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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION BY NEW UPLC METHOD FOR THE DETERMINATION OF TOLCAPONE 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 volmes 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 peak was found to be 0.998 respectively. All the analytical validation parameters were determined and found in the limit as per ICH guidelines.

Keywords: Tolcapone, 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 [4]. 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 [5].

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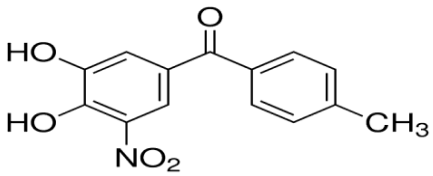
According to the van Demeter equation, as the particle size decreases to less than2.5 µm, there is a significant gain in efficiency, while the efficiency does not diminish at increased flow rates or linear velocities [6].

Therefore by using smaller particles, speed and peak capacity (number of peaks resolved per unit time in gradient separations) can be extended to new limits, termed Ultra Performance Liquid Chromatography or UPLC [7]. The technology takes full advantage of chromatographic principles to run separations. Using columns packed with smaller particles(less than2.5µm) and/or higher flow rates for increased speed, this gives superior resolution and sensitivity [8].

Review of Literature

Mohammad Hazara Begum A simple and selective LC method is described for the determination of Tolcapone tablet dosage forms [9].

Krishna and Shyamala A new method was established for simultaneous estimation of Tolcapone by RP-HPLC method [10].



Structure for Tolcapone

MATERIALS & METHODS

Table 1. Instrumentation

UV-Visible	Nicolet evolution 100
UV-Visible	Vision Pro
UPLC	Open lab EZ chrome
UPLC	Agilent Technologies
Ultra sonicator	Citizen, Digital Ultrasonic
pH meter	Global digital
Electronic balance	Mettler Toledo
UPLC Column	Zorbax SB Cyano

Table 2. Reagents and Chemicals

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Dihydrogen	AR Grade
Acetonitrile	HPLC Grade
Dipotassium hydrogen	AR Grade
Orthophosphoric acid	HPLC Grade

Working/Reference Standards

Tolcapone Gift samples obtained from Chandra Labs, Hyderabad.

MATERIALS & METHODS

Preparation of Standard Solution of Tolcapone

Accurately weighed about100mg of Tolcapone and transferred in to100ml of volumetric flask and added 70mL of diluents (Mobile phase used as diluents) and sonicated for 5min and diluted up to the mark with diluent (1000µg/mL)

Then Pipette out 5ml of this solution into 50ml volumetric flask and diluted volume up to the mark with same diluents [11, 12].

Preparation of Sample Solution of Tolcapone

Sample name: **TOLCAPONE**

20 Tablets were weighed and Crushed in motor and pestle and the fine powder of equivalent to 100mg of Tolcapone sample into a 100ml clean volumetric flask added about 70mL of diluents and sonicated up to 20 min for completely dissolved and diluted up to the mark with diluent & mixed well. The prepared solution was filtered through 0.45µ PVDF syringe filter.

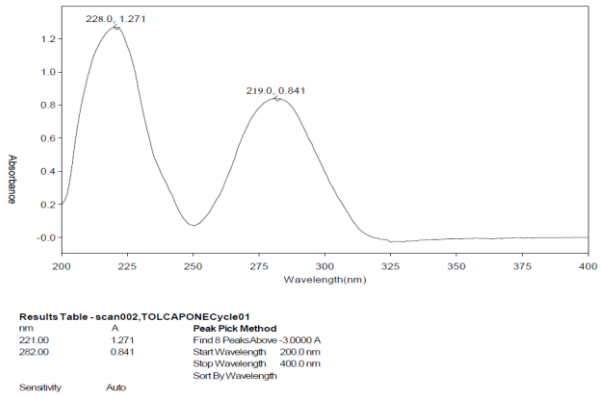
Pipette out 5ml of the above solution into 50ml volumetric flask and diluted volume up to the mark with same diluent.

CHROMATOGRAPHIC CONDITIONS

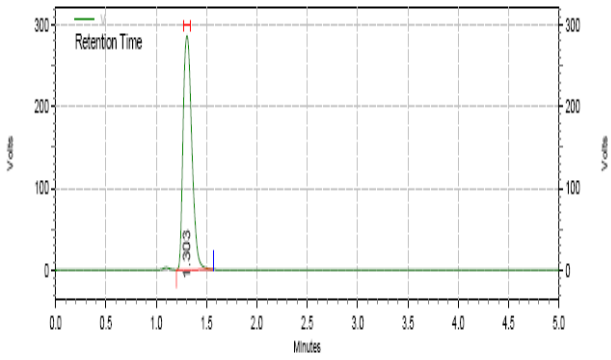
Column	Zorbax SB Cyano (50x2.0mm) 1.5µm
Flow rate	0.5mL /min
Mobile Phase	Ammonium acetate buffer pH 3.5: Acetonitrile (60:40)
Wavelength	230
Injection volume	10µL

RESULT AND DISCUSSION

Chromatogram for determination of working wavelength

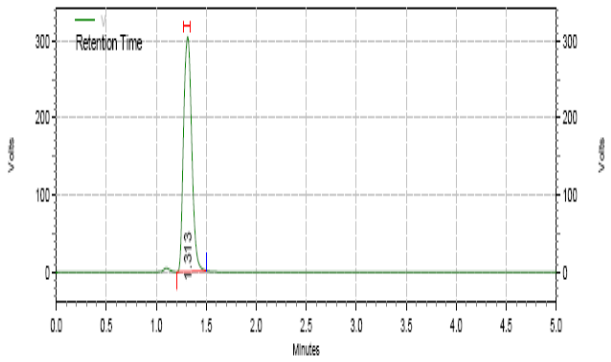


Chromatogram For Optimized Concentration



S.No.	Name	Rt (min)	Peak Area	Theoritical Plates	Tailing Factor	Resolution
1	TOLCAPONE	1.303	29015682	3040	1.4	-

Assay



Chromatogram of assay sample preparation

Table 3.Assay results

Tolcapone		
	Standard Area	**Sample Area**
Injection-1	29015682	29250675
Injection-2	28741403	29330439
Injection-3	28992855	29372959
Injection-4	28917696	29278538
Injection-5	29052205	29116653
Average Area	28943968.2	29269852.8
Standard deviation	110602.3	
%RSD	0.38	
Assay(%purity)	101.12	

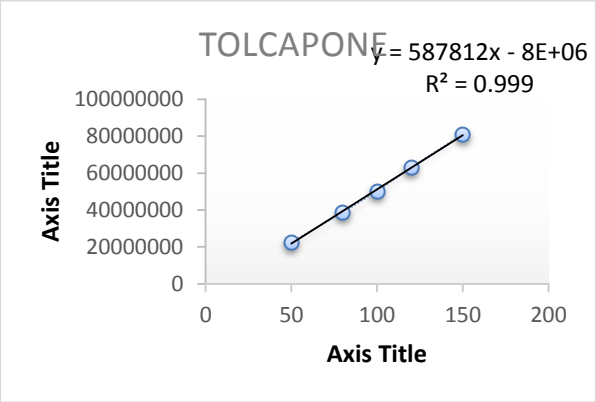
Table 4. Accuracy

Name of the Sample	**Standard Weight in mg**	**Area**	**Conc Added (µg/ml)**	**Conc Recovered (µg/ml)**	**%Recovery**	**Average**
50% Recovery_01	50	14336848	50	49.59	99.2	100.0
50% Recovery_02	50	14307397	50	49.49	99.0	
50% Recovery_03	50	14317645	50	49.52	99.0	
100% Recovery_01	100	29427560	100	101.78	101.8	
100% Recovery_02	100	29390329	100	101.65	101.7	
100% Recovery_03	100	29428703	100	101.79	101.8	
150% Recovery_01	150	43087855	150	149.03	99.4	
150% Recovery_02	150	43053777	150	148.91	99.3	
150% Recovery_03	150	43028928	150	148.83	99.2	

Table 5. Method Precision

Injection	**TOLCAPONE**	
	Area	**%Assay**
1	29367373	100.6
2	29359122	100.5
3	29351924	100.5
4	29299288	100.4
5	29327705	99.9
6	29368338	100.7
Average		100.4
SD		0.27
%RSD		0.3

Linearity



Robustness:

Table 6. Result of robustness study

Chromatographic changes		**Rt(min)**	**Tailing Factor**	**Theoretical Plates**	**%RSD for Standard areas**
Flow rate (mL/min)	0.5	1.743	1.4	3041	0.3
	0.6	1.050	1.4	3048	0.3
Temperature (°C)	20	1.313	1.4	3096	0.1
	30	1.310	1.4	3065	0.9

Table 6. Ruggedness

Intermediate precision/Ruggedness		
Name of the Standard	**Area**	**%Assay**
Intermediate Precision_01	29250675	100.1
Intermediate Precision_02	29330439	100.2
Intermediate Precision_03	29372959	100.6
Intermediate Precision_04	29278538	99.9
Intermediate Precision_05	29116653	99.6
Intermediate Precision_06	29286510	100.2
	Average	100.1
	Std Deviation	0.33
	%RSD	0.3
% RSD Between %Assay of both Analysts		0.2

DISCUSSION

Assay

The amount of Topiramate present in the taken dosage form was found to be 101.12 % respectively.

Accuracy

The percentage mean recovery of Topiramate is 100.00% respectively.

System Suitability

The % RSD for the retention times and peak area of Topiramate 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 Topiramate is 0.999.

Precision

Test results for Topiramate 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 Tolcapone 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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