RESEARCH ARTICLE

A Novel Stability Indicating RP-HPLC with PDA Approach for Estimation of Voclosporin in Bulk and Marketed Formulation

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ABSTRACT

An easy, specific, and reliable method for determining voclosporin has been established using the reversed phase-high performance liquid chromatography (RP-HPLC) approach. Chromatographic conditions included stationary phase C18 Kromasil (250 mm x 4.6 mm and 5 µm), solvent system (0.1% OPA: Acetonitrile) in a 60:40 ratio, 1-mL/min flow rate, and detection wavelength of 282 nm were opted to separate the voclosporin with retention time of 2.2 minutes. A linearity analysis was performed between 3.95 to 23.7 µg/mL, and the R2 value was found to be 0.999. Precision's %RSD was determined to be between 0.4 and 0.7. The limit of detection (LoD) and limit of quantitation (LoQ) values are 0.01 and 0.03 µg/mL, respectively. Using the aforesaid approach, the %assay of the marketed formulation was 99.07%. To test the stability representing characteristics of the suggested approach, forced degradation experiments of voclosporin were performed and %degradation was measured. Because the procedure was easy, precise, accurate, and cost-effective, it may be poted for regular analysis of quality control samples in industry.

Keywords: Voclosporin, Reversed phase-high performance liquid chromatography, C18 Kromasil, Specific, Stability indicating.

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INTRODUCTION

During the production process, pharmaceutical items that are being made should have suitable checks performed on them to determine their identity, strength, quality, and purity. The quality control unit then decides whether the products should be accepted or refused. The testing of pharmaceutical raw materials as well as final goods for the presence of contaminants and degradants is a crucial component of the course of developing new drugs and manufacturing them[1]. Validating analytical methods is an important stage in the process of designing novel dosage forms since it gives information regarding the analytical method's accuracy, precision, specificity and sensitivity stated in ICH.[2] HPLC has been shown to be the most accurate analytical technique. It is therefore the method of choice for both numeric and qualitative analyses of medical products. The basic idea is that a sample solution is put into a porous column, and a liquid is then forced through the column at a high pressure.[3] Patients who suffer from systemic lupus erythematosus are susceptible to developing a specific form of glomerulonephritis known as lupus nephritis.[4,5] Patients who suffer from systemic lupus erythematosus are more likely to experience renal failure, morbidity, and mortality as a direct result of lupus nephritis.[5,6] The therapy of lupus nephritis with vocosporin, a calcineurin inhibitor, may be found in.[4-10] Voclosporin blocks calcineurin, which prevents the activation of T cells by inhibiting the production of early inflammatory cytokines.[5-9] This results in the reduction of T-cell proliferation. This decreases the inflammation in the kidney, making it possible to treat lupus nephritis and preventing lasting damage to the kidney.[6-9] Voclosporin was approved by FDA in 2021.[10] As it is a new entity in pharmaceuticals, a competent liquid chromatographic method is required to assess the quality of Voclosporin in bulk and formulations. A literature survey revealed that, as of now no single analytical approach was reported for analysis of voclosporin. Hence, the development of a novel HPLC method was selected and validated. The chemical structure of voclosporin was shown in Figure 1.

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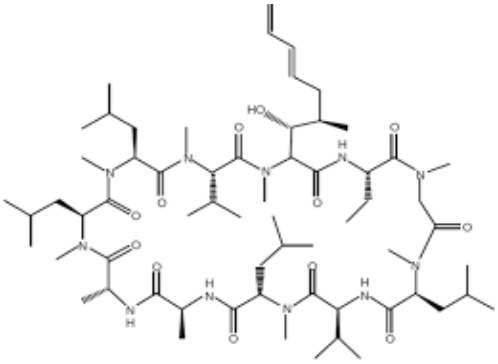

Figure 1: Voclosporin chemical structure

MATERIALS AND METHODS

Instruments

The chromatographic investigation was carried out with the assistance of a Waters HPLC separation module that was fitted with a PDA detector. The Empower 2 program was used to perform checks and processing on the signal being produced. A pH meter made by Mettler-Toledo was used to determine the values of the liquids' pH levels.

Chemicals and Reagents

A sample of voclosporin was sent by Spectrum Research Lab in Hyderabad. Orthophosphoric acid and acetonitrile were bought from Rankem Limited, India. The Millipore Milli Q Plus water treatment device was used to get water that was HPLC grade.

Preparation of Solutions

Standard solution **(***15.8 µg/mL of voclosporin)*

Accurately weighed 7.9 mg of voclosporin was placed in a 50 mL flask, dissolved with 30 Ml of diluent and sonicated for 5 minutes and the remaining portion was filled with the same diluent and labeled as a standard stock solution (158 µg/mL of voclosporin). One mL of stock solution further diluted to 10 mL to attain 15.8 µg/mL of voclosporin

Sample solution (15.8 µg/mL of voclosporin)

Capsules of powder equivalent to 7.9 mg of voclosporin was placed in a 50 mL volumetric flask, dissolved with 30 mL of diluent and sonicated for 5 minutes and the remaining portion was filled with same diluent and 0.45 µ filters were used to clean the mixture. Voclosporin concentration of 15.8 µg/mL was reached by diluting 1-mL of stock solution to 10 mL.

Method Development

It was done by various trials (Table 1) by altering the solvent system, buffers and columns, etc. Trial 5 was considered as optimized method (Figure 1) for the separation of voclosporin in bulk and formulation. Chromatographic conditions included stationary phase C18 Kromasil (250 mm x 4.6 mm and 5 µm), solvent system (0.1 % OPA: Acetonitrile) in a 60:40 ratio, flow rate of 1-mL/min, the detection wavelength of 282 nm, and column oven temperature of 30°C were opted to separate the voclosporin with retention time of 2.2 minutes.

Table 1: Various trails

Trail. No	*Column type*	*Mobile phase*	*Observation*
1	Altima(150 m m x 4.5 m m, 5 m.)	Water: Methanol (50:50)	Peak shape, USP plate count was not good and base line disturbances observed
2	Altima(150 m m x 4.5 m m, 5 m.)	0.1N KH2PO4: Acetonitrile (50:50)	Peak shape was not good and base line disturbances were observed
3	Azilent(150 m m x 4.5 m m,5 m).	0.1N KH2PO4: Acetonitrile (60:40)	Peak shape was not good and USP tailing was observed
4	Kromasil (250 mm x 4.5 m m, 6 m.)	0.1N KH2PO4: Acetonitrile (40:60)	peak shape was not good and base line disturbances, USP tailing were observed
5	Kromasil(25 0 mm x 4. 5 mm, 6 m.)	0.1% OPA: Acetonitrile (50:50)	Peak with good system suitability
Wavelength- 282 nm and Flow rate- 1.0 mL/min,			

Method Validation

The act of providing recorded evidence to indicate that an analytical technique is acceptable for its intended purpose is referred to as analytical method validation (or simply method validation). It entails carrying out a series of tests and assessments in order to analyze the performance characteristics of the technique, with the goal of ensuring that it is dependable, accurate, exact, and specific. [11,12]

System suitability parameters

After producing standard solutions of voclosorphin at a concentration of 15.8 µg/mL, the solutions were injected six times in order to establish system suitability characteristics. These metrics included tailing and plate count and relative standard deviation (RSD).

Linearity

It evaluates the capability of the approach to generate findings that are directly relative to the input concentration of the intended analyte while operating within a certain range of concentrations.[7] It was executed by analyzing the voclosporin solutions in the range of 3.95 to 23.7 µg/mL. The R^2 was calculated from the calibration curve.

Accuracy

It determines how closely the findings obtained by the procedure correspond to the actual value of the substance being analyzed.[8] It is determined by the usual standard

addition method, where 50, 100 and 150% levels of standard concentration solutions were added to separate individual sample solutions with predetermined concentrations. %Recovery of spiked concentrations was calculated for each level and an average was found.

Precision

Precision measures the reproducibility of the entire analytical technique.[9] Intraday (n = 6) and Inter day precision(n = 9) were tested by analysis of standard solution and the %RSD was calculated.

Robustness

It evaluates the capability of the method to produce consistent results regardless of relatively modest shifts in essential factors like as temperature, pH, or the procedure for sample preparation.[11] Robustness conditions like flow flow rate (± w10%), mobile phase (± 1 part) and temperature (± 5°C) were maintained and the standard solution was injected in triplicate. %RSD for resultant peak areas were assessed.

Specificity

It assesses the method's capacity to distinguish the substance desired from other elements in the sample mix. No interference peaks in a blank, degradation samples and placebo at RT of voclosporin. Therefore, this method was said to be specific.[12]

Forced degradation studies

To guarantee that the RP-HPLC technique is specific to the analyte and is capable of reliably detecting and quantifying degradation products, it is essential to undertake forced degradation experiments at an early stage in the method development process.[13-20] These studies should be carried out as soon as possible. These investigations give essential information on the drug's stability, degradation mechanisms, and possible contaminants, adding to an overall understanding of the drug's stability profile and assuring the safety of its patients.[16-19]

The current method's stability has been demonstrated through forced degradation testing.[13-16] After thoroughly combining equal parts of 1N HCl and stock solutions of standard voclosporin, the mixture was allowed to reflux on a water bath at 60°C for an hour. The resulting solution was cooled to room temperature, and then it was neutralized with 1N NaOH. In order to obtain a solution of 15.8 µg/mL of voclosorpin, additional dilution steps were carried out. To accomplish alkali and oxidative degradation in the same manner, different amounts of 1N NaOH and 10% H_2O_2 alkali were added to solutions. In order to carry out experiments on thermal and photo deterioration, 10 mL of a stock solution of voclosorin were placed in a temperature-controlling device that was maintained at 80°C with a relative humidity of 75% for 24 hours. The UV chamber was maintained at 254 nm with a dark control. In order to get a solution of 15.8 µg/mL of voclosorpin, the resulting solutions were further diluted.

In a similar manner, neutral degradation was accomplished by sonicating 10 mL of stock solution and 10 mL of water for 10 minutes. After waiting for 24 hours, a solution of voclosorpin with a concentration of 15.8 g/mL was produced by diluting the solution described above. The %degradtion of voclosporin for 24 hours at an interval of 6 hours was monitored for all kinds of stated degradations

Assay of marketed dosage form

Assay of the voclosporin in the commercial capsule was found by examining standard and sample solutions of 15.8 µg/mL of voclosporin, respectively.

RESULTS AND DISCUSSIONS

Method Validation

The results in Table 2 confirmed the approach's system suitability in accordance with ICH recommendations. The tailing or peak symmetry, theoretical plates and %RSD values were determined to be >2, <2000 and <2. A standard solution of the voclosporin working standard was made in accordance with the method, and it was injected into the HPLC apparatus five separate times. Standard chromatograms were used to test the system's suitability characteristics. This was done by calculating the system suitability parameter from six duplicate injections. All of these values were found to be within the acceptable range (Figure 2).

Linearity

To demonstrate the linearity standard solutions were injected with concentrations of about 3.95 to 23.7 µg/mL of voclosporin. The graph was plotted between concentration and peak area. The correlation coefficient was found to be 0.999 and the linearity plot was shown in Figure 3 and Table 3. The

Table 2: Result of system suitability test for voclosporin

S No	*Peak name*	*RT*	*Peak area*	*Plate count*	*Tailing*
1	Voclosporin	2.22	223537	5583	1.44
2	Voclosporin	2.22	224631	5467	1.40
3	Voclosporin	2.23	225855	5727	1.33
4	Voclosporin	2.23	225040	5824	1.39
5	Voclosporin	2.24	224292	5643	1.43
6	Voclosporin	2.24	223438	5655	1.42
Mean			224465.5		
SD			9205.9		
%RSD			0.4		

Table 3: Linearity data of voclosporin

Linearity level (%)	*Concentration (µg/mL)*	*Area*
0	0	0
25	3.95	564874
50	7.9	111821
75	11.85	168937
100	15.8	220577
125	19.75	274231
150	23.7	329031

limit of detection (LoD) and limit of quantitation (LoQ) was determined from a linearity plot and values are 0.01 and 0.03 µg/mL, respectively.

Accuracy

%Recovery of voclosporin in 50, 100, 150% levels of spiked samples was calculated as 99.86% and results were depicted in Table 4.

Precision

Intraday precision was performed by injection 100% standard for six times on same and %RSD was determined. Inter-day precision was analyzed by injecting standard solution for three times in a day for three days. The data was represented in Table 5.

Robustness

Small deliberate changes were made in the method by altering flow rate, mobile phase ratio and temperature could not influence the system suitability of the method. %RSD of the above conditions were represented in Table 6.

Table 4: Accuracy data voclosporin at various levels

%Level	*Amount added (µg/mL)*	*Amount recovered (µg/mL), (n = 3)*	*%Recovery (Mean)*
50%	7.90	7.95	100.63
100%	15.80	15.88	100.50
150%	23.70	23.50	99.15

Table 5: Intraday precision voclosporin at 15.8 µg/mL

Precision	*Mean*	*SD*	*%RSD*
Intraday (n = 6)	224470.2	914.24	0.40
Inter day (n = 9)	2255334	15257.35	0.68

Table 6: Robustness data of voclosporin (15.8 µg/mL) at various altered conditions

Parameter	*Peak area mean (n = 3)*	*SD*	*%RSD*
Flow minus (0.9 mL/min)	2281088	29654.14	1.3
Flow plus(1.1 mL /min)	2251590	9006.36	0.4
Mobile phase minus (61:39),	2255899	9023.596	0.4
Mobile phase plus (59:41)	2260262	4520.524	0.2
Temperature minus (25°C)	2248060	6744.18	0.3
Temperature plus (35°C)	2235107	11175.54	0.5

Table 7: FD results of voclosporin

Type of FD	*%Degradation of voclosporin*	*Purity angle*	*Purity threshold*
Acid	5.32	0.443	0.501
Alkali	4.91	0.524	0.589
Oxidation	3.93	0.293	0.381
Thermal	3.86	0.294	0.377
UV	2.83	0.253	0.375
Water	1.64	0.265	0.381

Forced degradation studies

The degradation of voclosporin was estimated to be about 20% based on the chromatograms and parameters data (Figure 4, Table 7). According to the forced degradation (FD) investigations, the %degradation of voclosporin was higher in acidic (5.32%), alkali (4.91%), and peroxide (3.93%) degradation tests when compared to the other stress settings. The obtained degradants and voclosporin have high peak purity due to higher purity threshold values than purity angles. The percentage degradation was determined by comparing the peak area of the control sample with the degraded sample. As a result, the described technique considers stability to be

Table 8: Assay of marketed formulation of voclosporin

Drug	*Peak*	*RT Min.*	*Area*	*Label claim (mg)*	*Amount found (mg)*	*Assay ± SD(%w/w) (n = 6)*	*%RSD*
Voclosporin	Standard	2.23	225855	7.9	7.83	99.11 ± 0.56	0.57
	Test	2.23	225040				

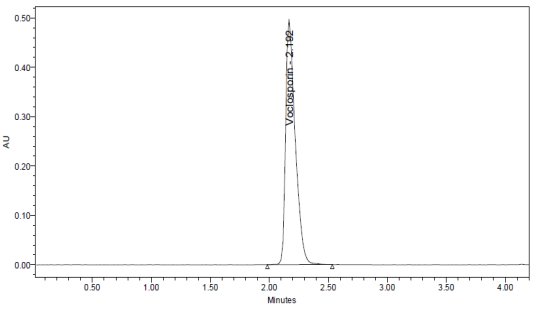

Figure 2: Optimized method chromatogram

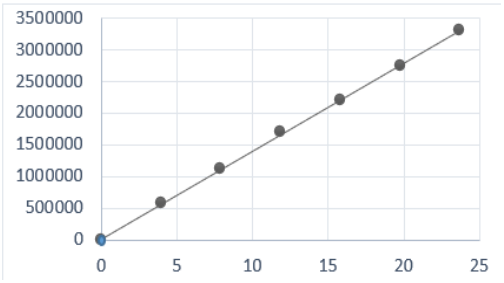

Figure 3: Linearity plot of voclosporin

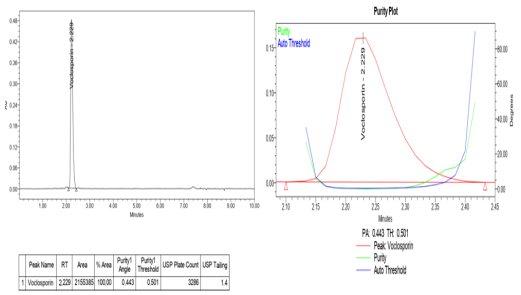

Figure 4: Chromatogram and purity plot of voclosporin in acid degradation condition

evaluating the stability of voclosporin API and tablet form. The %purity of voclosporin was determined to be 99.11 ± 0.56 (Table 8).

Overall, the function that RP-HPLC plays in the quality control department is crucial since it is responsible for delivering analytical data that is accurate, exact, and dependable.[17] This data is used for quality evaluation, method development, impurity profiling, stability testing, and regulatory compliance testing.[18] Because of its adaptability, sensitivity, and capacity to process complicated samples, it is an instrument that cannot be done without when it comes to verifying the safety of products.[19,20] In the stated approach that was predicted, a shorter RT (2.23 minutes) for voclosporin, and the method had a high sensitivity (LoD of 0.01 µg/mL and LoQ of 0.013 µg/mL). Those features indicate the method's inexpensiveness and good sensitivity. In order to determine whether or not voclosporin is stable, FD tests were carried out making use of the method described above. Pharmaceutical product quality must be consistently maintained and ensured, and this requires the use of stability-indicating HPLC techniques. In order to meet the regulatory requirements for drug approval, stability-indicating methods and stability data are required by regulatory bodies. It guarantees the safety and effectiveness of the API by facilitating the identification and measurement of degradation products. Through prolonged investigations, it makes it easier to assess product stability over the course of its suggested shelf life. In the course of developing and producing new products, it assists in addressing unforeseen impurities or degrading problems.

CONCLUSION

A simple, cost-effective, sensitive, exact, and responsive reverse phase method was set up in the study of voclosporin in pure powder and formulations. Investigating voclosporin under different FD settings proves that the process is stability-indicating and accurate. The planned method did a good job of separating voclosporin and possible degradants with a good enough resolution to make the method specific. The RT for favipiravir is shorter with the new method. So, the proposed method has a lot of support in the pharmaceutical industry.

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AUTHORS' CONTRIBUTIONS

The design and framework of the study, the collection and evaluation of data, and the production of the paper were all areas in which each author participated equally. Additionally, each author has read the produced manuscript and given their approval for it to be published.

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