $-3.3 \pm 6.0\%$ for tacrolimus, sirolimus, and cyclosporine, respectively.

Table 3
Method accuracy.

Compound	Accuracy	
	Intra-assay ($n = 8$)	Inter-assay ($n = 100$)
Tacrolimus		
LLOQ (2.5 ng/mL)	99.9 ± 3.8%	100.0 ± 8.6%
ULOQ (24 ng/mL)	100.9 ± 2.6%	101.2 ± 6.3%
Sirolimus		
LLOQ (2.5 ng/mL)	100.6 ± 11.1%	104.4 ± 11.9%
ULOQ (24 ng/mL)	99.0 ± 4.7%	99.0 ± 7.1%
Cyclosporine		
LLOQ (62.5 ng/mL)	104.5 ± 3.9%	103.9 ± 6.9%
ULOQ (600 ng/mL)	101.4 ± 3.9%	100.0 ± 5.4%

LLOQ, lower limit of quantification; ULOQ, upper limit of quantification.

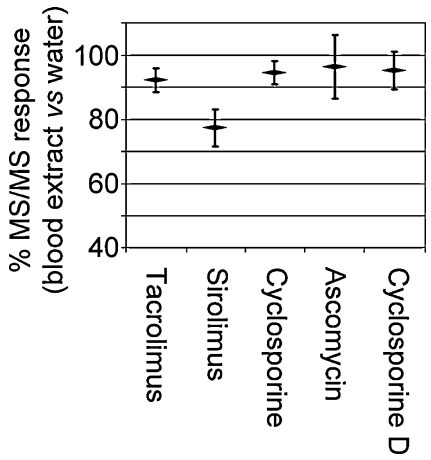

Fig. 2. Ion suppression effects. Immunosuppressants were added to blood extracts ($n = 15$ different patient extracts) or water extracts ($n = 10$). The LC–MS/MS responses obtained for the different compounds in blood samples compared to water samples are presented (mean ± standard deviation).

3.2. Recovery and matrix effects

The extraction efficiency was evaluated by comparing the MS/MS peak areas of processed samples of whole blood containing immunosuppressants ($n = 5$) with those of extracts of blank whole blood spiked with the expected concentration of each compound ($n = 5$). The calculated recoveries for tacrolimus, sirolimus, and cyclosporine were 95 ± 10%, 104 ± 6%, and 89 ± 6%, respectively (mean ± standard deviation).

The presence of ion suppression effects on the MS/MS response was determined by post-extraction spiking experiments. Extracts of blank whole blood ($n = 15$ different patient samples) and extracts of water ($n = 10$) were spiked with equivalent amounts of immunosuppressants before analysis by LC–MS/MS. The average MS/MS responses in the blood matrices compared to the water extracts were greater than 90% for all compounds, except for sirolimus (Fig. 2).

Interference in samples with high cyclosporine levels has been reported when using cyclosporine D as the internal standard [21]. This interference was presumed to be due to a metabolite of cyclosporine with the same mass as cyclosporine D. The presence of such an interference in our method was evaluated by analyzing patient samples containing high levels of cyclosporine ($n = 7$, range 825–2195 ng/mL cyclosporine) without adding cyclosporine D and no significant interference was observed (the mean background cyclosporine D levels in these samples was less than 1% of the cyclosporine D MS/MS response in samples with added internal standard).

3.3. Correlation with immunoassays

Results obtained with the LC–MS/MS method were compared to those obtained with immunoassays (chemiluminescent microparticule immunoassays on the Abbott ARCHITECT ci16200 analyzer) by analyzing patient samples in duplicate using both methodologies. Although the regression analyses showed that the methods were well correlated (Fig. 3A–C), a statistically significant positive proportional bias was observed for all immunoassays. Immunoassay results were overestimated compared to LC–MS/MS by a mean of 18.1% for tacrolimus, 41.4% for sirolimus, and 15.6% for cyclosporine (Fig. 3D–F), which is comparable to previously published biases for the Abbott Architect chemiluminescent immunoassays [22–25].

Table 4
Calculation of the amortization period.

Cost of immunoassay reagents per year (21,701 tests × $13.50)	$292,964
Cost of LC–MS/MS reagents per year (21,701 tests × $0.54)	$11,719
Average annual savings	$281,245
Cost of the two LC–MS/MS instruments	$625,000
Cost for the modification of the laboratory work space to receive the LC-MS/MS	$35,680
Cost of the annual service contract (for 4 years after the one year warranty)	$60,700
Amortization period 625,000 + 35,680 + [60,700 (x − 1)] = 281,245 (x)	x = 2.72 years (~33 months)

All prices are in Canadian dollars (CAD).

3.4. Financial aspects

The introduction of mass spectrometry in our laboratory took approximately two years, from the planning of the project to the analysis of the first clinical samples. The initial step was establishing a financial plan to submit to the biochemistry department and to the hospital management in order to get approval for funding. At the time, the cost per sample for our tacrolimus and cyclosporine immunoassays was $13.50 CAD, while the estimated cost per sample for the reagents, consumables, HPLC columns, and solvents required for the operation of the LC–MS/MS was established at $2.50 CAD. When including the projected cost of the annual service contract for the mass spectrometers, the anticipated savings over a 5-year period were $1,046,925 CAD, or $209,385 CAD/year (considering a predicted 22,558 tacrolimus and cyclosporine tests per year, like in 2005). Sirolimus tests were not included in the calculations since this drug was measured by HPLC, not by immunoassay, and that no significant difference in reagent cost per sample was expected when switching from HPLC-UV to HPLC–MS/MS. In addition, the medical technologists' salaries were not considered in the financial planning since the same number of technologists was required for the measurement of immunosuppressants before and after implanting the mass spectrometers.

After three years of routine operation, the financial analysis was updated in view of the real tacrolimus and cyclosporine test volumes (mean 21,701 tests per year from 2008 to 2010) and the definite cost per sample using LC–MS/MS ($0.54 CAN per sample including costs for standards, solvents, sample vials, pipet tips, guard columns, and nitrogen collision gas). The details of the calculations are presented in Table 4. The actual costs per sample for LC–MS/MS analysis of immunosuppressants are much lower than initially anticipated, partly because the chromatography was performed on an inexpensive guard column and that methanol was chosen instead of acetonitrile as the extraction and mobile phase solvent. Thus, the two LC–MS/MS systems were completely financed by the savings in reagent costs in approximately 33 months.

3.5. Training of personnel and routine operation

Mass spectrometers are complex instruments and proper training of the laboratory personnel is important to ensure the quality of the results produced. The medical technologists must perform the sample processing steps and adequately operate and supervise the instruments. Since immunosuppressants are analyzed daily, even over the weekends, 10 medical technologists were trained to operate the mass spectrometers, to perform routine maintenance, and also to solve minor problems. The trained technologists are not dedicated to the pharmacology laboratory, but they rotate in the core lab and sometimes in other specialized sectors. To facilitate

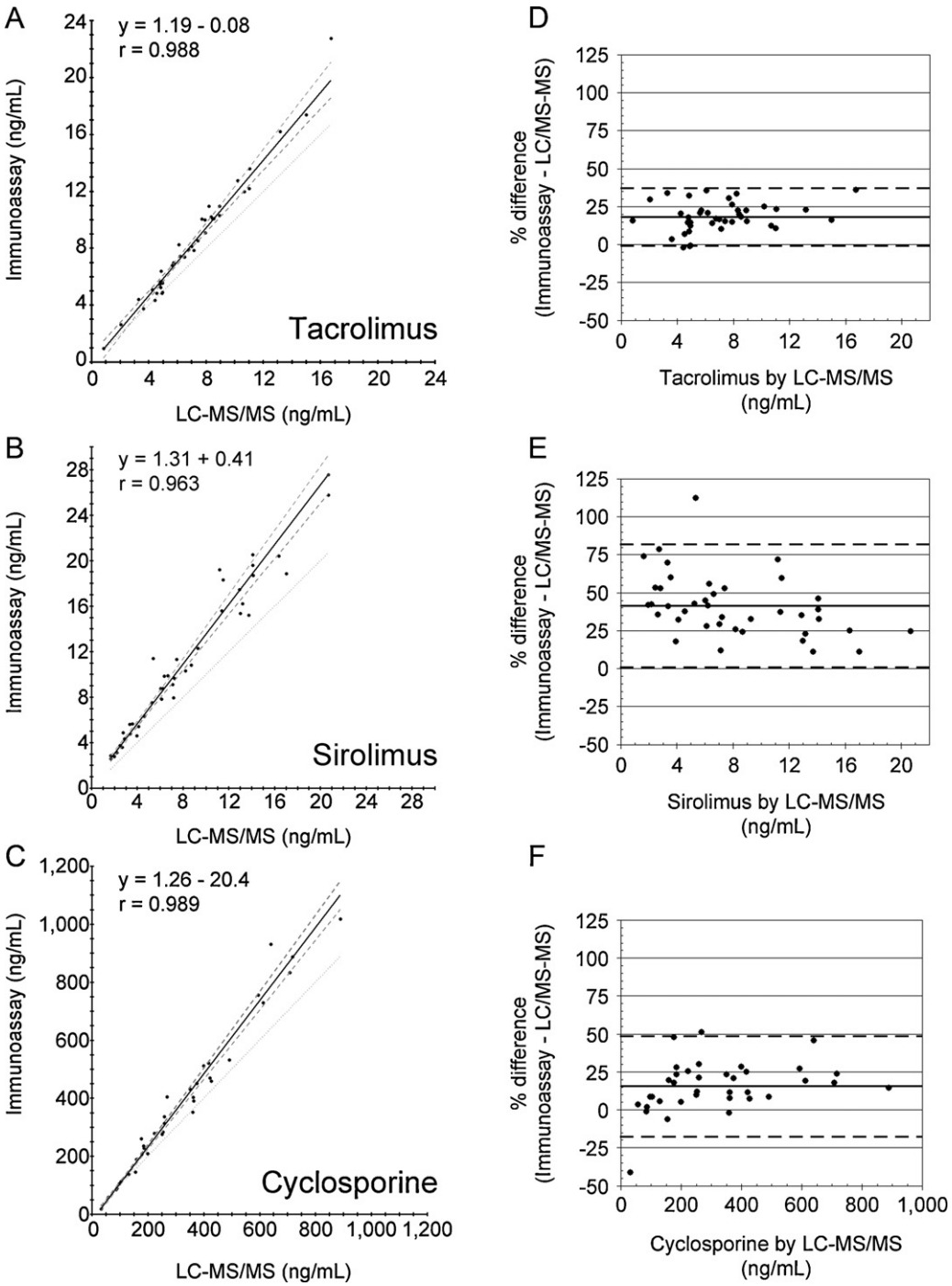

Fig. 3. Correlation between LC–MS/MS and immunoassays. The immunosuppressants were measured in duplicate using both methodologies ($n = 40$ for tacrolimus and sirolimus, and $n = 36$ for cyclosporine). (A–C) Weighed Deming regression analyses results are presented, with 95% confidence interval of the slope depicted as long dotted lines on each side of the regression line, and the identity line depicted as a short dotted line. (D–F) Bland–Altman plots representing the percentage difference of the immunoassay results compared to the LC–MS/MS results, with mean bias indicated by solid lines and the 95% confidence intervals by dotted lines.

maintenance of the instruments, checklists were prepared stating what tasks need to be done and at what frequency. The technologists perform most of the preventive maintenance, although more complex interventions are left to the laboratory coordinator.

In our laboratory, only two people are fully trained to troubleshoot complicated problems or to set up new methods: the clinical biochemist and the laboratory coordinator. However, this does not affect the routine operations because two identical HPLC–MS/MS systems have been installed, and if one instrument fails then the samples are analyzed on the other system until the laboratory coordinator, the clinical biochemist, or the vendor's technical service corrects the problem.

For the volume of immunosuppressant tests ordered in our laboratory, two medical technologists are needed to assure that the patient results are reported on the same day the samples are received (one person from 8:00 am to 2:00 pm each day, and another person from 8:00 am to 4:00 pm on weekdays). One of the technologists is in charge of performing the sample processing, while the other manages the mass spectrometers (performs maintenance, changes solvents, creates the worklist, checks the

calibration and the quality control results, verifies peak integrity, and inscribes patient results in the laboratory information system).

4. Discussion

The present paper describes the successful replacement of immunoassays by mass spectrometry for the simultaneous quantification of tacrolimus, sirolimus, and cyclosporine. Because of the minimal sample pre-treatment steps and the rapid chromatography run-time on the guard column, the developed method is well suited for routine quantification of immunosuppressant drugs in the high volume clinical laboratory setting. The method is technically simple, rapid, accurate, and precise (with inter-assay imprecision lower than 10–15%). The method also shows good extraction efficiency for all immunosuppressants and little matrix effects, except for sirolimus for which ion suppression effects are more pronounced. The observed ion suppression for sirolimus does not affect quantification of patient samples since it is relatively constant from sample to sample and can be corrected for by making the calibration curve in a whole blood matrix. In addition, replacing immunoassays by mass spectrometry lead to substantial savings in reagent costs, as also reported by others [26,27]. In our laboratory, the estimated annual savings reached > $250,000 CAD and permitted the complete financing of two HPLC–MS/MS systems over less then three years. In order to absorb the cost of the two mass spectrometers (including installation and annual service contracts) over a 5-year period, a minimum of 13,950 tests/year would have be needed (based on an estimated saving of $13 CAD per test).

Immunoassays are still largely used to quantify immunosuppressant drugs in clinical laboratories. The major advantage of such assays is their ease of operation, and also that they are performed on instrument platforms used for other immunoassays and which are sometimes already installed in the laboratory. However, the major inconvenient of these assays is the lack of specificity for the parent drug *vs.* drug metabolites or structurally related compounds, as exemplified by the fact that immunoassays tend to overestimate immunosuppressant drug levels when compared to chromatographic methods (see Section 3.3 and [16–19,22–26]). Since different immunoassays show variable degrees of specificity, assay-specific therapeutic ranges should be established and the long-term therapeutic monitoring of patients should be done using the same method.

On the other hand, mass spectrometric methods measure only the parent drug, and the results produced by these methods are thus more accurate. However, small differences between different laboratories using mass spectrometry may be observed, partly because no certified reference materials are available to prepare calibrators for tacrolimus, sirolimus, and cyclosporine. The major drawbacks of mass spectrometry in the clinical laboratory setting are the turnaround time constraints, the complexity of the instrumentation, and the requirements for highly trained personnel. In our experience, the training of medical technologists to operate mass spectrometers and to perform some of the routine maintenance is not a major challenge. The total training time allocated for a new technologist is 4 days, during which time the person learns how to use the mass spectrometry software, how to start the instrument, how to change the mobile phases, the columns, and the filters, how and when to tune the mass spectrometers, and how to prepare the patient samples for analysis. After the initial training, the new technologist is teamed with an experienced technologist for the first weeks so as to learn to solve rare problems when they arise. If needed, the clinical biochemist or the fully trained laboratory coordinator is always available to assist the technologists. Finally, for a routine assay like quantification of immunosuppressant drugs where a rapid turnaround time is required for a high volume of samples, a short chromatographic run-time should be favored, and the sample preparation should be simple and rapid. In addition, the installation of two identical HPLC–MS/MS systems should be considered in order to prevent service interruptions when preventive maintenance needs to be performed on one instrument, or in the case of a major problem necessitating intervention from the instrument manufacturer. Having two systems also allows for greater flexibility. In our laboratory, both instruments are used daily for analysis of immunosuppressant drugs, which accelerates the reporting of patient results. Also, the free instrument time (end of the day and overnight) is used to quantify other compounds (such as hypoglycemic agents, anticonvulsive drugs, antiviral drugs, and creatinine [28]) for clinical purposes or for research projects.

Many mass spectrometric methods for the measurement of immunosuppressant drugs have been described [27,29–38]. Most methods use electrospray ionization and quantify ammonium adducts of the immunosuppressants [27,29–35] while other methods monitor sodium adducts [37,38]. In our method, sodium adducts were forced by addition of sodium acetate to the mobile phases. Simple sample processing procedures are a major advantage when a high number of samples needs to be analyzed, and simple protein precipitation is frequently performed prior to analysis of immunosuppressant drugs, although solid phase extraction remains an option, either prior to the chromatography [33,37] or on-line [30,34,38]. The total chromatographic run-time is also trivial when results need to be reported on the same day the samples were received, but the quality of the results must still meet clinical needs. In such, the method described in the present paper shows comparable precision as most published methods, with better or comparable throughput. In addition, the present method uses a simple guard column for chromatographic retention instead of a much more expensive analytical column, which leads to significant cost savings without affecting the quality of the results, as evidenced by the monthly international proficiency testing scheme.

5. Conclusion

Mass spectrometry is now an integral part of the clinical laboratory. With support from the department and the hospital management, and with the hard work of laboratory personnel that are willing to learn new technologies, mass spectrometry can easily be introduced in specialized sections of clinical laboratories. The initial investment is rapidly compensated by the low cost of reagents per sample, with better quality results as a bonus. Also, the flexibility offered by HPLC–MS/MS systems to develop new assays quickly and independently from in vitro diagnostic companies is a great advantage and facilitates research collaborations with other clinical departments or with academic laboratories.

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