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# Establishment and Validation of a Stability-Indicating RP-HPLC Method for Quantitative Determination of Voclosporin in Bulk and Pharmaceutical Dosage Forms

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**Abstract**
A sensitive, specific, and precise RP-HPLC method was developed for estimating voclosporin in its capsule dosage form. Chromatographic separation was carried out using an Agilent C18 column (250 x 4.0 mm, 5 µm). The mobile phase consisted of 0.01N phosphate buffer and acetonitrile in a 70:30 ratio, with a flow rate of 1 mL/min. The column temperature was set at 30°C, and the detection wavelength was optimized at 220.0 nm. The retention time for voclosporin was 2.446 minutes. The % relative standard deviation (RSD) for voclosporin was 0.1%, and the repeatability precision % RSD was 0.7%. The recovery of voclosporin was 99.93%, and the assay result was 99.38%. The limits of detection (LOD) and quantification (LOQ) were 0.15 µg/mL and 0.47 µg/mL, respectively, based on the regression equation. The regression equation was determined to be y = 13283x + 2123. This method offers reduced retention and run times, making it efficient, economical, and suitable for routine quality control testing in pharmaceutical industries.

**Keywords**: HPLC, Voclosporin, Method Development, Validation.

## Introduction:

Voclosporin (fig. 1) is a calcineurin inhibitor approved for the treatment of lupus nephritis[1-7] (LN) in patients diagnosed with systemic lupus erythematosus (SLE). LN is a form of glomerulonephritis that occurs in individuals with SLE and is a major cause of renal failure, morbidity, and mortality in these patients. Within 10 years of an SLE diagnosis, 5–20% of patients with LN progress to end-stage kidney disease, a life-threatening condition. Early and accurate intervention for LN is crucial to improving clinical outcomes. The drug received FDA approval in 2021[7]. The method used for validation followed ICH Q2 guidelines, while

stability testing adhered to ICH Q1A guidelines. A few RP-HPLC and LC-MS methods have been reported for the estimation of voclosporin[8-10].

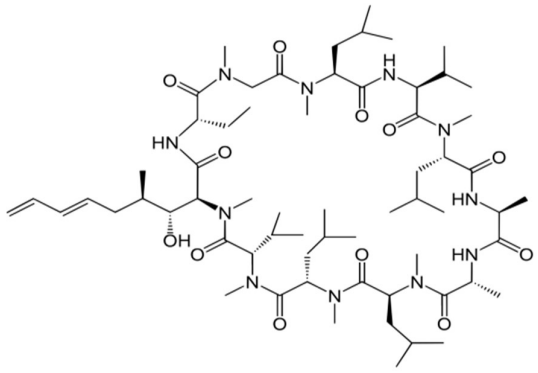


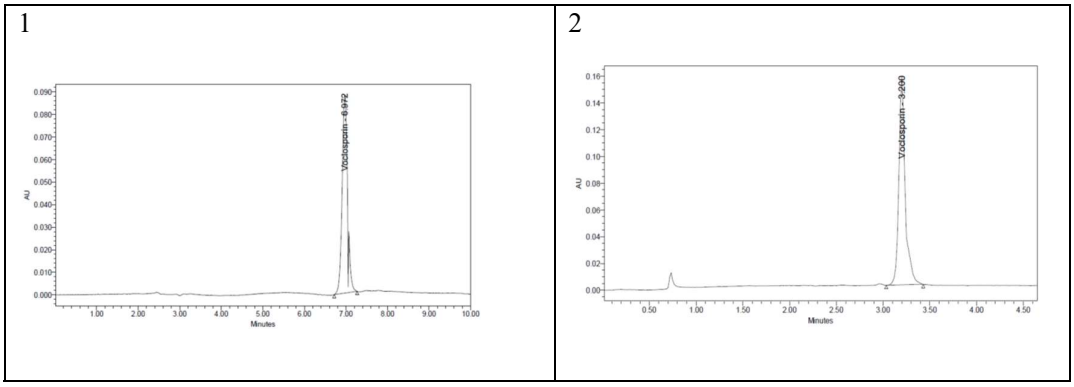
**Figure 1. Chemical Structure of Voclosporin**

## *Materials & Methods:*

The HPLC instrument utilized for this study was a water HPLC 2965 system equipped with an Auto Injector and a PDA Detector. The analysis was performed using Empower software. For measuring the absorbance of Voclosporin solutions, a UV-VIS spectrophotometer (PG Instruments T60) with a special bandwidth of 2 mm and 10 mm matched quartz cells was employed. The solvents and reagents used in the method included HPLC-grade methanol, HPLC-grade acetonitrile, HPLC-grade water, and glacial acetic acid, all of which were procured from Rankem Chemicals, Hyderabad.

### Chromatographic conditions:

During this chromatographic condition, number of trails are performed. Best trail selected for Voclosporin. (Fig. 2; Table 1)



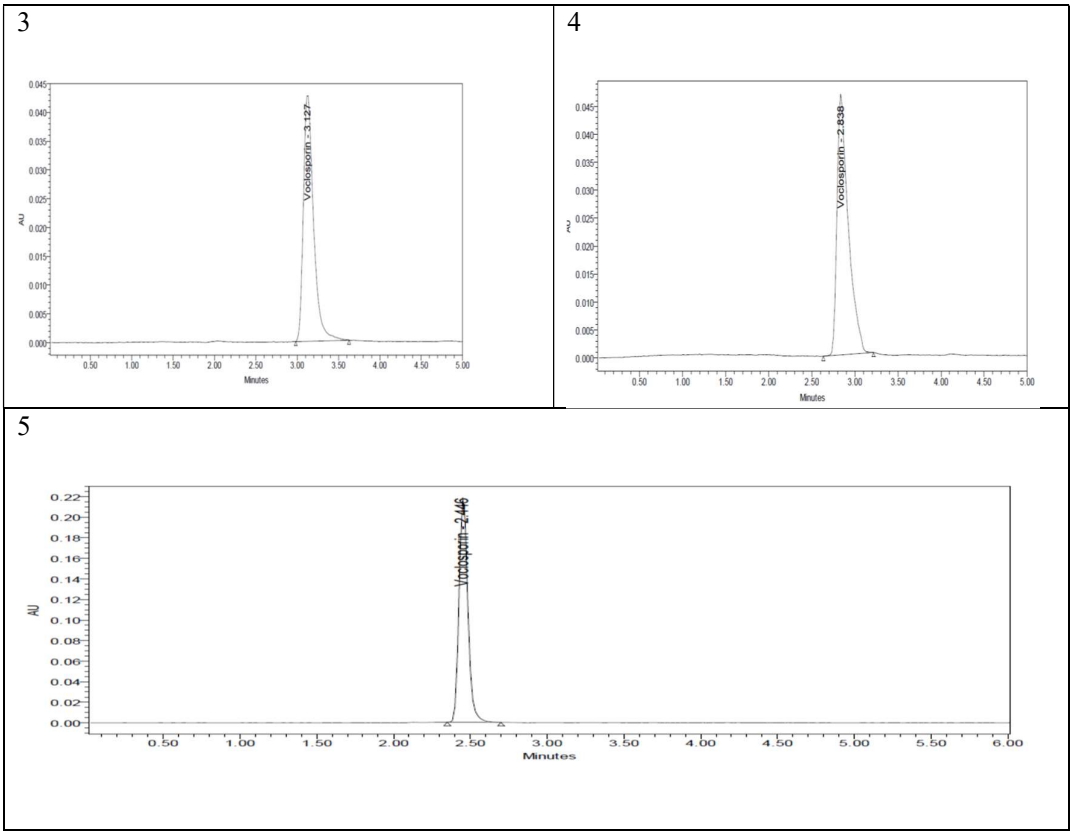



**Figure 2. Trail chromatograms (1-5)**

**Table 1. Trails for Optimisation of chromatographic condition**

| Trails | Column | Mobile Phase | Wavelength (nm) | Flow rate (ml/min) | Injection volume (µL) | Run Time (Minutes) | Observation |
|---|---|---|---|---|---|---|---|
| **Trail-1** | Kromasil $C_{18}$ (4.6 x 250 mm, 5.0 µm) | 0.1% OPA and Methanol (50:50) | 220 nm | 1.0 | 10 | 10 | Broad peak and system suitability variables were not within range |
| **Trail-2** | Kromasil $C_{18}$ (4.6 x 250 mm, 5.0 µm) | 0.1% OPA **:** Acetonitrile (50:50) | 220 nm | 1.0 | 10 | 5 | peak shape was not good and less USP plate count |
| **Trail-3** | BDS C18 (4.6 x 250 mm, 5.0 µm) | 0.1% OPA : Acetonitrile (50:50) | 220 nm | 1.0 | 10 | 5 | peak shape was not good and less USP plate count |
| **Trail-4** | BDS C18 (4.6 x 250 mm, 5.0 µm) | 0.01N $KH_2PO_4$: Acetonitrile (50:50) | 220 nm | 1.0 | 10 | 5 | Voclosporin peak shape and USP plate count were not good |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Trail-5** | Agilent C18 (4.6 x 250 mm, 5.0 µm) | 0.01N $KH_2PO_4$: Acetonitrile (70:30) | 220 nm | 1.0 | 10 | **5** | Peak has good resolution, tailing factor, theoretical plate count and resolution. |

**Preparation of Standard stock solutions:** Accurately weighed 7.9mg of Voclosporin transferred to 10 ml volumetric flask. 7.5 ml of diluents was added to flask and sonicated for 10 minutes. Flasks were made up with diluent and labelled as Standard stock solution. (790µg/ml of Voclosporin)

**Preparation of Standard working solutions (100% solution):** 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (79µg/ml Voclosporin)

**Preparation of Sample stock solutions:** 5 Capsules were weighed and the average weight of each Capsule was calculated, then the weight equivalent to 1 Capsule (243.8 mg Avg wt) was transferred into a 25 ml volumetric flask, 50 ml of diluent was added and sonicated for 25 min, further the volume was made up with diluent and filtered by UPLC filters. (360µg/ml of Voclosporin)

**Preparation of Sample working solutions (100% solution):** 2.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (79µg/ml of Voclosporin).

Optimized chromatographic conditions were mentioned in Table 2.

**Table 2. Optimised chromatographic conditions for Voclosporin**

| Parameters | Observation |
|---|---|
| Mobile phase | 0.01N $KH_2PO_4$: Acetonitrile (70:30) |
| Flow rate | 1 ml/min |
| Column | Agilent $C_{18}$ (4.6 x 250mm, 5.0µm) |
| Detector wave length | 220 nm |
| Column temperature | 30° C |
| Injection volume | 10.0 µL |
| Run time | 5.0 min |
| Diluent | Water and Acetonitrile in the ratio 50:50 |
| Retention time | 2.446 min |

**Validation:**

**System suitability parameters:**

The system suitability parameters were determined by preparing standard solutions of Voclosporin (79 ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. Results are tabulated in Table 3 and Chromatograms shown in Fig. 3.

**Table 3. System suitability parameters for Voclosporin**

| S no | Voclosporin | | |
|---|---|---|---|
| Inj | RT(min) | USP Plate Count | Tailing |
| 1 | 2.438 | 8419 | 1.19 |
| 2 | 2.440 | 8210 | 1.23 |
| 3 | 2.440 | 8221 | 1.23 |
| 4 | 2.444 | 8195 | 1.18 |
| 5 | 2.446 | 8241 | 1.17 |
| 6 | 2.449 | 8946 | 1.16 |

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

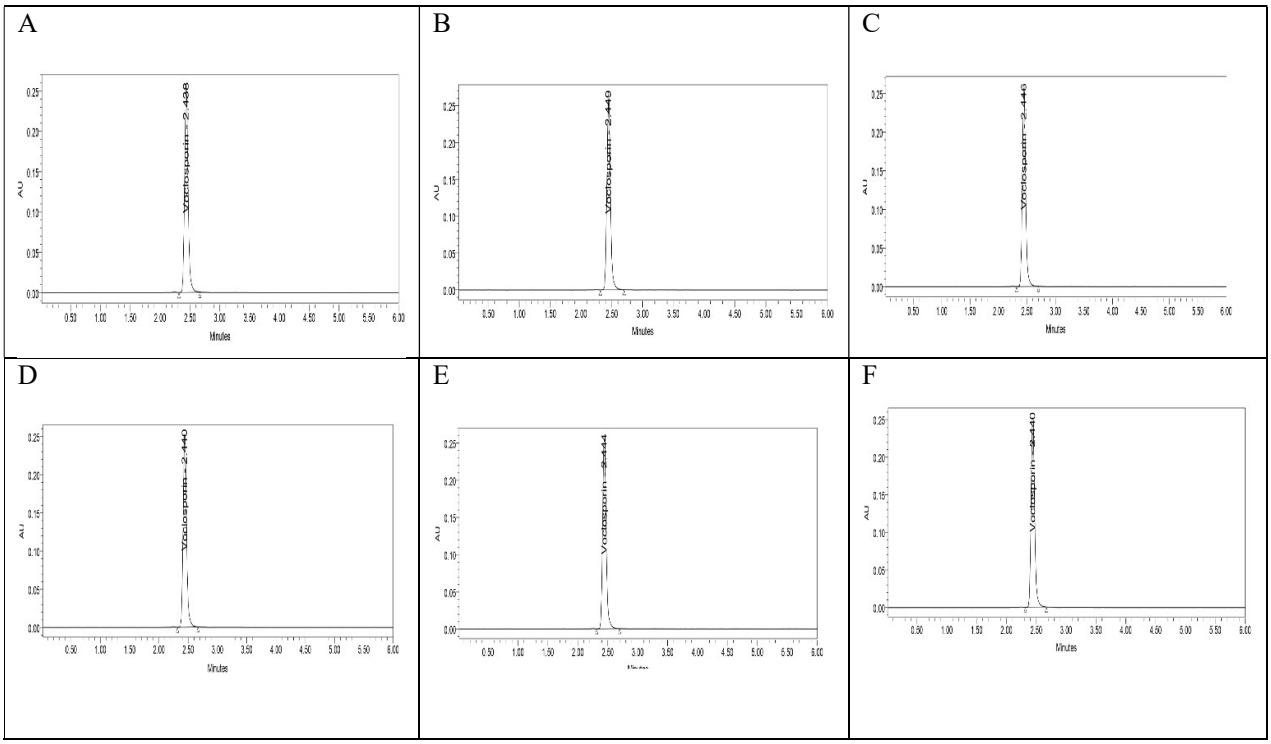



**Figure 3. System Suitability Chromatograms (A to F)**

**Discussion:** According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

**Specificity:**

Retention time of Voclosporin was 2.446 min. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific. Chromatograms shown in Fig. 4.

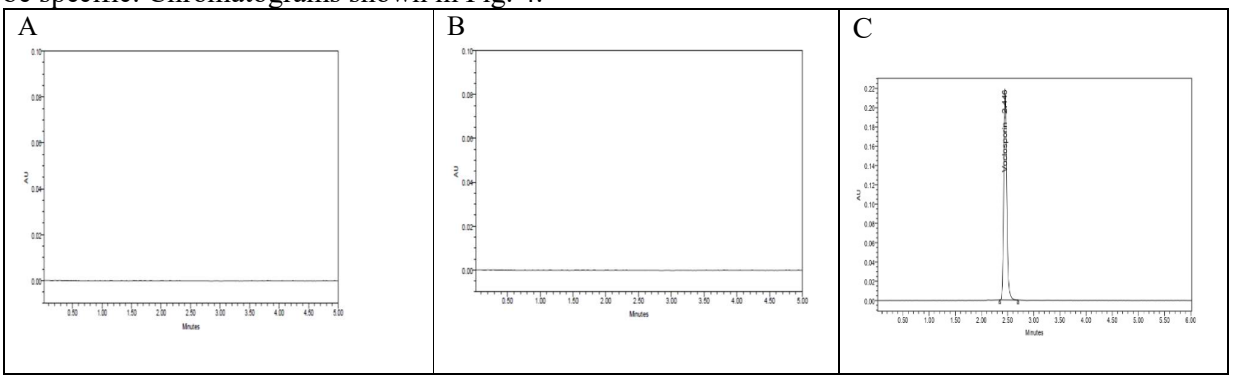



**Figure 4. Typical chromatograms A) Blank B) Placebo C) Standard chromatogram**

**Precision:**

**Preparation of Sample stock solutions:** 5 Capsules were weighed and the average weight of each Capsule was calculated, then the weight equivalent to 1 Capsule (243.8 mg Avg wt) was transferred into a 25 ml volumetric flask, 50ml of diluent was added and sonicated for 25 min, further the volume was made up with diluent and filtered by UPLC filters. (360µg/ml of Voclosporin)

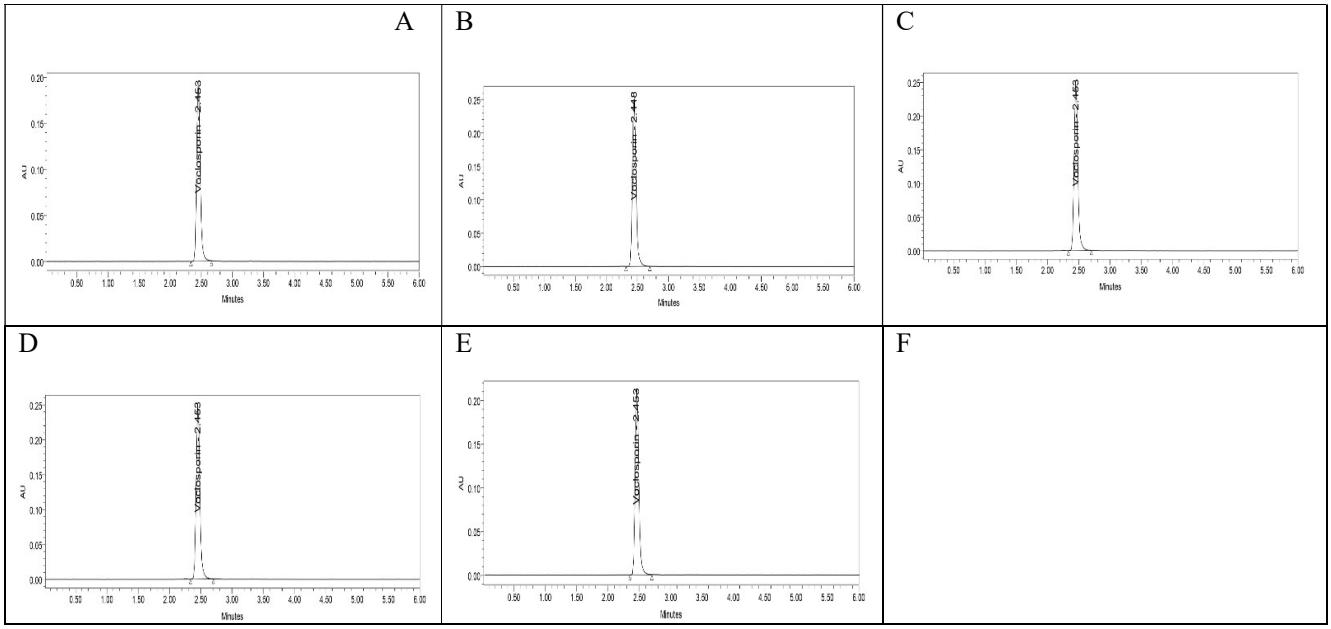
**Preparation of Sample working solutions (100% solution):** 2.5 ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluent. (79 µg/ml of Voclosporin)

The precision was determined by preparing test solution of Voclosporin (79 ppm) and the solutions were injected six times and the % RSD for the area of six standard injections results should not be more than 2%.

**Repeatability:** Six working sample solutions of 79 ppm are injected and the % Amount found was calculated and % RSD was found to be 0.7 %. Repeatability data for Voclosporin was shown in table 4 and chromatograms were shown in figure 5.

**Table 4. Repeatability data for Voclosporin**

| S. No. | Peak Area |
|---|---|
| 1 | 1059500 |
| 2 | 1049634 |
| 3 | 1038738 |
| 4 | 1057331 |
| 5 | 1051266 |
| 6 | 1046041 |
| AVG | 1050418 |
| STDEV | 7578.7 |
| % RSD | 0.7 |



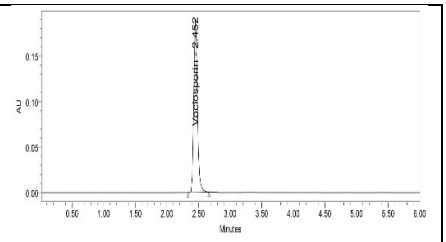


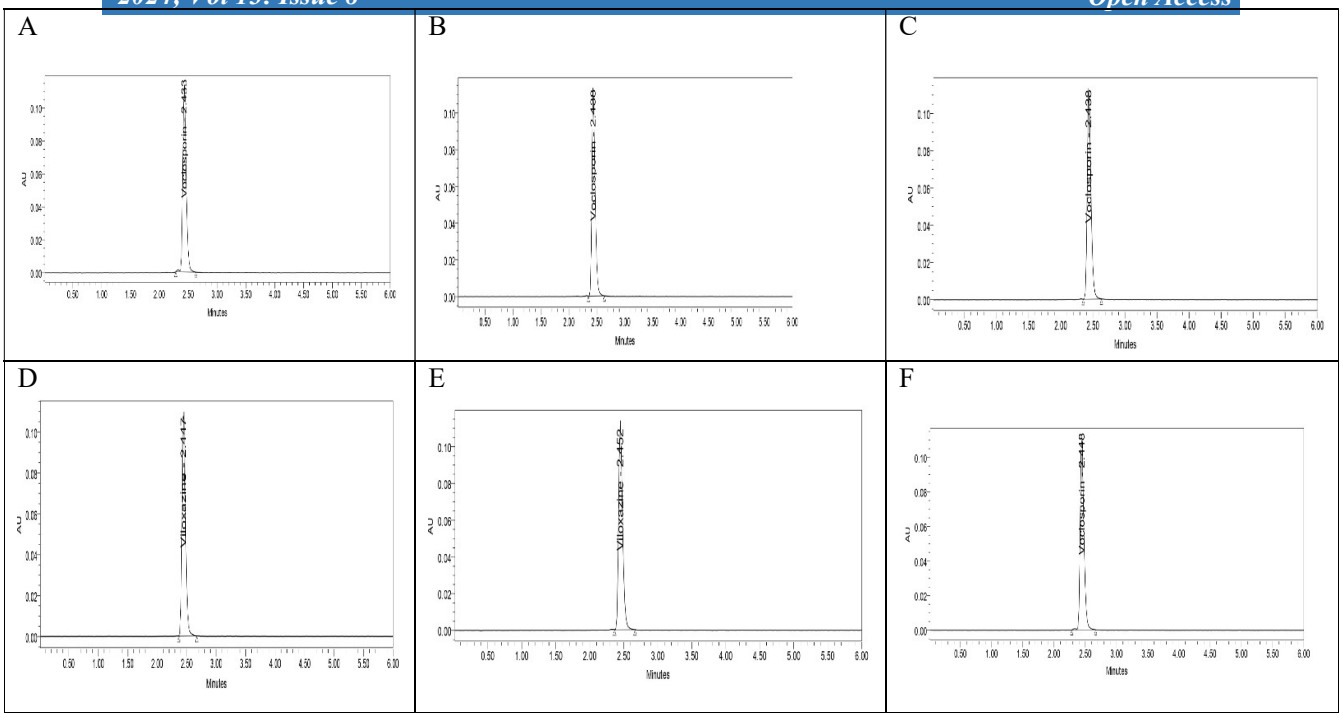

**Figure 5. Repeatability Chromatograms (A to F)**

**Intermediate precision:** six working sample solutions of 79 ppm are injected on the next day of the preparation of samples and the % amount found was calculated and and chromatogram were shown in figure 6. Intermediate precision data was shown in table 5.

**Figure 6. Intermediate precision Chromatograms (A to F)**

**Table 5. Intermediate precision data**

| S.No | Peak Area |
|---|---|
| 1 | 1010204 |
| 2 | 1012318 |
| 3 | 1013771 |
| 4 | 1015512 |
| 5 | 1010870 |
| 6 | 1015138 |
| AVG | 1012969 |
| STDEV | 2204.1 |
| % RSD | 0.2 |




**Discussion:** % RSD were calculated and obtained as 0.2 % for Voclosporin

**Sensitivity (Table 6):**

**Table 6. Sensitivity table of Voclosporin**

| Molecule | LOD | LOQ |
|---|---|---|
| **Voclosporin** | 0.15 | 0.47 |

LOD: Detection limit of the Voclosporin in this method was found to be 0.15µg/ml.

LOQ**:** Quantification limit of the Voclosporin in this method was found to be 0.47µg/ml

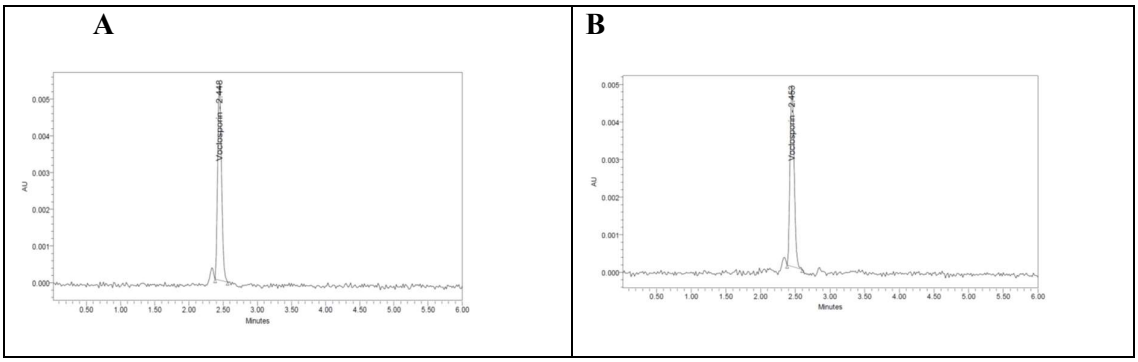



**Figure 7. Chromatograms of A. LOD B. LOQ for Voclosporin**

**Linearity:**

**Preparation of Standard stock solutions:** Accurately weighed 7.9mg of Voclosporin

transferred to 10 ml volumetric flask. 3/4 th of diluents was added to flask and sonicated for 10 minutes. Flasks were made up with diluent and labeled as Standard stock solution. (790µg/ml of Voclosporin)

**25% Standard solution:** 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (19.75µg/ml of Voclosporin)

**50% Standard solution:** 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (39.5µg/ml of Voclosporin)

**75% Standard solution:** 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (59.25µg/ml of Voclosporin)

**100% Standard solution:** 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (79µg/ml of Voclosporin)

**125% Standard solution:** 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (98.75µg/ml of Voclosporin)

**150% Standard solution:** 1.5ml each from two standard stock solutions was pipetted out and made up to 10ml (118.75µg/ml of Voclosporin)

To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 19.75ppm to 118.75ppm of Voclosporin. Plot a graph to concentration versus peak area. Correlation Co-efficient was found to be 0.999 and Linearity plot was shown in figure 8. Linearity data was shown in table 7 and chromatograms were shown in figure 9.

| Voclosporin | |
|---|---|
| Conc (µg /mL) | Peak area |
| 0 | 0 |
| 19.75 | 267076 |
| 39.5 | 523375 |
| 59.25 | 795317 |
| 79 | 1046469 |
| 98.75 | 1318446 |
| 118.5 | 1573153 |

**Table 7. Linearity Concentration and Response**

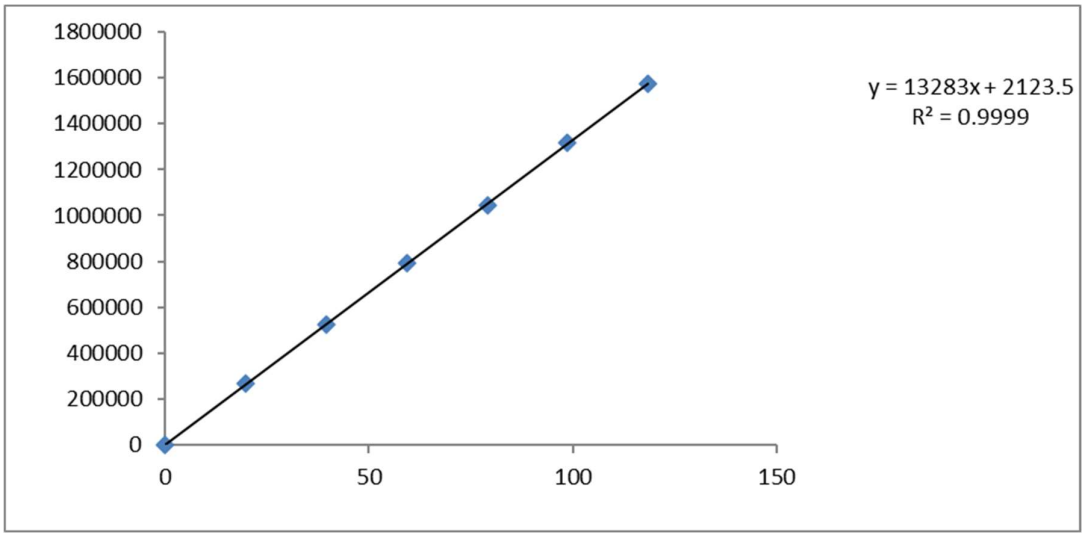


**Figure 8. Calibration curve of Voclosporin**

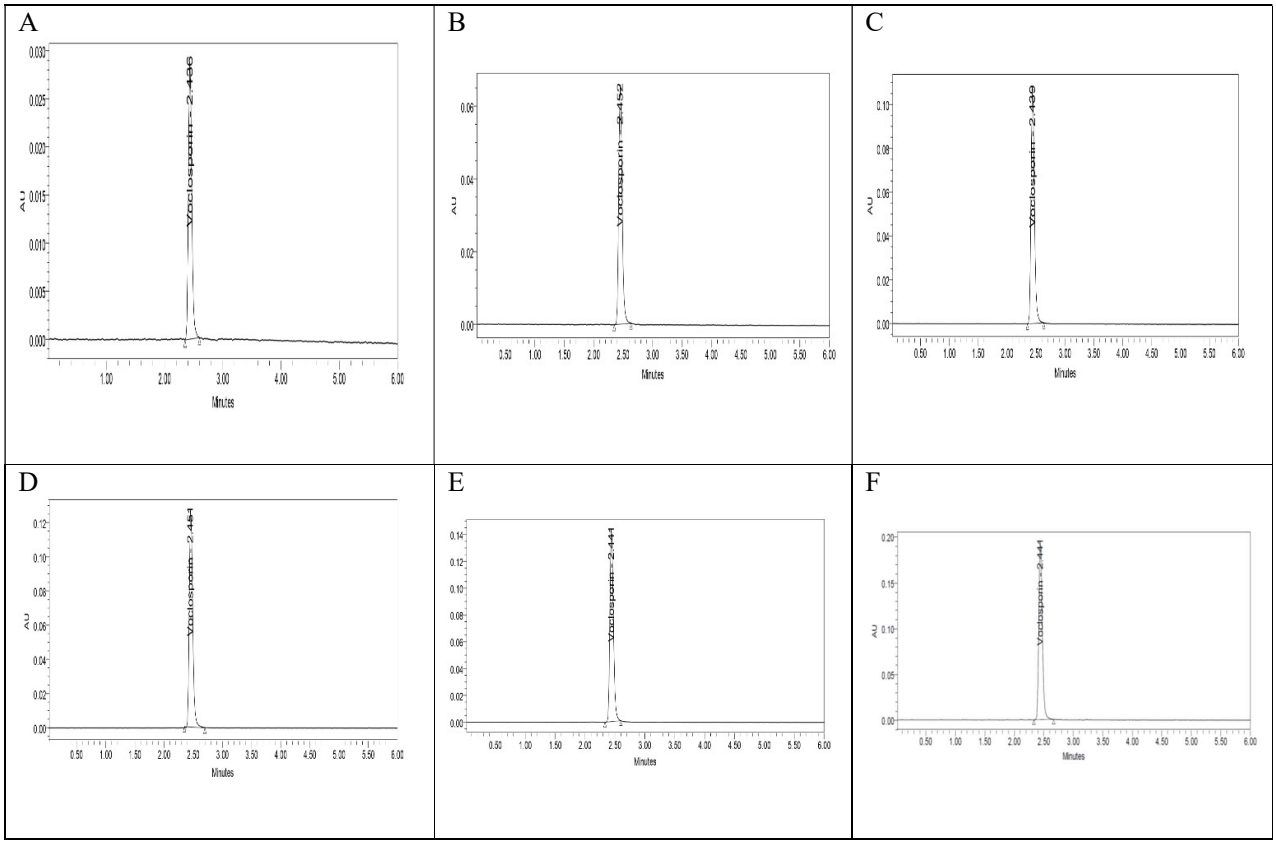



**Figure 9. Chromatograms showing the linearity A) 25% B) 50% C) 75% D) 100% E) 125% F) 150% Concentrations of Voclosporin**

**Accuracy:**

**Preparation of Sample stock solutions:** 5 Capsules were weighed and the average weight of each Capsule was calculated, then the weight equivalent to 1 Capsule (243.8 mg Awg wt) was transferred into a 25 ml volumetric flask, 50ml of diluent was added and sonicated for 25 min, further the volume was made up with diluent and filtered by UPLC filters. (360µg/ml of Voclosporin)

**Preparation of Standard working solutions (100% solution):** 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (79µg/ml Voclosporin)

**Preparation of 50% Spiked Solution:** 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

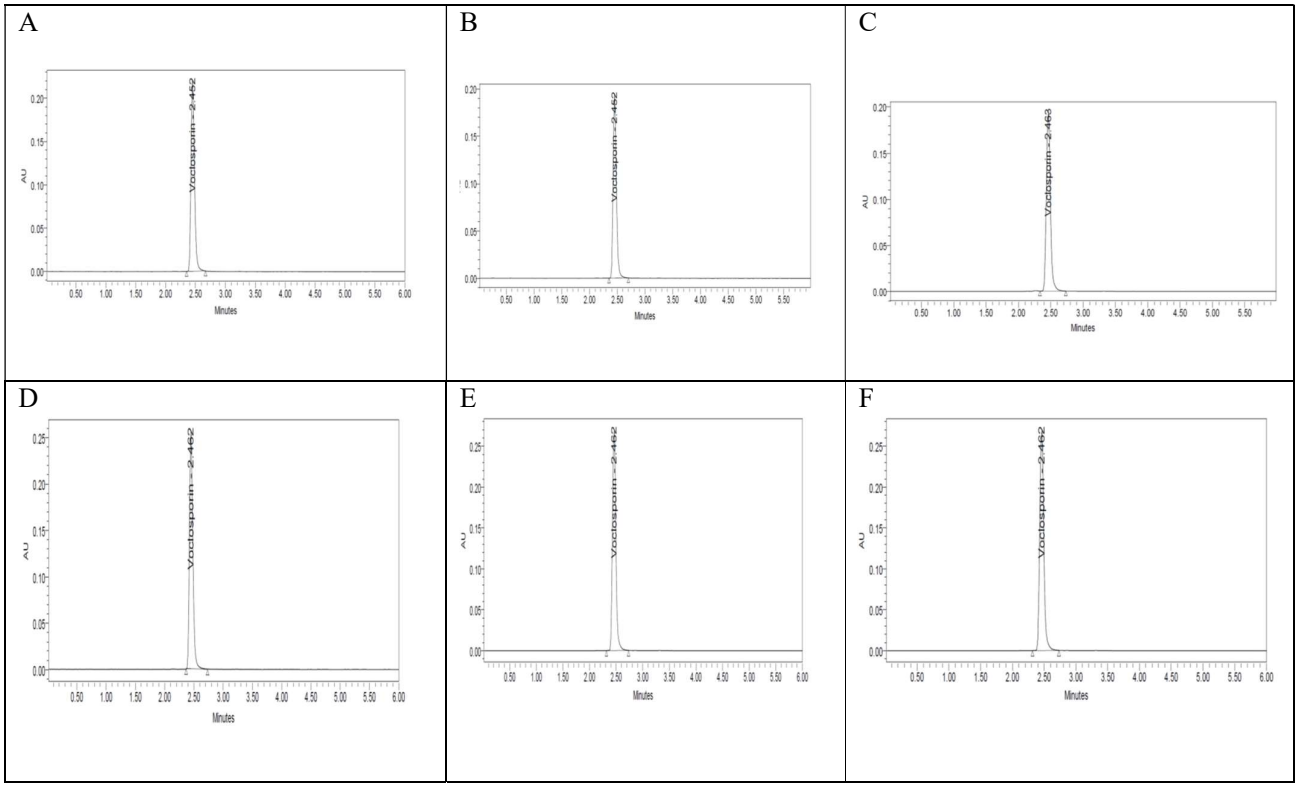
**Preparation of 100% Spiked Solution:** 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

**Preparation of 150% Spiked Solution:** 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Accuracy data of Voclosporin shown in table 8 and chromatograms were shown in figure 10.

**Table 8. Accuracy data**

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|
| 50% | 39.5 | 39.79 | 100.72 | 99.93% |
| | 39.5 | 39.61 | 100.28 | |
| | 39.5 | 39.60 | 100.26 | |
| 100% | 79 | 78.23 | 99.03 | |
| | 79 | 79.27 | 100.34 | |
| | 79 | 79.41 | 100.52 | |
| 150% | 118.5 | 118.13 | 99.68 | |
| | 118.5 | 118.55 | 100.05 | |
| | 118.5 | 116.70 | 98.48 | |



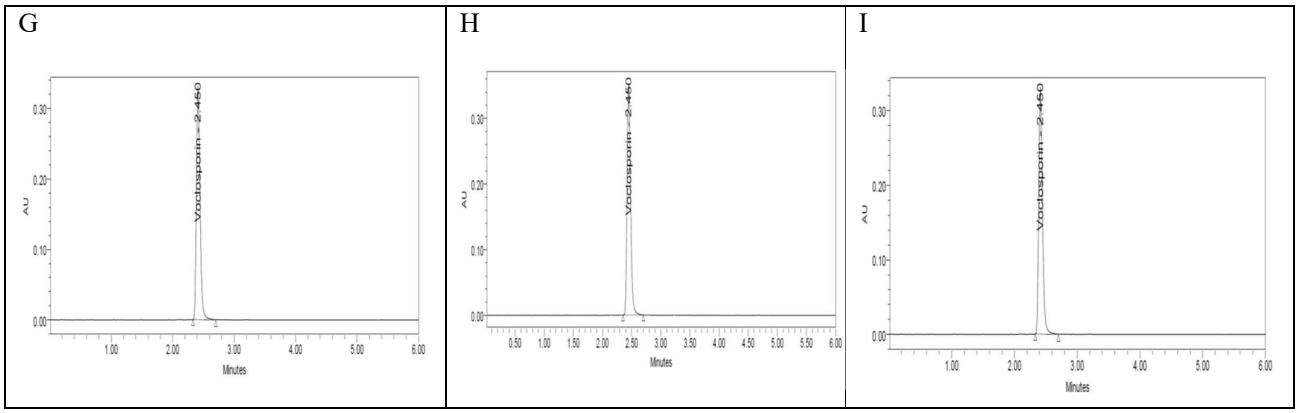



**Figure 10. Chromatogram representing accuracy levels at 50 % (A,B,C) , 100% (D,E,F) & 150% (G,H,I)**

**Acceptance Criteria:**
The % Recovery for each level should be between 98.0 to 102

**Robustness:**
Small Deliberate changes in the method are made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. % RSD of the above conditions are calculated. Robustness data was shown in table 9 and chromatograms were shown in figure11.

**Table 9. Robustness Data**

| S.no | Condition | %RSD of Voclosporin |
|---|---|---|
| 1 | Flow rate (-) 0.9ml/min | 0.1 |
| 2 | Flow rate (+) 1.1ml/min | 0.1 |
| 3 | Mobile phase (-) 75B:25A | 1.0 |
| 4 | Mobile phase (+) 85B:15A | 0.8 |
| 5 | Temperature (-) 25°C | 0.1 |
| 6 | Temperature (+) 35°C | 0.3 |

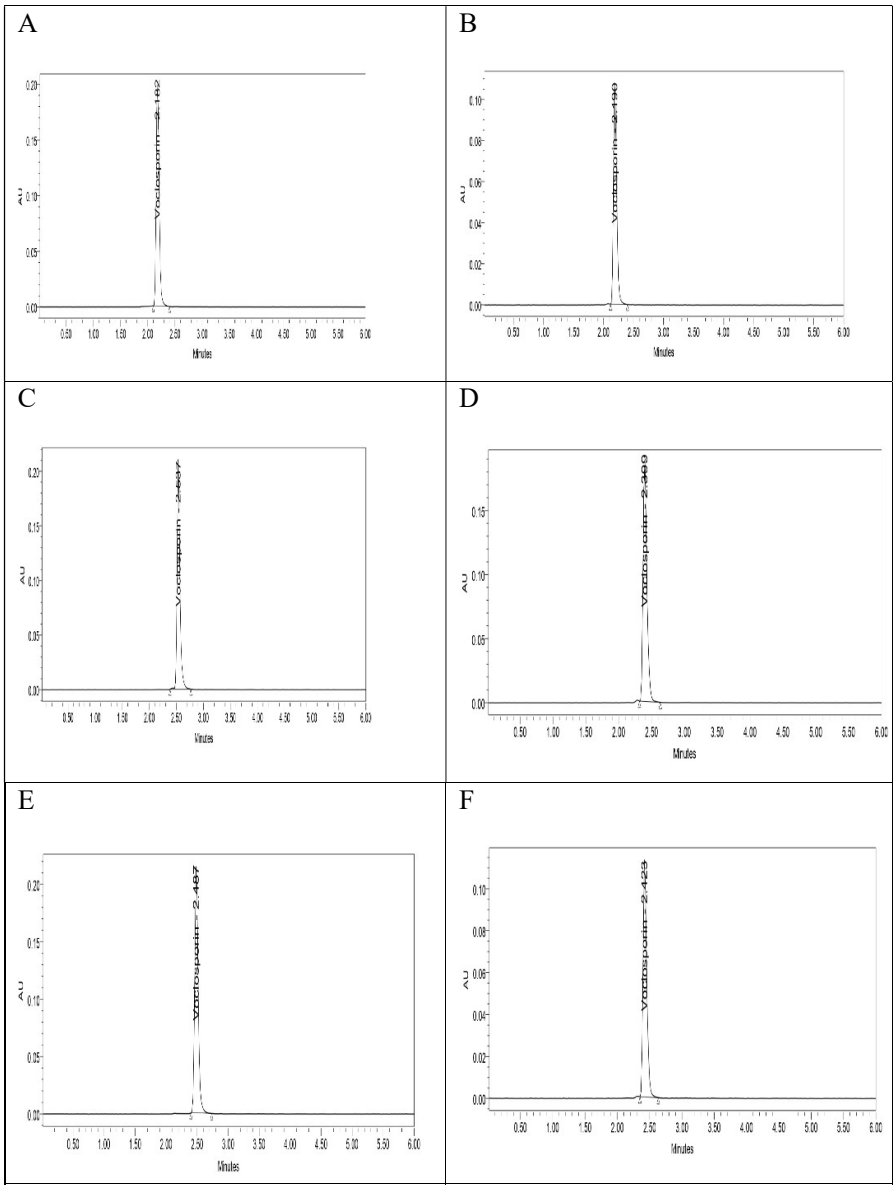



**Figure 11. Chromatogram representing the effect of A) Flow rate-0.9 ml/min B) Flow rate - 1.1 ml/min C) Mobile phase -75B:25A D) Mobile phase -85B:15A E) Temperature -25° C F) Temperature- 35° C**

**Assay of marketed formulation:**

Standard solution and sample solution were injected separately into the system and chromatograms were recorded and drug present in sample was calculated using before mentioned formula.

**Table 10. Assay of Formulation**

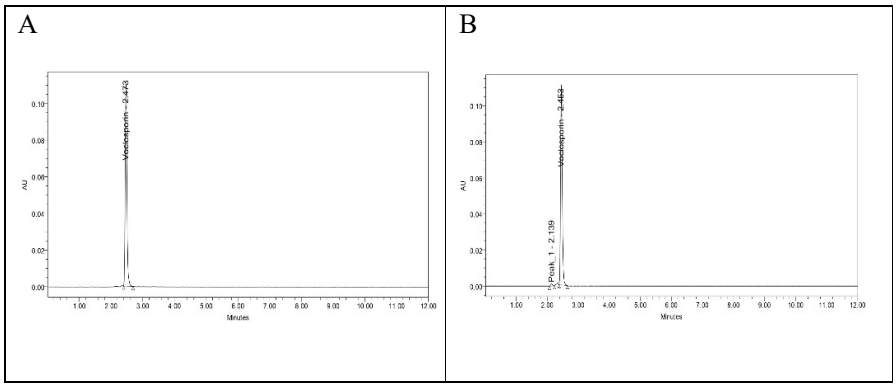
| Sample No | % Assay |
|---|---|
| 1 | 100.24 |
| 2 | 99.31 |

| | |
|---|---|
| 3. | 98.28 |
| 4. | 100.04 |
| 5. | 99.46 |
| 6. | 98.97 |
| AVG | 99.38 |
| STDEV | 0.7170 |
| %RSD | 0.7 |

**Degradation Studies:** Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation. Degradation studies data shown in table 11 and chromatograms were shown in figure 12.

**Table 11. Degradation Data of Voclosporin**

| S.NO | Degradation Condition | % Drug Degraded | %UN Drug Degraded |
|---|---|---|---|
| 1 | Acid | 5.52 | 94.48 |
| 2 | Alkali | 4.29 | 95.71 |
| 3 | Oxidation | 5.91 | 94.09 |
| 4 | Thermal | 3.86 | 96.14 |
| 5 | UV | 2.07 | 97.93 |
| 6 | Water | 0.87 | 99.13 |



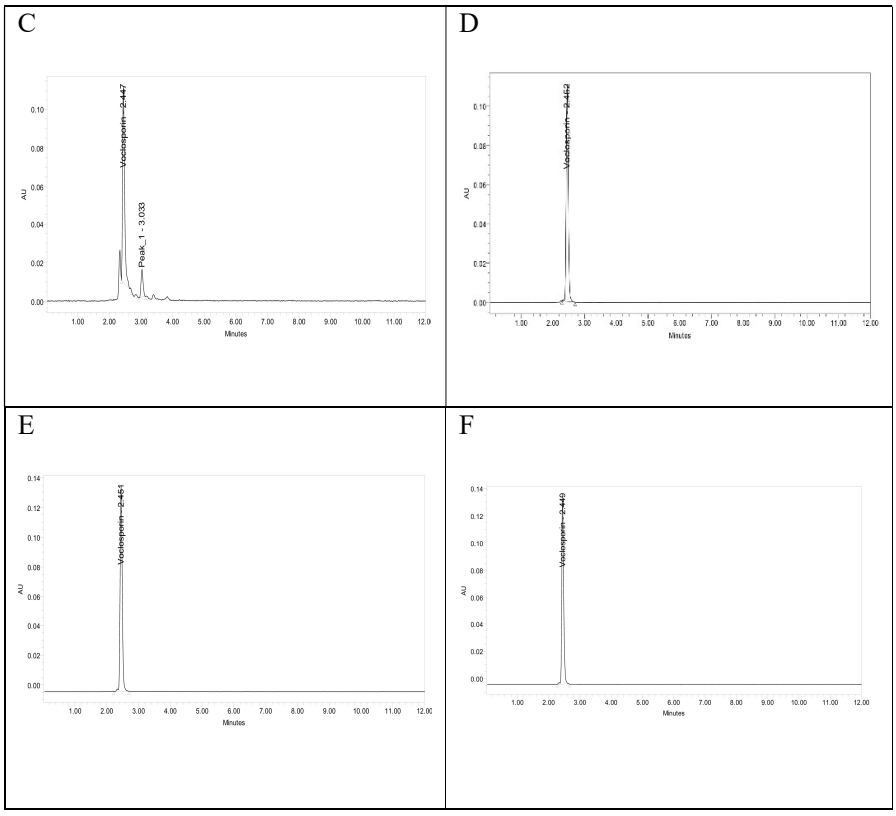



**Figure 12. Chromatograms showing forced degradation studies A) Acid degradation B) Alkali degradation C) Oxidation degradation D) Thermal degradation E) UV degradation F) Water degradation**

## Conclusion:

A simple, efficient, and highly sensitive reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Voclosporin in capsule formulations. The retention time for Voclosporin was found to be 2.446 minutes. The relative standard deviation (% RSD) of Voclosporin was calculated at 0.1, indicating excellent precision of the method. The repeatability precision % RSD for Voclosporin was 0.7. The recovery rate was 99.93%, demonstrating outstanding accuracy. The assay value of Voclosporin was 99.38%. The limit of detection (LOD) and limit of quantification (LOQ), based on the regression equation, were 0.15 and 0.47, respectively. This method provides a fast, reliable, and efficient approach, making it ideal for routine quality control in the pharmaceutical industry.

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