

This article was downloaded by:

On: 23 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597273>

### A Simple HPLC Method for the Determination of Cyclosporin A in Human Whole Blood

Su-Geun Yang<sup>a</sup>; So-Ra Park<sup>a</sup>; Dae-Duk Kim<sup>a</sup>; Suk-Jae Chung<sup>a</sup>; Chang-Koo Shim<sup>a</sup>

<sup>a</sup> Research Institute of Pharmaceutical Science and College of Pharmacy, Seoul National University, Seoul, Korea

**To cite this Article** Yang, Su-Geun , Park, So-Ra , Kim, Dae-Duk , Chung, Suk-Jae and Shim, Chang-Koo(2006) 'A Simple HPLC Method for the Determination of Cyclosporin A in Human Whole Blood', *Journal of Liquid Chromatography & Related Technologies*, 29: 3, 391 – 401

**To link to this Article:** DOI: 10.1080/10826070500452010

**URL:** <http://dx.doi.org/10.1080/10826070500452010>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

## A Simple HPLC Method for the Determination of Cyclosporin A in Human Whole Blood

Su-Geun Yang, So-Ra Park, Dae-Duk Kim, Suk-Jae Chung,  
and Chang-Koo Shim

Research Institute of Pharmaceutical Science and College of Pharmacy,  
Seoul National University, Seoul, Korea

**Abstract:** A simple high performance liquid chromatography method for the determination of cyclosporin A in human whole blood was developed. Human blood samples were deproteinated with a zinc sulfate saturated acetonitrile–water (1:1) solution, and centrifuged. The supernatant was transferred to clean tubes, evaporated, and reconstituted for an HPLC analysis. Cyclosporin D was used as an internal standard. Chromatography was carried out using XTerra<sup>®</sup> C<sub>18</sub> 150 × 4.6 mm 5 μm column (Waters, Watford, UK) maintained at 80°C, with a mobile phase consisting of acetonitrile–water–*t*-butyl methyl ether–phosphoric acid (55:40:5:1, v/v/v/v). The flow rate of the mobile phase was adjusted to 1 mL/min. The detection wavelength was set at 210 nm. Under these conditions, cyclosporin A and cyclosporin D were cleanly separated with no interfering endogenous peaks present. The calibration curve for cyclosporin A in human blood was linear over the concentration range examined (50 ~ 3000 ng/mL), with a correlation coefficient greater than 0.999. The lower limit of quantification (LOQ) approached 50 ng/mL. The intra- and inter-day variations were in the ranges of 0.48 ~ 13.33% for precision and 98.30 ~ 103.74% for accuracy, respectively. The applicability of this method was demonstrated in a pharmacokinetic study of cyclosporin A in human volunteers.

**Keywords:** Cyclosporin A, High performance liquid chromatography, Zinc sulfate solution, Deproteinaton, Pharmacokinetic study, Therapeutic drug monitoring

Address correspondence to Chang-Koo Shim, Department of Pharmaceutics, College of Pharmacy, Seoul National University, San 56-1, Shinlim-dong, Kwanak-gu, Seoul 151-742, Korea. E-mail: shimck@snu.ac.kr

## INTRODUCTION

The introduction of Cyclosporin A (CsA, Fig. 1) into clinical use in the 1980's resulted in a significantly improved survival rate of transplant patients,<sup>[1]</sup> and CsA still remains the cornerstone of many drug regimens in the area of transplantation. CsA is a cyclic oligopeptide consisting of 11 amino acids. Seven of the amino acids of CsA are N-methylated, and the four remaining protonated nitrogen atoms form intermolecular hydrogen bonds with carbonyl groups, contributing substantially to the rigidity of the cyclosporin skeleton.<sup>[2]</sup> As a result, CsA is remarkably hydrophobic and relatively insoluble in water. In line with this characteristic, the oral bioavailability of CsA is low (30%), varying between individuals (5 ~ 50%),<sup>[2]</sup> and greatly influenced by various factors such as the amount of bile juice secreted and the amount of fat in food, etc,<sup>[3]</sup> making the prediction of the extent of absorption of CsA in patients difficult. In addition, the therapeutic index of CsA is narrow.<sup>[4]</sup> The major toxicity of CsA in transplant recipients is nephrotoxicity, which is characterized by dose dependent and transient reduction in renal blood flow and glomerular filtration rate.<sup>[5]</sup> These characteristics require that an optimum dosage regimen be established (i.e., maintenance of efficient and constant blood concentration of CsA). As a result, therapeutic drug monitoring (TDM) of CsA in the blood has become an indispensable adjunct for the effective treatment of transplant patients with CsA.<sup>[6]</sup>

Various clinical laboratory methods for analysis of CsA have been developed. High performance liquid chromatography (HPLC), immunoassay based methods, and HPLC mass spectrometry (LC/MS) represent the most frequently used methods. Immunoassay based methods are generally simple compared to HPLC methods, but they may overestimate CsA levels due to the cross-reactivity of the antibody in the assay kit with CsA metabolites in the blood.<sup>[7,8]</sup> LC/MS methods have several advantages over other methods with respect to sensitivity and specificity. However, extensive multi-step extraction procedures and the time consuming chromatographic separation, as well as the high cost of various equipment,<sup>[9-11]</sup> limit the wide application of these methods in routine TDM and bioavailability tests in clinical laboratories. Moreover, potential interference by other molecules in the blood, which have the same ratio of mass/electric charge, continues to be a major problem in the analysis of CsA.

Although HPLC methods are considered by many scientists as the gold standard for the analysis of CsA, some drawbacks such as time consuming and tedious extraction procedures and insufficient chromatographical separation due to matrix interferences have not been completely solved<sup>[12-15]</sup>. Recently, HPLC methods with simplified sample preparation have been reported.<sup>[16,17]</sup> However, robustness of these methods seems to be controversial, due to insufficient separation of CsA<sup>[16]</sup> or instability of CsA during the deproteination with hydrochloric acid.<sup>[17]</sup> In the present study, therefore, an

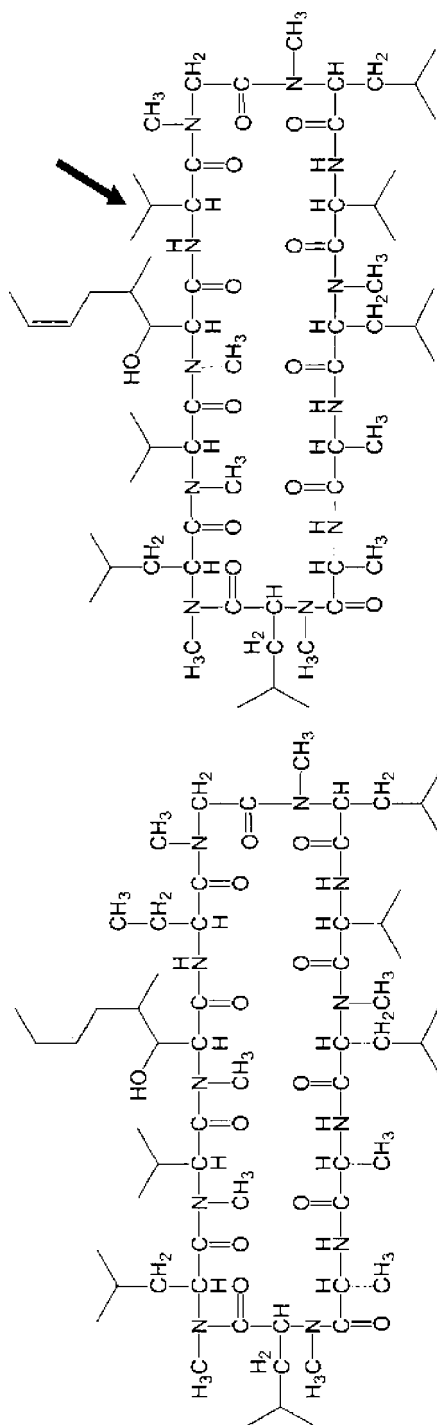


Figure 1. Structures of CsA and CsD (internal standard). The arrow indicates the structural differences between CsA and CsD.

HPLC method, which involves a simple sample preparation procedure and a clean chromatographic separation of CsA, was developed for its routine application to TDM and bioavailability tests.

## EXPERIMENTAL

### Chemicals and Reagents

CsA and cyclosporin D (CsD, Fig. 1) with a purity of over 98% were provided from CKD Pharm. Co. (Seoul, Korea). Acetonitrile and methanol were obtained from Fisher, Korea and zinc sulfate from Sigma, Germany. All other chemicals were reagent or HPLC grade.

### Instrumentation and Chromatographic Conditions

The HPLC system consisted of a 321 pump, 506 C interface (all from Gilson Inc., Middleton, WI), 2487 UV/VIS detector (Waters, Milford, MA), column oven (CTS-30, Young-Lin Instrument Co., Seoul, Korea) and the Unipoint program (Gilson Inc., Middleton, WI) for data processing. Samples were injected via a Gilson 234 autosampler equipped with a 100  $\mu$ L loop. XTerra<sup>®</sup> C<sub>18</sub> silica column (250 mm  $\times$  4.6 mm i.d., 5  $\mu$ m particle size, Waters, Milford, MA) was used for the chromatographic separation. The column temperature was maintained at 80°C. The mobile phase was composed of acetonitrile–water–*t*-butyl methyl ether–phosphoric acid (55:40:5:1, v/v/v/v) and delivered at a flow-rate of 1 mL/min. The detection wavelength was 210 nm.

### Extraction of CsA from Standard Samples

A stock solution of CsA with a concentration of 1 mg/mL in methanol was prepared by a 50 fold dilution of a 50 mg/mL methanolic solution of CsA with methanol. A methanolic solution of CsD, an internal standard (IS), at a concentration of 50  $\mu$ g/mL was also prepared by a 200 fold dilution of 10 mg/mL methanolic solution of CsD with methanol. The stock solution of CsA was appropriately diluted with methanol to give standard solutions of CsA in the concentration range of 0.5~30  $\mu$ g/mL.

A 100  $\mu$ L aliquot of each standard solution was spiked into 1.0 mL of blank human blood in a 5 mL polypropylene tube to yield a final concentration of 50, 100, 500, 1000, 1500, or 3000 ng/mL for CsA, and the resulting sample vortexed for 10 min. A 20  $\mu$ L aliquot of the internal standard solution (50  $\mu$ g/mL) was then added to each polypropylene tube, followed

by vortexing for another 10 min. A zinc sulfate-saturated acetonitrile:water (1:1) solution (1.5 mL) was added to each tube, and the tube was vortexed for 7 min and then allowed to stand for 10 min to achieve deproteination. The mixture was then centrifuged at 3000 rpm for 5 min. An aliquot (1.5 mL) of the supernatant was transferred to a clean tube and completely evaporated to dryness under vacuum, using a Speed Vac concentrator (Spin-vac, Hanil, Seoul, Korea). The residue was then reconstituted using 250  $\mu$ L of the mobile phase, and a 60  $\mu$ L aliquot was injected for HPLC analysis.

### Validation of the Method

Standard calibration curves were constructed by spiking drug free blood (1.0 mL) with 100  $\mu$ L of CsA stock solutions of various concentrations to yield final blood concentrations of 50, 100, 500, 1000, 1500, and 3000 ng/mL. The linear regression of the ratio of the peak areas of CsA and the internal standard versus the concentration was weighted by 1/X (reciprocal of the concentration), and the correlation coefficients were calculated. The lower limit of quantification (LOQ) was established when the accuracy was within the  $100 \pm 20\%$  range.

Inter-day variation was determined by analyzing a whole set of the standard samples (i.e., 50~3000 ng/mL) each day for five consecutive days. The intra-day variation was determined by analyzing five sets of standard samples (i.e., 50~3000 ng/mL) each day. Intra and inter-day assay precision was expressed by the coefficient of variation (CV, %) of the obtained data. The accuracy (%) of the method was estimated by comparing the observed concentrations with the nominal concentrations for nine sets of standard samples.

### Application

The assay method was applied to a bioavailability study for a CsA product, Sandimmune Neoral<sup>®</sup> (100 mg soft gelatin capsules, Novartis, Switzerland). Seven healthy male volunteers participated in the study. The volunteers were screened by physical examinations along with a series of laboratory tests. Two capsules of the formulation (i.e., 200 mg as CsA) were administered with 200 mL of water in a randomized sequence, under fasting conditions. Subjects were hospitalized (Kangnam Hospital, Seoul, Korea) at 9:00 p.m. 1 day before this study, and fasted 12 hours before and 4 hours after each drug administration. Eleven blood samples were taken from the antecubital vein before administration, and at 0.33, 0.66, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 8.0, and 12.0 h after the administration. The samples were stored frozen at  $-20^{\circ}\text{C}$  until analyzed.

## RESULTS AND DISCUSSION

The concentration of CsA should ideally be determined from whole blood samples, not from plasma samples, because more than 75% of the CsA is bound to erythrocytes.<sup>[18]</sup> During the past two decades, a number of HPLC methods have been extensively studied for use in an assay of CsA in blood samples.<sup>[12–15]</sup> In most cases, a short ultraviolet wavelength (e.g., 210 nm) was used for the detection of CsA in those methods, because CsA lacks chromophores. At this wavelength, however, many endogenous molecules also absorb ultraviolet light, thus interfering with the assay of CsA. In addition, the extraction of specific compounds from whole blood samples is generally complicated, compared to plasma samples, due to the potential coextraction of endogenous compounds from blood cells. Therefore, vigorous removal of potentially interfering endogenous compounds is required in such methods. Organic solvents such as n-hexane and diethyl ether are often used for this purpose. In our preliminary studies, however, we found that the recovery of CsA using such methods is highly dependent on the personal technique of the analyst and extraction conditions, such as shaking time and temperature. The selectivity and the reproducibility of the assay were found to be insufficient as well.

Direct column injection methods after a simple deproteination of blood sample have recently been reported.<sup>[16,17]</sup> Sample preparation time could be greatly reduced if these methods could be used. However, the fastidious setting of the analysis conditions is required for the separation of CsA by the method of Amini and Ahmadiani.<sup>[16]</sup> Wide application of their method appears to be limited by several factors. For example, the HPLC column should be only Shimpack CLC, other columns were not applicable, separation was completely lost when a guard column was used in these conditions, the composition of the mobile phase should be vigorously modulated, and storage conditions for the blood samples should be strictly kept.<sup>[16]</sup> Therefore, the recovery and the selectivity of their method were not sufficient in our preliminary examination, consistent with its limited application in routine TDM and bioavailability tests.

Khoschorur et al.<sup>[17]</sup> reported a two step deproteination method, in which acidic (usually hydrochloric acid) and basic solutions are serially added to blood samples for deproteination, followed by extraction with volatile solvents (e.g., diethyl ether). The selectivity of this analysis was found to be fairly good, but the robustness of the method appears to be controversial, due to the potential degradation of CsA under the acidic conditions used.<sup>[19]</sup>

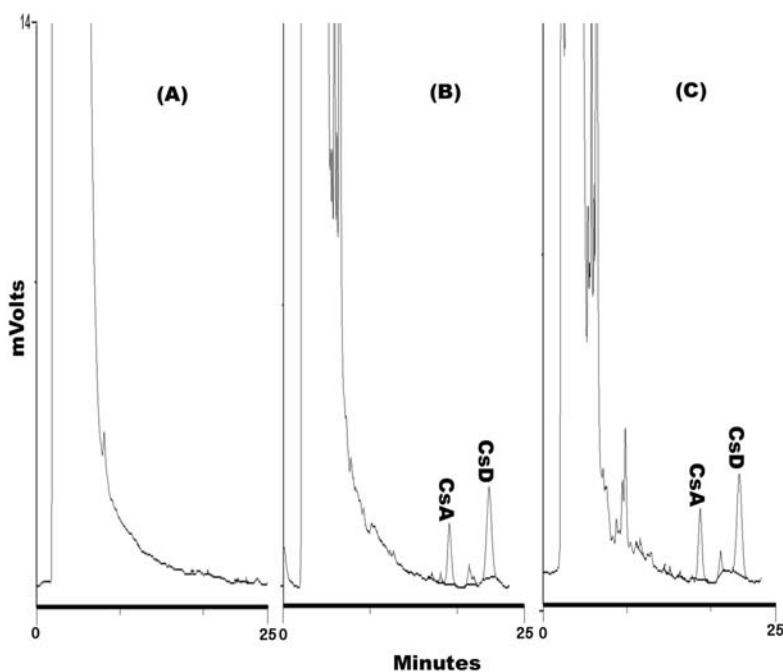
A zinc sulfate solution has been widely used for deproteination of plasma or urine samples containing xenobiotics.<sup>[20–22]</sup> In LC/MASS and HPLC analyses of CsA in blood samples, the zinc sulfate solution or solvent mixed zinc sulfate solution has been employed as well.<sup>[23–25]</sup> The use of zinc sulfate solution, however, should be followed by solvent extraction,

which often needs the employment of specific apparatus such as an on-line extraction apparatus for the LC/MASS analysis.

The method developed in the present study is characterized by no extraction of CsA after the deproteination of blood samples with the zinc sulfate solution. It needs only one step deproteination with the zinc sulfate saturated acetonitrile:water (1:1), evaporation of the supernatant, reconstitution of the residue with the mobile phase, and simple injection of aliquots to HPLC system.

Figure 2 shows typical chromatograms for a blank blood (A), a standard blood sample spiked with CsA and CsD (B), and a blood sample taken from a human subject (C) 2.5 h after an oral administration of two capsules of Sandimmune Neoral<sup>®</sup> (200 mg as CsA). The retention times for CsA and the internal standard (CsD) were *ca.* 16.8 and 21.0 min, respectively, and the chromatographic separation of CsA and CsD from other blood components was fairly good.

The calibration curves were linear over the concentration range examined (i.e., 50 to 3000 ng/mL). A good relationship with a highly significant correlation ( $r^2 > 0.999$ ) was consistently observed for every run. The slope, intercept and correlation coefficient of the calibration curves were



**Figure 2.** Typical HPLC chromatograms, (A) blank blood; (B) CsA blood standard of 500 ng/mL; (C) a volunteer sample 2.5 h after the administration of 200 mg CsA.



$8.72 \times 10^{-4} \pm 2.99 \times 10^{-5}$  (mL/ng),  $5.21 \times 10^{-3} \pm 2.54 \times 10^{-2}$  (peak-area ratio units), and  $0.999 \pm 0.001$  (mean  $\pm$  SD), respectively. The intercept value was not statistically different from zero, indicating that the assay parameter is directly proportional to the CsA concentration in whole blood.

Results of the validation of the present method are shown in Table 1. Precision and accuracy were estimated for blood CsA concentrations of 50~3000 ng/mL. The accuracy was over 97% for all concentrations, and the coefficients of variance (i.e., precision) were less than 8.12 and 13.33% for the concentration range for inter-day and intra-day assays. The LOQ of the present method, therefore, appears to be lower than the lowest concentration examined (i.e., 50 ng/mL). Considering the fact that most clinicians accept 50 to 150 ng/mL as a target trough level of CsA concentration to minimize allograft rejection and adverse effects,<sup>[26]</sup> the present method seems to be sufficiently sensitive for the routine TDM of CsA.

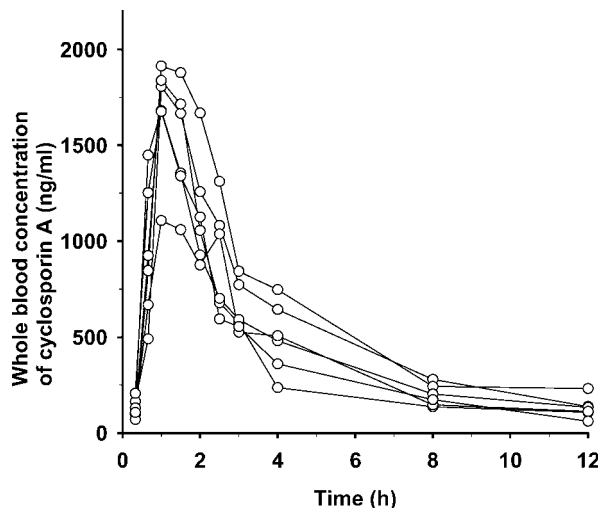
The applicability of the present method to human pharmacokinetic studies was demonstrated, as shown in Fig. 3, in which CsA in blood samples could be readily quantified within a time frame of 20 min (the first sampling time,  $140.8 \pm 54.8$  ng/mL) to 12 h (the last sampling time,  $120.2 \pm 58.3$  ng/mL) after the oral administration of Sandimmune Neoral<sup>®</sup> (200 mg as CsA).

## CONCLUSION

A simple and reproducible HPLC method for determining CsA in human blood was developed. The method is characterized by only one step deproteination with the zinc sulfate-saturated acetonitrile:water (1:1), evaporation of the supernatant, reconstitution of the residue with the mobile phase, and injection of aliquots to the HPLC system. The simplicity of the sample

**Table 1.** Precision and accuracy of the LC analysis of CsA in human blood

Concentration (ng/mL)	Accuracy (%, n = 9)	Precision (CV, %)	
		Intra-day (n = 5)	Inter-day (n = 5)
50	97.13	8.12	13.33
100	97.57	7.72	11.20
500	98.71	5.09	1.34
1000	103.74	3.44	2.04
1500	98.30	3.54	2.45
3000	99.94	0.67	0.48



**Figure 3.** Whole-blood concentration-time profiles of CsA after an oral administration of 200 mg of Sandimmune Neoral<sup>®</sup>, to seven healthy volunteers. Each data point represents individual concentration-time profiles.

preparation makes this method suitable for the rapid and routine analysis of CsA. The intra- and inter-day precision and accuracy results were within acceptable limits. The sensitivity is considered to be sufficient for the TDM of transplant patients and concentrations of CsA as low as 50 ng/mL could be accurately determined. Given the results of the validation and bioavailability study, it is reasonable to assume that the assay will also be suitable for use in pharmacokinetic studies involving humans.

#### ACKNOWLEDGMENT

This study was partly supported by a grant (M10414030004-04N1403-00410) from the Korea Science and Engineering Science (KOSEF).

#### REFERENCES

1. Kahan, B.D. Therapeutic drug monitoring of cyclosporine: 20 years of progress. *Transplant. Proc.* **2004**, *36*, 378–391.
2. Vine, W.; Bowers, L.D. Cyclosporine: structure, pharmacokinetics, and therapeutic drug monitoring. *Crit. Rev. Clin. Lab. Sci.* **1987**, *25*, 275–311.
3. Lindholm, A.; Henricsson, S.; Dahlqvist, R. The effect of food and bile acid administration on the relative bioavailability of cyclosporin. *Br. J. Clin. Pharmacol.* **1990**, *29*, 541–548.

4. Belitsky, P.; Dunn, S.; Johnston, A.; Levy, G. Impact of absorption profiling on efficacy and safety of cyclosporin therapy in transplant recipients. *Clin. Pharmacokinetics*. **2000**, *39*, 117–125.
5. Remuzzi, G.; Perico, N. Cyclosporine-induced renal dysfunction in experimental animals and humans. *Kidney. Int.* **1995**, *52*, S70–S74.
6. Andert, S.E.; Grimm, M.; Schreiner, W.; Muller, M.M. Therapeutic whole-blood levels of cyclosporin A in heart and lung transplantation. *Clin. Chim. Acta.* **1994**, *224*, 159–166.
7. Murthy, J.N.; Yatscoff, R.W.; Soldin, S.J. Cyclosporine metabolite cross-reactivity in different cyclosporine assays. *Clin. Biochem.* **1998**, *31*, 159–163.
8. Schutz, E.; Svinarov, D.; Shipkova, M.; Niedmann, P.D.; Armstrong, V.W.; Wieland, E.; Oellerich, M. Cyclosporin whole blood immunoassays (AxSYM, CEDIA, and Emit): a critical overview of performance characteristics and comparison with HPLC. *Clin. Chem.* **1998**, *44*, 2158–2164.
9. Mendonza, A.; Gohh, R.; Akhlaghi, F. Determination of cyclosporine in saliva using liquid chromatography-tandem mass spectrometry. *Ther. Drug. Monit.* **2004**, *26*, 569–575.
10. Christians, U.; Jacobsen, W.; Serkova, N.; Benet, L.Z.; Vidal, C.; Sewing, K.F.; Manns, M.P.; Kirchner, G.I. Automated, fast and sensitive quantification of drugs in blood by liquid chromatography-mass spectrometry with on-line extraction: immunosuppressants. *J. Chromatogr. B.* **2000**, *748*, 41–53.
11. Ceglarek, U.; Lembcke, J.; Fiedler, G.M.; Werner, M.; Witzigmann, H.; Hauss, J.P.; Thiery, J. Rapid simultaneous quantification of immunosuppressants in transplant patients by turbulent flow chromatography combined with tandem mass spectrometry. *Clin. Chim. Acta.* **2004**, *346*, 181–190.
12. Fletcher, S.J.; Bacchus, R.A. A simple improved method for the measurement of cyclosporin by liquid-liquid extraction of whole blood and isocratic HPLC. *Ann. Clin. Biochem.* **1988**, *25*, 510–515.
13. Chimalakonda, A.P.; Shah, R.B.; Mehvar, R. High performance liquid chromatographic analysis of cyclosporin A in rat blood and liver using a commercially available internal standard. *J. Chromatogr. B.* **2002**, *772*, 107–114.
14. Maguire, S.; Kyne, F.; UaConaill, D. An improved extraction procedure for an HPLC method for cyclosporin A in whole blood and plasma. *Ann. Clin. Biochem.* **1987**, *24*, 161–166.
15. Brozmanova, H.; Grundmann, M.; Safarcik, K.; Jegorov, A. High performance liquid chromatographic method for therapeutic drug monitoring of cyclosporine A and its two metabolites in renal transplant patients. *J. Chromatogr. B.* **2000**, *749*, 93–100.
16. Amini, H.; Ahmadiani, A. Simple determination of cyclosporine in human whole blood by high performance liquid chromatography. *J. Chromatogr. B.* **2003**, *795*, 209–214.
17. Khoschorur, G.; Semmelrock, H.J.; Rodl, S.; Auer, T.; Petek, W.; Iberer, F.; Tscheliessnigg, K.H. Rapid, sensitive high performance liquid chromatographic method for the determination of cyclosporin A and its metabolites M1, M17 and M21. *J. Chromatogr. B.* **1997**, *690*, 367–372.
18. Reichel, C.; von Falkenhausen, M.; Brockmeier, D.; Dengler, H.J. Characterization of cyclosporine A uptake in human erythrocytes. *Eur. J. Clin. Pharmacol.* **1994**, *46*, 417–419.
19. Kumar, M.; Singhal, S.K.; Singh, A. Development and validation of a stability indicating HPLC assay method for cyclosporine in cyclosporine oral solution USP. *J. Pharm. Biomed. Anal.* **2001**, *25*, 9–14.

20. Mason, J.L.; Hobbs, G.J. Simple method for the analysis of tenoxicam in human plasma using high performance liquid chromatography. *J. Chromatogr. B.* **1995**, *665*, 410–415.
21. Choo, K.S.; Kim, I.W.; Jung, J.K.; Suh, Y.G.; Chung, S.J.; Lee, M.H.; Shim, C.K. Simultaneous determination of loxoprofen and its diastereomeric alcohol metabolites in human plasma and urine by a simple HPLC-UV detection method. *J. Pharm. Biomed. Anal.* **2001**, *25*, 639–650.
22. Humphries, D.; Ruterbories, K.; Chan, C.; Narayanan, R. Development and validation of a liquid chromatography-tandem mass spectrometric method for the determination of alpha-methyltyrosine in human plasma. *J. Chromatogr. B.* **2004**, *810*, 229–234.
23. Rustum, A.M. Estimation of cyclosporin-A in whole blood by simple and rapid reversed-phase HPLC utilizing a salting-out extraction procedure. *J. Chromatogr. Sci.* **1990**, *28*, 594–598.
24. McMahon, L.M.; Luo, S.; Hayes, M.; Tse, F.L. High-throughput analysis of everolimus (RAD001) and cyclosporin A (CsA) in whole blood by liquid chromatography/mass spectrometry using a semi-automated 96-well solid-phase extraction system. *Rapid. Commun. Mass. Spectrom.* **2000**, *14*, 1965–1971.
25. Annesley, T.M.; Clayton, L. Simple extraction protocol for analysis of immunosuppressant drugs in whole blood. *Clin. Chem.* **2004**, *50*, 1845–1848.
26. Klintmalm, G.; Sawe, J.; Ringden, O.; von Bahr, C.; Magnusson, A. Cyclosporine plasma levels in renal transplant patients. Association with renal toxicity and allograft rejection. *Transplantation* **1985**, *39*, 132–137.

Received August 24, 2005

Accepted October 1, 2005

Manuscript 6712