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"Development and Validation of a High-Performance Liquid Chromatography Method for Voclosporin Analysis"

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ABSTRACT
The methodology was set up for synchronous estimation of a Voclosporin by RP-HPLC system. The chromatographic conditions were viably created for the unit of Voclosporin by using Inertsil - ODS C18 (250 x 4.6 mm, 5µ), stream is 1.0 ml/min, convenient stage extent was Methanol: Acetonitrile (45:55), recognizable proof wave length was 273 nm.
Keywords: Voclosporin, RP-HPLC, Acetonitril, Methanol and Water.

I. INTRODUCTION

HPLC is a form of liquid chromatography to quantify and analyze mixtures of chemical compounds. HPLC is subdivided on the basis of separation chemistry. All of these techniques can be used the same instrumentation.

Reverse Phase chromatography- In this type uses a non-polar (hydrophobic) stationary phase and a polar (usually including some water) mobile phase. This is the most common type of HPLC separation in use today.

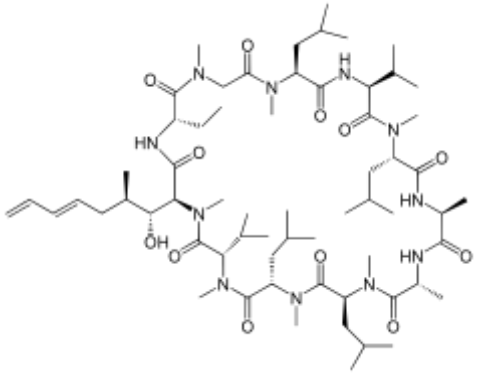
Normal phase chromatography- In this type uses a polar (hydrophilic) stationary phase and a non-polar (usually with no water) mobile phase. This was the type of separation to which the term "Chromatography" was first applied.

Reverse phase chromatography mechanism- The separation mechanism in reverse phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilized hydrophobic ligand, i.e., the stationary phase. Reversed phase chromatography is an adsorptive process by experimentaldesign, which relies on a partitioning mechanism to effect separation. The solute molecules partition (i.e., an equilibrium is established) between the mobile phase and the stationary phase.

Stationary phase- Any inert, non-polar substance that achieves sufficient packing can be used for reversed-phase chromatography. The most popular column is an octadecyl carbon chain (C18) bonded silica (USP classification L1) with 297 columns commercially available. This is followed by C8 bonded silica (L7- 166 columns), pure silica (L3-88 columns), cyano bonded silica (L10-73 columns) and phenyl bonded silica (L11 - 72 columns). Note that C18, C8 and phenyl are dedicated reversed phase packings while cyano columns can be used in a reversed phase mode depending on analyte and mobile phase conditions. It should be noted at this point that not all C18 columns have identical retention properties.

Column mobile phase- Mixtures of water or aqueous buffers and organic solvent are used to elute analytes from a reversed phase column. The solvents have to be miscible with water and the most common organic solvents used are acetonitrile, methanol or tetrahydrofuran (THF). Other solvents can be used such as ethanol, 2-propanol (iso-propyl alcohol). Elution can be performed isocratic (the water solvent composition does not change during the separation process) or by using a gradient (the water-solvent composition does change during the separation process).

DRUG PROFILE
Building:



Formula Chemical: $C_{63}H_{111}N_{11}O_{12}$
Molecular Strength: 1214.646g/mol
IUPAC:(3S,6S,9S,12R,15S,18S,21S,24S,30S,33S)-30-Ethyl-33-[(1R,2R,4E)-1-hydroxy-2-methylhepta-4,6-dien-1-yl]-1,4,7,10,12,15,19,25,28-nonamethyl-6,9,18,24-tetrakis(2-methylpropyl)-3,21-di(propan-2-yl)-1,4,7,10,13,16,19,22,25,28,31-

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undecaazacyclotritriacontane-2,5,8,11,14,17,20,23,26,29,32-undecone

Handling mechanism:

Through the suppression of calcineurin, voclosporin inhibits IL-2 production and T-cell mediated immunological responses, stabilizing podocytes in the kidneys. Voclospoprin is a cyclosporine an analog. It is identical to cyclosporine A (CsA) structurally with the exception of a change of amino acid in one location. This amendment changes voclosporin's binding to calcineurin. Inhibitors of cyclosporine T-lymphocytes reversibly. Lymphocine production and release are likewise inhibited. Cyclosporine A binds with cyclophiline to inhibit T-lymphocytes. A complex is produced of cyclophiline-cyclosporine which inhibits the serine-threonine phosphatase activity of calcineurines reliant upon Calcium and Calmodulins. In addition to calcineurin inhibition, many transcription factors needed for the induction of different cytokine genes such as IL-2, IFN-µ, IL-4 and GM-CSF are inhibited. In return, this decreases inflammation and is related to systemic lupus erythematosus in renal glomerulonephritis.

II. MATERIALS AND METHODS

Instruments-Instruments:

HPLC–Waters Model NO.2690/5 series Compact System Consisting of Inertsil-C18 ODS column.
UV spectrophotometer (Systronics)
Electronic balance (SARTORIOUS)
Sonicator (FAST CLEAN)

Substances containing chemicals:

Methanol HPLCGrade.
Buffer(KH2PO4)Hplc Grade.

Raw Equipment(Unprocessed Materials):

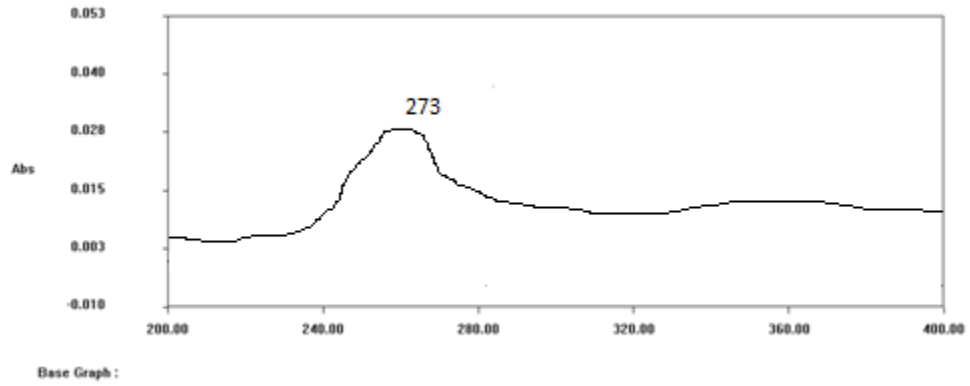
Voclosporin is working standard.

Stock Solution Preparation:Take 100mg Voclosporin working standard in 100ml V.F add methanol sonicate it 30minets,(That is 1000ppm solution).

Further Dilution (or) Trails Solution: Take 10ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minetsthat 100ppm solution)

SELECTION OF WAVE LENGTH:

Scan standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank. Voclosporin shows λmax at 273 nm.



DEVELOPMENT OF AN HPLC METHOD:

The goal of this study was to improve the assay technique for simultaneous quantification of Voclosporin on literature surveys. As a result, the trials detailed below show how the optimization was accomplished.

Trail: 1

Step Mobile: Degassed Methanol: Water55:45.

Chromatographic Conditions:

Flow rate: 1.0ml / min
Column: Inertsil-C18, plate ODS
Wave longitude detector :273 nm
Tempo in the column : Ambient
Size of injection : 20µl
Time to run: 6min
Retention time : 3.145

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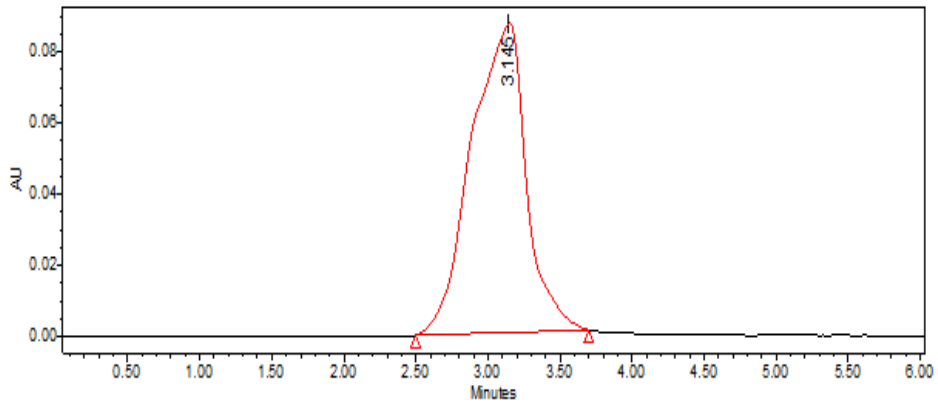

Fig1: Trial 1 chromatogram

Inference: got bold peak.

Trail: 2
Mobile Phase: Degassed Acetonitrile and methanol in the ratio of 30:70 V/V.
Chromatographic Conditions:
Flow rate : 1.0ml / min
Column: Inertsil-C18, plate ODS
Wave longitude detector :273 nm
Tempo in the column: Ambient
Size of injection : 20µl
Time to run : 6min
Retention time: 2.913

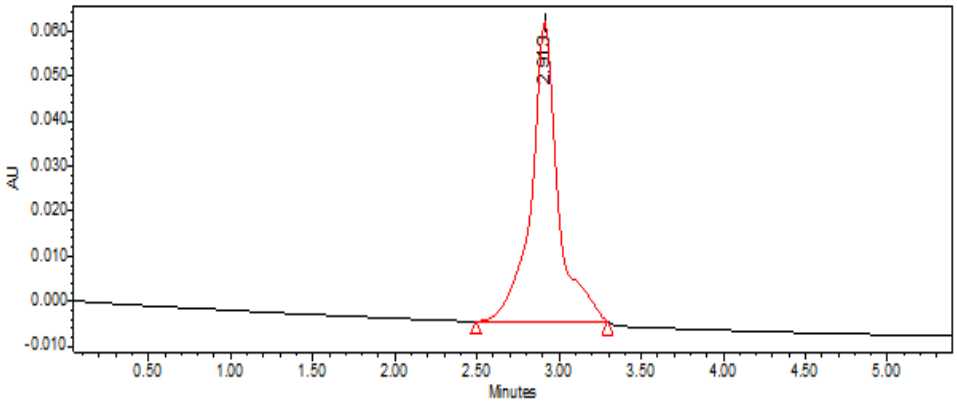

Fig 2: Trial 2 chromatogram:

Inference: Got noise base line.

Trail: 3
Mobile Phase: Degassed Acetonitrile and Water in the ratio of 40:60 V/V.
ChromatographicConditions:
Flow rate : 1.0ml / min
Column : Inertsil-C18, plate ODS
Wave longitude detector :273 nm
Tempo in the column: Ambient
Size of injection : 20µl
Time to run: 6min
Retention time : 3.071

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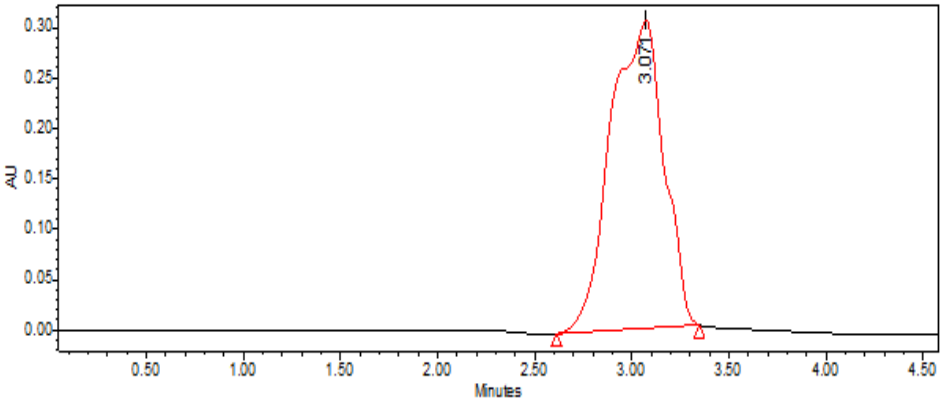

Fig 3: Trial3 chromatogram

Inference : Got peak tailing.

III. RESULTS AND DISCUSSIONS

ADVANCED METHOD (**OPTIMIZED METHOD)**

Mobile Phase:Methanol:Acetonitrile (45:55)V/V. Sonicate it 30minets, Filter this mobile phase through 0.45micron filter paper.

Optimized Method Stock Solution Preparation: Take 100mg Voclosporin working standard in 100ml V.F add methanol sonicate it 30minets, (That is 1000ppm solution).

Further Dilution (or) Optimized Method Solutions Preparation: Take 4ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minets(That 40ppm solution).

Chromatographic conditions:

Parameters	Method
Stationary phase (column)	Inertsil-ODS C_{18}(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol: Acetonitrile (45:55)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	6 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	273 nm
Drug RT (min)	2.921 min

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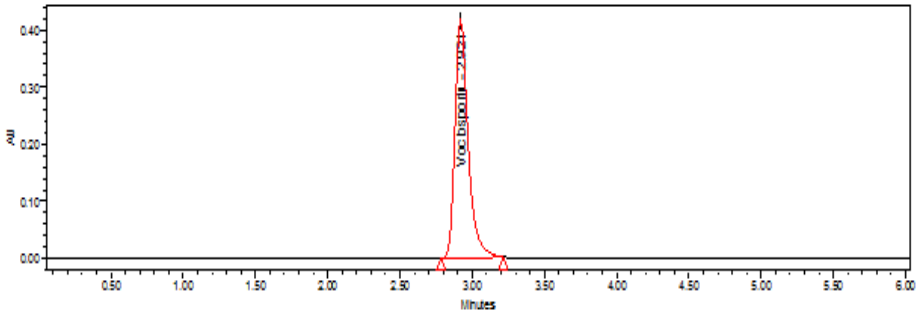

Fig 4: Standard chromatogram

Inference: Got chromatogram at a Rt of 2.921 for standard

S.NO	Name of the peak	Retention time(min)
1	Voclosporin	2.921

VALIDATION DATA

SYSTEM SUITABILITY:

A Standard solution was prepared by using Voclosporin working standard as per test method and was injected Five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Voclosporin, retention times and peak areas.

Validation Stock Solution Preparation:Take 100mg Voclosporin working standard in 100ml V.F add methanol sonicate it 30minets,(That is 1000ppm solution).

Validation Parameters Solutions Preparation: Take 2ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minets(That 20ppm solution).

Take 3ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minets (That 30ppm solution.

Take 4ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minets (That 40ppm solution.

Take 5ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minets (That 50ppm solution).

Take 6ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minets (That 60ppm solution).

Take 7ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minets (That 70ppm solution

TABLE-1: Data of System Suitability

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	2.922	1791232.12	11180	1.058
2	2.920	1791258.52	11156	1.052
3	2.922	1791211.45	11167	1.062
4	2.923	1791288.15	11139	1.060
5	2.921	1791198.69	11150	1.059
Mean	2.922786	1791237.78	11158	1.058
SD	0.00114	36.12691	-------	-------
% RSD	0.0370	0.00201	-------	-------

SPECIFICITY:

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

PRECISION:

Repeatability:

a. System precision: Standard solution prepared as per test method and injected five times.

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b. Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

OBSERVATION:
Test results are showing that the test method is precise. Refer tables 2 and 3 for system precision and for method precision.

Intermediate precision (analyst to analyst variability):
A study was conducted by two analysts as per test method

Repeatability:

(a) System precision

TABLE-2: Data of Repeatability (System precision)

	Injection	**Peak Areas of Voclosporin**	**%Assay**
Concentration 40ppm	1	1791322.45	100.23
	2	1791289.54	100.23
	3	1791270.36	100.23
	4	1791304.55	100.23
	5	1791298.69	100.23
Statistical Analysis	**Mean**	1791297.11	100.23
	SD	19.18875	0.00107
	% RSD	0.00107	0.00107

(b)Method precision:

TABLE-3: Data of Repeatability (Method precision)

	Injection	**Peak Areas of Voclosporin**	**%Assay**
Concentration 40ppm	1	1791284.64	100.23
	2	1791245.86	100.22
	3	1791242.35	100.22
	4	1791269.59	100.23
	5	1791258.63	100.53
	6	1791271.78	100.53
Statistical Analysis	**Mean**	1791262.14	100.33
	SD	16.27073	0.15751
	% RSD	0.000908	0.15699

Intermediate precision:
For**Analyst 1** ref: Table3.

Table4:
Data of Intermediate precision (Analyst 2)

	Injection	**Peak Areas of Voclosporin**	**%Assay**
Concentration 40ppm	1	1791313.13	100.23
	2	1791299.94	100.23
	3	1791288.99	100.23
	4	1791302.85	100.23
	5	1791307.45	100.53
	6	1791291.86	100.53

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Statistical Analysis	**Mean**	1791300.70	100.33
	SD	9.17101	0.15733
	% RSD	0.00051	0.15680

ACCURACY:

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Voclosporin into each volumetric flask for each spike level to get the concentration of Voclosporin equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Voclospori was calculated.

OBSERVATION:

$$\%\text{Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

TABLE-5: Data of Accuracy

Concentration % of spiked level	Area	Amount added (ppm)	**Amount found (ppm)**	**% Recovery**	**Statistical Analysis of % Recovery**	
50% Sample 1	895640.12	20	19.98	99.92	**MEAN**	99.92
50% Sample 2	895630.25	20	19.98	99.92		
50% Sample 3	895648.64	20	19.98	99.92	**%RSD**	0.0010
100 % Sample 1	1791239.99	40	40.09	100.22	**MEAN**	100.22
100 % Sample 2	1791248.31	40	40.09	100.23		
100% Sample 3	1791229.29	40	40.09	100.22	**%RSD**	0.00053
150% Sample 1	2686890.45	60	60.19	100.33	**MEAN**	100.33
150% Sample 2	2686875.22	60	60.19	100.33	**%RSD**	0.00045
150% Sample 3	2686899.63	60	60.19	100.33		

LINEARITY:

A Series of solutions are prepared using Voclosporin working standard at concentration levels from 20ppm to 70 ppm of target concentration.

TABLE6: Data of Linearity

Concentration (ppm)	**Average Area**	**Statistical Analysis**	
0	0	Slope	44543
20	895632.84	y-Intercept	5430
30	1343449.56	Correlation Coefficient	0.999
40	1791265.15		
50	2239081.56		
60	2686898.89		
70	3108214.95		

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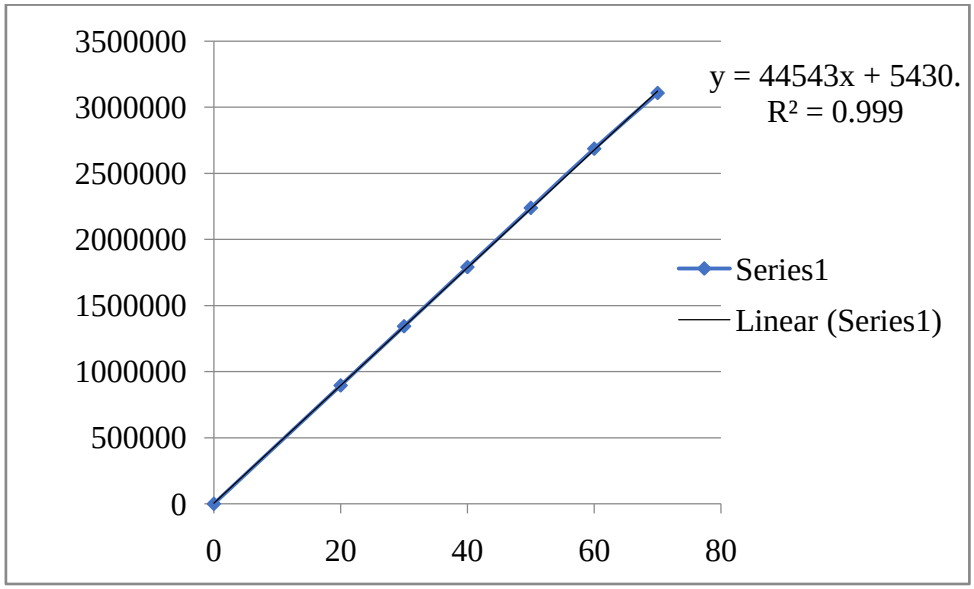

Fig: 41 Linearity Plot (Concentration Vs Response)

Ruggedness:

System to system variability:

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method.

Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability.

System to System variability:

Refer to Table3 for System 1

TABLEMENT: 7
Data on System Variability
System-2

S.NO:	Peak area	Assay % of Voclosporin
1	1791232.85	100.22
2	1791248.53	100.23
3	1791220.65	100.22
4	1791253.85	100.23
5	1791245.42	100.53
6	1791239.27	100.53
Mean	1791240.09	100.33
%RSD	0.00066	0.15747

Robustness:

Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and1.2ml/min. The systemsuitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow.

Voclosporin was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

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TABLE: 10 there's proof that flux rate variability has an impact

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	1781414.51	1.109		1791259.45	1.113		1801023.23	1.126
	1781444.19	1.113		1791268.45	1.115		1801054.56	1.128
	1781425.98	1.115		1791242.42	1.113		1801038.39	1.127
	1781433.89	1.119		1791272.35	1.114		1801069.25	1.127
	1781415.61	1.121		1791239.45	1.115		1801046.46	1.126
Avg	1781426.83	1.115	Avg	1791256.42	1.114	Avg	1801046.37	1.127
SD	12.54597	0.0047	SD	14.9302	0.001	SD	17.24614	0.0008
%RSD	0.000704	0.4280	%RSD	0.00083	0.0879	%RSD	0.00095	0.0742

LOD AND LOQ (LIMIT OF DETECTION AND LIMIT OF QUANTITATION):

From the linearity plot the LOD and LOQ are calculated:

$$LOD = \frac{3.3\,\sigma}{S}$$

$$= \frac{3.3 \times 36.12691}{44543} = 0.0026$$

$$LOQ = \frac{10\,\sigma}{S}$$

$$= \frac{10 \times 36.12691}{44543} = 0.0081$$

Market Sample:

Drug name	**Brand name**	**Company**
Voclosporin	Psorid-100	Biocon

$$\% \text{ Assay} = \frac{\text{Amount found}(x)}{\text{Amount added}} \times 100$$

X=y-c/m

Injection	**Peak Areas of Voclosporin**	**%Assay**
1	1791431.42	100.24
2	1791286.04	100.23
3	1791537.27	100.25
4	1791648.04	100.59
5	1791854.34	100.26
Mean	1791551.422	100.314
SD	215.7097	0.154693
% RSD	0.01204	0.154209

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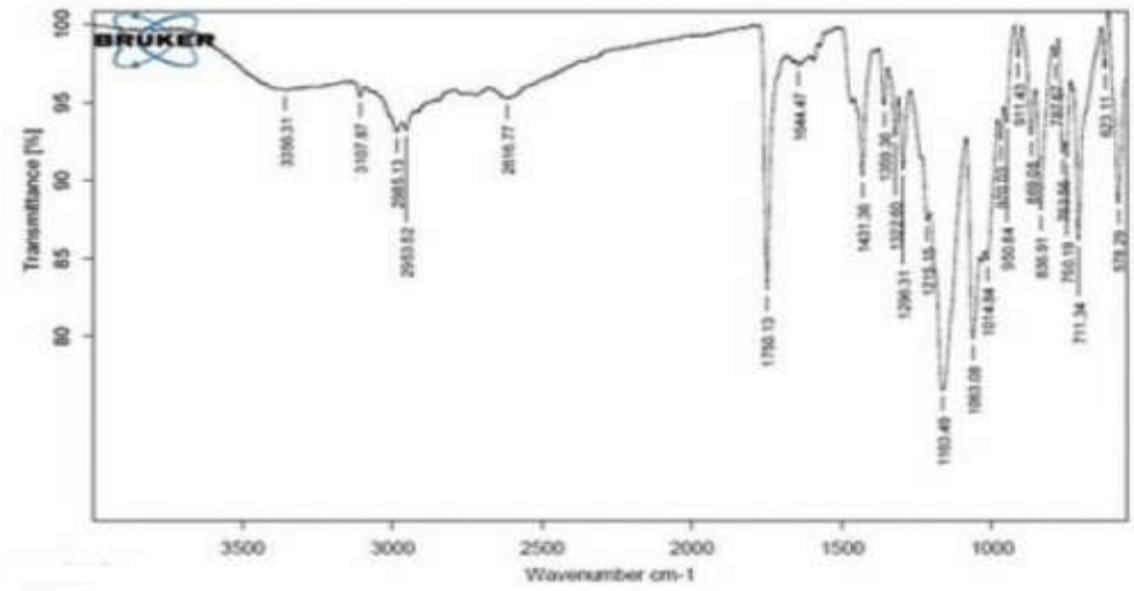
FTIR:-



FTIR Spectra for Voclosporin

IV. SUMMARY AND CONCLUSION:

Different parameters were studied to create the analytical approach. For starters, the maximum absorbance of Voclosporin was discovered to be 273 nm. The injection volume was set at 20µl, which resulted in a nice peak area. The Inertsil C18 column was employed in this work, and ODS picked a nice peak shape. The temperature of the ambient environment was determined to be adequate for the type of the medication solution. Because of the good peak area, adequate retention duration, and good resolution, the flow rate was set at 1.0ml/min. Different mobile phase ratios were investigated, however the mobile phase with a Methanol: Acetonitrile (45:55) ratio was chosen because to its symmetrical peaks and high resolution. As a result, the planned research made use of this mobile phase.

The accuracy of both the system and the procedure was determined to be precise and well within range. The correlation coefficient and curve fitting were discovered during the linearity investigation. For both medicines, the analytical approach was shown to be linear throughout a range of 20-70ppm of the target concentration. Both robustness and ruggedness tests were passed by the analytical. The relative standard deviation in both circumstances was excellent.

REFERENCES

[1]. V. Gupta, A.D. K. Jain, N.S. Gill, K. Gupta, Development and validation of HPLC method - a review , Int. Res J Pharm. App Sci., (2012);2(4) 17-25

[2]. Y. Kazakevich, R. Lobrutto, HPLC for Pharmaceutical Scientists, John Wiley & Sons, New Jersey, 2007.

[3]. S. Ahuja, H. Rasmussen, Development for Pharmaceuticals,Separation Science and Technology, Elsevier, New York [2007] Vol.8

[4]. M.S. Azim, M. Mitra, P.S. Bhasin, HPLC method development and validation: A review, Int. Res. J. Pharm. (2013);4(4):39-46.

[5]. B.V. Rao, G.N. Sowjanya1, A. Ajitha, V.U.M. Rao, Review on stability indicating hplc method development, World Journal of Pharmacy and Pharmaceutical Sciences, (2015);4(8)405-423.

[6]. M.S. Charde, A.S. Welankiwar, J. Kumar, Method development by liquid chromatography with validation, International Journal of Pharmaceutical Chemistry, (2014);04(02): 57-61.

[7]. S. Sood, R. Bala, N.S. Gill, Method development and validation using HPLC technique – A review, Journal of Drug

Discovery and Therapeutics, 2014; 2(22): 18-24.
[8]. M.W. Dong, Modern Hplc for practicing scientists, John Wiley & Sons, New Jersey, 2006.
[9]. P.K. Singh, M. Pande, L.K. Singh , R.B. Tripathi, steps to be considered during method development and validation for analysis of residual solvents by gas chromatography, Int. Res J Pharm. App Sci., (2013); 3(5):74-80.
[10]. B. Prathap, G.H.S. Rao, G. Devdass, A. Dey, N. Harikrishnan , Review on Stability Indicating HPLC Method Development, International Journal of Innovative Pharmaceutical Research, (2012); 3(3): 229-237.
[11]. B. Sriguru, N.P. Nandha, A.S.Vairale, A.V. Sherikar, V. Nalamothu, Development and validation of stability indicating HPLC method for the estimation of 5-Fluorouracil and related substances in topical formulation, Int. J. Res. Pharm. Sci. (2010) ; 1(2): 78-85.
[12]. C.K. Kaushal, B. Srivastava, A process of method development: A chromatographic approach, J. Chem. Pharm. Res. (2010) ; 2(2): 519-545.
[13]. N.Toomula, A. Kumar, S.D.Kumar, V.S. Bheemidi, Development and Validation of Analytical Methods for Pharmaceuticals, J Anal Bioanal Techniques. (2011); 2(5): 1-4.
[14]. K. Kardani, N. Gurav, B. Solanki, P. Patel, B. Patel, RP-HPLC Method Development and Validation of Gallic acid inPolyherbal Tablet Formulation, Journal of Applied Pharmaceutical Science. (2013); 3(5): 37-42.
[15]. B. Nigovic, A. Mornar, M. Sertic, Chromatography – The Most Versatile Method of Chemical Analysis, Intech (2012) 385-425.
[16]. T. Bhagyasree, N. Injeti, A. Azhakesan, U.M.V. Rao, A review on analytical method development and validation,International Journal of Pharmaceutical Research & Analysis, Vol (2014); 4(8): 444-448.
[17]. A,Shrivastava, V.B. Gupta, HPLC: Isocratic or Gradient Elution and Assessment of Linearity in Analytical Methods, J Adv Scient Res, (212); 3(2); 12-20.
[18]. V. Kumar, R. Bharadwaj, G.G., S. Kumar, An Overview on HPLC Method Development, Optimization and Validation process for drug analysis, The Pharmaceutical and Chemical Journal,(2015); 2(2) ; 30-40.
[19]. Validation of Analytical Procedures: Text and Methodology, International Conferences on Harmonization, Draft Revised (2005), Q2 (R1).
[20]. Validation of Compendial Procedures, United State Pharmacopeia, USP 36 NF, (2010) 27(2).
[21]. https://www.researchgate.net/publication/263919817_HPLC_method_development_and_validation_-_An_overview
[22]. https://en.wikipedia.org/wiki/Voclosporin
[23]. https://go.drugbank.com/drugs/DB11693