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Journal of Chromatography B, 850 (2007) 471–480
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Simultaneous LC–MS–MS determination of cyclosporine A, tacrolimus, and sirolimus in whole blood as well as mycophenolic acid in plasma using common pretreatment procedure

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Received 20 August 2006; accepted 18 December 2006
Available online 11 January 2007

Abstract

The purpose of the study was to develop rapid and simple procedure for simultaneous determination of cyclosporine A (CsA), tacrolimus (TCR), and sirolimus (SIR) in whole blood and mycophenolic acid (MPA) in plasma. Ascomycin (ASCO), cyclosporine D (CsD), and desmethoxysirolimus (DMSIR) were used as internal standards (IS) for TCR, CsA and MPA, and SIR, respectively. In the method development, six-level blood calibrators were used for CsA (range 47–1725 ng/ml), TCR (range 2.1–38.8 ng/ml), and SIR (range 2.4–39.6 ng/ml). Four-level calibrators were used for MPA (range 0.15–5.48 μg/ml). Four levels of quality control (QC) standards were used for blood samples, together with two levels of QC standards in plasma. All QC standards and calibrators were obtained from commercial sources. Sample preparation based on precipitation of 50 μl of sample in zinc sulfate–methanol–acetonitrile mixture containing IS, followed by centrifugation. HPLC was performed on ChromSpher π column, 30 mm × 3 mm, in ballistic gradient of ammonium formate buffer–methanol at 0.8 ml flow rate. Following gradient elution profile was applied: 0–1.2 min at 30% methanol (divert valve to waste), 1.21–3.1 min 97% methanol (divert valve to detector), 3.11–3.7 min 30% methanol (divert valve to waste). ESI–MS–MS (MRM) was done on TSQ Quantum instrument with ESI source in positive ion mode. Ammoniated adducts of protonated molecules were used as precursor ions for all analytes but MPA. For this compound sodium adduct was used. Following transitions were monitored: for CsA *m/z* 1220–1203; for CsD 1234–1217; for SIR 931.6–864.5 and 882.6; for DMSIR 902–834.5; for TCR 821.5–768.5 and 785.5; for ASCO 809.5–756; for MPA 343–211.6; for MPA-glucuronide 514–306 and 211.6. The limits of quantitation were: 1 ng/ml for TCR and SIR, 20 ng/ml for CsA, and 0.1 μg/ml for MPA. Post-column infusion experiments showed that no positive or negative peaks appeared after injection of matrix in the elution range of target compounds. General signal suppression caused by matrix ranged from 20–40%, and was caused mainly by zinc sulfate present in deproteinizing solution. Extracted samples were stable for 2 days at 4 °C and for at least 20 days at −20 °C. MPA was fully separated from its glucuronide, which was eluted at around 0.7–0.8 min and directed to the waste. Some mutual cross-contribution of CsD and CsA was observed (below 1%), other IS did not contribute to target compounds and vice versa. Observations of chromatograms from patients taken single therapy demonstrated that possible metabolites of CsA, TCR, or SIR did not interfere with target compounds or IS.

Keywords: Immunosuppressants; TDM; LC–MS–MS

1. Introduction

Therapeutic drug monitoring (TDM) of immunosuppressive drugs is a must, dictated by narrow therapeutic range of these compounds, low dose-concentration correlation, and necessity of continuous use after organ transplantation. To most common immunosuppressive drugs belong: cyclosporine A (CsA), tacrolimus (TCR), sirolimus (SIR), and mycophenolic acid (MPA). These drugs were monitored in our hospital using immunoassays or HPLC. However, the use of immunoassays is costly and the comparison of results with those obtained by more specific HPLC or liquid chromatography–tandem mass spectrometry (LC–MS–MS) procedures show generally overestimation, caused by limited selectivity of all assays. This was reported for TCR [1,2], MPA [3], SIR [4–8], and CsA [9].

For these reasons, the use of LC–MS–MS became very common in the last years, at first for single immunosuppressants

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doi:10.1016/j.jchromb.2006.12.048

Table 1
Concentrations of analytes in commercial calibrators and QC samples used

Material[a]	CsA (ng/ml)	SIR (ng/ml)	TCR (ng/ml)	MPA (μg/ml)
WB cal. 1	46.7	2.4	2.1	–
WB cal. 2	115	6.6	5.6	–
WB cal. 3	304	12.7	10.9	–
WB cal. 4	483	19.6	15.8	–
WB cal. 5	777	29.0	21.9	–
WB cal. 6	1937	49.4	38.8	–
WB QC 1	100	3.0	2.5	–
WB QC 2	240	9.6	6.5	–
WB QC 3	483	19.4	14.5	–
WB QC 4	1725	39.6	30.6	–
Pl cal. 1	–	–	–	0.15
Pl cal. 2	–	–	–	0.89
Pl cal. 3	–	–	–	3.15
Pl cal. 4	–	–	–	5.48
Pl QC 1	–	–	–	1.80
Pl QC 2	–	–	–	4.86

Pl, plasma; cal., calibrator; QC, quality control sample.
[a] WB, whole blood.

[1,9–11], later for multiple drugs [12–17]. The latter approach allowed simultaneous monitoring of several drugs, administered for the patient. Simultaneous determination of immunosuppressants was limited to whole blood samples. On the other hand, MPA is determined in plasma, and the assay procedures published for MPA differ widely from those used for other immunosuppressants. Recently, Annesley et al. [18] described LC–MS–MS method for determination of MPA and its glucuronide (MPAG), using the same SPE cartridges and mobile phase components as for determination of CsA, SIR, and TCR in the whole blood.

The purpose of the present study was to present a method for simultaneous determination of CsA, TCR, and SIR in whole blood as well as MPA in plasma, using the same pretreatment procedure and same analytical conditions. This procedure contributes to further simplification of the TDM of immunosuppressive drugs since it allows determination all drugs involved in one analytical procedure.

2. Experimental

2.1. Reagents, materials

Cyclosporine A and D (>99% purity) were kindly donated by Novartis International Pharmaceutical Ltd., Cork Ireland. Sirolimus (rapamycin, 100% purity) and desmethoxysirolimus (DMSIR) (32-desmethoxyrapamycin, 95.6% purity) were a gift from Wyeth Research, Monmouth Junction, USA. Tacrolimus (99.5% purity) was kindly donated by Fujisawa Pharmaceutical Co., Osaka, Japan. Ascomycin (ASCO) and mycophenolic acid (>98% purity) were supplied by Sigma–Aldrich. Mycophenolic acid glucuronide (containing 0.8% mycophenolic acid) was supplied by Analytical Services International Ltd., London, UK.

Methanol and water were of LC–MS–grade, supplied by Riedel-de Haen through Sigma–Aldrich. Other reagents were of analytical grade and supplied by various manufacturers. The 0.1 M aqueous zinc sulfate was used as deproteinizing solution.

Stock internal standard solution: Cs D, 200 μg/ml, ASCO, 20 μg/ml, DMSIR, 20 μg/ml in acetonitrile. This mixture was kept at −70 °C.

Working internal standard: stock internal standard was diluted *ex tempore* in necessary amount 1:1000 with the mixture of methanol and acetonitrile (1:1). CsD was used as IS for CsA and MPA, ASCO as IS for TCR, and DMSIR was used as IS for SIR. CsD was chosen as IS for MPA after comparison of signals stabilities and reproducibility with the alternative IS ascomycin. CsD as internal standard for MPA gave more stable signal. Additionally, the signal intensities of CsD and MPA were more compatible.

For calibration of Cs A, SIR, and TCR assay, commercially available whole blood calibrators (6 + 1 Multilevel Calibrator set) and quality control (QC) samples (Four Level Whole Blood Control) from Chromsystems Inc., Munich, Germany, were used. For the calibration of MPA plasma, calibrators from Recipe GmbH, Munich, Germany, and QC samples from Chromsystems were applied. All calibrators and QC samples were aliquoted to 50 μl portions in Eppendorf tubes and stored at −70 °C. The use of commercial calibrators eliminates important source of random errors, associated with the preparation of "in house" standards. The standards used were recently evaluated by Annesley [19] with very good results. Table 1 shows the concentrations of calibrators and QC standards.

2.2. Sample preparation

Sample preparation was based on the procedure described by Annesley et al. [16]. In 1 ml Eppendorf tube, 50 μl blood or plasma, 250 μl water, 250 μl 0.1 M zinc sulfate, and 500 μl of working IS solution were successively added, without intermediate mixing. The tubes were vortexed 30 s, left for 5 min,

and centrifuged at 16.000 × *g* for 5 min. Clear supernatant was collected to LC vial.

2.3. Instrument

LC–MS–MS measurements were done of TSQ Quantum triple quadrupole instrument, Surveyor quaternary pump and Surveyor AS autosampler (Thermo Instruments, San Jose, USA). Electrospray ionization (ESI) source in positive ionization mode was applied. Divert valve was used to protect the instrument from the early eluting matrix compounds.

2.4. HPLC conditions

Mobile phase consisted of methanol (A) and ammonium formate buffer 10 mM, pH 3.0 (B). The flow rate of 0.8 ml/min. Following gradient elution profile was applied: 0–1.2 min at 30% A (divert valve to waste), 1.21–3.1 min 97% A (divert valve to detector), 3.11–3.7 min 30% A (divert valve to waste).

Chromatographic oven temperature was set at 65 °C, autosampler tray temperature at 5 °C. CP ChromSpher π, column, 20 mm × 3 mm, with guard (Varian Chrompack, Netherlands) was used. Injection volume was 30 μl.

2.5. MS–MS conditions

Source fragmentation energy was set at 15 V, scan time was 0.1 s, scan width *m/z* 0.1. Acquisition time was 3.7 min. The instrument was set in multiple reaction monitoring mode. For quantitation and documentation, Xcalibur software together with LCQuan software incorporated in TSQ Quantum was used.

3. Results and discussion

3.1. Optimization

Syringe infusion experiments in pure methanol showed the prevalence of sodium adducts of SIR and TCR and MPA. In contrary, the experiments performed in mobile phase in full scan mode showed mainly the presence of ammoniated adducts of all analytes, with exception of MPA. On the base of comparative pilot experiments, ammoniated adduct ions were chosen as precursors for CsA, CsD, SIR, DMSIR, TCR, ASCO, and MPAG. In the case of MPA, sodiated molecule was taken as precursor. Monitored transitions to appropriate product ions are shown in the table (Table 2). In the case of SIR, TCR, and MPAG, two transitions were applied, in order to increase the sensitivity and selectivity of assay.

Table 2
Transitions monitored and collision energies (CE)

Compound	Precursor *m/z*	Product *m/z* (CE)
CsA	1220	1203 (24 V)
CsD	1234	1217 (22 V)
SIR	931.6	864.5 (20 V) 882.6 (10 V)
DMSIR	902	834.5 (18 V)
TCR	821.5	768.5 (24 V) 785.5 (20 V)
ASCO	809.5	756 (24 V)
MPA	343	211.6 (30 V)
MPAG	514	306 (20 V) 211.6 (44 V)

The elution gradient profile was chosen on the base of pilot experiments, which showed that the use of methanol concentration of 30% did not cause any losses of target compounds in the first 1.2 min, when the eluate was diverted to the waste. Only MPAG eluted during the first minute, as discussed later in the text.

3.2. Matrix effects

Possible influence of co-extracted matrix compound on detectability of target analytes was checked in two experiments. In first approach, all drugs were analyzed in:

- pure methanol,
- reconstituted extract of blank blood or blank plasma (four various samples were taken for each matrix),
- deproteinizing solution (zinc sulfate solution with methanol–acetonitrile).

Concentrations in final solutions were as follows (ng/ml): CsA 250, CsD 200, MPA 250, ASCO 20, SIR 25, DMSIR 20, TCR 25. Four injections of each sample were done. The results were expressed as mean percentage of peak area of drugs injected in pure methanol (Table 3). The comparison of peak intensities in matrix samples with peak intensities in methanol indicates that zinc sulfate solution is the main factor causing ionization suppression, which ranged from around 20 to around 40%. In the case of plasma extracts, observed peak intensities were practically identical as those in zinc sulfate solution. In blood extracts, the intensities were up to 2–20% higher than in pure deproteinizing solution. This phenomenon most probably was caused by partial binding of zinc sulfate with blood proteins

Table 3
Signal intensities (percentage of peak area values of drugs diluted in methanol) in various matrices

Sample	CsA	CsD	MPA	ASCO	TCR	SIR	DMSIR
Blood ($n=4$)	80	95	60	89	73	69	70
Plasma ($n=4$)	–	84	60	72	–	–	58
Deproteinizing solution	78	84	58	68	60	58	62

Mean values from four injections of each sample.

and confirms that this compound is responsible for the ionization suppression observed.

In the second experiment, a post-column infusion solution containing all analytes was performed. The concentration of drugs were as follows: CsA, CsD and MPA 100 μg/ml, SIR, TCR, DMSIR and ASCO 10 μg/ml. Infusion rate was 5 μl/min. Chromatographic conditions were as in the analytical method, only divert valve was not activated, and the whole eluate was directed to the mass spectrometer. Blank blood and plasma samples, prepared as usual, were injected and all transitions were monitored. As controls free of biological matrix, methanol–water mixture (1:1) and deproteinizing solution were injected. In this experiment, negative peak evidencing suppression of ionization was observed at 0.3 min in blood

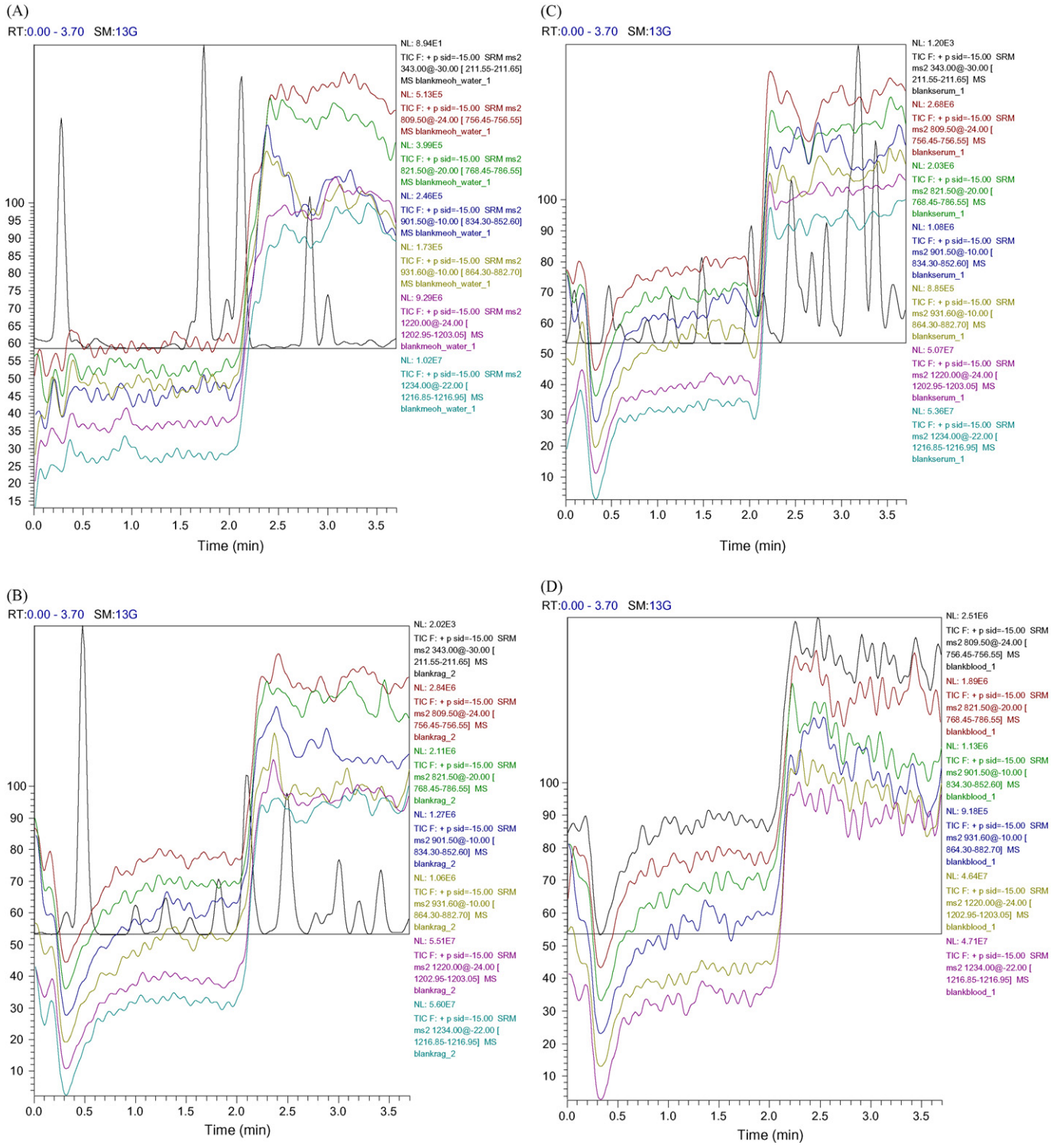

Fig. 1. Mass chromatograms of all analytes, taken after injection of methanol–water (A), blank plasma extract (B), deproteinizing solution (C), and blank blood extract (D) in post-column infusion experiment.

and plasma extract and in deproteinizing solution, but not in methanol–water. This observation confirms the statement that zinc sulfate is a main factor causing ionization suppression in extracted samples. At 2 min, an increase in signal intensities of all mass traces was observed, parallel to gradient profile. No positive or negative peaks were observed in the elution area. In mass trace of MPA some peaks of low intensities were observed, with retention times not interfering with MPA (Fig. 1).

Many authors [13–16] advocated additional purification of precipitated samples using various off-line or on-line solid phase extraction (SPE) procedures. In pilot experiments we observed that additional clean-up of precipitated samples with various SPE cartridges (IST Argonaut, IST Multimode, Bond Elut C18) did not assure better results in regard to sensitivity and matrix effects. On the other hand, regular cleaning of the instrument appeared critical for maintaining the sensitivity and preventing the appearance of random peaks originating from matrix. At daily injection of around 100 samples, the skimmer, tube lens, and transfer capillary should be cleaned twice a week.

3.3. Stability of samples and extracts

Aliquoted calibrators and QC samples were stored at −70 °C for several months and did not show any relevant change in signal intensities of target compounds. Calibrators and QC samples were extracted and tested on the same day. Then the extracts were stored for 2 days at 4 °C, and analyzed, then frozen and stored for next 20 days at −20 °C, thawed, and analyzed again (Table 4). The results showed good stability of analytes in extracts and were consistent with the findings of Holt et al. [8].

Table 4
Stability of analytes in extracts (expressed as percentage of the 1st value)

Drug	*C* (ng/ml)	After 2 days at 4 °C	After 20 days at −20 °C
CsA	47	108	106
	115	102	100
	304	107	108
	483	111	106
	777	101	99
	1937	103	99
TCR	2.1	110	98
	5.6	103	101
	10.9	80	96
	15.8	95	90
	21.9	95	92
SIR	6.6	98	99
	12.7	83	97
	19.6	101	102
	49.4	86	98
MPA	1800	92	90
	4860	93	90

3.4. Validation

The method was linear for all target compounds within the range of six calibrators used.

The lower limit of quantitation (LLOQ) was set at the concentrations for which an imprecision did not exceed 15%. LLOQ was established in 10 experiments, at different days, using lowest calibrators diluted 1:1 and 1:5 with blank blood or plasma. The LLOQ for TCR and SIR was 1 ng/ml, for CsA 20 ng/ml, and for MPA 0.1 μg/ml. Accuracy and day-to-day precision was studied by analysis of whole blood QC samples 1, 3, and 5 for CsA, SIR, and TCR and by analysis of plasma QC samples 1 and 2 for MPA. The analysis was done on 20 different days. The results of validation are presented in Table 5.

The sensitivity of the method is lower than some other published procedures of similar nature, e.g. of Streit et al. [12]. These authors, however, used less diluted sample and injected around 30-fold higher amount of material. Present procedure

Table 5
Results of method validation

Compound	Linearity range	Correlation, regression[a]	LLOQ	Accuracy and precision[a]	
				C	Found ± R.S.D.
CsA (ng/ml)	47–1937	$R = 0.9999$; $y = 0.004x + 0.056$	20	100	105 ± 9
				239	254 ± 7
				493	513 ± 8
				1859	1974 ± 10
TCR (ng/ml)	2.1–38.8	$R = 0.9998$; $y = 0.007x - 0.005$	1	2.47	2.45 ± 13%
				6.48	6.38 ± 12%
				13.9	14.1 ± 11%
				30.6	31.2 ± 12%
SIR (ng/ml)	2.4–49.4	$R = 0.9981$; $y = 0.009x + 0.015$	1	3.00	2.92 ± 14%
				10.2	10.2 ± 14%
				19.4	18.2 ± 12%
				39.6	40.5 ± 11%
MPA (μg/ml)	0.15–5.48	$R = 0.9996$; $y = 2.405x + 0.236$	0.1	1.80	1.90 ± 9%
				4.86	4.90 ± 6%

[a] Mean values from 50 day-to-day observations for CsA and TCR, and for 20 day-to-day observations for SIR and MPA. Data from daily QC samples. *C*, concentration, R.S.D., relative standard deviation.

Table 6
Absolute recoveries of analytes (mean value ± R.S.D. from three determinations)

Drug	Concentration	Recovery (%)
CsA (ng/ml)	47	109 ± 10
	304	101 ± 10
	1937	90 ± 6
SIR (ng/ml)	2.4	100 ± 35
	12.7	126 ± 30
	49.4	62 ± 26
TCR (ng/ml)	2.1	98 ± 25
	10.9	112 ± 20
	38.8	113 ± 21
MPA (μg/ml)	0.15	97 ± 8
	5.48	92 ± 6

has sufficient sensitivity and does not cause contamination of the instrument with high amount of matrix.

Recovery was tested at three concentration levels for CsA, SIR and TCR (calibrator 1, 3, and 6 and at two levels for MPA (calibrator 1 and 4). The recovery was calculated from signal intensity ratios of extracted drugs to drugs added to blank blood or plasma extracts. The results are shown in Table 6. A relatively broad variation in absolute recovery values for SIR and TCR were compensated by the use of internal standards, as was documented by achieving appropriate precision and accuracy levels.

Besides in house validation, the procedure was subjected to continuous external proficiency testing, organized by the College of American Pathologists (CAP) and by Analytical Services International Ltd. (UK). The example of recent results of proficiency testing programme, organized by CAP in July 2006 is given in Table 7. The evaluation of linearity was organized also by CAP (Programme LN31-A 2006). For CsA, the concentration range was from 56 to 1612 ng/ml and for TCR was from 2.2 to 34.8 ng/ml. Both assays were linear in full range tested.

3.5. Mass spectrometric selectivity

In the method applied, as well as in the most published LC–MS–MS procedures, target compounds and their internal standards were not chromatographically separated. This raised the question, whether the monitored product ion may "cross talk", causing mutual contribution to the signals measured.

This was examined on following way:

- Blank blood extract was pretreated in usual way, i.e. with precipitation solution containing internal standard mixture. In this experiment the possible contribution of internal standard product ions to target compounds might be seen.
- Blood containing high concentrations of target compounds (calibrator 6) was mixed with precipitation solution without internal standard mixture. In this experiment, the contribution of target compound product ions to internal standards might be observed.
- Blank plasma extract was pretreated routinely, and possible contribution of internal standard (CsD) to the mass trace of MPA was checked.

In the case of cyclosporines A and D a mutual contribution to signal intensities was observed; CsA contributed in around 0.6% to cyclosporine D, whether cyclosporine D contributed in around 0.9% to cyclosporine A (Fig. 2B). This phenomenon may influence the results; in the case of low CsA levels, some false increase may be expected, and in the case of very high CsA concentrations, the opposite may be observed. For other compounds, no peaks originating from target compounds were observed in chromatograms of internal standards, and vice versa.

The analysis of 10 blood samples obtained from subjects who did not receive any medication (blood bank samples) did not reveal any peaks occurring in mass traces of target compounds and internal standards (Fig. 2A).

Since most of examined clinical samples originated from patients receiving single therapy, it was possible to check the possible cross-interference of metabolites of target compounds. This was checked in 50 cases of patients individually receiving CsA, TCR, or SIR, respectively. In all cases, mass traces for all target compounds and internal standards were monitored, irrespective of therapeutic agent applied. No additional peaks in mass traces of non-applied compounds were observed.

3.6. Determination of MPA in the presence of MPAG

It is well known that MPA must be separated from MPAG due to breakdown of MPAG in the source [20,21]. In all published

Table 7
Results of external proficiency testing organized by College of American Pathologists (CSM-B 2006, peer group mass spectrometry)

Test, units	Our result	Mean	No.of labs	Acceptable range	Grade[a]
CsA (ng/ml)	734	677	40	356–998	Acc.
	217	189	40	113–266	Acc.
SIR (ng/ml)	27.2	18.5	51	8.1–29.0	Acc.
	8.5	11.5	52	5.1–17.9	Acc.
TCR (ng/ml)	16.4	17.4	39	10.6–24.2	Acc.
	7.9	10.1	39	5.6–14.7	Acc.
MPA (μg/ml)	4.4	5.69	13	3.1–8.3	Acc.
	2.6	3.10	13	1.8–4.4	Acc.

[a] Accepted.

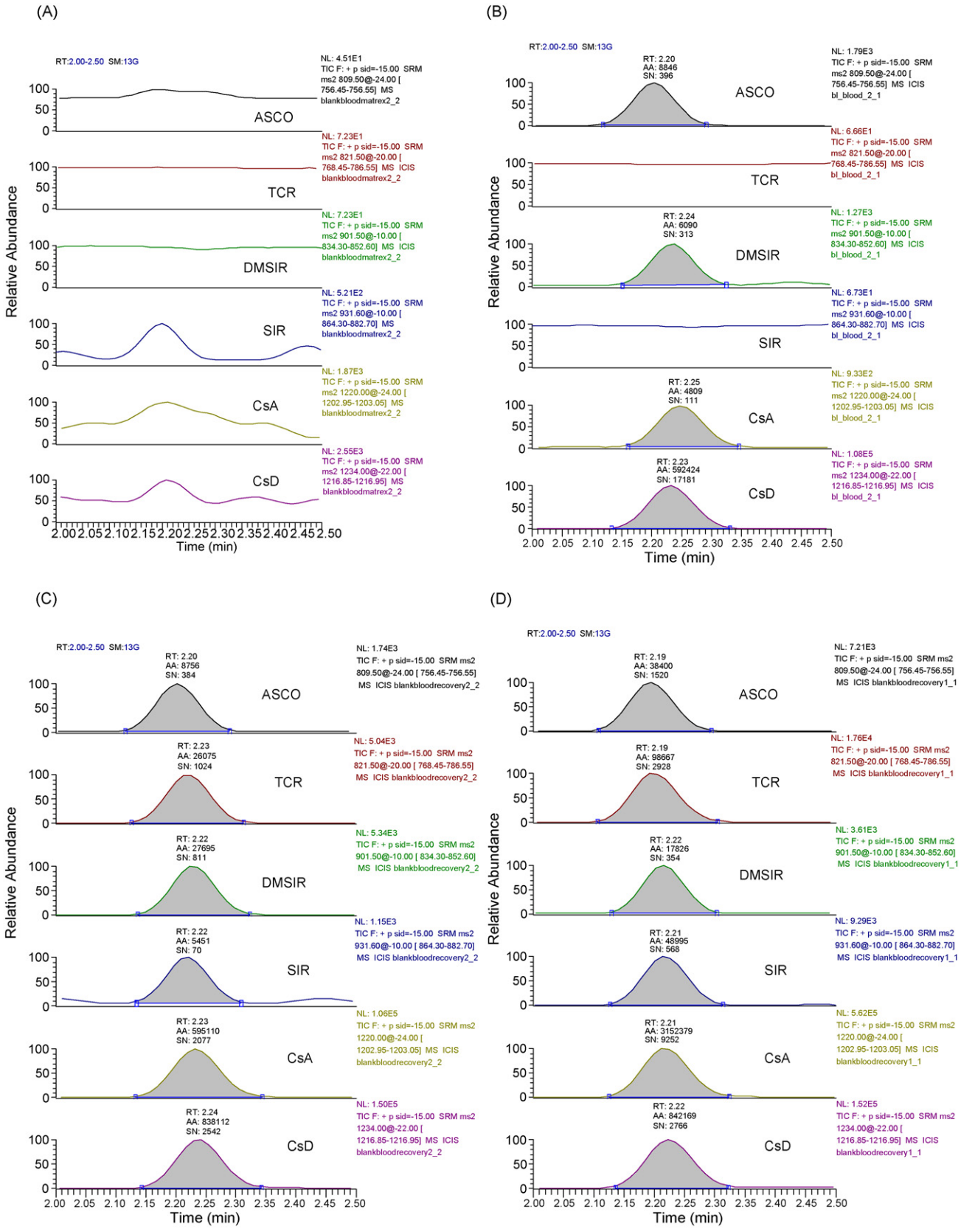

Fig. 2. Mass chromatograms of blank blood extract (A), blank blood with IS mixture (B), calibrator 3 (C), and calibrator 6 (D).

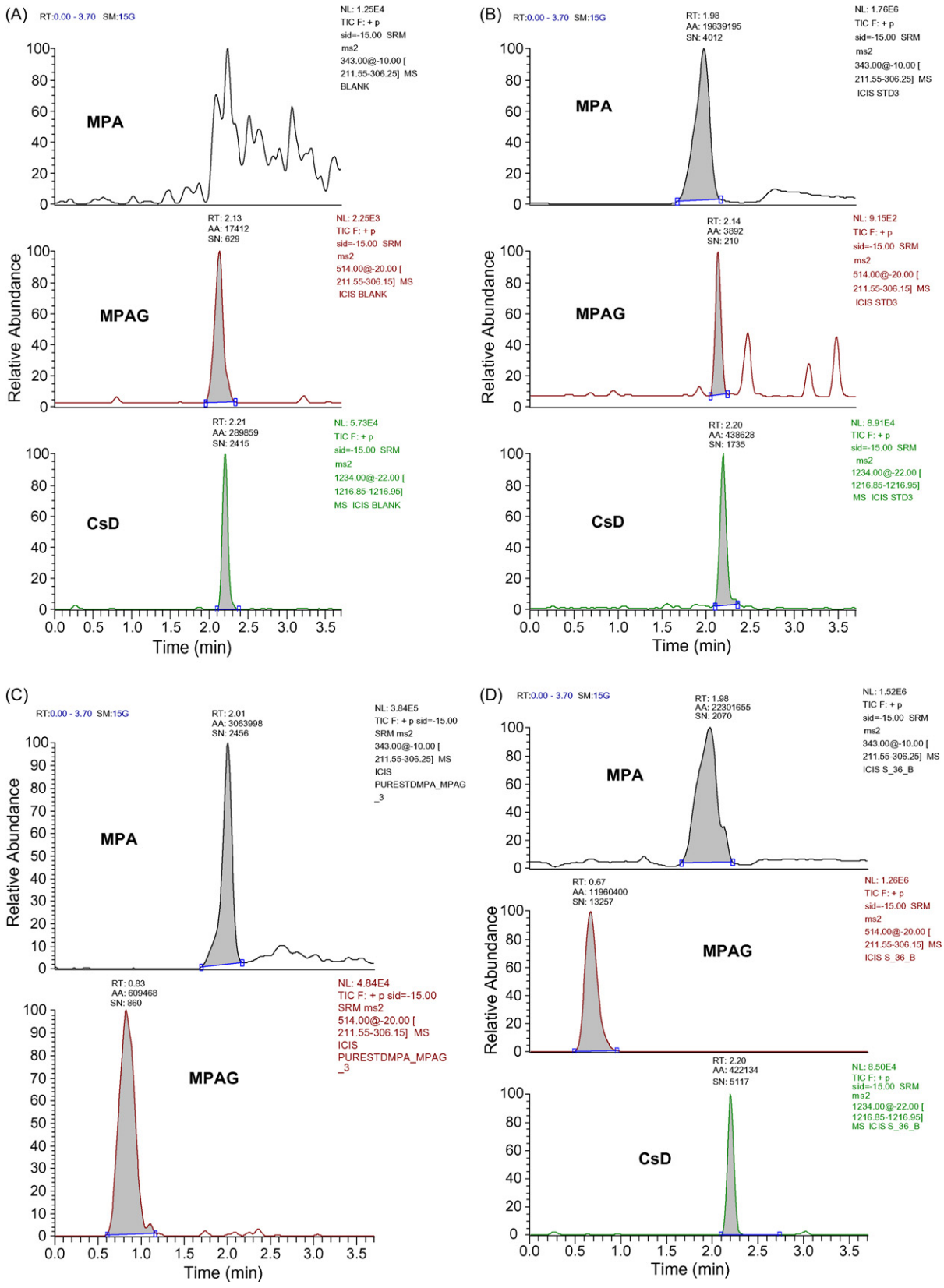

Fig. 3. Mass chromatograms of blank plasma extract (A), calibrator 4 for MPA (B), plasma standard containing MPA and MPAG, 1 μgm/l each (C), and patient plasma extract containing around 20 μg/ml MPAG and 6 μg/ml MPA (D). Upper trace, MPA; middle trace, MPAG; lowest trace, CsD (IS).

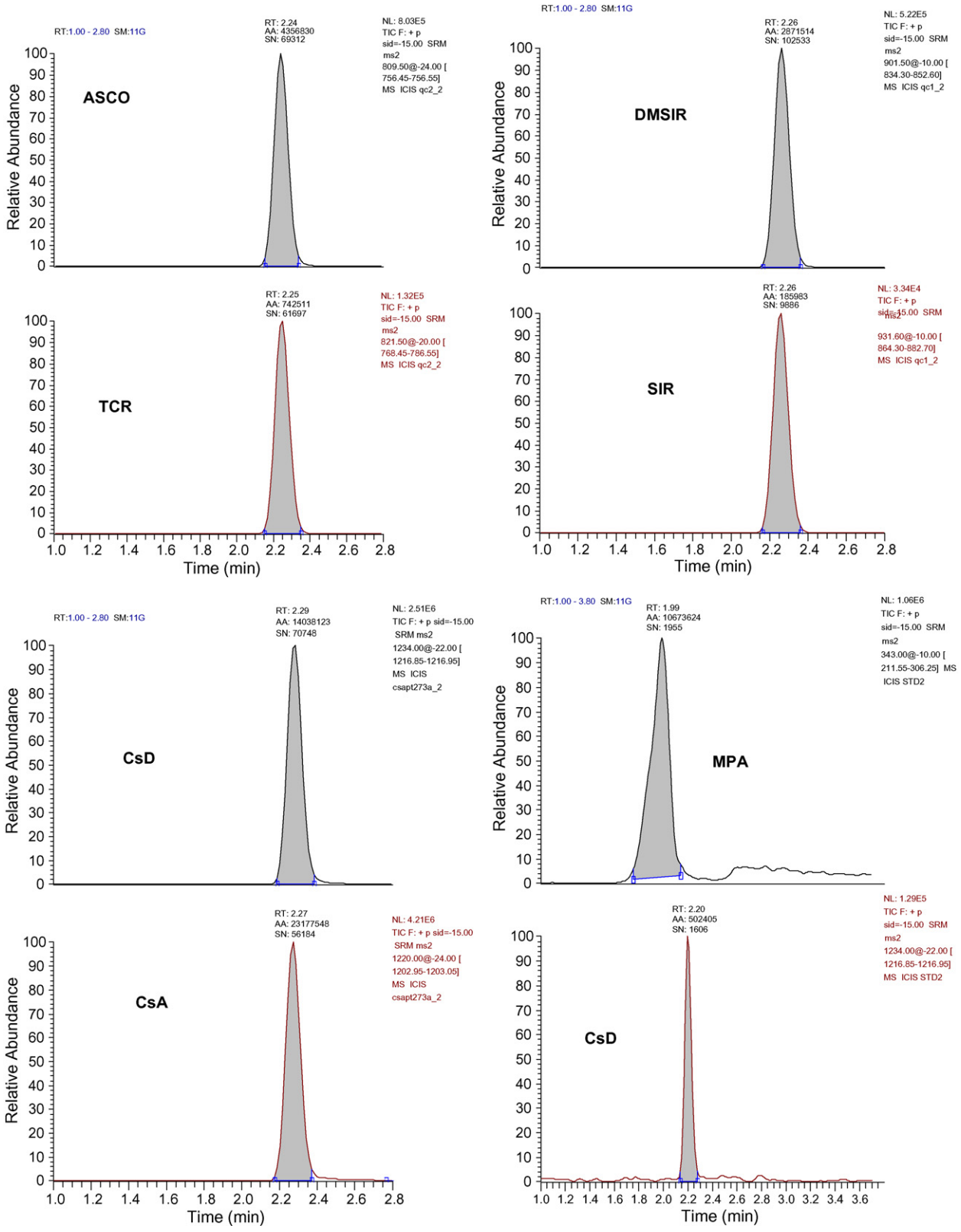

Fig. 4. Typical chromatogram showing mass traces of target compounds and internal standards in various clinical samples. The concentrations were: 34 ng/ml for TCR, 4.2 ng/ml for SIR, 260 ng/ml for CsA, and 8.60 μg/ml for MPA.

LC–MS–MS procedures [3,18,20,22], as well as in HPLC–UV procedure [23] these both compounds were fully separated. This was also achieved in the present method, as demonstrated on Fig. 3. MPAG eluted at Rt around 0.7–0.8 min, whereas the Rt of MPA was around 2 min. In the routine method conditions, MPAG was diverted to waste, and only MPA was measured. The experiments shown on Fig. 3 were performed without activation of the divert valve, in order to demonstrate the separation of MPA and MPAG.

4. Conclusions

Developed method assures sensitive and selective simultaneous determination of cyclosporine A, tacrolimus, and sirolimus in 50 μl of blood.

The pretreatment procedure is extremely simple and did not involve costly and time-consuming chromatographic clean-up step.

The determination of mycophenolic acid in plasma is possible in the same procedure, which also assures full separation from mycophenolic acid glucuronide. The method allows handling easily 50–100 samples per day and was implemented for routine use (Fig. 4).

References

[1] G.L. Lensmeyer, M.A. Poquette, Ther. Drug Monit. 23 (2001) 239.
[2] C.E. Staatz, P.J. Taylor, S.E. Tett, Ther. Drug Monit. 24 (2002) 607.
[3] A. Premaud, A. Rousseau, N. Picard, P. Marquet, Ther. Drug Monit. 28 (2006) 274.
[4] R.G. Morris, P. Salm, P.J. Taylor, P.J. Wicks, A. Theodossi, Ther. Drug Monit. 28 (2006) 164.
[5] F.B. Vicente, F.A. Smith, Y. Peng, S. Wang, Clin. Chem. Lab. Med. 44 (2006) 497.
[6] D. Wilson, F. Johnston, D. Holt, M. Moreton, J. Engelmayer, et al., Clin. Biochem. 39 (2006) 278.
[7] D. Zochowska, I. Bartlomiejczyk, A. Kaminska, L. Pachek, Transplant. Proc. 38 (2006) 78.
[8] C. Fillee, M. Mourad, J.P. Squiflet, J. Malaise, et al., Transplant. Proc. 37 (2005) 2890.
[9] B.G. Keevil, D.P. Tierney, D.P. Cooper, M.R. Morris, Clin. Chem. 48 (2002) 69.
[10] P.J. Taylor, C.E. Jones, P.T. Martin, S.V. Lynch, A.G. Johnson, S.M. Pond, J. Chromatogr. B 705 (1998) 298.
[11] D.W. Holt, T. Lee, K. Jones, A. Johnston, Clin. Chem. 46 (2000) 1179.
[12] F. Streit, V.W. Armstrong, M. Oellerich, Clin. Chem. 48 (2002) 955.
[13] U. Ceglarek, J. Lembcke, G.M. Fiedler, M. Werner, H. Witzigmann, J.P. Hauss, J. Thiery, Clin. Chim. Acta 346 (2004) 181.
[14] T. Koal, M. Deters, B. Casetta, V. Kaever, J. Chromatogr. B 805 (2004) 215.
[15] M.A. Poquette, G.L. Lensmeyer, T.C. Doran, Ther. Drug Monit. 27 (2005) 144.
[16] T.M. Annesley, L. Clayton, Clin. Chem. 50 (2004) 1845.
[17] P.J. Taylor, Ther. Drug Monit. 26 (2004) 215.
[18] T.M. Annesley, L. Clayton, Clin. Chem. 51 (2005) 872.
[19] T.M. Annesley, Clin. Chem. 51 (2005) 457.
[20] C.G. Patel, A.E. Mendonza, F. Akhlaghi, O. Majid, A.K. Trull, T. Lee, D.W. Holt, J. Chromatogr. B 813 (2004) 287.
[21] M. Vogeser, R. Zachoval, U. Spohrer, K. Jacob, Ther. Drug Monit. 23 (2001) 722.
[22] F. Streit, M. Shipkova, V.W. Armstrong, M. Oellerich, Clin. Chem. 50 (2004) 152.
[23] I.S. Westley, B.C. Sallustio, R.M. Morris, Clin. Biochem. 38 (2005) 824.