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Research Article

STABILITY INDICATING RP-HPLC METHOD FOR THE DEVELOPMENT AND VALIDATION OF VOCLOSPORIN IN BLUK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
For the purpose of estimating voclosporin using the RP-HPLC methodology in both bulk and pharmaceutical dose form, a straightforward, precise, and uncomplicated approach was established. The Agilent 150 stationary phase with dimensions of 4.8 µm × 5 µm is utilised in the chromatographic process. An optimised approach was used to finalise the following parameters: a detection wave length of 218 nm, a column temperature of 26 oC, a mobile phase consisting of 0.5% formic acid in water:acetonitrile (80:20) and a flow rate of 1.2 ml/min. The results showed that the retention duration of voclosporin was 2.153 minutes. The relative standard deviation (RSD) for the Voclosporin was determined to be 0.8%, and for the method precision, it was determined to be 0.7%.The percentage of recovery for voclosporin was 100.46%. The regression equation for Voclosporin is y = 22385x + 1431.2, and the LOD and LOQ values that were determined from it were 0.04, and 0.12, respectively. The new approach was easy and affordable, allowing it to be implemented for routine quality control tests in industries. It lowered retention times and run time.

Key Words: Voclosporin, Method development, Validation, RP-HPLC.

INTRODUCTION[1-10]

Voclosporin is an immunosuppressive medication mostly prescribed for the management of lupus nephritis, a severe renal inflammation resulting from systemic lupus erythematosus (SLE). This compound is classified as a calcineurin inhibitor (CNI), specifically designed to block the function of calcineurin, an essential enzyme responsible for activating T-cells and the immunological response. The immunosuppressive activity of voclosporin serves to mitigate the hyperactive immune responses that result in renal damage among individuals with lupus.
In January 2021, the U.S. Food and Drug Administration (FDA) granted regulatory approval to voclosporin as the initial oral therapy for lupus nephritis when used with standard-of-care medications such as mycophenolate mofetil (MMF) and corticosteroids. This development represents a notable progress in the treatment of lupus nephritis, which has traditionally depended extensively on non-specific immunosuppressive medications with restricted effectiveness and substantial adverse effects.

The mechanism of action of voclosporin is analogous to that of other calcineurin inhibitors, including cyclosporine and tacrolimus. Nevertheless, voclosporin has been specifically developed to enhance the pharmacokinetics and toxicity characteristics of these previous therapies. An important benefit of this therapy is its very predictable pharmacokinetic profile, which implies that its absorption and distribution in the body are more uniform. This phenomenon results in a decreased fluctuation in medication concentrations and diminishes

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the necessity for monitoring of therapeutic drugs, which was a significant drawback of previous Clinical Nutrition Interventions (CNIs).

The effectiveness of voclosporin in attaining full renal response and maintaining kidney function in patients with lupus nephritis was shown in landmark clinical trials such as AURORA 1 and AURA-LV. Voclosporin demonstrated in these trials a substantial reduction in proteinuria, a crucial marker of kidney injury, while also retaining a satisfactory safety profile.

Although voclosporin is a notable advancement in the management of lupus nephritis, it is not devoid of potential health hazards. Frequently seen adverse effects encompass hypertension, headache, and gastrointestinal disturbances. Furthermore, similar to other immunosuppressive treatments, there is a heightened susceptibility to infections, and prolonged usage can result in kidney damage. Thus, its usage must be meticulously supervised in clinical practice.

In summary, voclosporin has become a major asset in the arsenal for managing lupus nephritis, providing a more focused and efficient strategy in comparison to earlier therapeutic alternatives. The function of this substance in combination therapy is still developing, as current research investigates its long-term effectiveness and safety.

ANALYTICAL BACKGROUND[11]

Voclosporin is a calcineurin inhibitor for the treatment of lupus nephritis (LN) in patients diagnosed with systemic lupus erythematosus (SLE). It is chemically known as (3S,6S,9S,12R,15S,18S,21S,24S,30S,33S)-30-ethyl-33-[(1R,2R,4E)-1-hydroxy-2-methyl hepta-4,6-dien-1-yl]-1,4,7,10,12,15,19,25,28-nonamethyl-6,9,18,24-tetrakis(2-methylpropyl)-3,21-bis(propan-2-yl)-1,4,7,10,13,16,19,22,25,28,31-undecaazacyclotritriacontan-2,5,8,11, 14,17,20,23,26,29,32-undecone. By inhibiting calcineurin, voclosporin suppresses the production of IL-2 and the immunological responses mediated by T-cells, therefore stabilising podocytes in the kidneys.

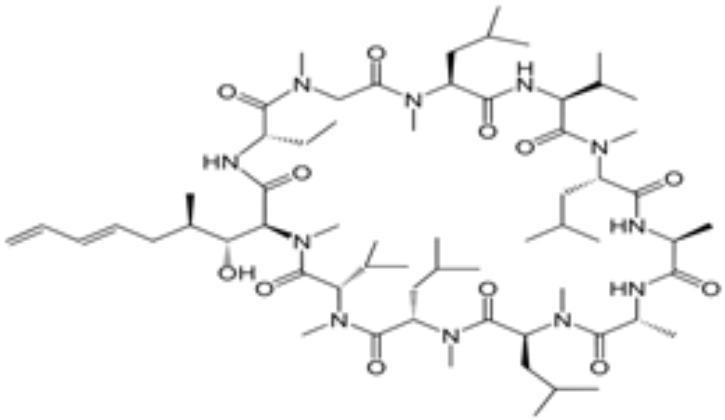

Figure 1 structure of Trilaciclib

High Performance Liquid Chromatography (HPLC) plays a crucial role in the validation of Voclosporin, In the review of literature, more economical methods were observed[11-12], hence a simple, cost-effective stability-indicating simultaneous estimation of Voclosporin by RP-HPLC in pharmaceutical dosage form must be developed and validated as per the guidelines of ICH (Q2 specification).

MATERIALS:

Voclosporin pure drug (API), Voclosporin formulation (Lupkynis), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

INSTRUMENTATION

The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Table 1: Chromatographic Conditions:

Mobile phase	Acetonitrile: 0.5% Formic acid (80:20 v/v)
Flow rate	1 ml/min
Column	Kromosil C18 (4.6 x 150mm, 5µm)
wave length	218 nm
Column temperature	26°C
Injection volume	10µL
Run time	6.0 min
Buffer	0.5% formic acid

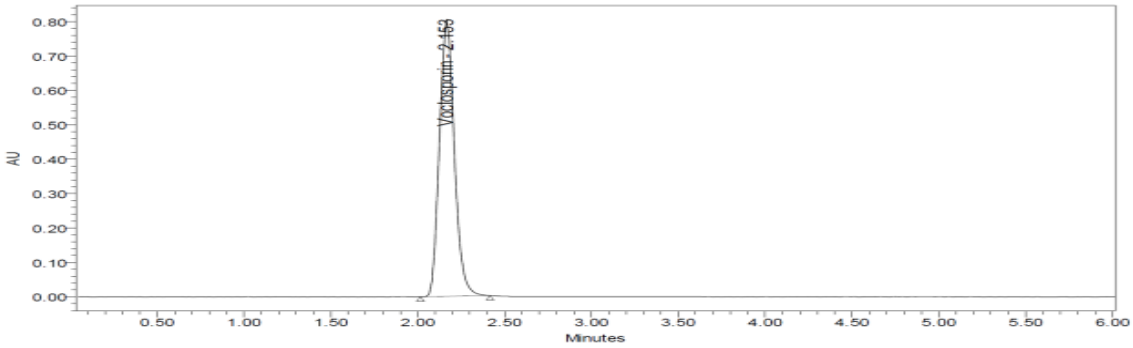

Figure 2: Optimized Chromatogram

Methods:

Preparation of Standard stock solutions: Accurately weighed 7.9mg of Voclosporin transferred 50 ml and volumetric flasks and 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (158µg/ml of Voclosporin)

Preparation of Standard working solutions (100% solution): 1ml of Voclosporin from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (15.8µg/ml of Voclosporin)

Preparation of Sample stock solutions: Remove, as completely as possible, the contents of NLT 10 Capsules. Mix the combined contents, and transfer a quantity, equivalent to 7.9mg of Voclosporin, to a 100-mL volumetric flask. Add Buffer to volume. Sonicate if necessary to ensure complete dissolution, further the volume was made up with diluent and filtered by HPLC filters. (79µg/ml of Voclosporin)

Preparation of Sample working solutions (100% solution): 2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (15.8µg/ml of Voclosporin)

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solution of Voclosporin (15.8ppm) and the solution were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity (Selectivity): Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific. Representative chromatogram is shown in Figure 4 and experimental data is given in Table 2

Table: 2 System suitability parameters for Voclosporin

S no	Voclosporin		
Inj	RT(min)	USP Plate Count	Tailing
1	2.152	8867	1.23
2	2.153	8867	1.23
3	2.154	8846	1.21
4	2.157	8845	1.23
5	2.157	8846	1.23
6	2.158	8868	1.24

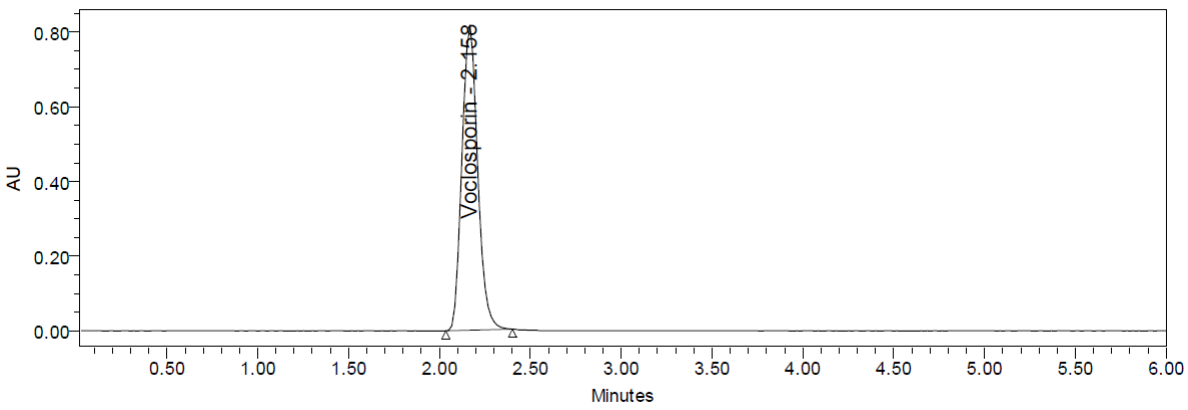

Figure 3: System Suitability Chromotogram of Voclosporin

Table 3: Specificity Data

Peak name	Rt	Area	USP plate count	Tailing
Voclosporin	2.158	354578	8847	1.2

Specificity:

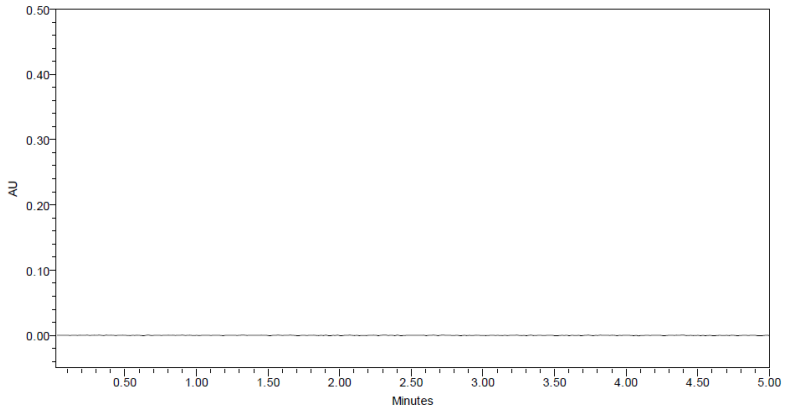

Figure 4 Chromatogram of blank.

The forced degradation conditions are mentioned in Table 3 and the results are mentioned in Table 4

Table 4: Forced degradation conditions for Voclosporin

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins
Base	2N NAOH	60^{0}c	30 mins
Oxdation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

From the results, degradation peaks were observed when the samples were exposed to acid. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table 5: Degradation profile results

Degradation Condition	% Drug Un Degraded	% Drug Degraded
Acid	98.61	1.39
Base	99.13	0.87
Oxidation	93.31	6.69
Thermal	98.02	1.98
Photolytic	98.31	1.69
Hydrolytic	99.12	0.88

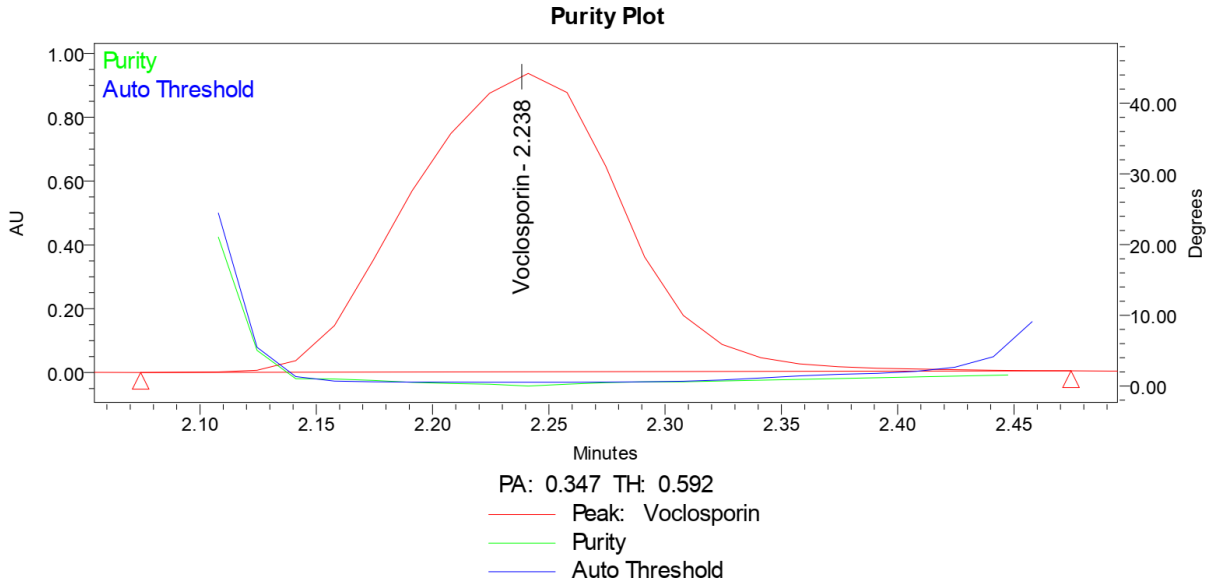

Figure 5: Purity Plots of Voclosporin

Limit of detection (LOD) The detection limit is considered as very low level of concentration of an analyte in a sample that can be detected, but not necessarily quantitated.
Limit of quantitation (LOQ): The limit of quantitation is considered as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy of the method.
The LOD values obtained for Voclosporin are listed in Table 5.

Table 6: Summary of limit of detection

Sample	Conc (µg/ml)
LOD	0.04
LOQ	0.12

Linearity: The linearity of the method was demonstrated for Voclosporin by analyzing the solutions ranging from 25% to 150% of the specification limit (Table 7). The correlation coefficient for Voclosporin was 0.999. This indicates good linearity (Figures 8).
Linearity:
Calibration data is given in table 4 and regression data in table 4 and calibration curve in figure 4, 5

Table 7: Calibration data of Voclosporin

Voclosporin	
Conc (µg/mL)	**Peak area**
0	0
3.95	89136
7.9	174600
11.85	272713
15.8	355860
19.75	442257
23.7	530857

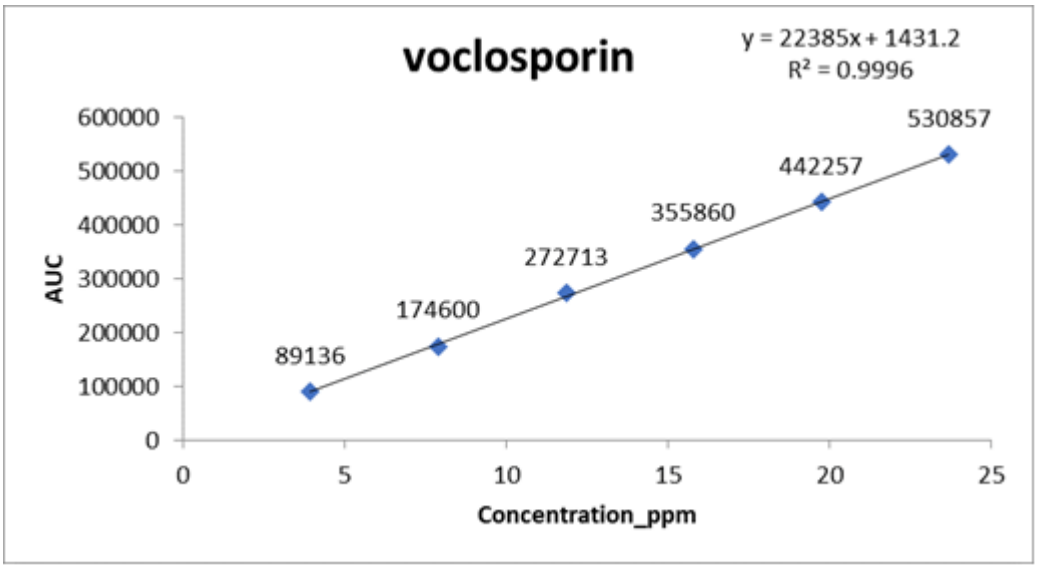

Figure 6: Calibration curve of Voclosporin

Table 8: regression data

Parameter	Metformin
Conc range (µg/mL)	3.95-23.7µg/ml
Regression Equation	y = 22385x + 1431.2
Co-relation	0.999

Accuracy: The accuracy of the method was determined by using solutions containing spiked samples of Voclosporin at 50%, 100% and 150% of the working strength. All the solutions were prepared in triplicate and analysed. The percentage recovery results obtained for each impurity was listed in Table 9

Table 9 Accuracy table of Voclosporin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% recovery
50%	7.9	7.88	99.78
	7.9	7.88	99.78
	7.9	7.94	100.51
100%	15.8	15.87	100.47
	15.8	15.87	100.47
	15.8	15.88	100.51
150%	23.7	23.92	100.93
	23.7	23.91	100.89
	23.7	23.88	100.76
Mean % recovery			**100.46**

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Voclosporin. Results of peak area are summarized in Table 10

Table 10 System precision table of Voclosporin

S. No	Area of Voclosporin
1.	353567
2.	359574
3.	352657
4.	358675
5.	354578
6.	354698
Mean	**355625**
S.D	**2824.8**
%RSD	**0.8**

Method Precision: The precision of the method was determined by analyzing a sample of Voclosporin). Data obtained is summarized in Table 11

Table 11 Repeatability table of Voclosporin

S. No	Area of Voclosporin
1.	850647
2.	853758
3.	850657
4.	859757
5.	850474
6.	857433
Mean	**853788**
S.D	**3989.2**
%RSD	**0.5**

Intermediate precision: It is differently from the repeatability, the precision obtained within a single laboratory over a longer period (generally at least several months) and considers more changes than repeatability. Data obtained is summarized in Table 12

Table 12 Intermediate precision table of Voclosporin

S. No	Area of Voclosporin
1.	354754
2.	354587
3.	359657
4.	354757
5.	359675
6.	354358
Mean	**356298**
S.D	**2612.9**
%RSD	**0.7**

Robustness: The chromatographic conditions were deliberately changed to evaluate the robustness of the existing method. To determine the robustness of method, system suitability solution is prepared as per methodology and injected into HPLC at different altered conditions to check the method's ability like flow rate (± 10%), column oven temperature (± 5°C) and Mobile phase (± 10%) from actual method conditions. No significant change is observed by changing flow, temperature, Mobile phase, and system suitability also complied as per methodology. The robustness results are summarized in Table 13.

Table 13 Robustness data for Voclosporin

Condition	%RSD of Voclosporin
Flow rate (-) 0.9ml/min	0.3
Flow rate (+) 1.1ml/min	0.6
Mobile phase (-) 30B:70A	0.8
Mobile phase (+) 40B:60A	0.7
Temperature (-) 27°C	0.5
Temperature (+) 33°C	0.8

Assay data: -

Lupkynis Tablet bearing the label claims Voclosporin7.9 mg. Assay was performed with the above formulation. Average % Assay for Voclosporin obtained was 99.89%. Assay data shown in table no 8.

Figure 7: Voclosporin marketed drug

Formula to calculate assay:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{10}{1} \times \frac{10}{5} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Avergage peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of sa,ple working standard taken in mg
P	Assay of sample working standard in % in dried basis
L.C	Label claim
FV	filled volume (1ml of a vail)

Table 14: Assay Data of Voclosporin

S.no	Standard Area	Sample area	% Assay
1	353567	354754	99.46
2	359574	354587	99.41
3	352657	359657	100.83
4	358675	354757	99.46
5	354578	359675	100.84
6	354698	354358	99.34
Avg	**355625**	**356298**	**99.89**
Stdev	**2824.8**	**2612.9**	**0.73**
%RSD	**0.8**	**0.7**	**0.7**

CONCLUSION

The Voclosporin HPLC analysis results establish that this approach is capable of accurately quantifying the concentration and purity of the medication. The consistent repeatability, sharp peak resolutions, and reliable retention lengths of this method make it highly suitable for routine quality control and pharmacokinetic research. HPLC is an essential tool for assessing the analytical characteristics of Voclosporin, ensuring its optimal efficacy and safety for clinical applications.

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