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Review Article

Quantification of Immunosuppressant's in Blood using LC-MS/MS

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Abstract

Numerous analytical advancements have been introduced for Therapeutic Drug Monitoring (TDM) of immunosuppressant's (cyclosporine a, tacrolimus, everolimus and serolimus). In recent years, liquid chromatography in tandem mass spectrometer has been the technique of choice for the determination of immunosuppressants. The automated on-line sample processing and purification technologies have simplified the sample purification and extraction process. In present review, the important liquid chromatographic as well as mass spectrometric parameters which can influence the determination of immunosuppressants are also discussed. It has been observed that extraction procedure, mobile phase composition, mobile phase gradient, column type, column temperature, cone voltage and analyte adduct ions have direct impact on the determination of immunosuppressants. Dried blood spot sampling for TDM of immunosuppressants has been suggested by several researchers. Present review also briefed some commercial analytical kits for immunosuppressants. On the basis of available literature, it was concluded that LC-MS/MS is the gold standard analytical technique for therapeutic monitoring of immunosuppressants.

Keywords: Immunosuppressants; Bio-analysis; LC-MS/MS; Cyclosporine; Tacrolimus; Everolimus; Sirolimus

Abbreviations

LC: Liquid Chromatography; MS/MS: Mass Spectrometer; LC-MS/MS: Liquid Chromatography in Tandem Mass Spectrometer; ESI: Electrospray Ionization; UPLC: Ultra-Performance Liquid Chromatography; TDM: Therapeutic Drug Monitoring

Introduction

The immunosuppressants such as cyclosporine A (Neoral®), tacrolimus (Prograf®), sirolimus (Rapamune®) and everolimus (Zortress®) have been indicated for the prophylaxis of organ rejection in patients receiving allogeneic organ transplants. The cyclosporine, tacrolimus, sirolimus and everolimus are poorly soluble in water. Their oral absorption is incomplete and has poor bioavailability. They are extensively metabolized by CYP3A isoenzyme system. Cyclosporine A primarily eliminated through biliary route. Fecal elimination predominates for tacrolimus, sirolimus and everolimus. All these four immunosuppressants extensively bound plasma proteins. The immunosuppressive action of these substances is primarily due to parent drug.

These aforesaid immunosuppressants are categorized as narrow therapeutic indexed drug substances. Their blood concentration below therapeutic level may leads to organ rejection (therapy failure) and the concentration above therapeutic level will be toxic. Therapeutic drug monitoring is recommended for all patients receiving treatment with any of these immunosuppressants. Over the years numerous analytical methods have been developed for the analysis of these immunosuppressants in biological fluids for pharmacokinetic studies and therapeutic monitoring. Many of these analytical methods determine only single analyte while there are methods which can analyze all four drugs simultaneously. The analytical methods for determination of these immunosuppressants should be accurate, precise, sensitive, highly selective and robust to avoid any wrong determination. The analysis of cyclosporine, tacrolimus, sirolimus and everolimus is a complex process, since these drugs are analyzed in biological fluids such as blood, plasma, urine and aqueous humour in very low concentrations; usually in ng/ml. Different types of detectors (e.g. ultraviolet detectors, mass spectrometers and electrochemical detectors etc.) have been used in liquid chromatographic methods. Liquid chromatography in tandem mass spectrometer has been considered as gold standard technique for the analysis of immunosuppressants in biological tissues and fluids. Nowadays antibody-based immunoassays for therapeutic drug monitoring are being replaced by liquid chromatography tandem mass spectrometer. The immunoassays have been reported to overestimate the cyclosporine A concentration in blood because of the cross reactivity of cyclosporine A metabolites [1,2]. Schutz et al. reported that in a liver recipient group CEDIA and the AxSYM showed a mean overestimation of CsA by 43% and 47%, respectively [1]. The other disadvantages of immunoassay methods are their high cost as compare to liquid chromatographic methods and their inability to simultaneously measure the two or more immunosuppressant's [3]. The liquid chromatographic techniques, especially LC-MS/MS provide higher analyte specificity, when compared with immunoassays. Simultaneous analysis of these immunosuppressants has been carried out by LC-MS/MS methods.

Hermann et al. enlisted the chromatographic parameters used in previously published papers for the estimation of cyclosporine A and

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its metabolites. The different investigators have used different set of chromatographic conditions: the column temperature varies between 50-80°C, the most commonly used columns are C8 and C18, the flow rate varies between 0.3-2.5 ml/min, the range of wavelength of detection was 206-230nm and the most common organic modifiers were Acetonitrile and methanol [4].

Biological samples

In pharmacokinetic investigations the immunosuppressants have been measured in various biological fluids. However, blood is the preferred biological fluid for therapeutic monitoring of immunosuppressants. There are several factors which make the blood as the preferred sampling fluid. One of these factors is: the high affinity of immunosuppressants for cellular components of blood. The higher concentrations of immunosuppressants have been observed in blood compared to plasma, so the blood is recommended as the commonly accepted biological matrix for their estimation [5,6]. Cyclosporine A is highly bound to red blood cells and plasma proteins; and during storage its distribution between red blood cells and plasma or serum is temperature dependent [7,8]. Therefore, the determination of cyclosporine A is preferred in whole blood to minimize the temperature dependent variations of cyclosporine A concentrations [8]. Koster et al. investigated that varying hematocrit value and analyte concentration influenced the extraction recoveries of sirolimus and everolimus; while there was no significant effect on the extraction recoveries of cyclosporine and tacrolimus [9]. Koster et al. also observed the positive as well as negative bias in the results when compared with the standardized hematocrit value (0.35 L/L). At the lowest hematocrit value (0.20L/L) and highest drug concentration (50µg/L) the sirolimus and everolimus showed −20% and −28% bias, respectively [9]. There are several analytical methods which can measure the immunosuppressants in other biological fluids. The cyclosporine A can be determined in blood, plasma, serum, bile and urine [10,11]. Szerkus et al. determined cyclosporine A in tissues (cornea, conjunctiva and eye globe) and fluids (lacrimal fluid and aqueous humor) of rabbit eye [12]. Guada et al. developed and validated an ultra-high performance LC-MS/MS method for the quantification of cyclosporine A in lipidnano-systems and mouse biological matrices such as whole blood, kidneys, lungs, spleen, liver, heart, brain, stomach and intestine; and the method was successfully applied for pharmacokinetic and biodistribution studies of cyclosporine A [13]. Belostotsky et al. investigated the tacrolimus distribution in the blood and saliva; and found a poor correlation between the drug levels in both biological fluids. In some of the cases the tacrolimus level was not detectable in saliva though the drug was present in the blood sample. Because of this poor correlation the sampling of saliva can't be considered for therapeutics monitoring of tacrolimus [14].

Amini and Ahmadiani reported that the interference increases in frozen blood samples and the number and intensity of interfering peaks gradually increased after long storage at 4 °C [15]. Several stability studies suggested that cyclosporine A, tacrolimus, sirolimus and everolimus can be measured in blood samples without stability concerns, if handled and stored properly. All these four immunosuppressants have been found stable in blood sample at room temperature for few days and at -80 °C for several months. The extracted samples (acetonitrile and methanol) were also stable in autosampler for a sufficient period of time (24 hrs to few days). The cycloaporine A is stable in blood samples for 2-3 months when stored at 1-4 °C [15].

Dried blood spot

Generally, the therapeutic drug monitoring of immunosuppressants is performed in venous blood samples. Recently, several investigations have been carried out on dried blood spot sampling method and it has been suggested as an alternative sampling method. Dried blood spot sampling is a newly evolving idea for therapeutic monitoring of immunosuppressants [16-21]. The dried blood spot sampling has several advantages over the conventional (venous) blood sampling. The patients can withdraw the capillary blood themselves using an automatic lancet. The first drop of blood is discarded and the second drop is applied to the sampling paper. To ensure the accuracy and uniformity of the method, the circles on the sampling paper are homogeneously and symmetrically filled with blood drop. The blood drop is dried on the sampling paper at room temperature and sealed packed properly. For extraction, the paper disks with a predetermined diameter (~ 8 mm) are punched out with the help of a whole puncher [16]. The dried blood spot method offers several advantages including small samples size (10-50 µL, preferably 30 µL bloods is applied as spot) and fewer hospital visits, since the sample can be posted to the laboratory [22-24]. Hinchliffe et al. developed a UPLC-MS/MS method for simultaneous estimation of cyclosporine A and tacrolimus from dried blood spot [23]. Rao et al. developed and validated a high-throughput LC- ESI-MS method for monitoring the sirolimus on a dried blood spot [24].

Wilhelm et al. and Hinchliffe et al. perform validation studies in which they compare the dried blood spot sampling methods with venous sampling method. In dried blood spot sampling method capillary blood was obtained from a finger prick with an automatic lancet and the drop of blood (~30 µL) was applied to sampling paper; while venous blood sampling was performed by venipuncture. The conclusions suggested that dried blood spot sampling method can be used as an alternative for conventional venous blood sampling in therapeutic monitoring of immunosuppressants [25,26]. Wilhelm et al. studied the influence of the hematocrit value on cyclosporine A concentration in dried blood sample, and observed that the difference in hematocrit value influences the analysis of cyclosporine A in the dried blood sample. The blood samples of high hematocrit value have high viscosity, and because of high viscosity the blood distribution on the paper is poor. The blood sample with higher hematocrit value (higher viscosity) will form smaller spot and center of the spot will be thick and have more drug concentration [27]. The perfect round spots with uniformity can be produced by spotting the blood in a fixed volume on the paper. The spots made with small volume of blood may influence the results if it is not sufficient to reach the margins of the fixed volume circle and the punched out part will show the white edges of unspotted paper [21]. Dried blood spot samples of everolimus were found stable for 34 days at 32 °C and for 3 days at 60 °C, while at 70 °C the everolimus was suggested unstable [19]. Sadilkova et al. investigated the stability of patient dried blood spot samples. Tacrolimus and cyclosporine A were found stable for at least five days at the temperature up to 60 °C, while degradation was observed in sirolimus at 60 °C within 24 h [20].

Extraction procedures

The protein precipitation and liquid-liquid extraction techniques have been used for extracting the immunosuppressants from biological fluids. Protein precipitation is the most commonly used technique, since it is simple and involve less cost. In protein precipitation technique blood sample has been mixed with $ZnSO_4$ solution and mixed properly by vortexing the mixture. The organic solvents such as methanol (MeOH) and Acetonitrile (ACN) are widely accepted protein precipitants. The $ZnSO_4$ solution has been added to whole blood sample to rupture the red blood cells and as a result to release the bound drug. Koster et al. found that use of $ZnSO_4$ provides good process efficiency for cyclosporine and tacrolimus while a poor efficiency was observed for sirolimus and everolimus [28]. Some of the protein precipitation based extraction methods for sirolimus and everolimus have used ZnSO4 solution in extraction procedure [3,29]. In general, the protein precipitated samples are centrifuged and the supernatant is removed and directly injected for the analysis. These protein precipitated samples can further be cleaned by loading and eluting on a solid phase extraction cartridges. In Solid Phase Extraction (SPE) the extraction cartridge (or extraction column) is conditioned with Solvent (s) and or buffer and then loaded with the supernatant of protein precipitated sample. Extraction column is flushed with the solvent system followed by analytes extraction with a suitable solvent system, and then extracted sample is injected into analytical column. The solid phase extraction can also be performed on-line. For on-line purification the liquid chromatography system is supported with multiport switching valve. The supernatant sample is injected into extraction column with suitable solvent system and then flushed at high flow rate. After flushing the switching valve is changed and the analytes are eluted from extraction column to the analytical column by using solvent system of higher organic phase. The supernatant of protein precipitated samples also has been cleaned by using turbulent flow chromatography (Cyclone 50 x 1 mm polymeric column, Cohesive Technologies, USA) supported with switching valve [30]. Numerous other advancements have been introduced in the sample extraction procedures. The automated and semi-automated online extraction procedures were developed for the extraction of immunosuppressants from blood. McMahon et al. uses semi-automated solid-phase extraction while Brignol et al. uses semi-automated high-throughput liquid/liquid extraction procedure for the extraction of cyclosporine A and everolimus [31,32]. Alvarez and Weiner purify the extracted supernatant of protein precipitated plasma samples on-line and directly injected into the LC-MS system [33]. Marinova et al. developed an automated protein precipitation-based whole blood sample preparation protocol for the quantification of sirolimus, everolimus and tacrolimus by using a liquid handling system [Tecan Freedom EVO 150 liquid handling platform (Tecan, Männedorf, Switzerland) equipped with an 8-channel liquid handling arm with standard fixed tips, one robotic plate-handling arm and one plate shaker (Te-Shake), and an additional component vacuum separation module (Te-VacS)] [29]. The supernatants of protein precipitated samples were then subjected to on-line solid phase extraction using column switching technique [29]. Hassell et al. demonstrated the application of Prelude TM Sample Preparation Liquid Chromatography (SPLC) system connected with a TSQ Vantage TM triple stage quadrupole mass spectrometer for measuring immunosuppressant's (cyclosporine A, tacrolimus, serolimus and everolimus) and chemotherapeutic drugs. The samples were cleaned online by turbo flow technology and the analytical separation was carried out on Prelude TM SPLC system [34]. MagSi-TDMPREP (amsbio) is a fully automated sample preparation kit. The kit eliminates the interfering proteins, phospholipids and salts from whole blood sample. The process of MagSi-TDMPREP involves the lysis of whole blood cells followed by addition of internal standard. The MagSi-TDMPREP beads are added to the sample and then protein precipitant is added. The precipitated protein are bound to the beads and removed by using magnetic separation. The supernatant can be directly injected into the LC-MS/MS [35,36].

Internal standards

For the analysis of drugs extracted from biological samples, it is preferred that internal standard and analyte should have physicochemical similarities [37]. Because of physicochemical similarities the internal standard and analyte will be extracted uniformlyin a simple solvent system and by a common procedure. The extraction efficiency of compounds having similar physicochemical properties will also be closely related. Further it may also simplify the elution of both compounds on a same type of analytical column with less complex mobile phase composition. The similarities or closeness in chemical properties (such as molecular weight and chemical structure) of analyte and internal standard also allows working on almost similar mass spectrometric parameters such as cone voltage, collision energy, type of ion adducts etc. For immunosuppressants estimation in biological fluid by LC-MS/MS, the most preferred internal standards are their isotope-labeled compounds (isotope-labeled compound viz. evelolimus-d4, sirolimus-d3, cyclosporine A-d4). The cyclosporine D also has been preferred as internal standard for the estimation of cyclosporine A. The other used internal standards include ascomycine, amiodarone, desmethoxyrapamycin and desmethyl sirolimus.

LC-MS/MS

For routine therapeutic monitoring of drug substances a sensitive, selective and rapid assay is required. The liquid chromatography (HPLC or UPLC) in tandem Mass Spectrometer (MS/MS) has been the technique of choice for pharmacokinetic investigations and therapeutic monitoring of immunosuppressant's.

Liquid chromatography: Over the years, several advancements have been introduced in the liquid chromatographic systems. The Ultra-Performance Liquid Chromatography (UPLC) is one of the recent technological advancements in the analytical field. UPLC analyzes the drugs in a shorter time when compared with traditional HPLC. Pedraglio et al. compared the performances of HPLC-MS/MS and UPLC-MS/MS in terms of sensitivity and resolution. UPLC significantly reduces the analysis time, decreases the mobile phase consumption, increases sensitivity and resolution [38,39]. Hsieh et al. developed an Ultra-Performance Hydrophilic Interaction Liquid Chromatography (UPHILIC) tandem mass spectrometer for the determination of everolimus in mouse plasma [40].

The C18 is the most commonly used analytical column for the elution of immunosuppressants. Amini and Ahmadiani suggested that C18 and C8 columns should be operated with higher percentage of acetonitrile (75%) at a temperature of 70 °C, while CN or TMS columns can be operated at lower temperature (60 °C) and lower

acetonitrile ratio (50%) [15]. Baldelli et al. reported a chromatographic method where analytes were eluted on C8 column (at 60 °C) for over 36 min (everolimus 9 min, cyclosporine A 21.3 min and cyclosporine D 32.2 min) by using a mobile phase consisted of 56% acetonitrile in water [41]. Apart from particle packed columns the cyclosporine A and sirolimus have been successfully eluted on monolithic column [33,24]. Rao et al. observed that monolithic column (Merck Chromolith Performance RP18e (25 x 4.6 mm)) generates much less back pressure (25 kg f) than the particulate column (444 kg f), while maintaining its efficiency [24]. Hatsis and Volmer demonstrated the applicability of cyano columns for simultaneous estimation of tacrolimus, sirolimus and cyclosporin A. The drugs were analyzed using selected reaction monitoring in negative electrospray ionization mode on a triple quadrupole mass spectrometer. According to Hatsis and Volmer, at 50 °C the chromatographic efficiency (theoretical plates per column) of tacrolimus, sirolimus and cyclosporin an on cyano column was much better than that on C18 column [42]. The sensitivity/ limit of detection in negative ionization mode has been reported to be higher than the positive ionization mode, with an argument that there are fewer product ions in negative ion mode than in positive ion mode [42].

The resolution and peak shape of cyclosporine A is significantly influenced by changing the column temperature [43]. Sawchuk and Cartier have optimized the column oven temperature. They also suggested that column life is shortened by increasing the temperature of column oven [43]. The tips for column longevity were suggested by Lensmeyer and Fields. The column life can be improved by careful cleaning the column and continuously maintaining the flow of mobile phase, and by using silica saturated column at lower column temperature, shorter run time and by using pre-column [44,45]. Ouyang et al. developed a liquid chromatographic method which can analyze the cyclosporine a in human blood at room temperature. The methods is said to improve detection limit, column life and peak broadening was also controlled. A mobile phase composition comprising isopropanol: acetonitrile: water (80:10:10) and flowing at 1ml/min is said to improve peak shape at column temperature of 25 °C [46]. Salm et al. developed a rapid LC-MS analytical method for the determination of CsA with a very short retention time between 0.5-1.0 min and total run time of 2 min [47]. The analytical column life was suggested at least 500 injections. Since the temperature of column oven was high (70 °C) so the limited column life is promised [47].

Hermann et al. evaluated the importance of essential chromatographic parameters such as percentage of organic modifier, column oven temperature, flow rate, pH of mobile phase and gradient steepness of mobile phase; in the determination of cyclosporin A and its metabolites, AM1, AM9 and AM4N. All parameters (except HCl addition) were found to be of significance to one or more response variables (resolution between AM1 and AM9, retention time of AM1/ AM9 and retention time of cyclosporine A) [4]. For the separation of analytes, the optimal chromatographic parameters were determined as: a reversed phase C8 column maintained at 80 °C, and mobile phase containing acetonitrile and water flowing in linear gradient (ACN 52.8 to 73%) at the rate of 0.8ml/min [4].

The preferred mobile phase composition for elution of immunosuppressants includes methanol and or acetonitrile as organic modifier and ammonium acetate buffer as aqueous medium. Formic acid in low percentage also has been used in mobile phase composition. In LC-MS/MS methods of immunosuppressants the mobile phase was pumped in gradient mode, though some isocratic LC-MS/MS methods were also developed.

Adducts: In LC-MS/MS methods the mass detector quantifies the analyte ions according to their mass/ charge (m/z) ratio. There are several modes and source of ionization in different mass spectrometers. Positive electrospray ionization is the commonly used mode of ionization for the analysis of small drug molecules. Generally, the positive electrospray ionization mode produces protonated species $[M+H]^+$. However for some molecules the formation of alkali metal ion adduct such as sodium-adduct $[M+Na]^+$ or potassium-adduct $[M+K]^+$ has been reported. The formation of these adducts depends upon the characteristics of the analyte molecules, sample processing, mobile phase composition and mass operating parameters. The alkali adducts are difficult to fragments into small species (daughter ions or fragments) and are unsuitable for selected reaction monitoring. Nozaki et al. reported an on-line ion suppression technique to eliminate alkali metal ion adducts. The tacrolimus solution was passed through ion suppressor device (ion chromatography system with a cation-exchange resin), and then directly introduced into the ion-trap mass spectrometer [48].

Different adducts for the same analyte have been observed under different solvent compositions. The direct infusion of methanol solution of sirolimus and tacrolimus showed prevalence of their sodium adducts, while the presence of mobile phase (methanol and ammonium formate buffer 10 mM, pH 3.0) in full scan mass spectrum showed dominance of ammoniated adducts of cyclosporine, sirolimus, and tacrolimus [49]. Chan et al. reported that isomeric active metabolites (13-O-demethylated (M1), 31-Odemethylated (M2) and 15-O-demethylated (M3) Tacrolimus) of tacrolimus demonstrated a strong ability to form ammonium-adduct and sodium-adduct ions. The sodium-adduct ions of the metabolites were easily formed in ion source but they were not found suitable for multiple reaction monitoring because of their poor fragmentation. So the ammonium-adducts were selected as precursor ions for multiple reaction monitoring [50]. The fragmentation of sodium adduct of everolimus is poor when compared with the fragmentation of ammonium-adducts of everolimus [51]. Sodium adduct of everolimus has been measured as single ion monitoring [52,53].The high intensity signal of sodium adducts of sirolimus and its metabolites were monitored in positive electrospray ionization mode [6]. Koseki et al. run a mass spectrum of cyclosporine A and its metabolites (AM1, AM4N, and AM9) in atmospheric pressure chemical ionization and in positive electrospray ionization mode. The sodium and ammonium adducts of cyclosporine A and its metabolites were observed in positive electrospray ionization mode, while the protonated ion peaks of cyclosporine A and its metabolites were observed with strong signal response in atmospheric pressure chemical ionization mode [54].

The several adducts of immunosuppressants have been analyzed. The ionic adducts which can be fragmented into daughter ions can be analyzed easily by using multiple reactions monitoring mode, while the hard adduct ion do not fragments easily into stable daughter ions

Table 1: Presenting mass spectrometric information for Immunosuppressant's.

Drug	Method	Detector & Mode	Ion analyzed	Instrument	Ref.
Cyclosporine A Tacrolimus Sirolimus Everolimus	UPLC/MS/MS	ESI, +ve MRM	$[M+NH_4]^+$	TQ-S triple-quadrupole mass spectrometer equipped with stepwave ion transfer optics	3
Cyclosporine A	LC-MS	ESI, +ve SIR	$[M+H]^+$	Waters Micromass ZQ™ 4000 single quadrapole mass spectrometer	4
Cyclosporine A	UHPLC–MS/MS	ESI, +ve MRM	$[M+NH_4]^+$	Acquity™ TQD (Triple Quadrupole Detector) mass spectrometer	13
Tacrolimus	LC-MS/MS	ESI,+ve MRM	$[M+NH_4]^+$	Quattro Micro™ mass spectrometer fitted with a Z spray ion source	17
Everolimus	LC-MS/MS	ESI,+ve MRM	$[M+NH_4]^+$	Quatro Micro™ mass spectrometer fitted with a Z spray ion source	19
Cyclosporine A Sirolimus Tacrolimus	LC–MS/MS	ESI, +ve MRM	$[M+NH_4]^+$	Quattro Micro™ triple quadrupole mass spectrometer	20
Cyclosporine A Tacrolimus Sirolimus Everolimus	LC-MS/MS	ESI,+ve MRM	$[M+NH_4]^+$	Quattro Micro™ mass spectrometer	21
Cyclosporine A Tacrolimus	UHPLC–MS/MS	ESI, +ve MRM	$[M+NH_4]^+$	Quattro Premier XE™ mass spectrometer	23
Sirolimus	LC-MS	ESI, +ve SIM	$[M+NH_4]^+$	Quadrupole time-of-flight (Q-TOF) mass spectrometer	24
Sirolimus, Everolimus Tacrolimus	LC-MS/MS	ESI,+ve MRM	$[M+NH_4]^+$	Agilent 6430 triple quadrupole	29
Cyclosporine A Tacrolimus Sirolimus	turbulent flow chromatography LC–MS/MS	Turbo Ion Spray +ve, MRM	$[M+NH_4]^+$	API 3000™ triple quadrupole mass spectrometer with Turbo Ion Spray	30
Cyclosporine A Everolimus	LC/MS	APcI,-ve & +ve SIM	$[M+H]^-$ $[M+H]^+$	Finnigan Model TSQ-700 triple quad rupole mass spectrometer	31
Cyclosporine A	UPLC–MS/MS	ESI, +ve MRM	$[M+H]^+$	Quattro Premier XE™ triple quadrupole mass spectrometer	39
Cyclosporine A Tacrolimus Sirolimus	LC-MS	ESI, -ve & +ve SRM	$[M+H]^-$ & $[M+Na]^+$	API 4000™ triple quadrupole mass spectrometer	42
Cyclosporine A	LC-MS/MS	ESI, +ve SRM	$[M+NH_4]^+$	API III triple quadruple mass spectrometer	47
Tacrolimus metabolites	LC-MS/MS	Turbo Ion spray +ve	$[M+NH_4]^+$	API 3000™ triple quadrupole mass spectrometer with Turbo Ion spray	50
Everolimus	LC-MS/MS	ESI,+ve SRM	$[M+NH_4]^+$	Quattro micro™ Quadrupole mass spectrometer	51
Cyclosporine A& metabolites	LC/MS	APCI, +ve SIM	$[M+H]^+$	TSQ® Quantum™ interfaced with atmospheric pressure chemical ionization	54
Cyclosporine A Sirolimus Tacrolimus	LC-MS/MS	APCI, +ve MRM	$[M+NH_4]^+$	API-2000™ mass spectrometer with atmospheric pressure chemical ionization source	55
Cyclosporine A, AM1, AM9, AM4N	UPLC-MS/MS	ESI, +ve MRM	$[M+H]^+$	Quattro Micro API triple quadrupole	59
Cyclosporine A Tacrolimus Sirolimus Everolimus	LC-MS/MS	API-MS/MS +ve, MRM	$[M+NH_4]^+$	API 3000™ (triplequadrupole) mass spectrometer equipped with TurboIonspray source	61
Cyclosporine A Tacrolimus Sirolimus Everolimus	UFLC-MS/MS	ESI,+ve MRM	$[M+H]^+$ & $[M+NH_4]^+$	API 3200™ triple quadrupole mass spectrometer with Turbo Ion Spray source	62
Cyclosporine A Tacrolimus Sirolimus Everolimus	LC-MS/MS	ESI, +ve MRM	$[M+NH_4]^+$	API 4000 ™ mass spectrometer	64
Cyclosporine A	LC–MS/MS	ESI, +ve MRM	$[M+NH_4]^+$	4000 QTRAP® mass spectrometer, equipped with a Turbo Ion Spray	65
Cyclosporine A	LC-MS	ESI, +ve MRM	$[M+NH_4]^+$	Quattro mass spectrometer fitted with a Z Spray ion source	66
Sirolimus	LC–MS/MS	turbo-ion spray +ve MRM	$[M+NH_4]^+$	API 2000™ triple quadrupole mass spectrometer	67
Tacrolimus Sirolimus	LC–MS/MS	ESI, +ve MRM	$[M+NH_4]^+$	Quattro Micro™ mass spectrometer fitted with a Z-spray ion source	68

Tacrolimus	LC–MS/MS	ESI, +ve SRM	$[M+NH_4]^+$	Quattro Micro™ mass spectrometer	69
Sirolimus Everolimus	LC–MS/MS	APCI -ve SRM	$[M+H]^-$ Deprotonated	TSQ Quantum™ Access Max™ triple quad rupole mass spectrometer with atmospheric pressure chemical ionization	70
Tacrolimus	LC/MS/MS	ESI,+ve MRM	$[M+NH_4]^+$	Quattro Micro™ mass spectrometer	71

Table 2: Presenting liquid chromatographic parameters for Immunosuppressant's.

Drug Name	Column Temp.	Mobile phase composition and Flow	Solid Phase extraction Column	Analytical Column	Ref.
Cyclosporine A Tacrolimus Sirolimus Everolimus	45 °C	A= 2mM NH_4Ac and 0.1% formic acid in water B= 2mM NH_4Ac and 0.1% formic acid in MeOH Flow = Gradient	Protein precipitation ($ZnSO_4$ and ACN)	Waters UPLC BEH C18 (50 mm ×2.1 mm) 1.7-µm column	3
Cyclosporine A	70 °C	MeOH (80%) 40mM NH_4Ac (20%), pH 5.1 Flow = Isocratic	C18 SPE cartridges (Sep Pak, 100 mg, Waters, Milford, USA)	Zorbax Bonus C18 reverse phase column (50 mm x2.1 mm I.D., 5µm, Agilent, Palo Alto, CA, USA)	12
Cyclosporine A	50 °C	A= 2mM NH_4Ac and 0.1% formic acid in water B= MeOH Flow = Gradient	protein precipitation (10% trichloroacetic acid solution and ACN)	Acquity UPLC® BEH C18 column (50 mm ×2.1 mm, 1.7 µm; Waters, USA)	13
Tacrolimus	60 °C	A= 2mM NH_4Ac, 0.1% formic acid in water B= 2mM NH_4Ac, 0.1% formic acid in MeOH Flow = Gradient	Venous Blood and DBS On-line SPE (Waters Oasis HLB cartridge column, (2.1mm×20 mm, 25µm)	Waters Atlantis dC18 (3.0× 100 mm, 5µm) column	17
Everolimus	60 °C	A= 2mM NH_4Ac, 0.1% formic acid in water B= 2mM NH_4Ac, 0.1% formic acid in MeOH	Dried Blood Spot SPE column (Waters Oasis HLB cartridge column, 2.1mm×20mm, 25µm).	Waters Atlantis dC18 3.0mm×100mm, 5µm	19
Sirolimus Tacrolimus Cyclosporine A	45 °C	A= 2mM NH_4Ac, 0.1% formic acid in water B= 2mM NH_4Ac, 0.1% formic acid in MeOH Flow = Gradient	Dried Blood Spot (Whatman 903 DBS cards)	Supelco (Sigma-Aldrich) Supelcosil LC-18-DB analytical column (3 × 33 mm, particle size 3 µm)	20
Cyclosporine A Tacrolimus Sirolimus Everolimus	55 °C	A= 0.05 % trifluoroacetic acid, 5 mM ammonium formate in water B= 0.05 % trifluoroacetic acid, 5 mM Ammonium formate in MeOH Flow = Isocratic	Dried Blood Spot (Whatman 903 sampling paper) Liquid liquid extraction	Symmetry Shield RP18, 50×4.6 mm, particle size 3.5 µm.	21
Sirolimus	45 °C	A= water (20%), 0.01% formic acid B= MeOH (80%), 0.01% formic acid Flow = Isocratic	Dried blood spot. Extracted in [MeOH: 0.1% (NH4)2SO4, 4:1 v/v]	Chromolith Performance RP18e, 25 x 4.6mm (Merck KgaA, Darmstadt, Germany)	24
Sirolimus, Everolimus Tacrolimus	60 °C	A= MeOH: W (98:2), , 2 mM ammonium formate, 0.1% formic acid B= 2 mM ammonium formate, 0.1% formic acid in water Flow = Isocratic/ Binary	Automatic protein precipitation and online sample cleaning by SPE column Zorbax Extend-C18 (2.1 mm × 12.5 mm, 5 µm, Agilent)	Zorbax Eclipse XDB-C18 column (4.6 mm ×50 mm, 1.8 µm particle size; Agilent)	29
Cyclosporine A Tacrolimus Sirolimus	40 °C	MeOH: W (97:3), 10mM NH_4Ac, and 0.1% acetic acid. Flow = Isocratic	turbulent flow column (Cyclone 50x1 mm polymeric column, Cohesive Technologies, USA)	50x2.1 mm(5 µm particle size) Phenyl-Hexyl-RP column from Phenomenex (Aschaffenburg, Germany).	30
Everolimus Cyclosporine A	55 °C	A= 20 mM NH_4Ac (25%) B= ACN (75%) Flow = Isocratic	Protein precipitation ($ZnSO_4$, ACN); SPE cartridges (SPEC PLUS 96-well C-18 AR 15 mg extraction plate)	4 X 125 mm Nucleosil 100, 5µm, C18 AB column	31

Cyclosporine A	43 °C	ACN (90%) NH_4Ac (10%), pH 5.1 Flow = Isocratic	Online SPE: Gilson ASPEC XL (West Beltline, Hwy, USA). Extraction cartridges (Oasis HLB, Waters Corporation, Milford, MA, USA),	Chromolith Performance RP-18e (10mm×4.6mm) (Merck KgaA, Darmstadt,Germany)	33
Cyclosporine A	50 °C	A= 0.1% formic acid in water B= 0.1% formic acid in ACN Flow = Gradient	Blood protein precipitation With acetonitrile LLE	Acquity UPLC BEH C18 (100mm x 2.1 mm, 1.7 µm)	39
Tacrolimus Sirolimus Cyclosporine A	50 °C	ACN: W (52:48), 0.1% acetic acid Flow = Isocratic	Protein precipitation	YMC™ CN column (150mm × 2.0mm i.d.) with 3µm particle size (Waters).	42
Tacrolimus metabolites	55 °C	A= 2mM NH_4Ac in water B= 2mM NH_4Ac in methanol with 0.1% formic acid Flow = Gradient	Liquid liquid extraction (methyl-t-butyl ether)	Genesis C18 (50mm×4.6mm(5µm) Grace Vydac (Hesperia, CA, USA).	50
Everolimus	55 °C	A= 2mM NH_4Ac, 0.1% formic acid in water B= 2mM NH_4Ac, 0.1% formic acid in MeOH Flow = Step-gradient	Protein Precipitation ($ZnSO_4$, ACN)	Waters TDM C18 cartridge column (10mm×2.1mm I.D.)	51
Cyclosporine and its metabolites	80 °C	A= 0.1% acetic acid B= MeOH Flow = Gradient	Direct analysis: Protein precipitation by methanol and ZnSO4	SymmetryC8 (4.6x75 mm, 3.5 µm) from Waters (Milford, MA, USA)	54
Cyclosporine A	75 °C	ACN: W Flow = Gradient	Strata-C18-E SPE-columns (500 mg/mL) (Phenomenex).	Reverse phase column (Hypersil MOS 250 X 4, 3 µm, Merck, Germany)	56
Cyclosporine A and its metabolites AM1, AM9, AM4N	50 °C	A= 2mM NH_4Ac, 0.1% formic acid in 5% acetonitrile B= 2mM NH_4Ac, 0.1% formic acid in 95% acetonitrile, v/v/v). Flow = Gradient	Protein precipitation ($ZnSO_4$, ACN, MeOH)	Acquity UPLC RP BEH (C18) column (1.7 **µ**m; 2.1x50 mm)	57
Cyclosporine A Tacrolimus Sirolimus Everolimus	60 °C	A= MeOH: W (97:3), 10mM NH_4Ac, 0.1% acetic acid . B= MeOH: W (50:50) Flow= Gradient	Perfusion-column (POROS R1/20, 2.1mm × 30 mm, 20µm particle size, Applied Biosystems, Darmstadt, Germany	Short phenyl-hexyl column (PhenomenexLuna 5 µm Phenyl Hexyl, 2mm × 50 mm, Aschaffenburg,Germany)	61
Cyclosporine A Tacrolimus Sirolimus Everolimus	60 °C	A= MeOH: W (50:50) B= MeOH: W (97:3) 10mM NH_4Ac, 0.1% acetic acid. Flow = Gradient	Perfusion column (POROS R1/20, 2.1 × 30 mm, 20 µm particle size, Applied Biosystems, Darmstadt, Germany)	Phenyl Hexyl RP column (Phenomenex Luna 5 µm particle size, 2 × 50 mm, Aschaffenburg, Germany)	62
Cyclosporine A, Tacrolimus, Sirolimus Everolimus	60 °C	MeOH: W (97:3), 10mM NH_4Ac, 0.1% acetic acid Flow = Isocratic	Poros R1/20, 2.1 mmD × 30 mm, 20 µm (Applied Biosystems, Darmstadt, Germany)	phenyl-hexyl reversed phase C18 column Zorbax Eclipse XDB (Agilent, Waghäusel, Germany) 3.0 × 75 mm, 3.5 µm	64
Sirolimus	50 °C	MeOH: W (80:20) 2 mmol/L NH_4Ac Flow = Isocratic	Liquid extraction	Column was a 15 cm X 4.6 mm (i.d.) Supelcosil LC-18-DB (5 µm particle size) ODS column	67
Tacrolimus Sirolimus	50 °C	A= MeOH: W (50:50) B= MeOH, 2 Mm NH_4Ac, 0.1% formic acid. Flow = Gradient	Protein precipitation ($ZnSO_4$, ACN)	C18 guardcolumn (5 µm, 4.0 mm length x 3.0 mm inside diameter, Phenomenex,Torrance, Calif)	68
Tacrolimus	55 °C	A= 2mM NH_4Ac, 0.1% formic acid in water B= 2mM NH_4Ac, 0.1% formic acid in MeOH Flow = Gradient	Protein precipitation in 96-round well plates, supernatant was directly injected	TDM C18 cartridgecolumn (10 mm ×2.1 mm, 10 µm, Waters)	69

Sirolimus Everolimus	70 °C	A= 10 mM NH_4Ac / MeOH (95:5) , 0.1% formic acid B= MeOH: ACN containing 10 mM NH_4Ac and, 0.1% formic acid C= CAN: 2-propanol: acetone (1:1:1) Flow = Gradient	Online: turbulent flow chromatography Extraction was done on cyclone column (0.5×50 mm),	Hypersil Gold C18 column with 1.9µm particle size (2.1×50mm, Thermo Fisher Scientific, Basel, Switzerland),	70
Tacrolimus	60 °C	A= 2mM NH_4Ac, 0.1% formic acid in water B= 2mM NH_4Ac, 0.1% formic acid in MeOH Flow = Step-gradient	MassTrak™ Immunosuppressants Kit	C18 reverse-phasecolumn (2.1×10 mm, MassTrak TDM C18 cartridge column)	71
Cyclosporine A	60 °C	ACN, MeOH and 0.2% NH_4OH (60:20:20) Flow = Isocratic	Liq-Liq extraction: ether:methanol (95:5)	Zorbax, Eclipse XDB, USA) C8 3.5 µm (2.1 x 50 mm)	72

and are directly analyzed in single ion recording mode. The commonly analyzed adduct for cyclosporine a, tacrolimus, sirolimus, and for everolimus is ammonium adduct. Volosov et al. reported that the use of ammonium acetate induced production of ammonium adducts [$NH4^+$] of the analytes (cyclosporine A, tacrolimus, sirolimus), but the ammonium adduct of cyclosporine A (m/z 1220) could not produce a stable abundant fragment of cyclosporine. So the intact cyclosporin ion (m/z 1202.7) was monitored as a "fragment" of its own ammonium adducts [55].

Metabolites: It is also important to determine the therapeutically active and or toxic metabolites while therapeutic monitoring of immunosuppressants. The cyclosporine is known to be nephrotoxic and hepatotoxic and its metabolites also have been suggested to have side effects. So it is important to measure the level of metabolites. Vollenbroeker et al. observed a positive correlation between cyclosporine and its metabolites with bilirubin concentration, while the negative correlation was found with creatinine. According to Vollenbroeker et al. the determination of cyclosporine metabolites may give an indication of body organ function, since the levels of these metabolites are correlated with clinically important parameters. HPLC-MS method developed by Vollenbroeker et al. can be used to obtain rapid information about the metabolism pattern of cyclosporine A in a patient [56]. The isomeric active metabolites (13-O-demethylated (M1), 31-Odemethylated (M2) and 15-O-demethylated (M3) Tacrolimus) of tacrolimus were determined by using LC-MS/MS method. The simultaneous determination of these metabolites was difficult because of their similar molecular weights (807.5 m/z) and similar structures. Chen et al. developed and validated a LC-MS/MS method for simultaneous determination of these three isomeric metabolites of tacrolimus in human whole blood and plasma. The m/z of the precursor ions for all three metabolites was selected as 807.5. The fragment selected for M1 has m/z 772.5 because of its abundance. The most abundant fragment ion of both M2 and M3 was at m/z of 754.5. Therefore the quantification of M2 and M3 was possible by eluting them in chromatographic column at different time intervals (different retention time). By this way these metabolites (M2 and M3) reaches the detector at different time intervals and were quantified without interference despite having same m/z [50]. Koseki et al. and Brozmanova et al. developed a LC/MS methods for simultaneous determination of cyclosporine A and its three main metabolites (AM1, AM4N and AM9) in human blood [54,57]. Hermann et al. evaluated the importance of essential chromatographic parameters for the determination of cyclosporin A and its metabolites, AM1, AM9 and AM4N [4].

Simultaneous analysis: There are some cases of organ transplant where the combined use of these immunosuppressants has been indicated (Zortress®, Rapamune®). The combined use of these immunosuppressants has been reported to improve the survival rate of transplant recipients and provides better immunosuppressive effect [58-60]. Therefore it is important to analyze these immunosuppressants simultaneously to avoid unnecessary delay and the cost of repeated analytical procedures.

Koal et al. reported a LC-MS/MS method for simultaneous determination of cyclosporine A, tacrolimus, sirolimus and everolimus. The blood samples were treated with protein precipitant and the supernatant was injected into solid phase extraction column. After purification the samples were transferred to HPLC column by switching valve. All four drugs were analyzed in a short run time of 2.5 minutes [61]. Karapirli et al. confirm the method of Koal et al. by simultaneously determining the cyclosporine A, tacrolimus, sirolimus and everolimus on a A Shimadzu Prominence series Ultra-Fast Liquid Chromatography system in tandem triple quadrupole mass spectrometer (API 3200 Applied Biosystems/MDS Sciex Concord, Canada) [62]. The samples were cleaned online by using a perfusion column (POROS R1/20, 2.1 × 30 mm, 20 µm particle sizes, Applied Biosystems, Darmstadt, Germany). The analytes were eluted on Phenyl Hexyl RP column (Phenomenex Luna 5 µm particle size, 2 × 50 mm, Aschaffenburg, Germany) [62].

Hetu et al. developed a rapid and economical LC-MS/MS method for simultaneous estimation of tacrolimus, sirolimus, and cyclosporine; and compared with chemiluminescent microparticle immunoassays. The immunoassay overestimated the immunosuppressants compared to LC–MS/MS by a mean of 18.1% for tacrolimus, 41.4% for sirolimus, and 15.6% for cyclosporine. Hetu et al. reported that the initial high cost of buying, installations and service contract of LC-MS/MS can be rapidly compensated by the very low costs of reagents and consumables. Hetu et al. also reported that in less than three years the savings generated by switching from immunoassays to mass spectrometry lead to a complete financing of two LC-MS/MS systems [63]. Ceglarek et al. developed a high-throughput turbulent flow chromatography–tandem mass spectrometric method for daily pre-

and post-dosage simultaneous monitoring of cyclosporin A (CsA), tacrolimus (FK 506) and sirolimus [30]. The (Tables 1 & 2) summaries the liquid chromatographic and mass spectrometric parameters used for the quantification of immunosuppressants, respectively.

Kit

The target concentrations of immunosuppressants are very low (mostly in nano-scale). The analytical methods for the quantification of immunosuppressants required to be highly selective and sensitive. Commonly available reagents and non-standardized procedures may lead to differences in inter and intra-laboratory results [71,73]. The harmonized and validated analytical procedures are expected to minimize the inter and intra-laboratory differences of results. The commercial kits are available for therapeutic monitoring of immunosuppressant by using LC-MS/MS. These kits are expected to minimize large inter-laboratory differences. Waters MassTrakTM Immunosuppressant's XE (IUO) kit [74,75], MassTox® immunosuppressant kit [76], ClinMass® complete Kit [77-79] are used for quantifying the immunosuppressants in blood. Napoli et al. carried out a multicenter evaluation study of LC/MS/MS MassTrak™ tacrolimus immunosuppressants kit (Waters Corporation, Milford, MA) and concluded that the kit is suitable for the monitoring of tacrolimus in kidney and liver transplant recipients [71]. The kit performance for precision; method comparison; linearity; interferences; limit of quantification; stability of tacrolimus in whole blood to repeated freeze/thaw, stability of tacrolimus in whole blood at critical temperatures, recovery, dilution and accuracy was evaluated [71]. MassTox® immunosuppressant kit for routine measurement of cyclosporine, tacrolimus, everolimus and sirolimus in whole blood is provided by chromsystems® [76]. MassTox® eliminated matrix effect with online sampling preparation using trap column technique [76]. ClinMass® complete Kit is provided for the determination of cyclosporine A, tacrolimus, sirolimus, and everolimus in whole blood by using LC-MS/MS [77-79]. The blood samples were purified on-line by using solid phase extraction cartridge (SPE-column). The ionic species were produced by using ESI positive ionization and the analytes were measured in multiple reactions monitoring mode [77].

Other methods

Jourdil et al. developed the first Laser Diode Thermal Desorption (LDTD) tandem mass spectrometry method for the analysis of cyclosporine A in blood. The LDTD-MS/MS method was ultrafast and sample analysis was done within 9 sec only. No chromatographic separation is required in this proposed method. The LDTD was used as a rapid sample introduction technique in conjunction with Atmospheric Pressure Chemical Ionization (APCI) and tandem Mass Spectrometry (MS/MS) [80]. The supernatant of protein precipitated samples was evaporated to dryness under nitrogen stream and then reconstituted in water and ethyl acetate. The 5µl of reconstituted sample were spotted on 96-well Laz Well TM plate. The sample was allowed to evaporate to dryness at room temperature before loading the plate into the LDTD apparatus [80]. The analysis was performed using S-960 LDTD ionization source from Phytronix Technologies coupled to an API 4000 triple quadruple mass spectrometer (ABSciex, Toronto, Canada) [80]. Mochizuki et al suggested that Electrochemical Detector (ECD) can be used as an alternative to mass spectrometers, since it is cheaper than mass spectrometers and highly sensitive as compared with UV detection. They developed and validated a simple and reliable reversed-phase HPLC method for determination of sirolimus using Electrochemical Detector (ECD). This method was successfully applied to monitor blood concentration of sirolimus in a liver transplant recipient [81].

Conclusion

Liquid chromatographic systems (HPLC, UPLC) in tandem mass spectrometer have been considered the gold standard analytical techniques for therapeutic monitoring of immunosuppressants. The LC-MS/MS methods have been found to be reliable, sensitive and specific. The successful application of dried blood spot sampling in therapeutic monitoring of immunosuppressants will be considered as a landmark achievement of bio-analytical procedures.

References

1. Schütz E, Svinarov D, Shipkova M, Niedmann PD, Armstrong VW, Wieland E, et al. Cyclosporin whole blood immunoassays (AxSYM, CEDIA, and Emit): a critical overview of performance characteristics and comparison with HPLC. Clin Chem. 1998; 44: 2158-2164.

2. Steimer W. Performance and specificity of monoclonal immunoassays for cyclosporine monitoring: how specific is specific? Clin Chem. 1999; 45: 371-381.

3. Tszyrsznic W, Borowiec A, Pawlowska E, Jazwiec R, Zochowska D, Bartlomiejczyk I, et al. Two rapid ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) methods with common sample pretreatment for therapeutic drug monitoring of immunosuppressants compared to immunoassay. J Chromatogr B Analyt Technol Biomed Life Sci. 2013; 928: 9-15.

4. Hermann M, Christensen H, Reubsaet JL. Evaluation of essential parameters in the chromatographic determination of cyclosporin A and metabolites using a D-optimal design. J Pharm Biomed Anal. 2002; 30: 1263-1276.

5. Yatscoff R, LeGatt D, Keenan R, Chackowsky P. Blood distribution of rapamycin. Transplantation. 1993; 56: 1202-1206.

6. Streit F, Christians U, Schiebel HM, Napoli KL, Ernst L, Linck A, et al. Sensitive and specific quantification of sirolimus (rapamycin) and its metabolites in blood of kidney graft recipients by HPLC/electrospray-mass spectrometry. Clin Chem. 1996; 42: 1417-1425.

7. Bowers LD, Singha J. A gradient HPLC method for quantization of cyclosporine and its metabolites in blood and bile. J. Liquid Chromatogr. 1987; 10: 411-420.

8. Sadeg N, Chuong PH, Claude JR, Hamon M. Micro method for liquid chromatographic determination of cyclosporin A in whole blood with use of a rapid extraction procedure. J Anal Toxicol. 1991; 15: 95-97.

9. Koster RA, Alffenaar JW, Greijdanus B, Uges DR. Fast LC-MS/MS analysis of tacrolimus, sirolimus, everolimus and cyclosporin A in dried blood spots and the influence of the hematocrit and immunosuppressant concentration on recovery. Talanta. 2013; 115: 47-54.

10. Christians U, Zimmer KO, Wonigeit K, Maurer G, Sewing KF. Liquid-chromatographic measurement of cyclosporin A and its metabolites in blood, bile, and urine. Clin Chem. 1988; 34: 34-39.

11. Yee GC, Gmur DJ, Kennedy MS. Liquid-chromatographic determination of cyclosporine in serum with use of a rapid extraction procedure. Clin Chem. 1982; 28: 2269-2271.

12. Szerkus O, Wolska E, Struck-Lewicka W, Siluk D, RadwaÅ„ska A, Wiczling P, et al. Development and validation of UHPLC method for the determination of cyclosporine A in biological samples. Biomed Chromatogr. 2014; 28: 802-809.

13. Guada M, Imbuluzqueta E, Estella-Hermoso de Mendoza A, Lana H, Dios-Viéitez MC, et al. Ultra high performance liquid chromatography-tandem mass spectrometry method for cyclosporine a quantification in biological

samples and lipid nanosystems. J Chromatogr B Analyt Technol Biomed Life Sci. 2013; 927: 164-172.

14. Belostotsky V, Adaway J, Keevil BG, Cohen DR, Webb NJ. Measurement of saliva tacrolimus levels in pediatric renal transplant recipients. Pediatr Nephrol. 2011; 26: 133-138.

15. Amini H, Ahmadiani A. Simple determination of cyclosporine in human whole blood by high-performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 2003; 795: 209-214.

16. Cheung CY, van der Heijden J, Hoogtanders K, Christiaans M, Liu YL, Chan YH, et al. Dried blood spot measurement: application in tacrolimus monitoring using limited sampling strategy and abbreviated AUC estimation. Transpl Int. 2008; 21: 140-145.

17. Hoogtanders K, van der Heijden J, Christiaans M, Edelbroek P, van Hooff JP, Stolk LM. Therapeutic drug monitoring of tacrolimus with the dried blood spot method. J Pharm Biomed Anal. 2007; 44: 658-664.

18. Hoogtanders K, van der Heijden J, Christiaans M, van de Plas A, van Hooff J, Stolk L. Dried blood spot measurement of tacrolimus is promising for patient monitoring. Transplantation. 2007; 83: 237-238.

19. van der Heijden J, de Beer Y, Hoogtanders K, Christiaans M, de Jong GJ, Neef C, et al. Therapeutic drug monitoring of everolimus using the dried blood spot method in combination with liquid chromatography-mass spectrometry. J Pharm Biomed Anal. 2009; 50: 664-670.

20. Sadilkova K, Busby B, Dickerson JA, Rutledge JC, Jack RM. Clinical validation and implementation of a multiplexed immunosuppressant assay in dried blood spots by LC-MS/MS. Clin Chim Acta. 2013; 421: 152-156.

21. den Burger JC, Wilhelm AJ, Chahbouni A, Vos RM, Sinjewel A, Swart EL. Analysis of cyclosporin A, tacrolimus, sirolimus, and everolimus in dried blood spot samples using liquid chromatography tandem mass spectrometry. Anal Bioanal Chem. 2012; 404: 1803-1811.

22. Edelbroek PM, van der Heijden J, Stolk LM. Dried blood spot methods in therapeutic drug monitoring: methods, assays, and pitfalls. Ther Drug Monit. 2009; 31: 327-336.

23. Hinchliffe E, Adaway JE, Keevil BG. Simultaneous measurement of cyclosporin A and tacrolimus from dried blood spots by ultra-high performance liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2012; 883-884: 102-107.

24. Rao RN, Maurya PK, Ramesh M, Srinivas R, Agwane SB. Development of a validated high-throughput LC-ESI-MS method for determination of sirolimus on dried blood spots. Biomed Chromatogr. 2010; 24: 1356-1364.

25. Wilhelm AJ, Klijn A, den Burger JC, Visser OJ, Veldkamp AI, Janssen JJ, et al. Clinical validation of dried blood spot sampling in therapeutic drug monitoring of ciclosporin A in allogeneic stem cell transplant recipients: direct comparison between capillary and venous sampling. Ther Drug Monit. 2013; 35: 92-95.

26. Hinchliffe E, Adaway J, Fildes J, Rowan A, Keevil BG. Therapeutic drug monitoring of ciclosporin A and tacrolimus in heart lung transplant patients using dried blood spots. Ann Clin Biochem. 2014; 51: 106-109.

27. Wilhelm AJ, den Burger JC, Vos RM, Chahbouni A, Sinjewel A. Analysis of cyclosporin A in dried blood spots using liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877: 1595-1598.

28. Koster RA, Dijkers EC, Uges DR. Robust, high-throughput LC-MS/MS method for therapeutic drug monitoring of cyclosporine, tacrolimus, everolimus, and sirolimus in whole blood. Ther Drug Monit. 2009; 31: 116-125.

29. Marinova M, Artusi C, Brugnolo L, Antonelli G, Zaninotto M, Plebani M. Immunosuppressant therapeutic drug monitoring by LC-MS/MS: workflow optimization through automated processing of whole blood samples. Clin Biochem. 2013; 46: 1723-1727.

30. Ceglarek U, Lembcke J, Fiedler GM, Werner M, Witzigmann H, Hauss JP, et al. Rapid simultaneous quantification of immunosuppressants in transplant patients by turbulent flow chromatography combined with tandem mass spectrometry. Clin Chim Acta. 2004; 346: 181-190.

31. McMahon LM, Luo S, Hayes M, Tse FL. 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.

32. Brignol N, McMahon LM, Luo S, Tse FL. High-throughput semi-automated 96-well liquid/liquid extraction and liquid chromatography/mass spectrometric analysis of everolimus (RAD 001) and cyclosporin a (CsA) in whole blood. Rapid Commun Mass Spectrom. 2001; 15: 898-907.

33. Alvarez C, Wainer IW. Development of an automatic solid phase extraction and liquid chromatography mass spectrometry method by using a monolithic column for the analysis of Cyclosporin A in human plasma. Talanta. 2009; 79: 280-283.

34. Hassell KM, Fair-Wandland SJ, Herman JL. Development and validation of methods for compounds of interest in therapeutic drug monitoring on the new Prelude SPLC LC-MS/MS system. Poster. ThermoFisher Scientific, 101 Constitution Blvd., Franklin, MA USA.

35. Automated clean-up for Therapeutic drug monitoring prior to LC-MS/MS analysis.

36. Van den Bossche D, Peersman N, Desmet K, Vermeersch P, Pauwels S. Deproteination of whole blood for LC-MS/MS using paramagnetic micro-particles. Clin Biochem. 2014; 47: 112-115.

37. Yang Z, Wang S. Recent development in application of high performance liquid chromatography-tandem mass spectrometry in therapeutic drug monitoring of immunosuppressants. J Immunol Methods. 2008; 336: 98-103.

38. Pedraglio S, Rozio MG, Misiano P, Reali V, Dondio G, Bigogno C. New perspectives in bio-analytical techniques for preclinical characterization of a drug candidate: UPLC-MS/MS in in vitro metabolism and pharmacokinetic studies. J Pharm Biomed Anal. 2007; 44: 665-673.

39. Jung HJ, Gwon MR, Park J, Seo JJ, Seong SJ, Kim EH, et al. Quantitative determination of cyclosporine in human whole blood by ultra-performance liquid chromatography with triple quadruple tandem mass spectrometry. Anal Sci. 2014; 30: 293-298.

40. Hsieh Y, Galviz G, Long BJ. Ultra-performance hydrophilic interaction liquid chromatography/tandem mass spectrometry for the determination of everolimus in mouse plasma. Rapid Commun Mass Spectrom. 2009; 23: 1461-1466.

41. Baldelli S, Zenoni S, Merlini S, Perico N, Cattaneo D. Simultaneous determination of everolimus and cyclosporine concentrations by HPLC with ultraviolet detection. Clin Chim Acta. 2006; 364: 354-358.

42. Hatsis P, Volmer DA. Evaluation of a cyano stationary phase for the determination of tacrolimus, sirolimus and cyclosporin A in whole blood by high-performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2004; 809: 287-294.

43. Sawchuk RJ, Cartier LL. Liquid-chromatographic determination of cyclosporin A in blood and plasma. Clin Chem. 1981; 27: 1368-1371.

44. Lensmeyer GL, Fields BL. Improved liquid-chromatographic determination of cyclosporine, with concomitant detection of a cell-bound metabolite. Clin Chem. 1985; 31: 196-201.

45. Kahn GC, Shaw LM, Kane MD. Routine monitoring of cyclosporine in whole blood and in kidney tissue using high performance liquid chromatography. J Anal Toxicol. 1986; 10: 28-34.

46. Ouyang J, Baeyens WR, Duan J, Delanghe J. Improvement of cyclosporin A determination in whole blood by reversed-phase high-performance liquid chromatography. Biomed Chromatogr. 2003; 17: 404-410.

47. Salm P, Taylor PJ, Lynch SV, Warnholtz CR, Pillans PI. A rapid HPLC-mass spectrometry cyclosporin method suitable for current monitoring practices. Clin Biochem. 2005; 38: 667-673.

48. Nozaki K, Tarui A, Osaka I, Kawasaki H, Arakawa R. Elimination technique for alkali metal ion adducts from an electrospray ionization process using an on-line ion suppressor. Anal Sci. 2010; 26: 715-718.

49. Bogusz MJ, Enazi EA, Hassan H, Abdel-Jawaad J, Ruwaily JA, Tufail MA. 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. J Chromatogr B Analyt Technol Biomed Life Sci. 2007; 850: 471-480.

50. Chen YL, Hirabayashi H, Akhtar S, Pelzer M, Kobayashi M. Simultaneous determination of three isomeric metabolites of tacrolimus (FK506) in human whole blood and plasma using high performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2006; 830: 330-341.

51. Taylor PJ, Franklin ME, Graham KS, Pillans PI. A HPLC-mass spectrometric method suitable for the therapeutic drug monitoring of everolimus. J Chromatogr B Analyt Technol Biomed Life Sci. 2007; 848: 208-214.

52. Vidal C, Kirchner GI, Wünsch G, Sewing KF. Automated simultaneous quantification of the immunosuppressants 40-O-(2-hydroxyethyl) rapamycin and cyclosporine in blood with electrospray-mass spectrometric detection. Clin Chem. 1998; 44: 1275-1282.

53. Christians U, Jacobsen W, Serkova N, Benet LZ, Vidal C, Sewing KF, et al. Automated, fast and sensitive quantification of drugs in blood by liquid chromatography-mass spectrometry with on-line extraction: immunosuppressants. J Chromatogr B Biomed Sci Appl. 2000; 748: 41-53.

54. Koseki N, Nakashima A, Nagae Y, Masuda N. Simultaneous quantitative determination of cyclosporine A and its three main metabolites (AM1, AM4N and AM9) in human blood by liquid chromatography/mass spectrometry using a rapid sample processing method. Rapid Commun Mass Spectrom. 2006; 20: 733-740.

55. Volosov A, Napoli KL, Soldin SJ. Simultaneous simple and fast quantification of three major immunosuppressants by liquid chromatography--tandem mass-spectrometry. Clin Biochem. 2001; 34: 285-290.

56. Vollenbroeker B, Koch JH, Fobker M, Suwelack B, Hohage H, Müller U. Determination of cyclosporine and its metabolites in blood via HPLC-MS and correlation to clinically important parameters. Transplant Proc. 2005; 37: 1741-1744.

57. Brozmanová H, Perinová I, Halvová P, Grundmann M. Liquid chromatography-tandem mass spectrometry method for simultaneous determination of cyclosporine A and its three metabolites AM1, AM9 and AM4N in whole blood and isolated lymphocytes in renal transplant patients. J Sep Sci. 2010; 33: 2287-2293.

58. Khanna AK. Mechanism of the combination immunosuppressive effects of rapamycin with either cyclosporine or tacrolimus. Transplantation. 2000; 70: 690-694.

59. Longoria J, Roberts RF, Marboe CC, Stouch BC, Starnes VA, Barr ML. Sirolimus (rapamycin) potentiates cyclosporine in prevention of acute lung rejection. J Thorac Cardiovasc Surg. 1999; 117: 714-718.

60. Kimball PM, Kerman RH, Kahan BD. Production of synergistic but nonidentical mechanisms of immunosuppression by rapamycin and cyclosporine. Transplantation. 1991; 51: 486-490.

61. Koal T, Deters M, Casetta B, Kaever V. Simultaneous determination of four immunosuppressants by means of high speed and robust on-line solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2004; 805: 215-222.

62. Karapirli M, Kizilgun M, Yesilyurt O, Gul H, Kunak ZI, Akgul EO, et al. Simultaneous determination of cyclosporine A, tacrolimus, sirolimus, and everolimus in whole-blood samples by LC-MS/MS. ScientificWorldJournal. 2012; 2012: 571201.

63. Hétu PO, Robitaille R, Vinet B. Successful and cost-efficient replacement of immunoassays by tandem mass spectrometry for the quantification of immunosuppressants in the clinical laboratory. J Chromatogr B Analyt Technol Biomed Life Sci. 2012; 883-884: 95-101.

64. Buchwald A, Winkler K, Epting T. Validation of an LC-MS/MS method to determine five immunosuppressants with deuterated internal standards including MPA. BMC Clin Pharmacol. 2012; 12: 2.

65. Mohamed R, Mercolini L, Cuennet-Cosandey S, Chavent J, Raggi MA, Peyrou M. Validation of a dried blood spot LC-MS/MS approach for cyclosporin A in cat blood: comparison with a classical sample preparation. J Pharm Biomed Anal. 2012; 66: 298-305.

66. Keevil BG, Tierney DP, Cooper DP, Morris MR. Rapid liquid chromatography-tandem mass spectrometry method for routine analysis of cyclosporin A over an extended concentration range. Clin Chem. 2002; 48: 69-76.

67. Holt DW, Lee T, Jones K, Johnston A. Validation of an assay for routine monitoring of sirolimus using HPLC with mass spectrometric detection. Clin Chem. 2000; 46: 1179-1183.

68. Wang S, Magill JE, Vicente FB. A fast and simple high-performance liquid chromatography/mass spectrometry method for simultaneous measurement of whole blood tacrolimus and sirolimus. Arch Pathol Lab Med. 2005; 129: 661-665.

69. Taylor PJ, Franklin ME, Tai CH, Pillans PI. Therapeutic drug monitoring of tacrolimus by liquid chromatography-tandem mass spectrometry: is it truly a routine test? J Chromatogr B Analyt Technol Biomed Life Sci. 2012; 883-884: 108-12.

70. Mueller DM, Rentsch KM. Sensitive quantification of sirolimus and everolimus by LC-MS/MS with online sample cleanup. J Chromatogr B Analyt Technol Biomed Life Sci. 2010; 878: 1007-1012.

71. Napoli KL, Hammett-Stabler C, Taylor PJ, Lowe W, Franklin ME, Morris MR, et al. Multi-center evaluation of a commercial Kit for tacrolimus determination by LC/MS/MS. Clin Biochem. 2010; 43: 910-920.

72. Kanduru SV, Somayaji V, Lavasanifar A, Brocks DR. An analytical method for cyclosporine using liquid chromatography-mass spectrometry. Biomed Chromatogr. 2010; 24: 148-153.

73. Wilson DH, Sepe D, Barnes G, Johnston F. Inter-laboratory differences in sirolimus results from six sirolimus testing centers using HPLC tandem mass spectrometry (LC/MS/MS). Clin Chim Acta. 2005; 355: 211-213.

74. Juenke JM, Smith J, Thomas RL, Bradshaw T, Brown PI, Johnson-Davis KL. An everolimus method comparison between Waters MassTrak™ XE (IUO) kit and an LC-MS/MS method in renal transplant patients.

75. Johnson-Davis KL, Juenke JM, Thomas RL, Bradshaw T. Everolimus method comparison between Waters MassTrak™ Immunosuppressants XE (IUO) kit and an in-house laboratory developed LC-MS/MS method in renal transplant patients. Ann Clin Lab Sci. 2015; 45: 27-31.

76. Tandem mass spectrometry in clinical diagnostics.

77. Solutions for clinical diagnostics.

78. Cosmobio Searching.

79. Willkommen bei RECIPE!

80. Jourdil JF, Picard P, Meunier C, Auger S, Stanke-Labesque F. Ultra-fast cyclosporin A quantitation in whole blood by Laser Diode Thermal Desorption-tandem mass spectrometry; comparison with High Performance Liquid Chromatography-tandem mass spectrometry. Anal Chim Acta. 2013; 805: 80-86.

81. Mochizuki N, Suka E, Matsumoto K, Akimoto O, Ohno K, Shimamura T, et al. Liquid chromatographic method for the determination of sirolimus in blood using electrochemical detection. Biomed Chromatogr. 2009; 23: 267-272.

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