IJPSR (2024), Volume 15, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 17 May 2023; received in revised form, 12 July 2023; accepted, 21 November 2023; published 01 January 2024

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ASCOMYCIN CONTENT IN TACROLIMUS API BY USING RP HPLC

M. Mahesh *, L. Hari Kumar Naik and K. Sai Lakshmi

Department of Pharmaceutical Analysis, JNTUA-Oil Technological and Pharmaceutical Research Institute, Jawaharlal Nehru Technological University Anantapur (JNTUA), Ananthapuramu - 515002, Andhra Pradesh, India.

Keywords:

Ascomycin, Tacrolimus, Validation, ICH guidelines and HPLC

Correspondence to Author:
M. Mahesh

Assistant Professor,
Department of Pharmaceutical Analysis, JNTUA-Oil Technological and Pharmaceutical Research Institute, Jawaharlal Nehru Technological University Anantapur (JNTUA), Ananthapuramu - 515002, Andhra Pradesh, India.

E-mail: meghavath9@gmail.com

ABSTRACT: A quick and affordable analytical method validation for the determination of Ascomycin content in Tacrolimus API by HPLC UV-detector was developed with respect to the accuracy, precision, linearity, selectivity, robustness, limit of quantification, limit of detection, according to ICH guidelines. The extraction of samples was performed using HPLC –LC Solutions Chemstation system manufactured by WATERS by using X-TERRA, C18 (4.6 x 150mm, 3.0µm) in the Presence of mobile phases A&B composed of 6 ml orthophosphoric acid and Acetonitrile: Tert-butyl Methyl Ether (81:19) in the ratio of 4: 1 and 1: 4. The estimated samples were then analysed using an UV visible code 2487 manufactured by Waters. With a linear calibration curve spanning from the mean recovery of method precision and system precision 140765 and 142973 and %of relative standard density is found to be 0.30% and 0.87%. With retention time of Ascomycin 29.5mins and Tacrolimus 33.4 min. Column oven temperature of 60 ^{0}C at a flow rate of 1.5mL/min and wavelength is found at 220 nm the total run time is 60mins with an injection volume of 20µL. The method is validated and is found to be accurate and precise and robustness. The r^2 is 0.9995 and the LOD is 0.133 µg/ml LOQ value is found to be 0.450µg/ml.

INTRODUCTION: A substance intended for the diagnosis, mitigation, prevention, or treatment of diseases in humans or animals, as well as for changing any bodily structure or function, is referred to as a drug [1]. By treating ailments, medications help advance human civilisation. Most drugs used nowadays are synthetic in nature. They are generated in large quantities and employed in pharmaceutical formulations for their medicinal benefits [2].

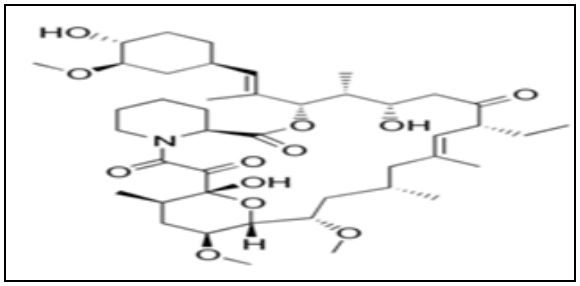
QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.15(1).145-52

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.15(1).145-52

In pharmacological therapy, safety and efficacy are two crucial considerations. The pharmacological-toxicological profile of a medicine and the negative effects brought on by contaminants in bulk and dose forms are used to assess the drug's safety. The adverse pharmacological or toxicological effects of medication contaminants frequently outweigh any therapeutic benefit from their administration [3].



Molecular Weight: 792.01g/mol.

FIG. 1: STRUCTURE OF TACROLIMUS

Today, many labs employ assay techniques like liquid chromatography-tandem mass spectrometry (LCMS/MS), which more precisely quantify certain Tacrolimus quantities. Only a few methods, including RP-HPLC, UPLC, and mass spectrometry approaches, have been reported for the detection of tacrolimus in pharmaceutical dosage forms, according to a study of the literature [4-8].

In the current study, by employing RP HPLC, we hope to produce and validate the ascomycin content in tacrolimus API. Better retention duration, incredibly sharp peak shapes, and symmetrical peak shapes are all advantages of the suggested RP-HPLC method's usage of a low-cost solvent solution. The proposed method has received approval based on ICH criteria. Structure of Tacrolimus shown in **Fig. 1.**

MATERIALS AND METHODS:

Materials: All the chemicals, reagents, instruments and working standards are mentioned in the **Table 1, 2** & **3** which are carried in the present study.

TABLE 1: LIST OF CHEMICALS & REAGENTS

Sl. no.	Chemicals	Grade	Manufacturer
1	Acetonitrile	HPLC	Merck , Mumbai, India
2	Ortho phosphoric acid	AR	Fischer scientific Mumbai, India
3	Water	HPLC	Merck , Mumbai, India
4	Tert-butyl methyl Ether	HPLC	Ramkem

TABLE 2: INSTRUMENT AND EQUIPMENT'S

Sl. no.	Instruments	Model No	Manufacturer
1	HPLC –LC Solutions Chemstation	2695 Alliance Series	WATERS
2	UV Detector	UV visible code 2487	WATERS
3	Digital balance	XSR 205	Mettler Toledo
4	Ultra Sonicator	----------	Rudolf

TABLE 3: LIST OF WORKING STANDARDS

Sl. no.	Name	Manufacturer
1	Tacrolimus	Gifted by TEYRO labs
2	Ascomycin	Synzeal

Methods: Reverse Phase HPLC

Methodology:

Diluent Preparation: Acetonitrile: Water (70:30). Measure accurately 1400 mL of HPLC grade Acetonitrile and 600 mL of HPLC grade water using a glass measuring cylinder and transfer into a 2 L mobile phase reservoir, sonicate for about 15 minutes to degas the diluent and use.

Standard Stock Solution Preparation: (300 µg / mL of Ascomycin): Weigh about 30 mg of Ascomycin working standard and 100 mg of Tacrolimus working standard into a 100 mL volumetric flask, add 50 mL of diluent and sonicate to dissolve completely. Then make the volume up to the mark with the diluent and mix well.

Standard Solution Preparation: (15 µg / mL of Ascomycin): Transfer 2.5 mL of standard stock solution in to a 50 mL volumetric flask, make the volume up to the mark with the diluent and mix well.

Sample Solution Preparation: Weigh about 30 mg of Tacrolimus test sample into a 10 mL volumetric flask to this add 5 mL of diluents and sonicate to dissolve completely. Then make the volume up to the mark with the diluent and mix well.

System Suitability Solution Preparation: (30 µg / mL): Weigh 30mg of Tacrolimus standard in 10 ml volumetric flask add some Diluent and shake well and sonicate to dissolve and make up to the volume. Dilute 1.0 mL of the above solution to 100 mL with diluent. Then transfer to amber colour vial and keep at ambient temperature for 3 hours then inject.

Preparation of Solutions for Linearity: From standard stock solution prepare respective concentrations were mentioned below **Table 4.**

TABLE 4: LINEARITY LEVELS

S. no.	Linearity level	Volume to be taken from stock solution (mL)	Final volume of dilution (mL)	Concentration (µg / mL)
1	LOQ	0.50	50	3.0

2	50% Linearity	1.25	50	7.5
3	80% Linearity	2.00	50	12.0
4	100% Linearity	2.50	50	15.0
5	120% Linearity	3.00	50	18.0
6	150% Linearity	3.75	50	22.5

Solution A: 6ml ortho phosphoric acid. Solution B: Acetonitrile: Tertiary butyl methyl ether 81:19.

Preparation of Solution A 0.1% Ortho Phosphoric Acid: 6ml ortho phosphoric acid: Pipette out 0.1ml OPA and add 100ml of water into a 100 ml of reagent bottle.

Preparation of Solution B Acetonitrile: Tert. Butyl Methyl Ether (81:19): 810.0ml of acetonitrile and 190.0ml of tertbutylmetyl ether make up the volume for 1000 ml transfer into a reagent bottle.

Preparation of Mobile Phase:

Mobile Phase A: Solution A: Solution B (4:1) 4:1 ratio of solution A and solution B collected in 500.0 ml reagent bottle and sonicate in ultra sonic water bath for 15-30 min.

Mobile Phase B: Solution A: Solution B (1:4) 1:4 ratio of solution A and solution B collected in 500.0 ml reagent bottle and sonicate in ultrasonic water bath for 15-30min.

Mobile Phase Optimization: During optimization different composition and proportions of mobile phases are tried such as methanol: water and acetonitrile: water. In these mobile phase system suitability tests was not satisfactory. Gradient programming for Mobile phase A containing solution A: Solution B (4:1) and for mobile phase B containing solution A and B (1:4). And the flow rate was 1.5ml/min details mentioned in **Table 5** it gave all System Suitability Test Satisfactory Results so this mobile phase was chosen for analysis of Ascomycin.

TABLE 5: GRADIENT PROGRAM

Gradient Program			
Time in Min	**Flow**	**Mobile Phase A (%)**	**Mobile Phase B (%)**
00	1.5	72	28
30	1.5	72	28
53	1.5	15	85
54	1.5	72	28
60	1.5	72	28

Method Development: The analytes were conducted on an analytical column X-TERRA, C18 (4.6 x 150mm, 3.0µm) in the Presence of mobile phases A&B composed of 6ml ortho phosphoric acid and Acetonitrile: Tert-butyl Methyl Ether (81:19) in the ratio of 4:1 and 1:4. The diluent is used as Acetonitrile: water (70:30).

The estimated samples a retention time of Ascomycin 29.5 min and Tacrolimus 33.4 min shown in **Fig 2**. Sample temperature and column oven temperature of 2°C and 60 °C at a flow rate of 1.5 mL/min and wavelength is found at 220 nm the total run time is 60 min with an injection volume of 20µL.

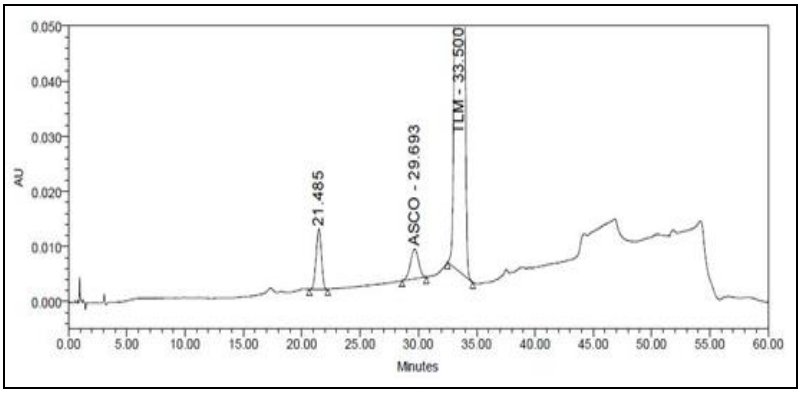


FIG. 2: METHOD DEVELOPMENT CHROMATOGRAM

RESULTS AND DISCUSSION

Results: Method Validation Parameters:

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The separation between the peak of Ascomycin and Tacrolimus and any blank interference is studied as specificity by injecting all the components individually and mixed at the specification limit. The results were tabulated in 3 and chromatogram shown in 3 & 4.

TABLE 6: PEAK NAME WITH RETENTION TIME

S. no.	Peak Name	Retention time
1	Blank	-------
2	Ascomycin	29.5
3	Tacrolimus	33.4

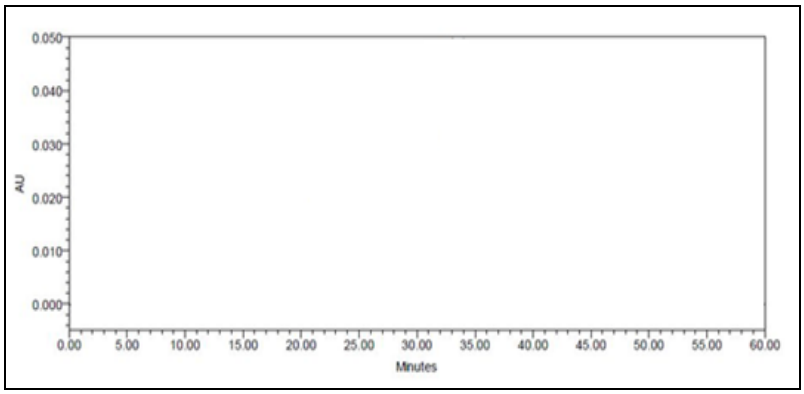


FIG. 3: BLANK CHROMATOGRAM

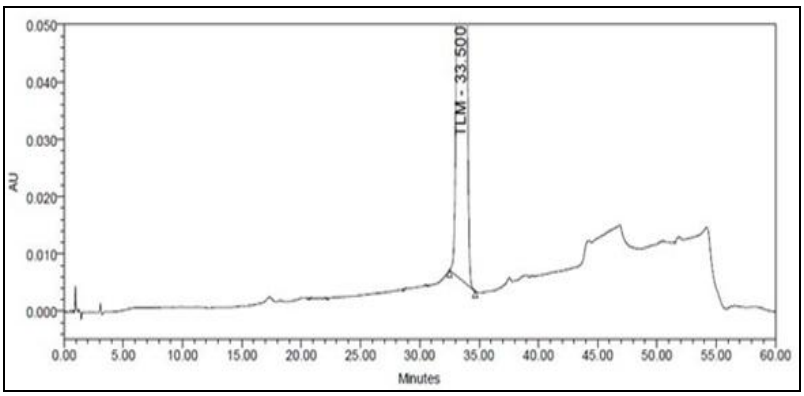


FIG. 4: CHROMATOGRAM OF TACROLIMUS

System Suitability: System suitability parameters are performed by injecting prepared standard solution in six times and measured the parameters like theoretical plates, retention time, tailing factor and %RSD. The results show in **Table 6.**

TABLE 7: RESULTS OF SYSTEM SUITABILITY

Sl. no.	Characteristic	Ascomycin
1	Resolution	3.5
2	Theoretical plates	5193
3	Tailing factor	01.41
4	Retention time	29.5 min

Linearity: It is ability of developed method to obtain test results shown in **Table 8** that are directly proposed to the sample concentration over a given range, Calibration standard of covering the range 3-22.5µg/ml were prepared with the suitable dilution made from stock solution. The calibration curve was plotted between peak response and concentration of the sample and calibration curve shown **Fig. 5.**

TABLE 8: RESULTS OF LINEARITY

Linearity		
Injection No.	**Conc.(µg/ml)**	**Area Response**
1	3.0	26485
2	7.5	66897
3	12.0	109102
4	15.0	140523
5	18.0	172256
6	22.5	210235
Intercept		3328.2
Slope		9531.6
Correlation Coefficient (R^2)		0.999

Calibration Curve:

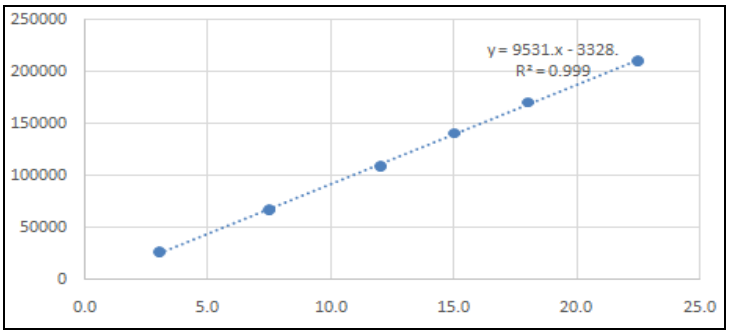


FIG. 5: CALIBRATION CURVE OF ASCOMYCIN

Accuracy: The accuracy was performed for this method by conducting the recovery studies were carried out by adding three different concentration level 50%, 100%, 150% respectively. The % recovery was found and tabulated in 3.4 to be in the range 95.29-100.29%. Chromatograms of different concentration levels show in **Fig. 6, 7** and **8.**

TABLE 9: RESULTS OF ACCURACY

Accuracy Results					
S. no.	**Solution**	**Sample weight**	**Area Response**	**%Recovery**	**Mean % Recovery**
1.	Control sample	30.1028	1086	NA	NA
2.	LOQ Accuracy-1	30.1138	27965	97.0755	95.29
3.	LOQ Accuracy-2	30.1138	28035	95.0737	
4.	LOQ Accuracy-3	30.1138	143256	95.2883	
5.	100% Accuracy-1	30.1138	140862	100.5195	100.29
6.	100% Accuracy-2	30.1138	144025	99.2046	
7.	100% Accuracy-3	30.1138	206823	101.1522	
8.	150% Accuracy-1	30.1138	210985	97.1586	98.40
9.	150% Accuracy-2	30.1138	210856	99.0701	
10.	150% Accuracy-3	30.1138	27965	98.9719	

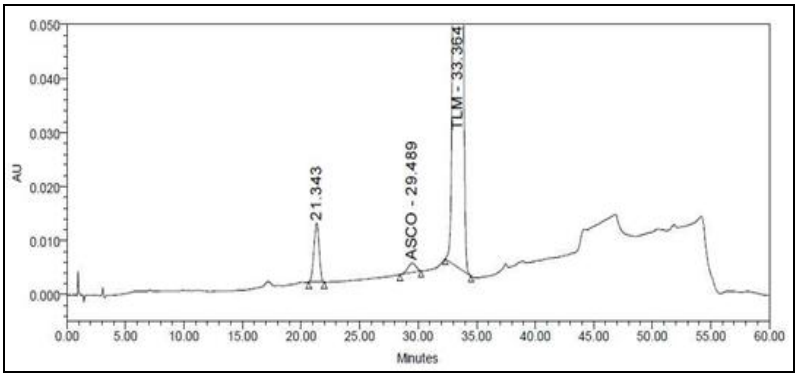


FIG. 6: CHROMATOGRAM SHOWING 50% ACCURACY RECOVERY

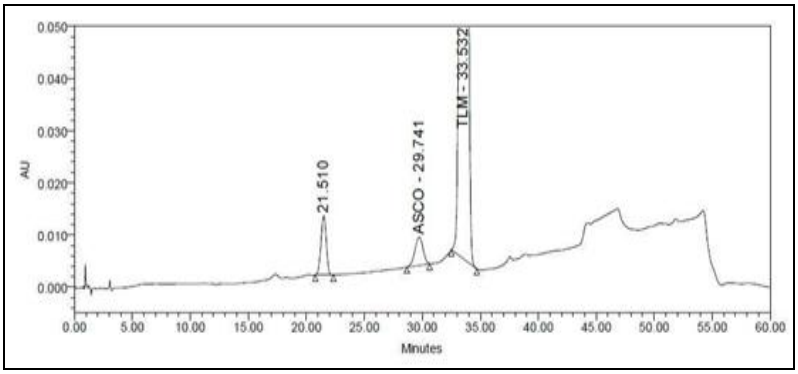


FIG. 7: CHROMATOGRAM SHOWING 100% ACCURACY RECOVERY

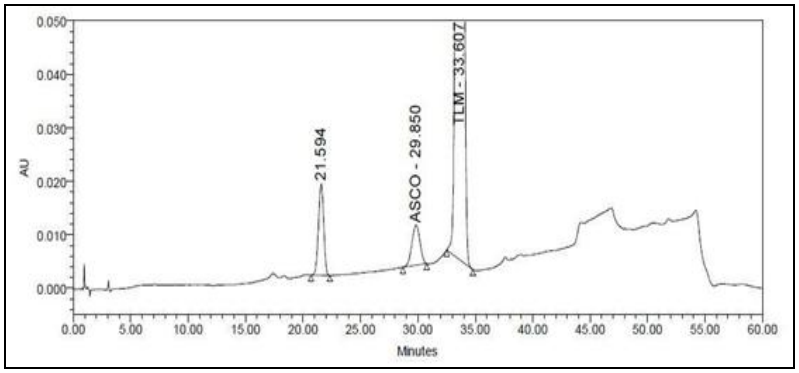


FIG. 8: CHROMATOGRAM SHOWING 150% ACCURACY RECOVERY

Precision: Method precision is determined by analysing a sample solution and six sample preparations from a homogenous mixture to which the Ascomycin impurity is spiked at the specification limit. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. The results shown in the **Table 10.**

TABLE 10: METHOD PRECISION RESULTS

	Method Precision	System Precision
Injection No.	**Area Response**	**Area Response**
1	140502	142538
2	140836	144326
3	141562	142832
4	140561	143256
5	140762	140862
6	140368	144025
Mean	140765	142973
STDEV	426.2987	1239.5829
%RSD	0.30	0.87

Limit of Detection & Limit of Quantification: Limit of detection (LOD) and limit of quantification (LOQ) of Dacomitinib were determined from the calibration curve method. The results were tabulated in 3.6.

TABLE 11: RESULTS FOR DETECTION AND QUANTIFICATION LIMITS

Sl. no.	Method	Range (µg/ml)	Linear regression	R^2	LOD (µg/ml)	LOQ (µg/ml)
1	HPLC UV	3.0-22.5	y =9545.7126x-4027.2635	0.9986	0.133	0.450

Robustness: Robustness of the method was determined by small deliberate changes in the method parameters and measuring the effect on the method by monitoring system suitability test. The obtained results shown in **Table 12.**

TABLE 12: RESULTS OF ROBUSTNESS STUDIES

Robustness conditions	% RSD	Tailing factor	Theoretical plates
pH	1.24	1.35	4950
Temp (35°C)	0.42	1.13	5423
Flowrate	0.35	1.77	3589

CONCLUSION: The Reverse phase HPLC Method for the determination of Ascomycin content in Tacrolimus is simple, precise, specific, and accurate and less time consumption could be recorded for analysis. The method was reliable in terms of system suitability, linearity, precision, accuracy, LOD, LOQ, recovery, and robustness. All the verification parameters were within the range according to ICH guidelines. So, this method can be applied to stability-indicating studies. In this manner, future works can be useful to carry out

Impurity profile studies and Pharmacokinetic studies.

ACKOWLEDGEMENT: We are grateful to Teyro labs private limited for providing gift sample.

Authors Contribution Statement: Mahesh designed the whole study including Selection of drug, literature collection, and plan of work at JNTUA-Oil Technological and Pharmaceutical Research Institute and prepared the manuscript. Harikumar Naik conducted method development and validation in JNTUA-Oil Technological and Pharmaceutical Research Institute. Sai Lakshmi prepared the part of the manuscript. All the authors read and approved the final version of the manuscript.

CONFLICTS OF INTEREST: Conflict of interest declared none.

REFERENCES:

1. Drug Dictionary.com Unbridge Vol.1.1, Random house 20 September 2007.
2. Araya AA and Tasnif Y: Tacrolimus. [Updated 2023 May 29]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK544318/
3. Gauthier Rosé, Naïma Tafzi, Souleiman El Balkhi, Jean-philippe Rerolle, Marilyne Debette-Gratien, Pierre Marquet, Franck Saint-Marcoux and Caroline Monchaud: New perspectives for the therapeutic drug monitoring of tacrolimus: Quantification in volumetric DBS based on an automated extraction and LC-MS/MS analysis. Journal of Chromatography B 2023; 1223: 1-7. doi.org/10.1016/j.jchromb.2023.12372.
4. Florian Lemaitre, Caroline Monchaud, Jean-Baptiste Woillard, Nicolas Picard and Pierre Marquet: Summary of the recommendations of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) on the therapeutic drug monitoring of Tacrolimus. Therapie 2020; 75(6): 681-685. doi: 10.1016/j.therap.2020.06.004.
5. Arkadiusz Kocur, Dorota Marszałek, Jacek Rubik, Agnieszka Czajkowska and Tomasz Pawiński: Therapeutic Drug Monitoring of Tacrolimus Based on Volumetric Absorptive Microsampling Technique (VAMS) in Renal Transplant Pediatric Recipients LC-MS/MS Method Development, Hematocrit Effect Evaluation, and Clinical Application. Pharmaceutics 2023; 15(1): 299. doi.org/10.3390/pharmaceutics15010299.
6. Xiaojun Yang, Mengqi Zhang, Mengna Ren, Sui Mao, Rajendra Dhakal, Nam-Young Kim, Yanwei Cao, Yuanyue Li and Zhao Yao: Ultra-fast and high-sensitive tacrolimus solution detection based on microwave biosensor. Sensors and Actuators A: Physical 2023; doi.org/10.1016/j.sna.2023.114282
7. Magdalena Bodnar-Broniarczyk, Karola Warzyszyńska, Katarzyna Czerwińska, Dorota Marszałek, Natalia Dziewa, Maciej Kosieradzki and Tomasz Pawiński: Development and Validation of the New Liquid Chromatography-Tandem Mass Spectrometry Method for the Determination of Unbound Tacrolimus in the Plasma Ultrafiltrate of Transplant Recipients. Pharmaceutics 2022; 14(3): 632. doi.org/10.3390/pharmaceutics14030632.
8. Sudhir K Sadul, A. Perumal, T. Somanath and Kalaivani: Development and Validation of Method for the Estimation of Tacrolimus Crystalline API in Tacrolimus Extended Release Capsules 0.5mg, 1mg and 5mg by Power X-ray diffractometer (PXRD). Research J Pharm and Tech 2020; 13(11): 5375-5381. doi: 10.5958/0974-360X.2020.00940.3
9. Patel S, Aundhia C, Seth A, Shah N, Gohil D, Parmar G, Desai S and Pandya K: "Development and Validation of UV-Visible Spectrophotometric Method for Estimation of Tacrolimus in Bulk and Pharmaceutical Nanoparticles", Journal of Pharmaceutical Research International 2021; 33(62): 238–242. doi: 10.9734 / jpri /2021/v33 i62B35192.
10. Juliana Dinéia Perez, Danielle Sanches Aragãoa, Fernanda Aparecida Ronchia, Andreia Cristina Febbaa, Claudia Felipe Rosso, Hélio Tedesco-Silva Junior, José Osmar Medina de Abreu Pestana and Dulce Elena Casarini: Simultaneous Determination of Everolimus, Sirolimus, Tacrolimus, and Cyclosporine-A by Mass Spectrometry. Transplantation Proceedings 2020; 52(5): 1402-1408.doi.org/10.1016/j.transproceed.2020.01.077
11. Patel S, Aundhia C, Sen AK, Zanwar A and Pandya K: Development and validation of first order derivative spectro-photometric method for simultaneous estimation of tacrolimus and berberine hydrochloride. International Journal of Pharmaceutical Sciences And Research 2021; 12(4): 2296-2300.doi.org/10.13040/I JPSR.0975-8232.12(4).2296-00.
12. Babu ARS, Thippeswamy B and Vinod AB: Determination of Tacrolimus in Rat Whole Blood Utilizing Triple Quadrupole LC/MS. J Anal Bioanl Techniques 2011, 2: 2; 118. DOI: 10.4172/2155-9872.1000118
13. Qingping Shi, Jianchun Li and Feng Ding: International Journal of Chem Tech Research. https://www.sphinxsai.com/2012/oct-dec/chempdf/CT=43(1543 1552).
14. Fruman DA, Klee CB, Bierer BE and Burakoff SJ: Calcine urinphosphatase activity in Tlymphocytesis inhibited by FK506 and cyclosporine A. PNAS 1992; 89: 3686–3690.doi: 10.1073/pnas.89.9.3686.PMID: 1373887.
15. Yuan J, Chen JQ, Xie ZY, Zhai JJ and Zhou SY: Determination of tacrolimus in rabbit aqueoushumor by liquid chromatography electrospray ionization tandem mass spectrometry. Journal of Chromatography B 2008; 868: 34–41. PMID: 18479984. DOI: 10.1016/j.jchromb.2008.04.028
16. Zhao W, Fakhoury M, Baudouin V, Storme T, Maisin A, Deschênes G and JacqzAigrain E: Population pharmacokinetics and pharmacogenetics of once daily prolonged-release formulation of tacrolimus in pediatric and adolescent kidney transplant recipients. Eur J Clin Pharmacol 2013; 69(2): 189–195.PMID: 22706623, DOI: 10. 1007/s00228-012-1330-6.
17. Similer R, Walsh G, Mattaliano RJ, Guziewicz N and Perez-Ramirez B: Maximizing data collection and analysis during formulation of Biotherapeutic Proteins. Bioprocess International 2008; 6(10): 38-45.
18. Connor E, Sakamoto M, Fujikawa K, Law T and Rifai N: Measurement of whole blood sirolimus by an HPLC assay using solid-phase extraction and UV detection. Ther Drug

Monit 2002; 24: 751–756. PMID: 12451293, DOI: 10.1097/00007691-200212000-00012.
19. Koal T, Deters M, Casetta B and 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 2004; 805: 215–22. PMID: 15135093, DOI: 10.1016/j.jchromb.2004.02.040
20. Koal T, Asperger A and Efer J: Simultaneous determination of a wide spectrum of pesticides in water by means of fast on-line SPE-HPLC-MS-MS—a novel approach. Chromatographia 2003; 57(1): 93–101. doi.org/10.1007/BF02492090
21. Christians U, Braun F, Kosian N, Schmidt M, Schiebel HM, Ernst L, Kruse C, Winkler M, Holze I and Linck A: High performance liquid chromatography/mass spectrometry of FK 506 and its metabolites in blood, bile, and urine of liver grafted patients. Transplant Proc 1991; 23(6): 2741-4. PMID: 1721262.
22. Goto T, Kino T, Hatanaka H, Nishiyama M, Okuhara M, Kohsaka M, Aoki H and Imanaka H: Discovery of FK-506, a novel immunosuppressant isolated from *Streptomyces tsukubaensis*. Transplant Proc 1987; 19(5-6): 4-8. PMID: 2445072.
23. Sehgal SN: Rapamune (*Sirolimus, rapamycin*): an overview and mechanism of action. Ther Drug Monit 1995; 17(6): 660-5. doi: 10.1097/00007691-199512000-00019. PMID: 8588237.
24. Oellerich M and Armstrong VW: Two-hour cyclosporine concentration determination: an appropriate tool to monitor neoral therapy. Ther Drug Monit 2002; 24(1): 40-6. doi: 10.1097/00007691-200202000-00008. PMID: 11805721.
25. Kahan BD, Keown P, Levy GA and Johnston A: Therapeutic drug monitoring of immunosuppressant drugs in clinical practice. Clin Ther 2002; 24(3): 330-50; discussion 329. doi: 10.1016/s0149-2918(02)85038-x. Erratum in: Clin Ther 2002; 24(7): 1223. PMID: 11952020.
26. Saag KG, Emkey R, Schnitzer TJ and Brown JP: Alendronate for the prevention and treatment of glucocorticoid-induced osteoporosis. Glucocorticoid-Induced Osteoporosis Intervention Study Group. N Engl J Med 1998; 339(5): 292-9. doi: 10. 1056/ NEJM 199807303390502. PMID: 9682041.
27. Napoli KL: A practical guide to the analysis of sirolimus using high-performance liquid chromatography with ultraviolet detection. Clin Ther 2000; 22: 14-24. doi: 10.1016/s0149-2918(00)89019-0. PMID: 10823370.
28. Oellerich, Michael MD, Hon MD; Armstrong and Victor W: The Role of Therapeutic Drug Monitoring in Individualizing Immunosuppressive Drug Therapy: Recent Developments. Therapeutic Drug Monitoring 2006; 28(6): 719-725. DOI: 10.1097/FTD.0b013e31802c5cf5
29. Dubbelboer IR, Pohanka A, Said R, Rosenborg S & Beck O: Quantification of tacrolimus and three demethylated metabolites in human whole blood using LC–ESI–MS/MS. Therapeutic Drug Monitoring 2012; 34(2): 134–142. https://doi.org/10.1097/FTD. 0b013e31824b0bfb
30. Wallemacq PE and Lesne M: New automated high-performance liquid chromatographic analysis of cyclosporin A and G in human serum. Journal of Chromatography B: Biomedical Sciences and Applications 1987; 413: 131-140. https://doi.org/10.1016/0378-4347(87)80220-7
31. Jusko WJ and D'Ambrosio R: Monitoring FK 506 concentrations in plasma and whole blood. Transplant Proc 1991; 23(6): 2732-5. PMID: 1721260.
32. Lauerma AI, Granlund H and Reitamo S: Use of the newer immunosuppressive agents in dermatology. Bio Drugs 1997; 8(2): 96-106. doi: 10.2165/00063030-199708020-00003. PMID: 18020498.
33. Liang SL, Breaud A, Dunn W and Clarke W: Comparison of the CEDIA and MEIA assays for measurement of tacrolimus in organ transplant recipients. Clin Chim Acta 2008; 396(1-2): 1-6. doi: 10.1016/j.cca.2008.06.007. Epub 2008 Jun 12. PMID: 18606158.
34. Komolmit P and Davies MH: Tacrolimus in liver transplantation. Expert Opin Investig Drugs 1999; 8(8): 1239-54. doi: 10.1517/13543784.8.8.1239. PMID: 15992148.
35. Iwasaki K: Metabolism of tacrolimus (FK506) and recent topics in clinical pharmacokinetics. Drug Metab Pharmacokinet 2007; 22(5): 328-35. doi: 10.2133/dmpk.22.328. PMID: 17965516.
36. Goto T, Kino T, Hatanaka H, Nishiyama M, Okuhara M, Kohsaka M, Aoki H and Imanaka H: Discovery of FK-506, a novel immunosuppressant isolated from *Streptomyces tsukubaensis*. Transplant Proc 1987; 19(5-6): 4-8. PMID: 2445072.
37. Lamb KE, Lodhi S & Meier-Kriesche HU: Long-term renal allograft survival in the United States: A critical reappraisal. American Journal of Transplantation 2011; 11(3): 450–462. https://doi.org/10.1111/ j.1600-6143.2010.03283.x
38. Kho M, Cransberg K, Weimar W and van Gelder T: Current immunosuppressive treatment after kidney transplantation. Expert Opin Pharmacother 2011; 12(8): 1217-31. doi: 10.1517/14656566.2011.552428. Epub 2011 Feb 2. PMID: 21284571.
39. Wallemacq PE: Therapeutic monitoring of immunosuppressant drugs. Where are we? Clin Chem Lab Med 2004; 42(11): 1204-11.
40. Korecka M, Solari SG and Shaw LM: Sensitive, high throughput HPLC-MS/MS method with on-line sample clean-up for everolimus measurement. Ther Drug Monit 2006; 28(4): 484-90. doi: 10.1097/00007691-200608000-00002. PMID: 16885715.

How to cite this article:
Mahesh M, Naik LHK and Lakshmi KS: Analytical method development and validation of ascomycin content in tacrolimus API by using RP HPLC. Int J Pharm Sci & Res 2024; 15(1): 145-52. doi: 10.13040/IJPSR.0975-8232.15(1).145-52.