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DEVELOPMENT AND VALIDATION OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR VOCLOSPORIN ANALYSIS

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ABSTRACT

A methodology was established for the synchronous estimation of Voclosporin using an RP-HPLC system. The chromatographic conditions were effectively developed for Voclosporin quantification utilizing an Inertsil ODS C18 column (250 x 4.6 mm, 5µ). The flow rate was set to 1.0 ml/min, with a mobile phase composition of Methanol: Acetonitrile (45:55). The detection wavelength was set at 273 nm.

Keywords: Voclosporin, RP-HPLC, Acetonitril, Methanol and Water.

INTRODUCTION

High-Performance Liquid Chromatography (HPLC) is a versatile technique used to quantify and analyze chemical compound mixtures. HPLC employs various separation chemistries, all utilizing similar instrumentation.

Reverse Phase Chromatography: Reverse phase chromatography employs a non-polar (hydrophobic) stationary phase and a polar (often containing water) mobile phase. This method is the most widely used in modern HPLC separations.

Normal Phase Chromatography: In contrast, normal phase chromatography uses a polar (hydrophilic) stationary phase and a non-polar (typically water-free) mobile phase, historically the original form of chromatography.

Mechanism of Reverse Phase Chromatography: In reverse phase chromatography, separation hinges on hydrophobic interactions between the solute molecules in the mobile phase and the immobilized hydrophobic ligand of the stationary phase. It operates through an adsorptive process where solute molecules partition between the mobile and stationary phases.

Stationary Phase: For reversed-phase chromatography, any inert, non-polar material that packs adequately can serve as the stationary phase. The most prevalent is octadecyl carbon chain (C18) bonded silica, followed by C8 bonded silica, pure silica, cyano bonded silica, and phenyl bonded silica columns, each offering varying column counts and applications.

Mobile Phase Composition: Mixtures of water or aqueous buffers with organic solvents like acetonitrile, methanol, or tetrahydrofuran (THF) are employed to elute analytes from a reversed-phase column. These solvents must be water-miscible, with acetonitrile and

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methanol being the most common choices. Elution can occur isocratically (constant water-solvent composition) or via gradient (changing water-solvent composition).

This introduction outlines the fundamental principles and operational aspects of HPLC, highlighting the versatility and critical parameters involved in chromatographic separations.

DRUG PROFILE

Building:

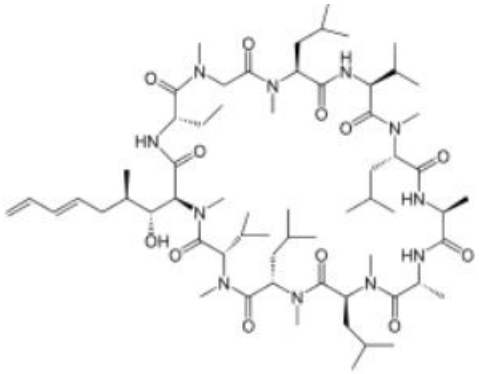

Figure 1: Structure of Voclosporin

Handling Mechanism:

Voclosporin suppresses IL-2 production and T-cell mediated immune responses by inhibiting calcineurin, thereby stabilizing podocytes in the kidneys. Structurally analogous to cyclosporine A (CsA), voclosporin differs by a single amino acid substitution that alters its binding affinity to calcineurin. Similar to cyclosporine A, voclosporin reversibly inhibits T-lymphocytes by forming a complex with cyclophilin, which blocks the serine-threonine phosphatase activity of calcineurin dependent on calcium and calmodulin.

In addition to calcineurin inhibition, voclosporin also inhibits several transcription factors necessary for inducing various cytokine genes such as IL-2, IFN-μ, IL-4, and GM-CSF. This dual action reduces inflammation associated with conditions like systemic lupus erythematosus and renal glomerulonephritis.

MATERIALS AND METHODS

Instruments:

- HPLC: Waters Model NO.2690/5 series Compact System equipped with an Inertsil-C18 ODS column.
- UV spectrophotometer: Systronics model.
- Electronic balance: SARTORIOUS.
- Sonicator: FAST CLEAN.

Chemicals and Reagents:

- Methanol, HPLC Grade.
- Buffer (KH_2PO_4), HPLC Grade.

Raw Materials:

- Voclosporin as the working standard.

Stock Solution Preparation:

- Prepare a 1000 ppm solution of Voclosporin by dissolving 100 mg of the working standard in 100 ml of volumetric flask (V.F.) using methanol. Sonicate for 30 minutes.

Further Dilution (Trail Solution):

- Take 10 ml of the above stock solution and dilute it to 100 ml with methanol in a volumetric flask (V.F.). Sonicate for 10 minutes to obtain a 100 ppm solution.

Selection of Wavelength:

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- Scan the standard solution of Voclosporin in the UV spectrophotometer from 200 nm to 400 nm in spectrum mode, using the diluent as a blank.
- Voclosporin exhibits maximum absorbance (λmax) at 273 nm.

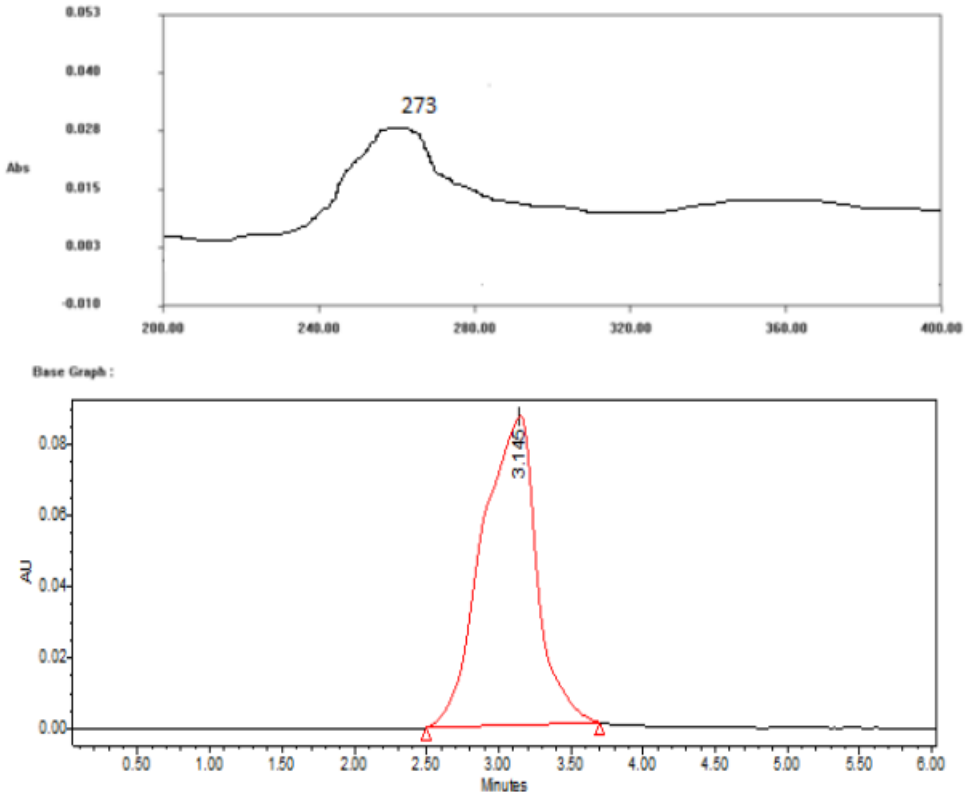

Fig2: Trial 1 chromatogram

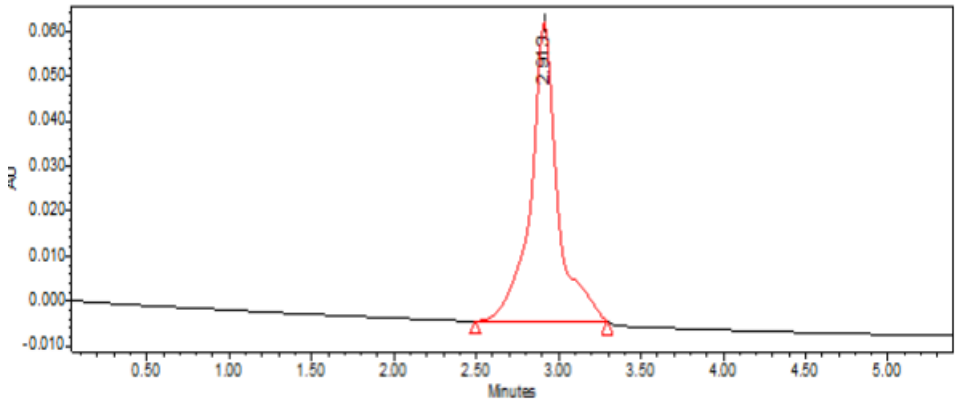

Fig 3: Trial 2 chromatogram:

Chromatographic conditions:

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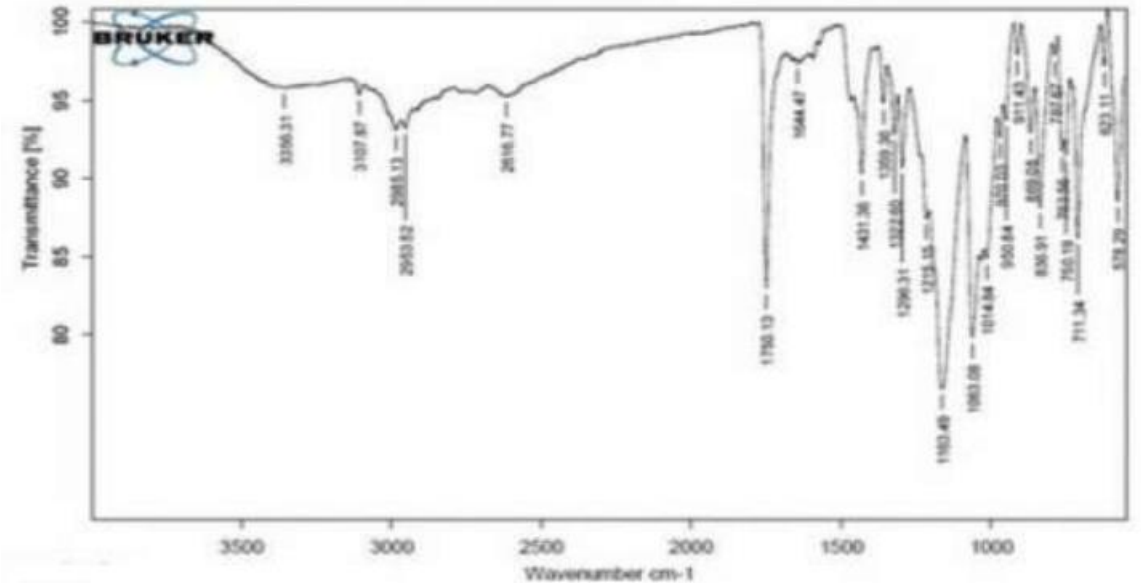
Parameters	Method
Stationary phase (column)	Inertsil-ODS C_{18}(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol: Acetonitrile (45:55)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	6 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	273 nm
Drug RT (min)	2.921 min

FTIR:-



FTIR Spectra for Voclosporin

CONCLUSION:

Various parameters were meticulously examined to establish the analytical method for Voclosporin. The maximum absorbance of Voclosporin was identified at 273 nm. An injection volume of 20 µl yielded a well-defined peak area. The use of an Inertsil C18 column provided optimal peak shape, and the ambient temperature was suitable for the medication solution. With satisfactory peak area, retention time, and resolution, a flow rate of 1.0 ml/min was selected. Several mobile phase ratios were evaluated, with the Methanol (45:55) ratio chosen for its symmetrical peaks and high resolution, which guided the study design. The accuracy of both the system and the procedure was confirmed to be precise and within acceptable limits. Linearity investigations revealed high correlation coefficients and well-fitted curves across a concentration range of 20-70 ppm for both medications. The analytical method passed robustness and ruggedness tests, showing excellent relative standard deviations in both scenarios.

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