e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

International Journal of Pharmaceutical Research & Analysis
www.ijpra.com

Research article

METHOD DEVELOPMENT, VALIDATION AND FORCED DEGRADATION STUDY OF VOCLOSPORIN BY UV SPECTROMETRIC METHOD

Yasotha Selvan*, Kamalakannan Dhanabalan, R.Manivannan, Kowshik Srinivasan, Maneesh P Shaji Shaji Joseph, Mohanraj Selvam, Muthuraman Muthusamy, Sumithraj Ravichandran

Dept. of Pharmaceutical Analysis, Excel College of Pharmacy, Komarapalayam – 637303, Tamil Nadu, India.

ABSTRACT

The assay of an absorbing substance may be quickly carried out by preparing a solution in a transparent solvent and measuring its absorbance at a suitable wavelength. The wavelength normally selected is a wavelength of maximum absorption (λ max), where small errors in setting the wavelength scale have little effects on the measured absorbance. Aim of the present study is to develop an accurate, precise, sensitive, reproducible, analytical technique and forced degradation study to determine the Voclosporin in pharmaceutical dosage forms. Voclosporin is a calcineurin inhibitor for the treatment of lupus nephritis (LN). Lupus nephritis (LN) is a type ofglomerulonephritis occurring in patients with systemic lupus erythematosus (SLE). The linearity data for Voclosporin is given in capsule; the linearity plots of Voclosporin at 222 nm. The regression equation and correlation coefficient forthe calibration cure of Voclosporin at 222 nm were found linear in the concentration range of 1.9 - 11.8 µg/mL at wavelength. Simple, precise and accurate UV spectrophotometric method were developed and validated as per ICH guidelines for the estimation of Voclosporin in capsule dosage form.

Keywords: UV spectrophotometric method; Voclosporin; Method Validation.

INTRODUCTION

ICH QIA-testing of stability for new drug molecules and their products Intrinsic stability of drug is determined using these guidelines QIA Guidelines of Section 2.1.2 of QIA guidelines (under section ICH QIA-testing of stability for new drug molecules and their products) [1]. These guidelines are helpful in designing methods for determining the stability of drugs. According to QIA, degradation depends on respective drug molecules and the nature of drug products. In this various type of conditions are explained such as humidity (75% relative humidity), oxidation, photolysis, and diverse range of pH (solution/suspension) [2].

Corresponding Author
Yasotha Selvan
Department of Pharmaceutical Analysis, Excel College of Pharmacy, Komarapalayam – 637303, Tamil Nadu, India.

Email : **yasothaselvan@gmail.com**

MATERIALS AND METHODS

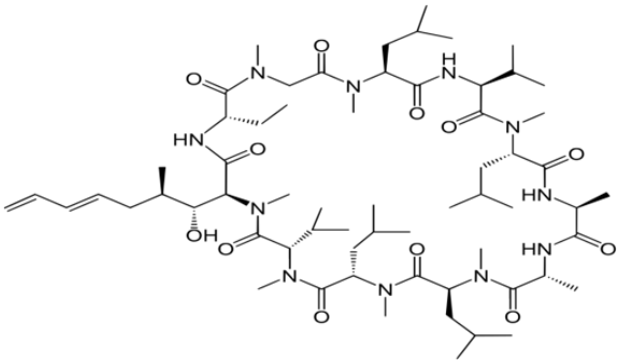
Drug Profile

Voclosporin is a calcineurin inhibitor for the treatment of lupus nephritis (LN). Lupus nephritis (LN) is a type ofglomerulonephritis occurring in patients with systemic lupus erythematosus (SLE). LN is a significant cause of renal failure, morbidity, and death in patients with SLE. Within 10 years of being diagnosed with SLE, 5-20% of those suffering from LN develop end-stage kidney disease, a fatal condition.

CAS No: 515814-01-4
Chemical Formula: $C_{63}H_{111}N_{11}O_{12}$
Molecular Weight: 1214.6 g/mol
Solubility: Soluble in water and ethanol

Melting point: >129 ° C
Boiling point: 1303.8±65

Structure



Mechanism of action [3]

Through the inhibition of calcineurin, voclosporin blocks IL-2 expression and T-cell mediated immune responses, stabilizing podocytes in the kidneys. Voclospoprin is a cyclosporine A analog. It is structurally similar to cyclosporine A (CsA) with the exception of an amino acid modification in one region. This modification changes the binding of voclosporin to calcineurin. Cyclosporine inhibitors reversibly inhibit T-lymphocytes.

Selection of Solvent

Solubility of the drug was checked in solvents like distilled water, ethanol, methanol, chloroform, acetone, acetyl nitrate.

Preparation of Placebo Solution

Weigh accurately about 104.1 mg of Voclosporin placebo and transfer in to a 100 ml volumetric flask. Add 30 ml of diluent and sonicate to dissolve. Dilute up to mark with diluent. Transfer 1 ml of this solution in to 10 ml volumetric flask and dilute up to mark with diluent and mix. Filter through 0.45µm nylon membrane filter.

Preparation of Voclosporin Standard Solution

Weigh accurately about Voclosporin working standard equivalent to 7.9 mg of Voclosporin transfer in to a 100 ml volumetric flask. Add 30 ml of diluent and sonicate to dissolve. Dilute up to mark with diluent. Transfer 1 ml of this solution in to 100 ml volumetric flask and dilute up to mark with diluent and mix. Filter through 0.45µm nylon membrane filter.

Preparation of Test Solution

Determine the average weight of 10 Capsule, Separate the API from of Capsule Shell. Weigh accurately about 112 mg of Voclosporin Tablet sample powder (equivalent to 7.9 mg of Voclosporin) and transfer in to a 100 ml volumetric flask. Add 30 ml of diluent and sonicate todissolve. Dilute up to mark with diluent. Transfer 1 ml of this solution in to 10 ml volumetric flask and up to mark with diluent and mix. Filter the solution through 0.45µm nylon membrane filter. (Dilution scheme: 112 mg (equivalent to 7.9 mg of Voclosporin) → 100.0 ml / 1.0 ml → 10.0 ml) Average Weight of Capsule: 25.0 mg.

Data evaluation

Record each Spectrum. All the Samples will be processed at the wavelength provided in the method and selectivity will be demonstrated with regards to non-interference from diluent blank with Voclosporin spectrum.

METHOD VALIDATION

Linearity and Range

Linearity of Voclosporin was determined at 6 levels with concentrations of 1.9, 3.9, 5.9, 7.9, 9.8, 11.8 µg/mL of Voclosporin prepared from stock solution. The absorbances of the solution was measured at 222 nm [4].

The calibration curves of absorbance Vs concentration were plotted for drug. Regression analysis was performed using least square method to generate the equations for the calibration line. Linearity was established from the correlation coefficient obtained for each calibration line.

ACCURACY

The accuracy of the proposed method was assessed by recovery studies which were carried out at three different levels i.e., 50%, 100% and 150%. A know amount of standard drug solution was added to the pre-analyzed sample solution at three different levels, absorbance was recorded. The % recovery was then calculated [5].

PRECISION

Intra-day precision

Standard stock solutions were taken in a 10 mL volumetric flasks and final volume was made up to the mark with buffer. The absorbances of these solutions were individually measured thrice within a day and recorded [6].

Inter-day precision

Standard stock solutions were taken in 10 mL volumetric flasks and volumes were made up to the mark with buffer. The absorbances of these solutions were individually measured thrice in three days and recorded.

Limit Of Detection (LOD) and Limit Of Quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) of Voclosporin for single point method were determined by using standard deviation of the response and slope approach as defined in ICH guidelines [7].

LOD=3.3xσ/S LOQ=10xσ/S

Robustness and Ruggedness

Prepare two test solutions of the same lot of Voclosporin in tablet as per analytical method. Measure the absorbance of this solution along with diluent blank solution and system suitability solution [8].

Degradation Study

Acid Degradation Studies

To 1 mL of stock solution Voclosporin, 1 mL of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain solution and solutions were adsorbed into the system and the spectrum were recorded.

Alkali Degradation Studies

To 1 mL of stock solution Voclosporin, 1 mL of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 10µg/ml solution and were adsorbed into the system and the spectrum were recorded to assess the stability of sample.

Oxidation

To 1 ml of stock solution of Voclosporin, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0c. For UV-VIS study,the resultant solution was diluted to obtain 10µg/ml solution and was adsorbed into the system and the spectrum were recorded to assess the stability of sample.

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105°C for 1 h to study dry heat degradation. For UV-VIS study, the resultant solution was diluted to 10 µg/ml solution and were adsorbed into the system and the spectrum were recorded to assess the stability of the sample.

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the Standard Stock Solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m^2 in photo stability chamber. For UV-VIS study, the resultant solution was diluted to obtain 10 µg/mL solutions and were adsorbed into the system and the spectrum were recorded to assess the stability of sample.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60º. For UV-VIS study, the resultant solution was diluted to 10 µg/mL solution and were adsorbed into the system and the spectrum were recorded.

RESULTS AND DISCUSSION

Selction of Solvent

Voclosporin showed maximum solubility in distilled water and ethanol (50:50v/v) hence it was selected as the solvent (diluents) for further studies.

Selection of Wavelength

Standard solution of 7.9 µg/mL of Voclosporin exhibited maximum absorbance at 222 nm showed in fig.no:1.

Linearity and Range

The linearity data for Voclosporin is given in capsule; the linearity plots of Voclosporin at 222 nm is shown in below figure 2. The regression equation and correlation coefficient for the calibration cure of Voclosporin at 222 nm were found linear in the concentration range of 1.9 -11.8 µg/mL at wavelength. The calibration curve was established by plotting the absorbance Vs concentration. Linear concentration were found and described by the regression equations.

For nanometer 222 nm: $y = 0.0633x + 0.0039$, $R^2 = 0.9996$

Where y is the absorbance of Voclosporin and x is the concentration in µg/mL, Voclosporin, r^2 is the correlation coefficient.

The beers law is obeyed in the concentration range of 1.9-11.8 µg/mL also calibration curve was reputed by plotting the absorbance Vs concentration. Linear concentration was found and described by the regression equation.

Table 1. Results of linearity of standard

S.No	Concentration (µg/mL)	Absorbance (nm)
1	1.9	0.126
2	3.9	0.253
3	5.9	0.374
4	7.9	0.506
5	9.8	0.633
6	11.8	0.742

Table 2. Recovery data of Voclosporin

Concentration µg/mL	Single Point Method		
	Amount Recovered	%Recovery	%RSD
5 µg/mL	3.95	99.73	0.77
10 µg/mL	7.91	99.74	
15 µg/mL	11.78	99.33	

Table 3. Precision data of Voclosporin

Day	Single Point Method		
	Voclosporin		
	Mean	Standard Deviation	% RSD
Interday	99.88	0.27	0.27
Intraday	99.99	0.12	0.12

Table 4. Robustness data of Voclosporin

Sample	Wavelength	Mean	Standard Deviation	%RSD
Voclosporin	220nm	0.51	0.00	0.28
	224nm	0.50	0.00	0.28

Table 5. Ruggedness data of Voclosporin

Absorbance of Voclosporin	Mean	Standard Deviation	%RSD
220nm	0.51	0.00	0.01
224nm	0.50	0.00	0.08

Table 6. Overall Results for validation

Parameters		Voclosporin	Limit
Assay (% mean assay)		99.97%	95-105%
Specificity		Specific	No interference of any peak
Intraday precision %RSD		0.27	NMT 2.0%
Interday precision %RSD		0.12	NMT 2.0%
Accuracy %recovery		99.97%	98-102%
LOD		0.055	NMT 3
LOQ		0.167	NMT 10
Robustness	**Wavelength Plus**	0.28	%RSD NMT 2.0
	Wavelength Minus	0.28	
Ruggedness	**Analyst 1**	0.01	
	Analyst 2	0.08	

Table 7. Analysis Degradation data of Voclosporin

S.no	Standard Area	Sample area	% Assay
1	0.506	0.486	95.97
2	0.505	0.489	96.56
3	0.506	0.493	97.35
4	0.507	0.497	98.14
5	0.506	0.502	99.13
6	0.508	0.505	99.72
Avg	**0.506**	**0.495**	**99.93**
Standard deviation	**0.001**	**0.01**	**0.19**
%RSD	**0.23**	**1.49**	**0.19**

Table 8. Degradation Data of Voclosporin

S.No	Degradation Condition	% Undegraded	%Drug Degraded
1	Acid	95.97	4.02
2	Alkali	96.56	3.43

3	Peroxide	97.35	2.64
4	Thermal	98.14	1.85
5	UV	99.13	0.86
6	Water	99.72	0.27

Fig. 1. UV spectrum of Voclosporin at 222nm

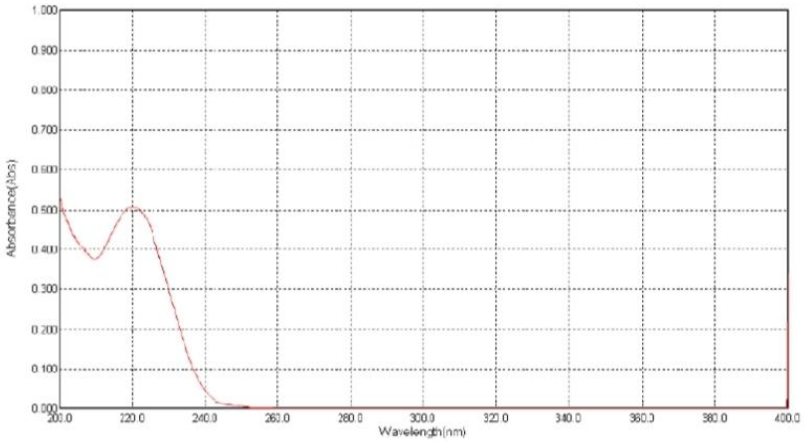


Fig. 2. Linearity graph of Voclosporin standard

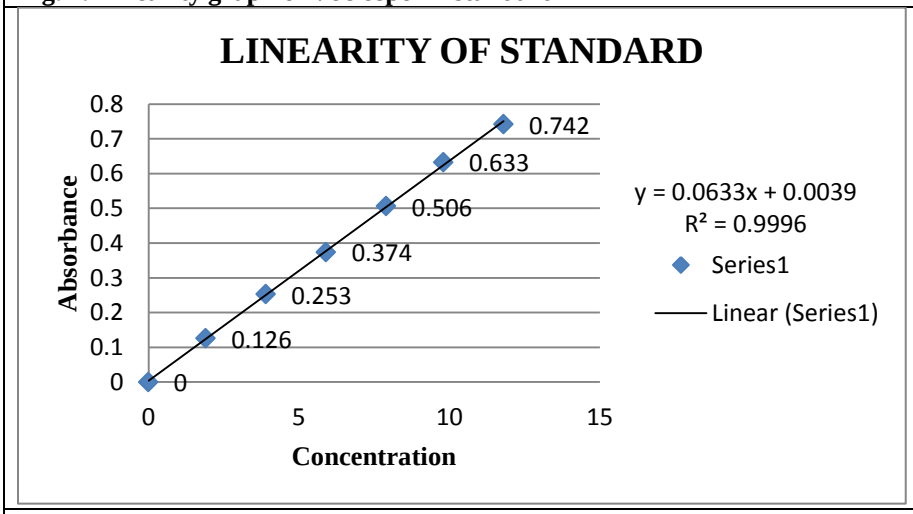
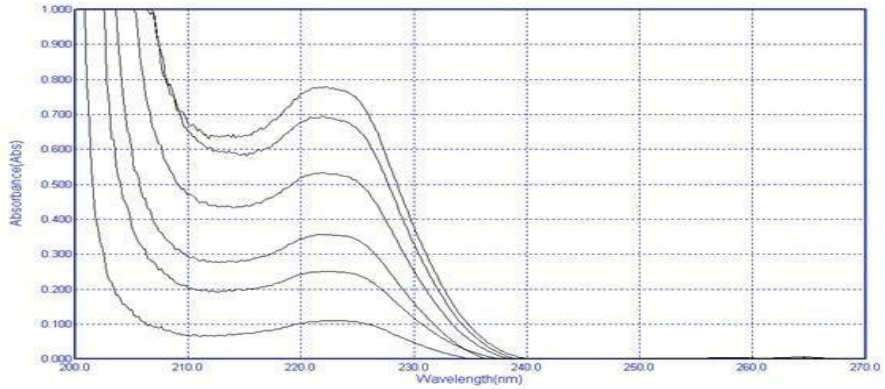


Fig. 3. Linearity overlay spectrum



ACCURACY

The % recoveries for Voclosporin in single point UV method absorption ratiomethods are tabulated. The spiked drugs were recovered at concentration range indicating that the developed method was accurate.

PRECISION

The developed method was found to be precise as the %RSD values for the intra- day and inter-day precision studies were within acceptable criteria less than 2%.

Forced Degradation Studies

Degradation studies were performed with the formulation and the degraded samples were scanned. Assay of the scanned samples was calculated and all the samples passed the limits of degradation.

Assay: Sample, bearing the label claim voclosporin 7.9mg. Assay was performed with the above formulation.

Degradation

The analysis of the same lot of Voclosporin capsule 7.9mg was carried out at different conditions of wavelength. The system suitability was found to meet the pre- established criteria at all the conditions and the degradation shows in acid, base and peroxide.

The analytical method meets the pre-established acceptance criteria for robustness study as per Method.

CONCLUSION

Simple, precise and accurate UV spectrophotometric method were developed and validated as per ICH guidelines for the estimation of Voclosporin in capsule dosage form.

From the solubility profile, distilled water & ethanol was chosen as a common solvent for the estimation of Voclosporin. The sample solution of 7.9 µg/mL of std stock solution of Voclosporin in distilled water& ethanol was prepared and the solution was scanned in UV region in the wavelength range from 200-400 nm by using distilled water & ethanol (50:50 v/v) as a solvent. The overlay spectra of Voclosporin was recorded. From the spectra, Voclosporin shows maximum absorbance at 222 nm.

Percentage of RSD for intraday and inter day precision studies for the drug was well within the acceptable range (<2%) indicating that the method have excellent repeatability andreproducibility. The percentage relative standard deviation for precision and accuracy was found to be low, which indicates that the method have considerable accuracy and precision. Percent recovery for Voclosporin was found in the range of 99.33% to 99.74% with standard deviation well below two indicating accuracy of the method. Recovery greater than 98% with low standard deviation justifies the accuracy of the method. Intraday and interday precision studies were carried out by analyzing capsule formulation by this method. The proposed method is found to be simple, precise, accurate and sensitive. Therefore, can be uses as a quality control tool for the estimation of drug from their dosage form in quality control laboratory.

The system suitability was found to meet the pre-established criteria at all the conditions and the degradation shows in acid (4.02%), alkaline (3.43%) peroxide (2.64%), thermal (1.85%), UV (0.86%), water (0.27%).

REFERENCES

1. ICH Harmonised Tripartite Guideline stability teasting; Photostability testing of newdrug substances and products Q1 B.
2. Dharti Patel, Miral Patel, Keyur Ahir, Sumer Singh. A review artilcle on development on forced degradation and stability indicating studies fpr drug substance and drug product. *Journal of pharmaceutical sciences and bioscientificResearch*. 9(2), 2019, 165-172.
3. Von V, Helene J Aus S. Forced degradation studies-comparison between ICH, EMA, FDA and WHO guidelines and ANVISAs resolution RDC 53/2015; (2015).
4. Farah Iram, Huma Iram, Azhar Iqbal. Forced Degradation studies. *Journal of Analytical & Pharmaceutical Research*. 3(6), 2016, 1-5.
5. M.Kats. Regulatory Considerations and implementation. *Biopharm International*. 18(7), 2005, 32-42.
6. Blessy M, Ruchi D. Development of forced degradation and stability indicating studies of drugs. *Journal of pharmaceutical analysis*. 4, 2014, 159-165.
7. Russell Handy et al., Development and validation of a LC/MS/MS method for quantifying the next generation calcineurin inhibitor, voclosporin, in human whole blood, *J Chromatogr B Analyt Technol Biomed Life Sci*. 15;874(1-2), 2008, 57-63.
8. Neha Desai et al., Analytical Method Development and Validation for Simultaneous Estimation of Curcumin and Cyclosporine by Rp-HPLC. *International Journal of Pharmacy and Pharmaceutical Sciences*. 11(2), 2019, 28-33.