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DETERMINATION OF VOCLOSPORIN AND FORCED DEGRADATION STUDY BEHAVIOUR BY RP-HPLC IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Russian botanist Tswett invented chromatography as a separation technique. He describes in detail the separation of pigments, the coloured substances by filtration through column, followed by developments with pure solvents. The aim of this research works is to develop a novel, accurate, precise,cost – effective analytical method by using RP-HPLC method , for the selected immunosuppressive agent (voclosporin). Voclosporin is a calcineurin inhibitor for the treatment of lupus nephritis (LN) in patients diagnosed with systemic lupus erythematosus (SLE). Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50. A Standard solution of Voclosporin working standard was prepared as per procedure and was injected five times into the HPLC system. Chromatographic conditions used are stationary phase Kromasil (250mm*4.6mm 5µ), Mobile phase0.01N Kh2po4 Buffer: Acetonitrilein the ratio of 60:40 and flow ratewas maintained at 1ml/min, detection wave length was282nm, column temperature was set to30^{o}Cand diluent was mobile phaseConditions were finalized as optimized method.

Keywords: Voclosporin, Forced Degradation Study, RP-HPLC.

INTRODUCTION

Qualitative Inorganic Analysis seeks to establish the presence of a inorganic compound in a sample or given element [1]. Quantitative analysis seeks to establish the amount of a compound in a sample or given element. Qualitative Organic Analysis seeks to establish the presence of a given functional group or organic compound in a sample..

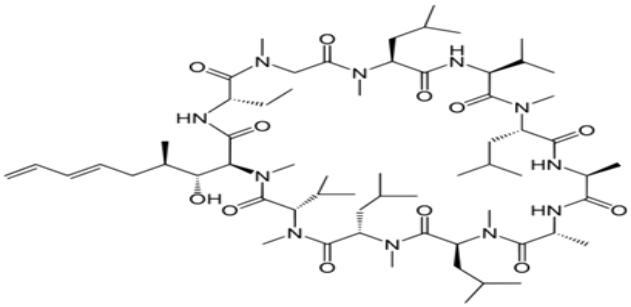
MATERIALS AND METHODS

DRUG PROFILE

Voclosporin:

Voclosporin is a calcineurin inhibitor for the treatment of lupus nephritis (LN) in patients diagnosed with systemic lupus erythematosus (SLE). Lupus nephritis (LN) is a type of glomerulonephritis occurring in patients with systemic lupus erythematosus (SLE). LN is a significant cause of renal failure, morbidity, and death in patients with SLE [2].

CAS No: 515814-01-4
Chemical Formula: C63H111N11O12
Molecular Weight: 1214.6 g/mol



Structure

Solubility: Soluble in water and ethanol
Melting point: >129 ° C
Boiling point: 1303.8±65

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.

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Methods:

Diluent

Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Preparation of Standard stock solutions

Accurately weighed 7.9mg of Voclosporin transferred 50ml and volumetric flasks, 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 ofVoclosporin).

Preparation of Standard working solutions (100% solution)

1ml of Voclosporin from each 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

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 50 ml volumetric flask, 20ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (158 µg/ml of Voclosporin).

Preparation of Sample working solutions (100% solution)

1ml 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 solutions of Voclosporin (15.8ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined [4]. The % RSD for the area of six standard injections results should not be more than 2%.

Specificity

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.

Precision:

Preparation of Standard stock solutions

Accurately weighed 7.9mg of Voclosporin transferred 50ml and volumetric flasks, 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 (15.8 µg/ml of Voclosporin).

Preparation of Sample stock solutions

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 50 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters.(158 µg/ml of Voclosporin)

Preparation of Sample working solutions (100% solution)

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (15.8µg/ml of Voclosporin)

Linearity

Preparation of Standard stock solutions

Accurately weighed 79 mg of Voclosporin transferred 50ml and volumetric flasks, 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).

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (3.95µg/ml of Voclosporin).

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (7.9µg/ml of Voclosporin)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (11.85µg/ml of Voclosporin)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (15.8µg/ml of Voclosporin)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (19.75µg/ml of Voclosporin)

150% Standard solution: 1.5ml each from two standard stock solutions was pipettede out and made up to 10ml. (23.7µg/ml of Voclosporin)

Accuracy:

Preparation of Standard stock solutions

Accurately weighed 7.9mg of Voclosporin transferred 25ml and volumetric flasks, 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).

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

Robustness [5]

Small deliberatechanges in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation

0.25ml of Standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.1ml Voclosporin, were transferred to 10ml volumetric flasks and made up with the same diluents.

LOQ sample Preparation

0.25ml of Standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.3ml Voclosporin, were transferred to 10ml volumetric flasks and made up with the same diluents.

Degradation studies [6]

Oxidation

To 1 ml of stock solution of Voclosporin, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 15.8µg/ml solutionand10µlwereinjectedintothe system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies

To 1 ml of stocks solution Voclosporin, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c.The resultant solution was diluted to obtain15.8µg/ml solution and10µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

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 15.8µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 15.8µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the 5000µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 15.8µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxingthedruginwaterfor6h r s at a temperature of 60º. For HPLC study, the resultant solution was diluted to 15.8µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Assay Methodology

Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system. And percent purity was found out by following formulae.

RESULTS AND DISCUSSIONS

SYSTEM SUITABILITY

A Standard solution of Voclosporin working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from five replicate injections are within range.

Precision:

Repeatability

Six working sample solutions of 15.8ppm are injected and the % Amount found was calculated and %RSD was found to be 0.5.

Repeatability Chromatogram Intermediate precision

Five working sample solutions of 15.8ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.7.

Linearity

To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 3.95ppm to 23.7ppm of Voclosporin. Plot a graph to concentration versus peak area. Slope obtained was 56983Y-Intercept was9911.6and Correlation Co-efficient was found to be 0.999.

Accuracy

Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recovery was calculated as 99.75.

LOQ

Quantification limit of the Voclosporinin this method was found to be 0.159µg/ml.

Robustness

Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. % RSD of the above conditions are calculated.

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.

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.

Table 1. Repeatability data

S.No	Peak Area
1	849958
2	854345
3	846554
4	844959
5	853933
6	845728
AVG	849246
STDEV	4158.8
%RSD	0.5

Table 2. Intermediate precision data

S.No	Peak Area
1	848254
2	846920
3	848983
4	840231
5	842194
6	843015
AVG	844933
STDEV	3596.4
%RSD	0.4

Table 3. Linearity Concentration and Response

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	3.95	246037
50	7.9	458883
75	11.85	685586
100	15.8	913068
125	19.75	1137384
150	23.7	1355133

Table 4. Accuracy data

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	7.9	23.7	23.7	99.75%
	7.9	23.7	23.7	
	7.9	23.7	23.7	
100%	15.8	15.77	99.82	
	15.8	15.72	99.49	

	15.8	15.74	99.60	
150%	23.7	23.85	100.64	
	23.7	23.51	99.21	
	23.7	23.71	100.05	

Table 5. Robustness Data

Parameter	%RSD
Flow Minus	0.5
Flow Plus	0.5
Mobile phase Minus	1.0
Mobile phase Plus	1.2
Temperature minus	0.7
Temperature plus	0.3

Table 6. Assay of Formulation

Sample No	%Assay
1	99.33
2	99.84
3	98.93
4	98.75
5	99.80
6	98.84
AVG	99.25
STDEV	0.4860
%RSD	0.49

Table 7. Degradation Data of Voclosporin

S.No	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	7.05	0.267	0.346
2	Alkali	5.96	0.312	0.340
3	Oxidation	5.69	0.296	0.341
4	Thermal	4.34	0.310	0.349
5	UV	2.75	0.298	0.384
6	Water	0.75	0.345	0.423

Table 8. Summary Table

Parameters	Voclosporin		LIMIT
Linearity &Range(µg/ml)	13.95-23.7 µg/ml		R< 1
Regressioncoefficient	0.999		
Slope(m)	56983		
Intercept(c)	9911.6		
Regression equation(Y=mx+c)	y = 56983x + 9911.6		
Assay(% mean assay)	99.25%		90-110%
Specificity	Specific		No interference of any peak
System precision %RSD	0.6		NMT 2.0%
Method precision%RSD	0.5		NMT 2.0%
Accuracy %recovery	99.75%		98-102%
LOD	0.053		NMT 3
LOQ	0.159		NMT 10
Robustness	**FM**	0.5	%RSD NMT 2.0
	FP	0.5	
	MM	1.0	
	MP	1.2	
	TM	0.7	
	TP	0.3	

Fig. 1. Blank Chromatogram

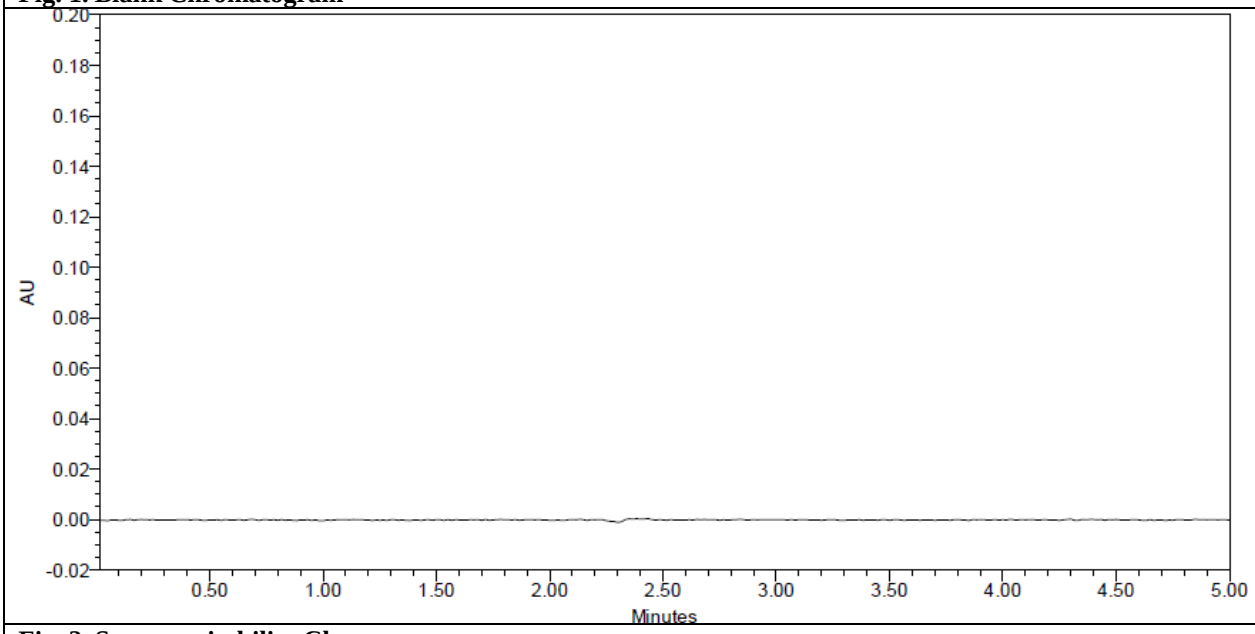


Fig. 2. System suitability Chromatogram

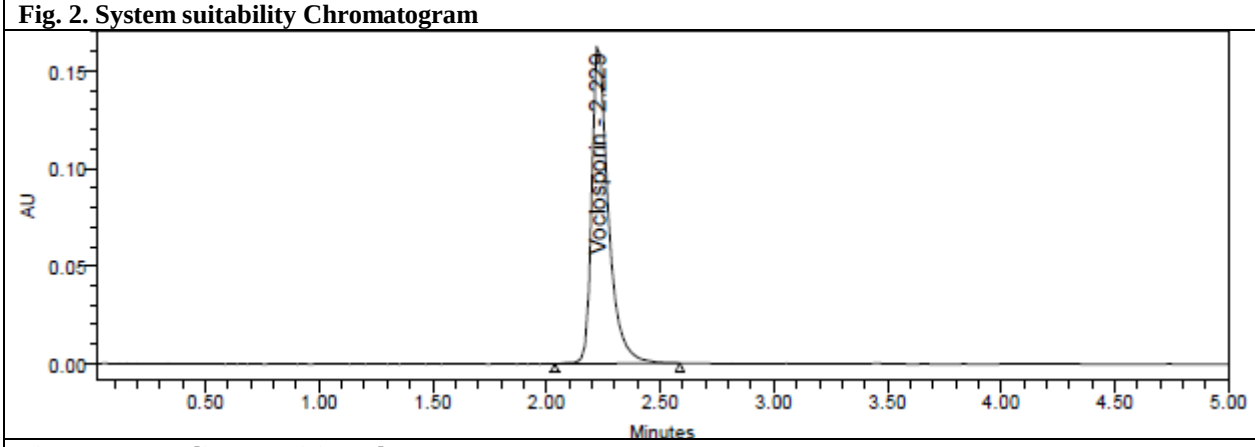


Fig. 3. Intermediate precision Chromatogram

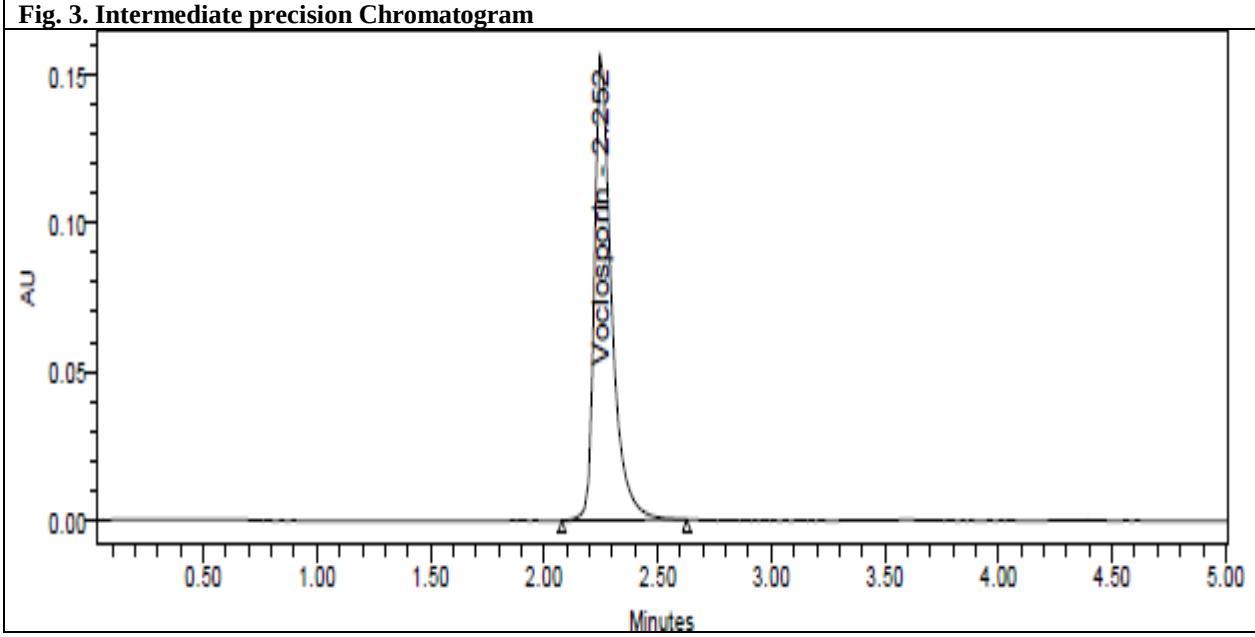


Fig. 4. Linearity 25% Chromatogram

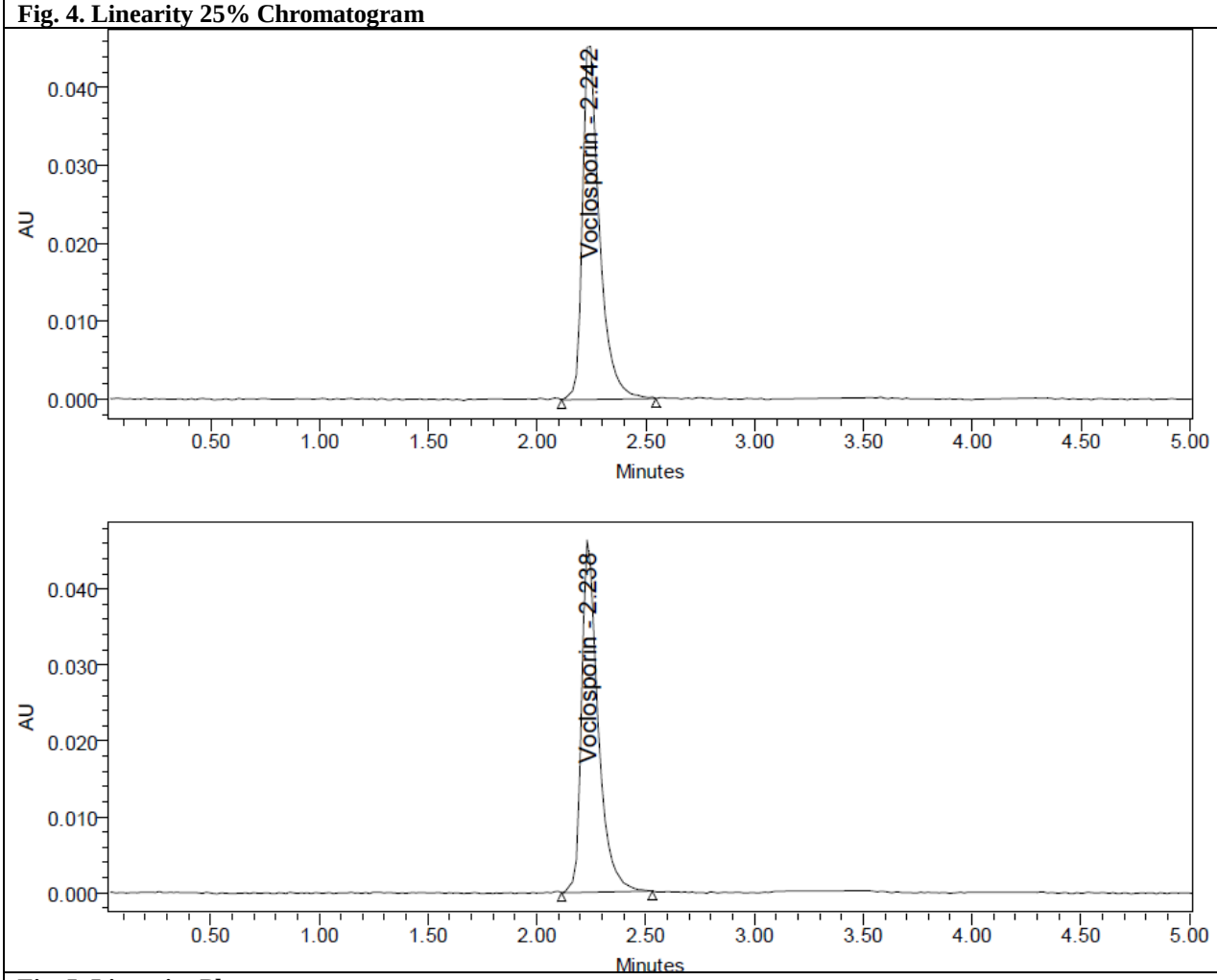


Fig. 5. Linearity Plot

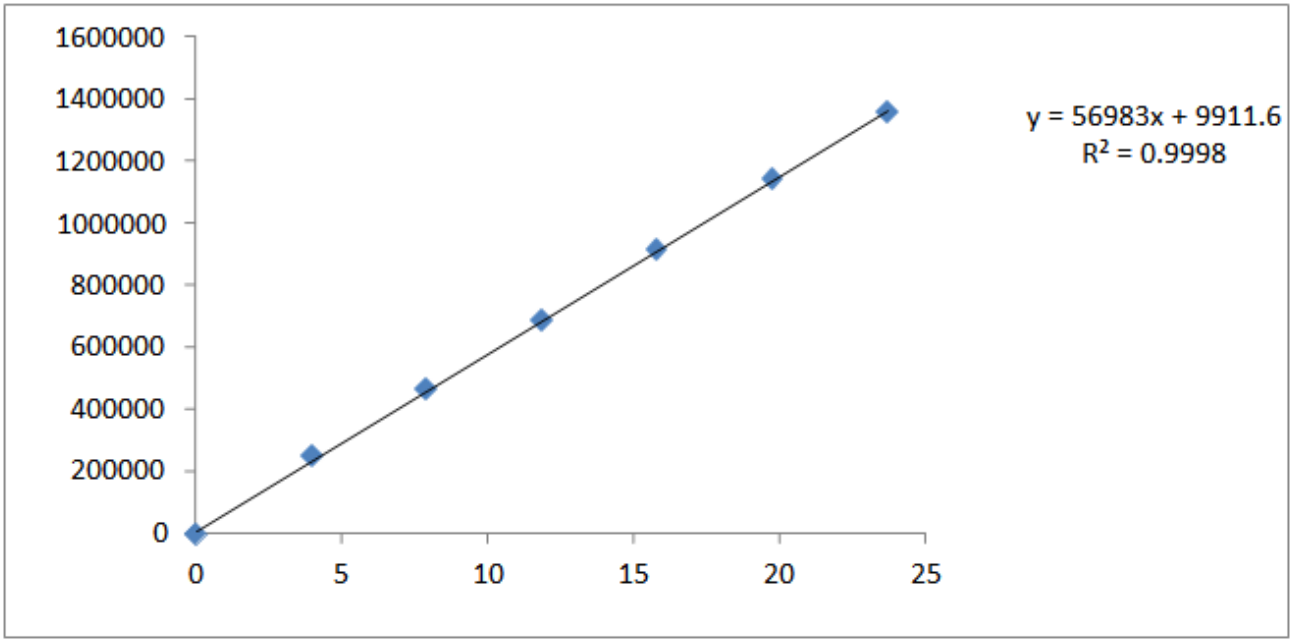


Fig. 6. Accuracy 100% Chromatogram

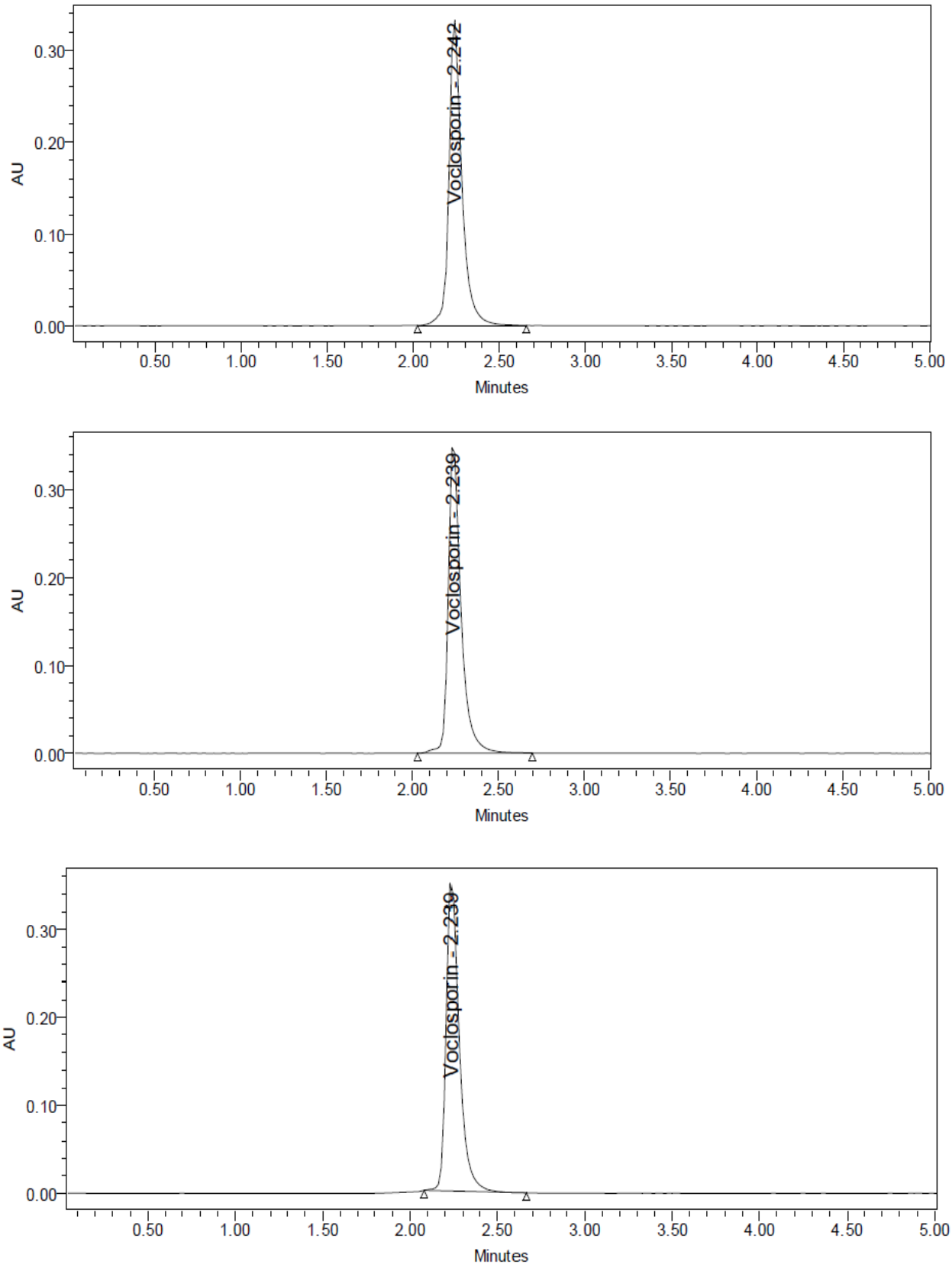


Fig. 7. LOD Chromatogram of Voclosporin

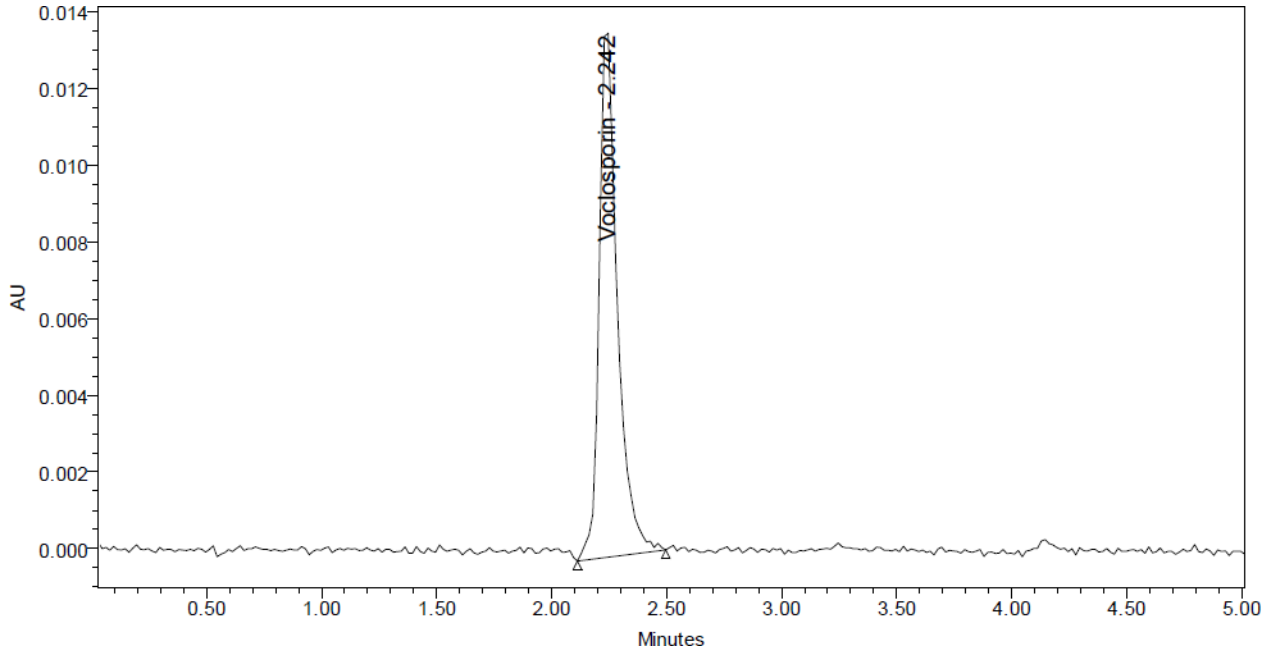
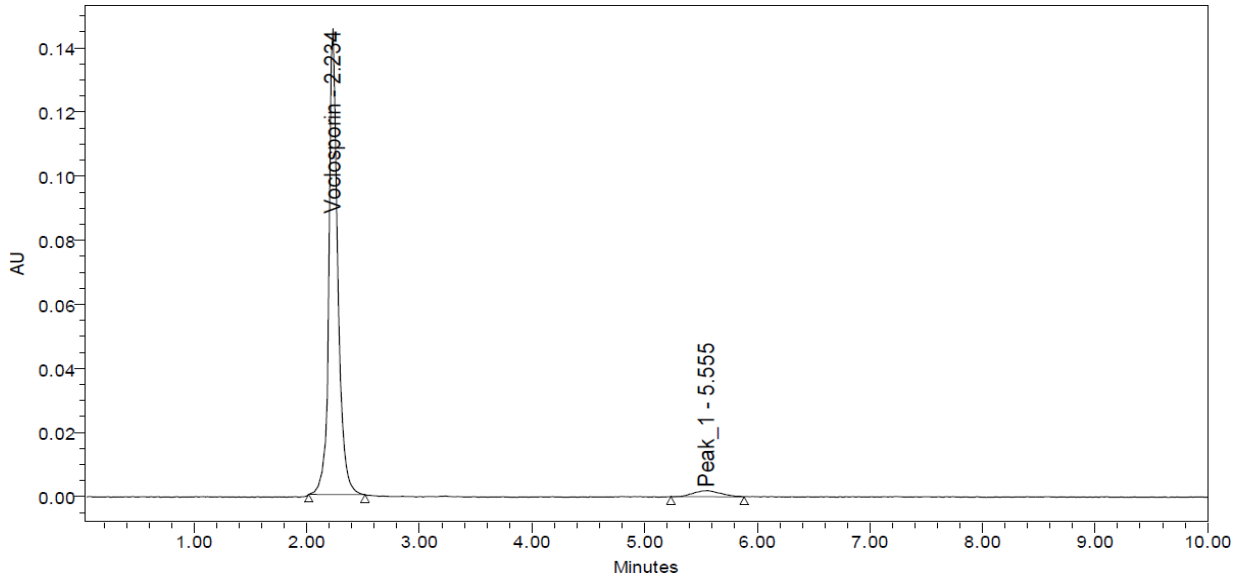


Fig. 8. Acid degradation chromatogram



CONCLUSION

Chromatographic conditions used are stationary phase Kromasil (250mm*4.6mm 5□), Mobile phase0.01N Kh2po4 Buffer: Acetonitrilein the ratio of 60:40 and flow ratewas maintained at 1ml/min, detection wave length was282nm, column temperature was set to30^{o}Cand diluent was mobile phaseConditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between25% to150 % levels, R^2 value was found to be as 0. 999. Precision was found to be 0.6 for repeatability and 0.5 for intermediate precision. LOD and LOQ are 0.053µg/ml and 0.159µg/ml respectively. By using above method assay of marketed formulation was carried out99.25% was present. Degradation studies of Voclosporin were done, in all conditions purity threshold was more than purity angle and within the acceptable range.Full length method was not performed; if it is done this method can be used for routine analysis of Voclosporin.

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