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BEST ANALYTICAL APPROACHES FOR QUANTIFICATION OF CYCLOSPORIN IN HUMAN WHOLE BLOOD -A REVIEW FOCUSED ON HPLC & LC-MS METHODS

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ABSTRACT:

Analytical methodologies for the determination of cyclosporin have evolved significantly over the years, providing clinicians and researchers with powerful tools for therapeutic drug monitoring and pharmacokinetic studies. Among the various techniques available, High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS) stand out as the most reliable and widely used methods. Other techniques, such as Gas Chromatography (GC), Enzyme-Linked Immunosorbent Assay (ELISA), Capillary Electrophoresis (CE), and Tandem Mass Spectrometry (MS/MS), also have their unique advantages and applications for cyclosporin determination. However, HPLC and LC-MS remain at the forefront due to their robustness, sensitivity, and ability to analyze a wide range of cyclosporin concentrations accurately. Advancements in instrumentation and software have further improved the precision and efficiency of these methodologies. Researchers and clinicians now have access to faster analysis times, reduced sample volumes, and improved data processing capabilities. Additionally, ongoing research continues to explore new sample preparation techniques and the development of novel stationary phases and mobile phases to enhance sensitivity and specificity. Overall, analytical methodologies for cyclosporin determination have come a long way, playing a crucial role in optimizing therapy, improving patient outcomes, and advancing our understanding of cyclosporin pharmacokinetics and pharmacodynamics. As technology continues to evolve, these methodologies will undoubtedly remain essential tools in clinical and research settings. This review explains several published methodologies and the instrumental conditions, which have been applied to quantify the cyclosporine within the last decade.

KEYWORDS: Cyclosporin, accuracy, sensitivity

1. INTRODUCTION:

Cyclosporin, is synthetically 30-ethyl-33-[(E)- 1-hydroxy-2-methylhex-4-enyl]-1,4,7,10,12,15,19,25,28-nonamethyl-6,9,18,24-tetrakis(2-methylpropyl)-3,21-di(propan-2-yl)1,4,7,10,13,16,19,22,25,28,31undecazacyclotritriacontane2,5,8,11,14,17,20,23,26,29,32-undecone[1]. Since its first clinical application in the late 1970s, cyclosporine has undoubtedly revolutionized transplant medicine by increasing rates of acute rejection and early graft survival. A pure form cyclosporine was synthesized in 1973, Cyclosporin was first discovered in Norway in 1969[2]. Cyclosporine is an immunomodulatory calcineurin inhibitor that is used to treat a variety of inflammatory and autoimmune conditions as well as prevent organ transplant rejection. Cyclosporine is a calcineurin inhibitor that is well-known for its immunomodulatory properties, which help treat a variety of inflammatory and autoimmune conditions and prevent organ transplant rejection [3]. It was isolated from Beauveria nivea, a fungus. Cyclosporine, which was initially produced by Sandoz and received FDA approval for use in 1983, is now available in a variety of products manufactured by Novartis (formerly Sandoz). Cyclosporin has the molecular weight of 1202.635 and has a chemical formula C62H111N11O12[4]. People who have received a heart, kidney, or liver transplant take cyclosporine to prevent rejection of their transplanted organs. In most cases, it is taken with other medications to help your new organ work normally. Cyclosporine is a member of the immunosuppressant class of drugs. It helps your body accept the new organ as if it were your own by weakening the immune system [5]. The purpose of this study was to provide an overview of the analytical methods used in the last ten years to find Cyclosporin and its metabolites in biological samples, such as HPLC and LC-MS/MS.

2. PHARMACODYNAMIC:

T cell survival is improved by cyclosporine's potent immunosuppressive effects on organ and bone marrow transplant recipients. Graft versus host disease, Allograft rejection, and inflammatory autoimmune disease are serious immune-mediated reactions that are prevented and controlled by this medication. Hyperlipidemia, gingival hyperplasia, and hypertrichosis are cyclosporine's most notable side effects. It is also up for debate whether or not this drug causes nephrotoxicity [6].

3. MECHANISM OF ACTION:

T cell activation is inhibited by cyclosporine, an inhibitor of calcineurin. Inside of cells, it forms a complex known as cyclosporine-cyclophilin by binding to the receptor cyclophilin-1. After that, this complex stops calcineurin from activating and dephosphorylating the NF-AT (nuclear factor of activated T cells) that normally trigger inflammatory responses. Other factors that are related to the function of T helper cells and the development of thymocytes are also reduced when NF-AT is inhibited [7].

4. SAMPLE TREATMENT PROCEDURE:

The most crucial step in selecting an analytical method for measuring cyclosporin in various samples is selecting a sample preparation technique. The direct injection of biological samples into the liquid chromatographic column reduces the analysis's recovery, sensitivity, and specificity as well as causes column obstruction. In any case, direct infusion of the serum or test was accounted for in some studies [8]. Solid phase extraction (SPE), Protein precipitation (PP), liquid-liquid extraction (LLE), and dispersive SPE (dSPE) have all been utilized in the majority of the experiments to control the effects of the matrix [9]. Acetonitrile, Methanol, trichloroacetic acid, perchloric acid, silver nitrate, and a mixture of water and organic solvents were used to extract proteins from biological samples, which were then quickly centrifuged. One of these substances that had a significant impact on protein precipitation was silver nitrate. Analytes are deliberately dispersed between the solid and liquid phases in SPE, a method. C18 is the most commonly used sorbent for SPE purification of samples. It is injected into syringes, disks, or cartridges and is influenced by vacuum pressure. Target components trapped in the solid phase are removed from the sample matrix using numerous

volumes of solvent with the reasonable solvent the analytes get purified and washed from solid phase [10]. Off-line or on-line SPE are both options. When compared to offline SPE, online SPE offers complete analysis, automation, low contamination, and higher sensitivity. Ion exchange SPE can extract charged molecules from a solution. Ion exchange SPE is based on the electrostatic interaction between the positively charged groups of the chemical bound to the surface of the silica with the charged carboxylate functional groups of cyclosporin. Ion exchange SPE is based on the electrostatic interaction between the positively charged groups of the compound bonded to the surface of the silica and the charged carboxylate functional groups of cyclosporin. The separation of the anionic and cationic materials by the bonded silica cartridges of liquid chromatography-strong cation exchanger (LC-SCX), Liquid chromatography-strong anion exchanger (LC-SAX) and liquid chromatography-weak cation exchanger (LC-WCX). The LC-SCX substance consists of silica with aliphatic sulfonic acid groups bonded to the surface. These groups are used to extract LC-SCX without using recovery elution or recovery. An aliphatic carboxylic acid group that is bonded to the surface of the silica is used in LC-WCX to recover a weak cationic species [11]. Mixing an aqueous sample with an immiscible solvent is the basis of LLE. Target analytes were extracted by selectively partitioning the analytes and impurities between the phases after the phases were separated [12]. The disadvantages of LLE methods include the requirement for solvent removal, the use of evaporation, and the use of large quantities of organic solvents with high purity.

5. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TECHNIQUE:

By reviewing the literature from 2019 to 2023, it is revealed that HPLC equipped with UV visible, fluorescence, and mass spectrometer identification has been the most widely used procedure for the investigation of MTX and its metabolites, despite the fact that fluorimetry, evaporative light dispersing identifier (ELSD), and electrochemical location (ECD) could also be combined with LC. The wavelength range of 205 to 215 nm is mostly reported wavelength for HPLC & UV-visible detection. Chromatographic techniques have made use of numerous stationary and mobile phases; However, the majority of HPLC tests were carried out on C18 columns. Some researchers used LC techniques with pre- or post-column derivatization. Pre-column derivatization involves adding reagents to the samples prior to injecting them into HPLC; post-column derivatization involves adding reagents to the elution between the column and the detector.

1. Sushma M, Vikram H, et al, 2022. In the liquid crystalline nanoparticles (LCNs), cyclosporine (CsA) was eluted with a retention time of 4.55 minutes using novel RP-HPLC. Specificity, system suitability, linearity, precision, accuracy, and robustness were then examined for the method. The created strategy was viewed as ideal to measure Cyclosporine (CsA) in nano detailing and other showcased plans as well. The strategy exhibited linearity scope of 1 to 64g/ml. Cyclosporine (CsA) had a recovery rate of between 98 and 100 percent. Excipients and mobile phase solvents were found to have no effect on the developed method's precision or specificity [13].

2. Huang Y, Tang H. et al, 2022. First, the chromatographic behaviors of cyclosporine A and sirolimus under identical chromatographic conditions on a biological liquid chromatography (BioLC) and traditional liquid chromatography (TraLC) column were systematically examined in this study. An example of entire blood with a volume of 50 µL was ready by protein precipitation with 1 mol/L sodium hydroxide and afterward removed into 500 µL of ether: 95:5, v/v methanol The organic layer was removed and evaporated at 50 °C under a stream of nitrogen after 10 minutes of centrifugation at 14.000 r/min. After that, the residue was reconstituted for use in 200 mL of methanol. On the ZORBAX 300SB C8 column, cyclosporin A and sirolimus were separated by isocratic elution [14].

3. Desai NE, Momin M. et al, 2019. To create and approve a fast opposite stage elite execution fluid chromatography (RP-HPLC) technique for the assessment of curcumin and cyclosporine in the container measurement structure. Curcumin and cyclosporine had retention times of 3.073 and 6.373 minutes, respectively, and their correlation coefficients (R2) were 0.9993 and 0.998. Curcumin's and cyclosporine's responses were linear at concentrations of 8-48 g/ml and 4-24 g/ml, respectively. The method's satisfactory accuracy was demonstrated by the values of the percent recovery that ranged from 97 to 103 percent. The precision of the method can be inferred from the fact that the precision study's relative standard deviation (RSD) values were less than 2. As a result, routine quality control and analysis of curcumin and cyclosporine fixed-dose combination pharmaceutical dosage forms and API can be easily carried out using this approach [15].

6. LIQUID CHROMATOGRAPHY- MASS SPECTROSCOPY (LC-MS):

LC-MS has one of the most outstanding strategies for the investigation of immunosuppressive medications because of its low LODs. The application of the mobile phases and flow rate in LC-MS is constrained in a number of ways. Methanol or acetonitrile mixtures with water serve as the mobile phase frequently in RP-HPLC. Ammonium acetate, acetic acid, and formic acid, all common volatile additives, were used to improve analyte separation. The mass spectrometer and the HPLC eluent were communicated by number of ionization sources. Electrospray ionization (ESI) and barometrical tension synthetic ionization (APCI) are the two ionization sources used frequently; However, in this review, ESI is the most frequently used ion source. It is critical to determine the mass ions created in the ion source or noticed in a mass spectrum in order to fragment these ions and study the fragmentations. Most studies on LC-MS/MS evaluation of cyclosporin and its metabolites are done in MRM mode to distinguish and quantify the analytes by both its atomic particle and identifiable sections. MRM detection has the highest sensitivity and specificity.

1. Srividya V. Kanduru *et al*, 2009. An internal standard (IS) for amiodarone in liquid chromatography-mass spectrometry analysis of cyclosporin in rat plasma. The IS and drug were extracted from 100 L of rat plasma using liquid-liquid extraction with 4 mL of 95:5 ether and methanol. After removing the natural layer, the accumulation was recreated using 500 µL of water. Then, the aqueous layer was transferred to LC-MS sample vials and a volume of 10mL was injected. The ions used to measure CyA and IS had retention times of 3.35 and 4.72 minutes, respectively, at m/z 1202.8 and 645.9. The mean error and CV percentage were 19%. The revealed examine technique showed high proportions of linearity, responsiveness, dependability and accuracy, permitting its appropriateness in pharmacokinetic studies in rodent [16].

2. Huo Y, Zhang S, Pan C *et al*, 2023. Cyclosporine A and Tacrolimus were evaluated by liquid chromatography tandem mass spectrometry (LC-MS/MS) utilizing a simple protein precipitation technique without zinc sulfate as a pretreatment. The influence of matrix was eliminated. This method's linearity, matrix effect, carry over, precision, and accuracy have all been investigated. The relationship coefficient (R2) was significantly higher than 0.995. Cyclosporine and tacrolimus had intraday and between-day accuracy of less than 10%. It is appropriate in routine clinical diagnostics for therapeutic medication monitoring of tacrolimus and cyclosporine A [17].

3. Yuan YS, Liao JM *et al*, 2023. To detect CSA in whole blood using CSA-d12 as the internal standard in the study, a straightforward LC-MS/MS procedure with a high throughput was developed and validated. To produce whole blood samples, we employed a modified one-step protein precipitation technique. Injection volume was 10 µl. In positive ion mode, an electrospray ionization source was utilized. In the multiple reaction monitoring (MRM) mode with a dwell time of 120 milliseconds, precursor/production pairs were used at 1,232.0/1,215.2 m/z for CSA and 1,219.9/1203.0 m/z for CSA-d12. In the current study, the CSA concentrations discovered by our LC-MS/MS approach were compared to those found by other methods in nephropathy patients with comparable eGFR. The LC-MS/MS method that was presented in this study appeared to perform better than other methods that had been described in previous studies because it had a lower detection limit and a wider detection range in actual clinical applications [18].

4. Zijp TR, van Hateren K *et al*, 2023. Using isotope dilution, UPLC-MS/MS system measure the analytes like tacrolimus, sirolimus, everolimus, and cyclosporin A in whole blood with a RT of 1.5-minute. For Execution Capability, 1101 clinical examples were estimated on the double LC-MS/MS framework notwithstanding the IS framework, during a time of one month, and the outcomes were looked at utilizing Passing Bablok relapse and Tasteless Altman examination. With regression equation of slope 0.98–1.02x and intercepts of -0.11–0.88 g/L, there was excellent agreement for all four analytes. There was a slight bias between the systems, with mean differences ranging from -0.93 to 1.43 percent. Currently, the technique is used in clinical routine, with daily peak intensities of more than 180 injections [19].

5. YUAN YS, Kang CM, *et al*, 2022. For the purpose of detecting CSA in whole blood, a novel LC-MS/MS method was developed and proven to work. A modified one-step protein precipitation method was used to prepare samples. For chromatographic separation, a (50 × 2.1 mm × 2.7 µm) C18 column and a mobile phase flow rate of 0.5 mL min-1 were utilized. In the positive ion multiple reaction monitoring mode, an electrospray ionization source was utilized for mass spectrometric detection. The mass spectrometer was connected to two HPLC systems, and each sample took 1.75 minutes to run. Clinical reportable range, linear range, and limit of quantification were 5,85, 5,85, and 5,85 ng·mL-1 respectively. The matrix effect and specificity were both acceptable. According to the clinical data, our modified LC-MS/MS method had a lower limit of detection and a much wider detection range than the methods that had been studied before [20].

6. Antunes NJ, Kipper K, *et al*, 2021. In this study quantification of cyclosporin (CYC), tacrolimus (TAC), sirolimus (SIR), and everolimus (EVE) in human whole blood for TDM was determined by using UHPLC-MS/MS assay method. The method was evaluated by analyzing blood samples from patients and/or samples from quality control [proficiency testing (PT) scheme]. For CYC, TAC, SIR, and EVE, the method was linear from 23.75 to 1094.0, 1.3 to 42.4, 1.3 to 47.0, and 1.2–41.6 µg/mL, respectively. The results for reproducibility within and between assays were less than 11%. An in-house validated approach based on protein precipitation and liquid-liquid extraction produced findings equivalent to those achieved previously by PT and patient sample quantification. Cyclosporin, tacrolimus, sirolimus, and everolimus were all tested for stability in human whole blood. When utilized to assess CYC, TAC, SIR, and EVE in whole blood within their respective calibration ranges, this approach provided good analytical results [21].

Table 1: Review of HPLC and LC-MS/MS

Matrix	Analyte	Sample Preparation	IS	Column	Mobile Phase	Flow rate	Detector	LOQ	Linear Range	% Recovery	Run time
LC-MS Rat plasma	cyclosporin	Liquid-liquid extraction	Amiodarone HCL	C8 3.5um (2.1×50mm) pre column- c8 3.5um (2.1×10mm)	Acetonitrile, methanol and 0.2% NH_4OH (60:20:20)	0.2ml/min	ESI	50ng/ml	R^2>0.995		8.5 min
LC-MS/MS blood sample	cyclosporin	Protein precipitation	EMA guidelines	2.6um c18 100Å 50×2.1mm	A-2Mm ammonium acetate with 0.1% formic acid (v/v) in water B-2mM ammonium acetate with 0.1% formic acid v/v in methanol	0.3ml/min	ESI	50ng/ml^{-1}	R^2>0.995	LQC-95.8% MQC-89.9% HQC-93.1%	4.5 min
LC-MS/MS Whole blood	Cyclosporin	Protein precipitation	-	C18 (2.1×50mm, 2.7um) guard column-(2×2.1mm)	Gradient elution (buffer A: 2mM ammonia acetate and 0.1% formic acid in water; buffer B: methanol	0.5ml/min	ESI	5.85 ng/ml	R^2>0.99 5.85-1, 890.00ng/ml	85 to 115%	4.3 min
LC-MS/MS Whole blood	Cyclosporin	Protein Precipitation	EMA united states food & drug administration	C_{18} 2.6 um 50x2.1mm	Mobil A-0.02mol\L ammonium formate buffer B-methanol	1.00ml\ min	ESI	-	11.0-965.2 ug \1	99.99%	2.3 min
RP – HPLC drug	Powdered		USP & ICH guidelines	C_{18} (4.6x100mm,5 um	Acetonitrile & 10mM ammonium	0.8ml\min	-	0.15ug\ml	1-64ug\ml	98-100%	10 min

	cyclosporin				acetate in 90;10 (v/v)						
LC/MS whole blood	Cyclosporin	Protein precipitation	-	C_{18} (sigma-Aldrich, 50x2.1mm, 2.7um particles) guard column-Phenomenex 2x2.1mm	Organic phase (methanol) water phase (2mmol/l ammonium acetate & 0.1% formic acid in ultrapure water	0.5ml/min	ESI	5.85ng/ml	5.85-1890.00ng/ml	85% to 115%	3 min
HPLC whole blood	CyclosporinA	Protein precipitation	-	250mmx50um	Acetonitrile, water (70:30 v/v)	1ml/min	-	30ng/ml	30-2000ng/ml R^2=0.997	83.5%	-
UHPLC-MS/MS whole blood	Cyclosporin	Protein precipitation	EMA guidlines	C_{18} -1.7um particle size 1.7um, 2.1mm, 5mm	A – 2mM ammonium acetate with 0.1% formic acid (v/v) in deionized water B- 2mM ammonium acetate with 0.1% formic acid (v/v) in methanol	300ul/min	Triple quadrupole mass spectrometer.	12ul/l	12-1094 R^2>0.99	77.32 (9.02)%	5 min
LC-MS/MS Whole blood	Cyclosporin-A	Protein precipitation	EMA & FDA guidlines	2.6um phenylhexyl 50x2.1mm	A-0.1 formic acid and 10mM ammonium formate in water B-90:10 acetonitril: water also contain 0.1% Formic acid and 10mM ammonium formate	0.8ml/min	ESI and an optimized multiple reaction monitoring (MRM) algorithm to detect compounds.	20ng/ml	-	QCH- 97.5 QCL-107.0	-
LC-MS/MS Whole blood	Cyclosporin-A	Protein precipitation	Purchased from sigma Aldrich, Inc	Affinity column (phenomenex, luna 5um, 2mmx50mm)	A- 97% methanol with 0.3mM ammonium acetate	A-0.405ml/min B-0.5ml/min	ESI	9ng/ml	R^2>0.995 Range of 20 to 30ng/ml	83.87% &126.6%	2.5 min

					&0.003% acetic acid B-50% methanol &50% H_2O						
LC-MS/MS Whole blood	Cyclosporin-A	Protein precipitation	-	50×2×1mm	A-0.1%(v/v) formic acid, 10mM ammonium formate in water B-0.1%(v/v) formic acid, 10mM ammonium formate in methanol	A-0.5ml/min B-0.5ml/min	ESI	20ng/ml	-	-	3.5 min
HPLC-MS/MS Whole blood	Cyclosporin-A	Protein precipitation	FDA guide lines	Col. Kinetex, 2.6um phenylhexyl; 2.1x50mm	A-20m mol.L^{-1} ammonium formate&0.1% formic acid in water B-20m mol.L^{-1} ammonium formate & 0.1% formic acid in methanol	1ml/min	ESI	5ug/L	5-2000ug/L	57.4%	3.5 min
LC-MS/MS Whole blood	Cyclosporin-A	Protein precipitation	-	C18 column (Shimadzu, 4.6 × 100 mm, 5 µm particles)	water phase (2 mmol/L ammonium acetate and 0.1% fromic acid, solvent A) and methanol phase (2 mmol/ L ammonium acetate and 0.1%	1ml/min	ESI	-	R^2= 0.9993	87.4-109.6%	-

					formic acid, solvent B)						
RP-HPLC drug	cyclosporin	-	USP/ICH-Q2R1 guidelines.	Eclipse XDB-C_{18} (4.6x150mm,5 um)	Acetonitrile: water: methanol (50:10:40v/v/v)	0.5ml/min	-	0.5575 ug/ml	4=24ug/ml R^2= 0.9986	95.68±0.63 %	10 min
UPLC/ MS/MS Whole blood	CyclosporieA	1.Osmotic burst (OB) method 2.Divalent metal ions (DMI) method		C18 column (2.0 × 50 mm, 1.6 m)	A- 5mM ammonium acetate solution containing 0.1 % formic acid B- 500mM ammonium acetate:methanol (1:99, v/v) with 0.1 % formic acid	0.3 ml/min	ESI	Less than 15%	R^2= 0.997-0.999	88.3 %	4 min

7. Deprez S, Stove CP, *et al*, 2021. The goal of this study was to evaluate the DBS-MS 500, which was linked online to an LC-MS/MS system, for TDM of immunosuppressants using DBS. Two techniques for quantifying tacrolimus, sirolimus, everolimus, and cyclosporin A in DBS and whole blood were devised and verified in accordance with worldwide recommendations. Both procedures were accurate and reproducible throughout a calibration range of 1 to 50 ng/mL for tacrolimus, sirolimus, and everolimus, and 20 to 1500 ng/mL for cyclosporin A, with biases of less than 11% (20% for the LLOQ) and CVs of less than 14% (with a single exception) (20% for the LLOQ). Reproducible (CV 15%) IS-compensated relative recovery values were After drying for one month, the stability experiment at RT revealed that there were no decreases in concentrations, indicating that DBS's extractability did not deteriorate [22].

8. Perez JD, Aragão DS, *et al*, 2020. To validate this study, 235 combinations of four immunosuppressive medications in patient blood were analyzed. In a single extraction, the four immunosuppressants could be measured simultaneously. CyA was measured between 9 and 1000 ng/mL, while TAC, SIR, and EVE were measured between 2 and 50 ng/mL. The method's accuracy ranged from 83.87 percent to 126.6% (coefficient of determination [R^2] > 0.995). For the lower limit of quantification, 15% of variation was validated. 20 percent of patients treated with EVE had concentrations between 6 and 6.9 ng/mL, 28 percent treated with SIR had concentrations between 4 and 4.9 ng/mL to TAC, 22 percent treated with CyA had concentrations between 5 and 5.9 ng/mL, and 50 percent treated with CyA had concentrations between 20 and 30 ng/mL [23].

9. Pablo AH, Breaud AR, *et al*, 2020. A technique for determining the levels of everolimus, tacrolimus, sirolimus, and cyclosprine A in whole blood. With run-to-run precision, accuracy, limit of quantitation, carryover, and linearity are some of the key parameters that must be included in method validation. Worthiness measures for approval ought to be laid out before information assortment for approval [24].

10. Krnáč D, Reiffová K, *et al*, 2019. This method is of simultaneous determination of immunosuppressants drugs like Cyclosporine A, Tacrolimus, Sirolimus, and Everolimus this method has been developed and validated. It is quick, simple method, and robust. Methanol and 0.5 mol of protein precipitate were used to make a 50µL whole blood sample. L-1 ZnSO4. With a sufficient linearity range, the method demonstrated adequate accuracy and precision. With MRM 1219,7 → 1201,7, the linear range for CsA was 0.1–100 µg/l has a therapeutic range that can go as high as 300 µg/ml. For the stability study the prepared solutions of Cyclosporine A, Sirolimus, Everolimus, and Tacrolimus, kept for 24 hours at room temperature and 7 days in an autosampler at 10 °C. The developed method can be used for TDM (therapeutic drug monitoring) and it is applicable for routine use in clinical laboratories with suitable HPLC–MS/MS method [25].

11. Gong ZS, Wu ZH, Xu SX, Han WN, *et al*, 2019. An LC-MS/MS method was developed for the determination of everolimus, sirolimus, cyclosporine A, and tacrolimus. Blood samples were prepared using a single-step protein precipitation. The newly developed method was systematically evaluated and validated according to established guidelines for quantifying these four immunosuppressant drugs in human whole blood. The lower limits of the measuring interval (LLMI) were 5 ng/mL for cyclosporine A and 0.5 ng/mL for sirolimus, tacrolimus, and everolimus. Direct connection coefficients were all > 0.999. IS-standardized grid rectification factor was within 0.88-1.17. For the four immunosuppressant drugs, the average spiked recoveries of five replicates were between 87.4 and 109.6% [26].

12. Wu L, Ye Z, Zhang X, Cheng Y, *et al*, 2021. In this study, a UPLC-MS/MS method was developed and validated for determining CsA in whole blood samples using various physical pretreatment techniques. Techniques such as the use of divalent heavy metal ions (zinc and copper), sonication, freeze-thaw, and osmotic burst were evaluated based on the properties of erythrocytes. The results showed that the osmotic burst technique was highly effective for drug extraction from erythrocytes. Following validation, the osmotic burst (OB) method and the DMI (Zn^{2+}) method were applied to 103 clinical blood samples. Using the Bland-Altman method to analyze the results from both methods, it was found that 95.1 percent of the samples had differences within 20 percent of the mean. Consequently, the differences between the DMI (Zn^{2+}) and OB pretreatment methods are considered insignificant. Therefore, the OB method can be used as an efficient pretreatment technique for determining CsA in whole blood samples [27].

7. CONCLUSION:

This review provides information about cyclosporin analysis from 2019 to 2023**.** According to a literature review the different analytical procedures, like HPLC utilizing various detector systems and UV-visible spectrophotometry, are most commonly employed to quantify cyclosporin in biological materials. The comparison of different analytical methods is provided in Table 1. As proven, LC-MS with high selectivity and low LOQ is the most often used technology for cyclosporin detection in biological materials. HPLC was shown to be an accurate technique for measuring cyclosporin. The drug cyclosporin has low water solubility and due to its polarity of the molecule the drug is typically tested using reversed-phase HPLC techniques with a C18 silica stationary phase and a mixture of polar solvents as a mobile phase, typically H2O and methanol or acetonitrile. Compared to UV-visible detectors, electron spray ionization detectors are more sensitive. Test handling was finished by strong stage extraction technique. An easy and simple sample pretreatment method was done. Like protein precipitation, and two common extraction techniques are solid-phase extraction and liquid–liquid extraction frequently used. Fluid extraction consumes a lot of reagents and affects the climate, though the extraction segment of strong stage extraction is generally costly. Dried blood spot (DBS) is a method which allows for more frequent and convenient monitoring of accurate linearity, precision, and stipulates lower LLMI for the instrument and higher requirements for sample pretreatment.

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