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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION BY NEW RP-UPLC METHOD FOR THE DETERMINATION OF VOXILAPREVIR IN TABLET DOSAGE FORM

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ABSTRACT

A simple accurate, precise rapid isocratic RP-UPLC method development for the simultaneous estimation of Voxilaprevir in tablet dosage form. The chromatographic system was carried on Acquity BEH C18 (50*3.0mm. 1.7µm) using mobile phase consisting a mixture of 60 volumes of Methanol of 20 volumes of 0.1% Orthophosphoric acid, 20 volumes of Acetonitrile with detection of 245 nm. The retention time of Voxilaprevir was found to be 1.328 min calibration curve was linear over the concentration range of Voxilaprevir, the correlation coefficient for both peaks were found to be 0.998 respectively. All the analytical validation parameters were determined and found in the limit as per ICH guidelines.

Keywords: Voxilaprevir, RP-UPLC.

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INTRODUCTION

Chromatography is a non-destructive procedure for resolving a multi-component mixture of traces, minor or constituents in to individual fractions. It is a method of separating a mixture of components in to individual components through a porous medium under the influence of solvent [1-3]. UPLC refers to Ultra Performance Liquid Chromatography. UPLC brings dramatic improvements in sensitivity, resolution and speed of analysis can be calculated. It has instrumentation that operates at high pressure than that used in HPLC & in this system uses fine particles (less than 2.5µm) & mobile phases at high linear velocities decreases the length of column, reduces solvent consumption & saves time.

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According to the van Deemter equation, as the particle size decreases to less than2.5 µm, there is a significant gain in efficiency, while the efficiency does not diminish at increased flow rates or linear velocities [4-6]

Review of Literature

MD. Abdul Sattar, A. Suneetha. RP-HPLC Method Development and Validation for Velpatasvir and Voxilaprevir by Simultaneous Determination in Bulk and Their Pharmaceutical Dosage Forms [7].

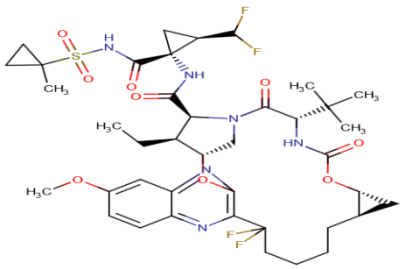
Marakada Sridevi, T. Siva Rao and Challa Gangu Naidu. Development and validation of liquid chromatographic method for simultaneous determination of Sofosbuvir, Velpatasvir and Voxilaprevir in in fixed tablet dosage form [8].

J. Sandya Rani and N. Devanna. Development and validation of RP-HPLC for simultaneous estimation of Sofosbuvir, Velpatasvir and Voxilaprevir in in bulk and tablet dosage form [9].

MD. Abdul Sattar, A. Suneetha. RP-HPLC Metthod Development and Validation for Velpatasvir and Voxilaprevir by Simultaneous Determination in Bulk and Their Pharmaceutical Dosage Forms [10]

Drug profile

Voxilaprevir is a Direct-Acting Antiviral (DAA) medication used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with Hepatitis C Virus (HCV) [11-14].



Structure for Voxilaprevir

MATERIALS & METHODS

Table 1. Instrumentation

UV-Visible Spectrophotometer	Thermo scientific
UPLC	Agilent 1290 Infinity with PDA
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Thermo scientific
Electronic balance	Shimadzu
Column	Acquity BEH C18 (50*3.0mm. 1.7µm)

Table 2. Reagents And Chemicals

Potassium Dihydrogen ortho phosphate Dipotassium hydrogen orthophosphate	Rankem/ AR Grade
Acetonitrile	Merck/ HPLC Grade
Water	Merck/ HPLC Grade
Methanol	Merck/ HPLC Grade
O-Phosphoric acid	Rankem/ AR Grade

Working/Reference Standards

Voxilaprevir Gift samples obtained from Madras pharmaceuticals, Chennai

MATERIALS & METHODS

Preparation of Standard Solution of Voxilaprevir

Weighed about 10 mg of VOXILAPREVIR & transferred in to a 100mL volumetric flask, then added 70mL of diluent, sonicated for 3min. Made final volume up to mark with the diluents &mixed well (100µg/ml). Taken 5mL of standard stock solution and transferred in to 50mL volumetric flask then diluted up to mark with diluents & mixed well (10µg/ml).

Preparation of Sample Solution of Voxilaprevir

Sample name: VOXILAPREVIR

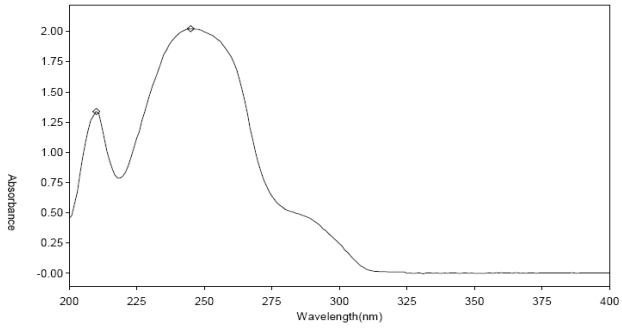
Weigh 10 Tablets then crush with mortar and pestle then weigh a quantity of powder equivalent to 10 mg of VOXILAPREVIR & transferred in to a 100mL volumetric flask, then added 70mL of diluent, sonicated for 3min. Made final volume up to mark with the diluents &mixed well (100µg/ml). Taken 5mL of standard stock solution and transferred in to 50mL volumetric flask then diluted up to mark with diluents & mixed well (10µg/ml)

Table 3. Chromatographic Conditions

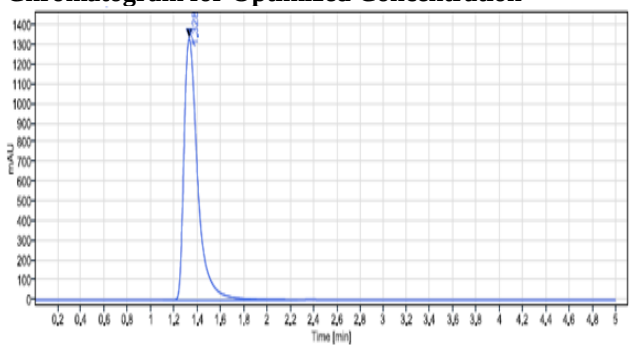
Mobile phase	Methanol:0.1% Orthophosphoric acid: Acetonitrile (60:20:20) v/v
Column	Acquity BEH C18 (50*3.0mm. 1.7µm)
Flow rate	0.5mL/min
Column temperature	Room temperature (20-25°C)
Sample temperature	Room temperature (20-25°C)
Wavelength	245 nm
Injection volume	10 µL
Run time	5 min

RESULT AND DISCUSSION

Chromatogram for determination of working wavelength

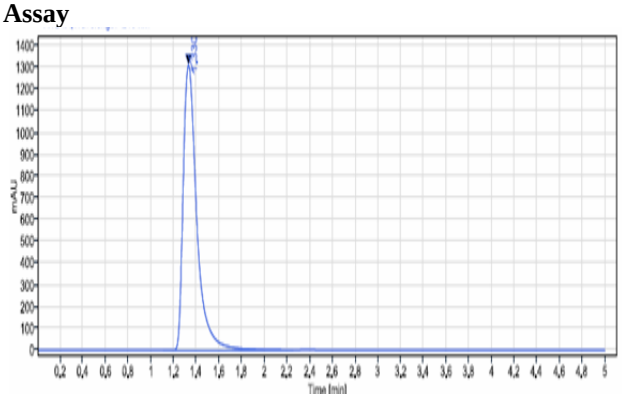


Chromatogram for Optimized Concentration



S. No.	Name	Rt (min)	Peak Area	Theoretical Plates	Tailing Factor	Resolution
1	Voxilaprevir	1.328	10941.77	5825	1.35	-

Assay



Chromatogram of Assay sample preparation

Assay Results

VOXILAPREVIR		
	Standard Area	**Sample Area**
Injection-1	10943.45	10888.68
Injection-2	10978.27	10915.4
Injection-3	10952.19	10914.46
Injection-4	10945.54	10933.13
Injection-5	10941.57	10939.09
Average Area	10952.20	10918.15
Standard deviatuion	15.11	
%RSD	0.14	
Assay(%purity)	99.69	

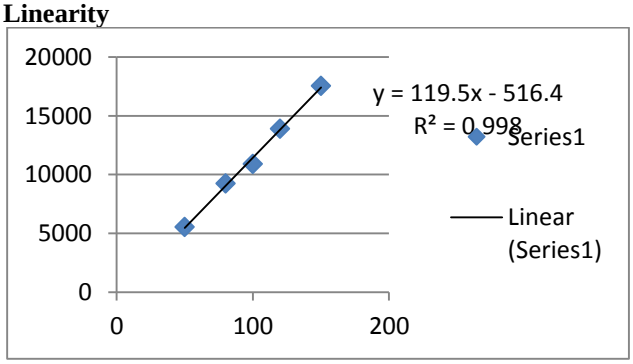
Accuracy

% Recovery Level	Area	Concentration Added	Concentration Recovered	%Recovery	Average
50%_01	7004575	250	252.18	100.9	100.5
50%_02	7020900	250	252.77	101.1	
50%_03	7002470	250	252.11	100.8	
100%_01	13910853	500	500.83	100.2	
100%_02	13902676	500	500.53	100.1	
100%_03	13701006	500	493.27	98.7	
150%_01	21010188	750	756.42	100.9	
150%_02	21026894	750	757.02	100.9	
150%_03	21021825	750	756.84	100.9	

Method Precision

METHOD PRECISION		
S.No.	**RT**	**AREA**
1	1.329	10952.11
2	1.329	10949.92
3	1.33	10953.23
4	1.329	10937.69
5	1.331	10937.81
6	1.33	10948.59
AVG	1.3297	10946.5583
SD	0.0008	7.0134
%RSD	0.061	0.064

Linearity



ROBUSTNESS

Result of Robustness Study

Chromatographic changes		Retention time(min)	Tailing Factor	Theoretical Plates
Flow rate (mL/min)	0.8	1.520	1.38	5827
	1.2	1.185	1.31	5830
Temperature (°C)	35	1.335	1.32	5890
	45	1.336	1.35	5812

Ruggedness

VOXILAPREVIR	**%Assay**
Analyst 01	99.51
Analyst 02	99.69
%RSD	0.28

DISCUSSION

Assay

The amount of Voxilaprevir present in the taken dosage form was found to be 99.69 % respectively.

Accuracy

The percentage mean recovery of Voxilaprevir is 100.50% respectively.

System Suitability
The % RSD for the retention times and peak area of Voxilaprevir were found to be less than 2%.

Linearity and Range
The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Voxilaprevir is 0.998.

Precision
Test results for Voxilaprevir are showing that the %RSD of Assay results are within limits.

Robustness
The system suitability parameters were within limit at all variable conditions.

Ruggedness
The %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

CONCLUSION

The validated method is found to be Specific, Linear, Precise, Accurate, Robust and Rugged for the estimation of Voxilaprevir in tablet dosage form. Hence it is concluded that the assay method is found to be valid in terms of reliability, precision, accuracy and specificity for routine analysis as well as for stability analysis.

ACKNOWLEDGEMENT
Nil

CONFLICT OF INTEREST
No interest

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