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An Automated MALDI Mass Spectrometry Approach for Optimizing Cyclosporin Extraction and Quantitation

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A combinatorial extraction method and an automated matrix-assisted laser desorption/ionization (MALDI) mass spectrometry procedure were used to improve the clinical analysis of the immunosuppressant drug cyclosporin A. Cyclosporin extracts from whole blood were analyzed by MALDI and electrospray ionization (ESI) mass spectrometry, allowing for their identification and quantification. Due to limitations associated with the current multistep cyclosporin extraction procedure from whole blood, a combinatorial approach was devised to optimize this extraction. Optimization was performed by generating an array of solvent systems to be used for extraction from blood, and an automated analysis was carried out on a MALDI mass spectrometer to identify successful extractions. The first generation of experiments revealed four binary solvent systems to be effective for cyclosporin extraction (hexane/EtOH, ACN/H_2O, ACN/MeOH, and hexane/$CHCl_3$). A new array based on these solvent systems was generated, and a second iteration of these experiments was then performed. In the second generation of experiments, hexane/$CHCl_3$ (70:30) was found to provide the most effective single-step extraction of these solvent systems for cyclosporin and its metabolites. The limits of detection were determined to be 15 ng/mL in whole blood for ESI/MS and MALDI-MS and could also be used for identifying major drug metabolites. In addition to applying this combinatorial approach to extraction procedures, this experimental design could easily be extended to examine other approaches, such as optimizing chemical reactions and screening inhibitors in enzymatic reactions.

Mass spectrometry is a powerful tool for the analysis of drugs and may be a viable clinical alternative to other analytical methods, such as immunoassays or high-performance liquid chromatography (HPLC). While immunoassays can simultaneously measure for a drug and its metabolites, this approach typically has low reproducibility and reliability[1,2] and provides little to no selectivity between a drug and its metabolites. This is a major limitation, since metabolites often have biological activity similar to that of the parent compound yet are indistinguishable by immunoassays.[3,4] And although HPLC is relatively selective and accurate, the sensitivity is very compound-dependent, and the method development can be time-consuming. In comparison, mass spectrometry has demonstrated its utility in monitoring, characterizing, and quantifying a variety of low molecular weight drugs and their metabolites with greater selectivity, accuracy, and efficiency.[1,5−11]

The analysis of cyclosporin A (CsA), an immunosuppressant provided to liver transplant patients, requires close monitoring of its concentration in a patient's blood to ensure adequate immune suppression. A lipophilic cyclic peptide, CsA represents one of the most clinically tested drugs, whose quantification from whole blood is typically accomplished by fluorescence polarization immunoassay (FPIA), radio immunoassay, enzyme-linked immunosorbent assay (ELISA), or HPLC. As an alternative, mass spectrometry offers a variety of advantages over these techniques; as stated above, it is mass-specific, rapid, and sensitive for picomole to femtomole quantities of material. Recently, Muddiman et al.[1,5] reported using matrix-assisted laser desorption mass spectrometry (MALDI-MS) in the detection and quantification of CsA in blood with results comparable to those obtained with HPLC.

While the advantages of using mass spectrometry for the analysis of CsA are clear, a major obstacle lies in the extraction procedure. The "ether" extraction steps[12] initially used in this study, while effective at extracting CsA, required an inordinate time commitment (typically 50 min to 1 h) for completion. To overcome this problem, a procedure was designed to optimize extraction by employing mass spectrometry and combinatorial chemistry.

RESULTS AND DISCUSSION

These experiments used mass spectrometry and combinatorial chemistry to develop an efficient extraction method for cyclosporin from whole blood samples and investigated mass analysis as a

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(1) Muddiman, D. C.; Gusev, A. I.; Proctor, A.; Hercules, D. M.; Venkataramanan, R.; Diven, W. *Anal. Chem.* **1994**, *66*, 2362−2368.
(2) Rustum, A. M. *J. Chromatogr. Sci.* **1990**, *28*, 594−98.
(3) Rosano, T. G.; Freed, B. M.; Cerilli, J.; Lempert, N. *Transplantation* **1986**, *42*, 262−267.
(4) Freed, B. M.; Rosano, T. G.; Lempert, N. *Transplantation* **1987**, *43*, 123−127.
(5) Muddiman, D. C.; Gusev, A. I.; Langner, K. S.; Proctor, A.; Hercues, D. M.; Tata, P.; Venkataramanan, R.; Diven, W. *J. Mass Spectrom.* **1995**, *30*, 1469−1479.
(6) Taylor, P. J.; Jones, A.; Balderson, G. A.; Lynch, S. V.; Norris, R. L.; Pond, S. M. *Clin. Chem.* **1996**, *42*, 279−285.
(7) Poon, G. K.; Raynaud, F. I.; Mistry, P.; Odell, D. E.; Kelland, L. R.; Harrap, K. R.; Barnard, C. F.; Murrer, B. A. *J. Chromatog. A* **1995**, *712*, 61−66.
(8) Pacifici, R.; Pichini, S.; Altieri, I.; Caronna, A.; Passa, A. R.; Zuccaro, P. *J. Chromatog. B, Biomed. Appl.* **1995**, *664*, 329−334.
(9) Straub, R. F.; Voyksner, R. D. *J. Chromatogr.* **1993**, *1993*, 167−181.
(10) Rideout, D.; Bustamante, A.; Siuzdak, G. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10226−10229.
(11) Crowther, J.; Adusumalli, V.; Mukherjee, T.; Jordan, K.; Abuaf, P.; Corkum, N.; Goldstein, G.; Tolan, J. *Anal. Chem.* **1994**, *66*, 2356−61.
(12) Garraffo, R.; Lapalus, P. *J. Chromatogr.* **1985**, *337*, 416−422.

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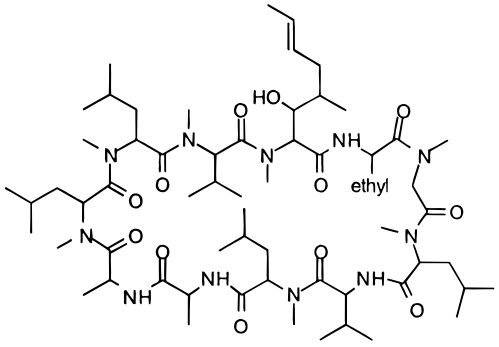
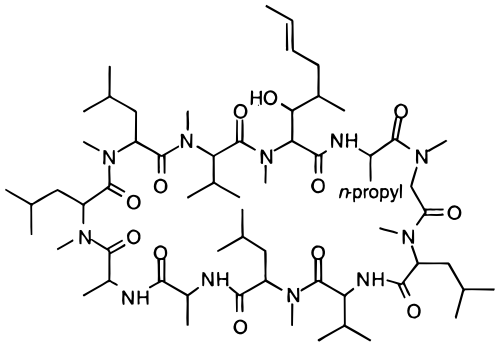


Cyclosporin A



Cyclosporin G

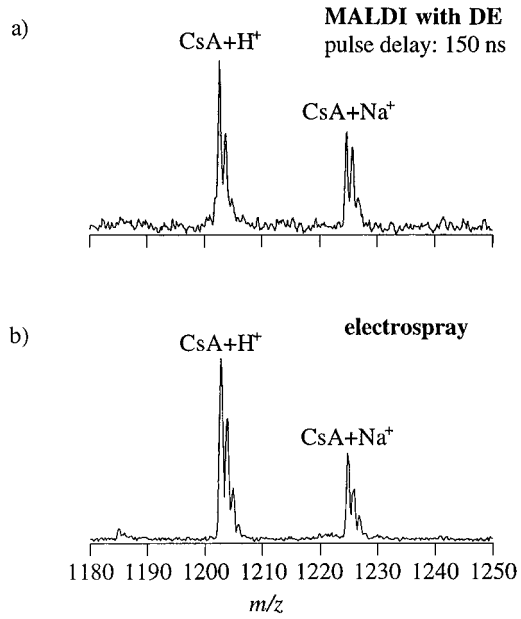


Figure 1. Mass spectrum (a) of CsA extracted from whole blood (spiked with CsA) using matrix-assisted laser desorption/ionization (MALDI) with a delayed extraction time-of-flight analyzer, comparable to (b) mass data obtained by electrospray ionization with a quadrupole.

clinical tool by examining its resolving power,[13] quantitation capabilities, and metabolite identification. In the past, the resolving power of MALDI mass spectrometers presented a significant limitation in accurately identifying drugs and their metabolites.[5] This issue was addressed in the analysis (Figure 1) of CsA using ESI with a quadrupole mass analyzer and MALDI using time-of-flight analysis with a delayed extraction[14] ionization source. The data in Figure 1 clearly indicate that MALDI with delayed extraction and ESI have sufficient resolving power to distinguish isotopes, as well as contaminating species or metabolites differing in mass by only 1 Da.

A Combinatorial Approach toward Optimizing Extraction Conditions. Extraction is a necessary part of the analysis procedure for mass spectrometry, HPLC, and immunoassays. Mass spectrometry, in particular, requires samples that are relatively salt-free. The amount of time required for this extraction[12] represents a serious limitation in automating CsA analysis by mass spectrometry and, in general, is a rate-limiting step in obtaining mass analysis of many drugs from a biological medium. Given this, efforts were directed toward creating a more simplified and efficient extraction protocol.

Extraction procedures are typically developed through trial and error, relying on chemical insight as well as a fortuitous application of solvents. In order to develop a single-step methodology for extracting CsA from whole blood, a combinatorial approach for identifying effective extraction conditions was incorporated using chemical insight based on the polarity of CsA. Analysis was performed using an automated mass spectrometry-based approach. The new CsA extraction procedure was then examined (using cyclosporin G as an internal standard) with ESI-MS and MALDI-MS to test and compare the clinical utility of these mass analysis techniques and to determine the effectiveness of the new one-step extraction procedure.

Optimizing the extraction procedure would increase the efficiency of CsA analysis; however, performing the optimization experiments can involve days of searching for the best solvent conditions. As an alternative to manually performing these tests, we took an approach that employed both combinatorial chemistry and automated mass spectrometry. In our method, seven solvents, including acetonitrile, hexane, chloroform, methanol, ethanol, a sodium hydroxide solution, and water, were mixed in different ratios to create 36 binary solvent systems. An extraction was performed with each binary solvent system on blood samples spiked with CsA. The experiment then involved adding 200 μL of solvent to 100 μL of blood, vortexing for 2 min, centrifuging for 1 min, extracting the organic layer, evaporating the solvent, and finally redissolving in 40 μL of methanol to facilitate ESI analysis. The time course of the extraction procedure required 5−10 min, depending on how evaporation was facilitated. The sample was then submitted (in duplicate) for MALDI mass analysis.

Automated MALDI Analysis. Because MALDI-MS is better suited for the simultaneous analysis of multicomponent mixtures than ESI-MS and the MALDI matrix effects of 2,5-dihydroxybenzoic acid (DHB) are minimal above m/z 500, MALDI-MS with the DHB matrix was used for the automated testing of these biological extracts. In addition, while LC/MS has many advantages, such as low background noise and no matrix interference, it was not used because it would significantly reduce the efficiency of this approach. A MALDI mass spectrometer equipped with automated, multisampling capabilities was used to facilitate data collection and analysis. The organic layer for each sample was placed on a MALDI sample plate (with a capacity for 100 samples) and was then loaded into the mass spectrometer and analyzed using a computer-controlled algorithm.

(13) Siuzdak, G. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11290−11297.
(14) Vestal, M. L.; Juhasz, P.; Martin, S. A. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 1044−1050.

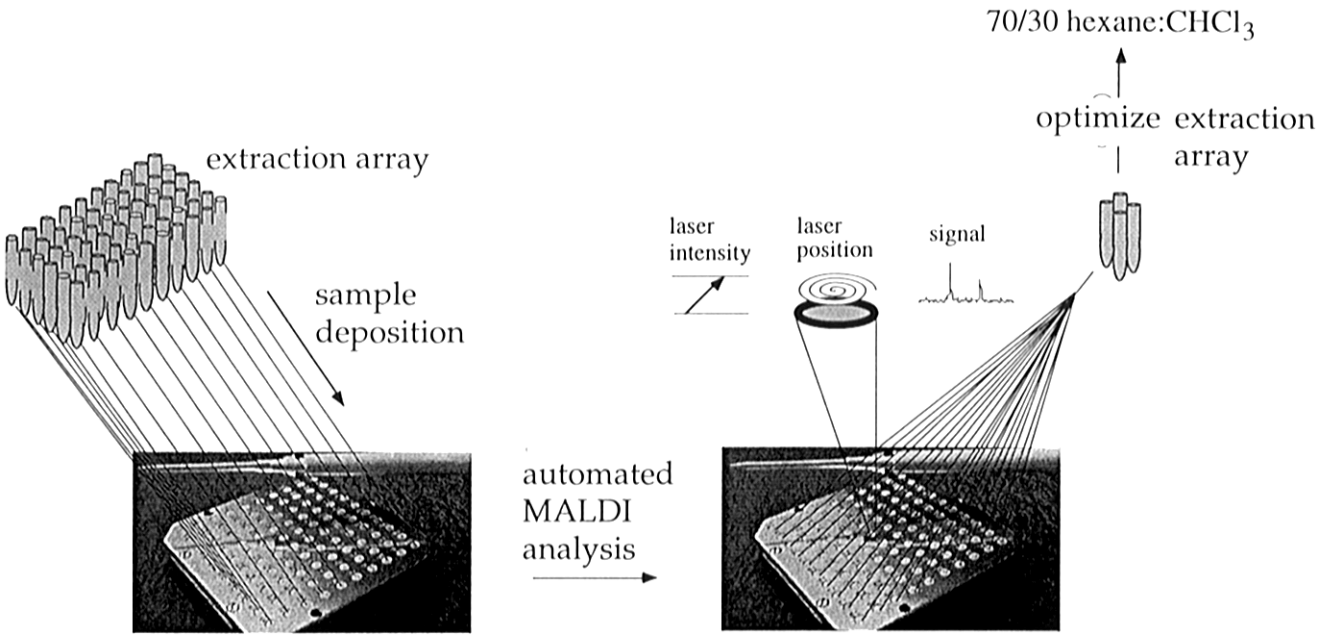


Figure 2. Automated MALDI mass spectrometry experiments, performed as a function of laser position (within each well) and laser intensity for each extracted CsA sample. The first iteration of these extraction experiments generated four useful solvent systems, and the second generation of these experiments produced an effective 70:30 hexane/$CHCl_3$ extraction solvent system.

The automation of the MALDI analyses was designed to search for a CsA signal from each well corresponding to each extraction system. These analyses were driven by a computer-controlled procedure to monitor for the CsA ion signal as a function of laser position within each sample well and as a function of laser intensity. To accomplish this, the computer workstation automatically adjusted laser intensity and searched the sample well until a signal (within the specified mass range and intensity threshold) was obtained. Each parameter (laser intensity, step size in well, and m/z range) was adjusted on the basis of a careful preselection of autosampler options from the manual analysis of CsA to minimize time of the analysis and maximize the signal quality.

The laser intensity was initially set at a minimum energy setting and then allowed to increase up to a maximum (from ~2 to 50 μJ/pulse, as controlled by a variable neutral density filter). Step sizes were made in five increments, resulting in an increase in laser intensity of approximately 10 μJ/pulse per step. The laser intensity was increased until an acceptable data signal was acquired, whereby if no signal was observed, the laser beam was repositioned on the well and MALDI analysis resumed at the lower laser power. To adjust the laser position on the MALDI sample plate, a preprogrammed spiral search pattern was used which began in the center of each circular well and spiraled outward in 0.2 mm increments. For each sample well analysis, only signals that reached a specified intensity were saved, and once this signal was observed, the analyses would automatically move to the next well. On average, the total time spent for each sample well analysis was 140 s. This included acquisition time for averaging 64 scans and a delay for adjusting laser intensity and repositioning the sample plate.

Following the first set of experiments using the MALDI-automated analysis procedure (Figure 2), four extraction solvent systems (hexane/EtOH, ACN/H_2O, ACN/MeOH, and hexane/$CHCl_3$) proved to be reasonably effective in extracting CsA. Similar to employing combinatorial chemistry in chemical synthesis, another iteration was performed to further optimize these extraction conditions, in which the four systems were tested at ratios of 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, and 10:90. In these second generation experiments, it was found that the solvent hexane/$CHCl_3$, at a ratio of 70:30, provided the optimum extraction conditions. With this solvent system, we set out to compare this method to the previous extraction method (ether extraction) in terms of reliability, quantification, and metabolite identification.

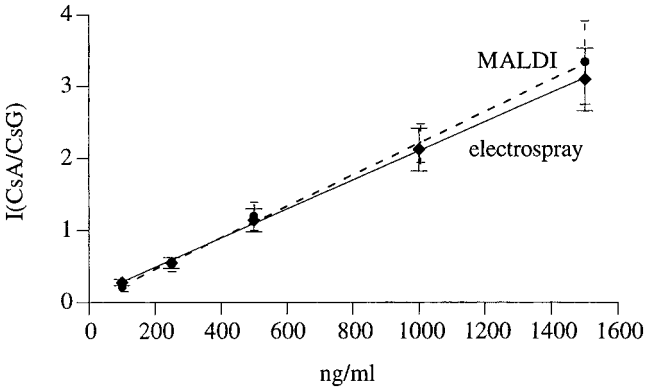


Figure 3. Standard curve of CsA with CsG as an internal standard generated with MALDI, $y = 0.0800 + 0.00220x$ ($R = 0.999$), and electrospray, $y = 0.0803 + 0.00203x$ ($R = 0.999$).

Quantitation and Metabolite Analysis. Both ESI and MALDI were employed to examine the new CsA extraction procedure and the ether extraction (ESI-MS because it represents an important method in quantitative drug analysis). First, calibration curves were obtained using the ether extraction (a plot of the ratio of $\sum_{int}[CsAH^+ + CsANa^+]/[CsGH^+ + CsGNa^+]$ vs CsA concentration) with both instruments. The data from this exhibited a linear relationship in the concentration range that was tested (0−1500 ng/mL). The equations of the lines were $y = 0.0575 + 0.00212x$ ($R = 0.999$) and $y = 0.0835 + 0.00208x$ ($R = 0.999$) for electrospray and MALDI, respectively, and the relative standard

Table 1. Results Obtained from the ESI-MS and MALDI-MS Analysis of Standard Blood Samples

CsA standard (ng/mL)	ether extraction[12]				30% hexane/70% $CHCl_3$ extraction			
	ESI		MALDI		ESI		MALDI	
	(ng/mL) (CV)	error	(ng/mL) (CV)	error	(ng/mL) (CV)	error	(ng/mL) (CV)	error
100	96 (4%)	4%	92 (9%)	8%	98 (8%)	2%	94 (4%)	6%
250	262 (4%)	5%	279 (10%)	12%	231 (3%)	8%	238 (1%)	5%
500	446 (1%)	11%	499 (1%)	0.2%	523 (8%)	5%	543 (4%)	9%
1000	1002 (3%)	2%	1037 (2%)	4%	1009 (4%)	1%	961 (4%)	4%
1500	1520 (2%)	1%	1471 (8%)	2%	1404 (7%)	6%	1514 (5%)	1%

deviation of the slope was 2% for both. The new extraction generated standard curves for CsA that were comparable with those from the ether extraction method (Figure 3). Here, the relative standard deviations of the slope were 1.2% for electrospray and 2.5% for MALDI, with a correlation coefficient (R) of 0.999. Errors increased at higher concentrations of CsA because the internal standard was maintained at 200 ng/mL to provide higher accuracy at low concentrations of CsA; therefore, it is likely that this created greater variations at higher concentrations. Analyses were also performed to determine the lower levels of CsA detection from the blood samples with MALDI-MS, experiments which resulted in a lower limit of detection of 15 ng/mL.

Following the generation of a calibration curve, five standard blood samples were obtained from a kit used in clinical analysis and tested with both the new and ether extraction procedures (Table 1). The results for each sample were the averages of approximately 25 data points obtained over a period of 5 min whose relative deviations were calculated by the mean concentration with respect to the standard sample concentration. The accuracy of the new combinatorial extraction and the ether extraction was, on the average, ±5%. The precision was indicated by the coefficient of variation (CV), which is the percentage of standard deviation of the mean. This CV ranged from 1 to 4% for electrospray and from 1 to 10% for MALDI with the ether extraction, and from 3 to 8% for electrospray and from 1 to 5% for MALDI with the new extraction procedure. In general, electrospray mass analysis produced slightly less variation than MALDI mass analysis.

It is known that metabolites interfere with the immunoassay quantitative analysis of CsA due to the cross-reaction with the assay.[2,15,16] The inability to effectively distinguish metabolites from the parent drug has also been experienced in mass spectrometry. Muddiman et al. reported that, when using a reflectron time-of-flight mass spectrometer to analyze CsA and its metabolites, a significant overlap between metabolite, internal standard, and CsA (2 Da mass difference) was observed.[1,5]

In our study, the interference was resolved by the sufficiently high mass resolution of the quadrupole ESI and delayed extraction MALDI mass spectrometers. The patient's blood samples were analyzed by both MALDI and ESI, as shown in Figure 4. The ion at m/z 1203 is $[CsA + H]^+$, m/z 1217 is $[CsG + H]^+$, m/z 1219 is a protonated CsA metabolite (M8, M17, and/or M18), m/z 1225 is $[CsA + Na]^+$, m/z 1235 is a metabolite of CsA (M10 and/or M16), m/z 1239 is $[CsG + Na]^+$, and m/z 1241 is the hydroxylated metabolites (M8, M17, and/or M18).[17,18] The ion

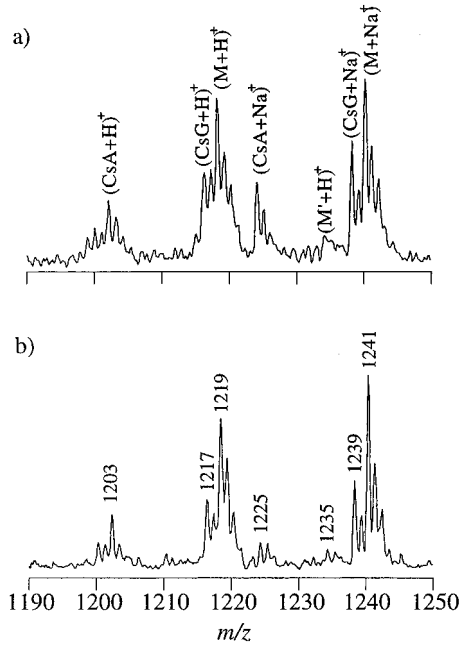


Figure 4. (a) MALDI mass spectrum of a patient blood extract containing CsA spiked with CsG. The extraction procedure was performed using the traditional multistep extraction procedure.[12] (b) MALDI spectrum of a patient blood extract containing CsA spiked with CsG using the single-step extraction procedure developed with the combinatorial approach. M (M8, M17, and/or M18) and M′ (M10 and/or M16) represent metabolites of CsA.

at m/z 1241 may also have a small contribution due to the K^+ adduct of CsA; however, this contribution is less than a couple of percent of the total peak due to the low concentration of K^+. In both the ESI and MALDI mass spectral experiments, cyclosporin metabolites were resolved from the parent drug and detected in significant quantities.

The quantitative data generated from the mass analysis of the patient's blood are listed in Table 2 for both extraction methods and are compared with the results from a clinical fluorescence polarization immunoassay method (FPIA). The concentration of each sample (the mean of three replications) served as the clinical result. In all cases, the mass spectral results were lower than clinical results. The highest difference between the two was 40%, which is much higher than standard curve statistics. However, FPIA assay does overestimate the concentration of CsA due to the cross-reaction with the CsA metabolites. In fact, the concentrations of CsA obtained from FPIA can be 90% higher than those obtained using an HPLC-based assay.[19] These results are con-

(15) Blick, K. E.; Melouk, S. H.; Fry, H. D.; Gillum, R. L. *Clin. Chem.* **1990**, *36*, 670.
(16) Burckart, G. J.; Starzl, T.; Williams, L.; Sanghvi, A.; Gartner, C.; Venkataramanan, J. R.; Zitelli, B.; Malatack, J.; Urbach, A.; Diven, W.; Ptachcinski, R.; Shaw, B.; Iwatsuki, S. *Transplant. Proc.* **1985**, *17* (1), 1172.
(17) Maurer, G.; Loosli, H.; Schreier, E.; Keller, B. *Drug. Disp. Metab.* **1984**, *12*, 120−127.
(18) Maurer, G.; Lemaire, M. *Transplant. Proc.* **1986**, *18 (Suppl. 5)*, 25−34.

Table 2. Results Obtained on Patient Blood Samples Using the Fluorescence Polarization Immunoassay (FPIA), as Well as ESI-MS and MALDI-MS with Both Extraction Procedures

clinical blood samples, FPIA assay (ng/mL)	ether extraction[12]		30% hexane/70% $CHCl_3$ extraction	
	ESI (ng/mL)(CV)	MALDI (ng/mL) (CV)	ESI (ng/mL) (CV)	MALDI (ng/mL) (CV)
120	86 (3%)	82 (1%)	95 (19%)	88 (9%)
195	149 (16%)	155 (11%)	152 (19%)	141 (5%)
213	165 (8%)	164 (7%)	153 (4%)	137 (11%)
250	155 (17%)	154 (4%)	179 (8%)	191 (13%)
457	308 (7%)	303 (4%)	298 (1%)	277 (11%)

sistent with the previously reported results.[1,19,20]

CONCLUSION

MALDI and electrospray ionization mass spectrometry are accurate and sensitive analytical techniques that have utility for the clinical analysis of CsA and potentially for other drugs. In the quantitative analysis of CsA, both MALDI and ESI provide accuracy and selectivity far exceeding those of existing immunoassay methods. For instance, MALDI and ESI offer a reliable means of identifying and quantifying metabolites that can have biological activity comparable to that of the parent drug,[3,4] suggesting that MALDI has utility as a pharmacokinetic tool. The sensitivity of mass spectrometry (15 ng/mL) also exceeds that of immunoassays (25 ng/mL).

The combinatorial approach toward optimizing extraction conditions which was developed and implemented in this study improved the overall efficiency of the mass analysis procedure by a factor of 5. Using this approach, we identified a 70:30 hexane/$CHCl_3$ binary solvent system which efficiently extracts CsA and its metabolites directly from blood. This approach also has potential for optimizing chemical reactions and monitoring enzymatic/inhibitor reactions.

EXPERIMENTAL SECTION

MALDI/MS. The cyclosporin measurements were performed on a Voyager-Elite time-of-flight mass spectrometer with delayed extraction (PerSeptive Biosystems, Inc., Framingham, MA). Samples were mixed with 2,5-dihydroxybenzoic acid (~0.2 M in 50:50 CH_3CN/0.5%TFA−H_2O) at a 1:1 ratio. Two microliters of sample−matrix mixture solution were deposited into a well of the MALDI sample plate and inserted into the ionization source of the instrument. Samples were irradiated with a nitrogen laser (Laser Science Inc.) operated at 337 nm. The laser beam was attenuated by a neutral density filter. Ions produced by MALDI were energetically stabilized using a delayed extraction period of 150 ns and then introduced into the MS with an acceleration voltage of 20 kV.

ESI/MS. The ESI/MS experiments were carried out in an API 100 Perkin-Elmer Sciex single-quadrupole mass spectrometer. Samples were introduced into the source through a silica capillary at a flow rate of 4.0 μL/min controlled by a Harvard Apparatus, Inc. syringe pump. The nebulizer gas was optimized and set at a rate of 1.6 L/min, and an electrospray potential of 4200 V was applied in the interface sprayer. A curtain gas of ultrapure nitrogen was pumped into the interface at the rate of 1.2 L/min to aid desolvation of the charged droplets and to prevent particulate matter from entering the analyzer region. Ions generated from the ion evaporation process entered the analyzer through the vacuum interface by the help of entrance optics.

Combinatorial Experiments. Thirty-six different solvent solutions, ACN/hexane, ACN/H2O, ACN/MeOH, ether/100 mM NaOH, hexane/EtOH, and hexane/$CHCl_3$, with solvent:solvent ratios set at 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100, were used. The second generation experiments expanded the concentration range of the solvent systems that were found useful. In each experiment, a volume of 200 μL of each solution was added to 100 μL of whole blood (containing 300 ng/mL CsA for the first and 160 ng/mL CsA for the second), mixed by vortexing for 2 min, and then centrifuged for 1 min at 5000*g*. The upper layer was removed and evaporated, redissolved in 40 μL of methanol, and then analyzed (in duplicate) using a MALDI-MS autosampling program.

"Combinatorial" Extraction. A volume of 10 μL of 4 μM CsG was added to 100 μL of a whole blood sample and vortexed with 200 μL of hexane/$CHCl_3$ (7:3) solvent system for 2 min. The mixture was then centrifuged for 1 min at 5000*g*. The upper layer was recovered and evaporated under vacuum. The sample was redissolved in 40 μL of methanol and subjected to MS analysis.

Fluorescence Polarization Immunoassay (FPIA). These experiments were performed according to the detailed procedure described by Abbott Laboratories in their TDxFLx immunosupressant drug assay.[21]

Stock Solutions. The cyclosporin A stock solution was obtained from Abbott Laboratories as a kit of standard samples typically used for FPIA. The cyclosporin G (Sandoz Corp.) stock solution was prepared by dissolving 3.2 mg of cyclosporin G in 4 mL of methanol and further diluting with MeOH to 4 μM.

Preparation of Standard Curve Solution. The whole blood calibrators were obtained from Abbott Laboratories in the concentrations of 0, 100, 250, 500, 1000, and 1500 ng/mL.

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(19) Tjiandra-Maga, B.; Verbesselt, R.; Scharpé, S.; Verkerk, R.; Lamber, W. E.; Liedekerke, B. V.; Leenheer, A. D. *J. Clin. Chem. Clin. Biochem.* **1990**, *28*, 53−57.

(20) Burckar, G. J.; Hain, A.; Diven, W.; Venkataramanan, R.; Starzl, T. E. *Transplant. Proc.* **1990**, *22*, 1319−1321.

(21) TDxFLx Immunoupressant Drug Assays−Cyclosporin Monoclonal Whole Blood No. 9797-60, Abbott Laboratories, Abbott Park, IL 60064.

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