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High Performance Liquid Chromatographic Method for Determination of Tolcapone In Pharmaceutical Dosage Forms

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Abstract: *A simple, reproducible and efficient High performance liquid chromatographic method is developed for the determination of Tolcopone in pharmaceutical dosage forms. An Inertsil column,C18(150x4.6 ID) 5µm and mobile phase consisting of a mixture of 40 volumes of mixed phosphate buffer and 60 volumes of acetonitrile were used for separation and quantification. Analysis were run at a flow rate 1.0ml/min.The UV detection wavelength was set at 239nm.The developed method was validated according to literature and found to be linear. Robustness, specificity, precision, accuracy, linearity, LOD and LOQ was validated using this method.*

Key Words: *HPLC, Tolcapone, Method development, Mobile phase, Acetonitrile.*

1. INTRODUCTION:

Tolcapone is chemically known as 5-(4-methylbenzoyl)- 3-nitrobenzene-1,2-diol. Its chemical formula and molar mass were $C_{14}H_{11}NO_5$ and 273.2408 g/mol. Tolcapone is a drug that inhibits the enzyme catechol-O-methyl transferase (COMT). It is used in the treatment of Parkinson's disease as an adjunct to levodopa/carbidopa medication. It is a yellow, odourless, non-hygroscopic, crystalline compound8 . Tolcapone is associated with a risk of hepatotoxicity. The structure of Tolcopone is shown in the fig.1.

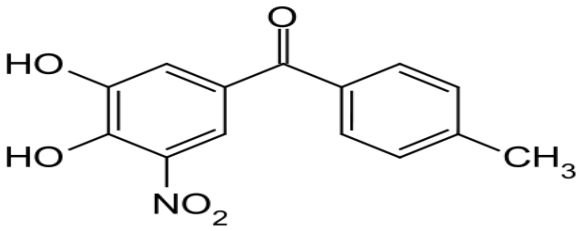

Fig 1: Structure of Tolcopone

Mechanism of action:

The precise mechanism of action of tolcapone is unknown, but it is believed to be related to its ability to inhibit COMT and alter the plasma pharmacokinetics of levodopa, resulting in an increase in plasma levodopa concentrations. The inhibition of COMT also causes a reduction in circulating 3-OMD as a result of decreased peripheral metabolism of levodopa. This may lead to an increase distribution of levodopa into the CNS through the reduction of its competitive substrate, 3-OMD, for transport mechanisms. Sustained levodopa concentrations presumably result in more consistent dopaminergic stimulation, resulting in greater reduction in the manifestations of parkinsonian syndrome.

Materials and Methods

Preparation of standard stock solution of TOLCAPONE

50mg of Tolcapone was weighed in to 500ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.The optimized conditions are shown in the table 1.

Table 1: Optimized chromatographic conditions

Mobile phase	Mixed Phosphate buffer (KH2PO4 +K2HPO4):Acetonitrile 40:60
Column	INERTSIL column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25°C)
Sample temperature	Room temperature(20-25°C)
Wavelength	239nm
Injection volume	20 µl
Run time	10 min
Retention time	3.99min

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 10mg of tolcapone in 10 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase From above stock solutiond 125µg/ml of tolcapone is prepared by diluting 1.25ml of tolcapone to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution:

5tablets (each tablet contains 12.5mg of tolcapone) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions tolcapone (125µg/ml) were prepared by dissolving 12.5mg of tolcapone and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 125µg/ml of tolcapone was made by adding 1ml & 1.25ml of stock solution to 10 ml of mobile phase.

Linearity and range

Preparation of mixed standard solution

Weigh accurately 10mg of tolcapone in 10ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Tolcapone is 0.999. The relationship between the concentration and Tolcapone is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.The linearity values are given in the table 2 and linearity graph is shown in the fig 2.

Table 2 : linearity of Tolcapone

S.No.	Conc.(µg/ml)	Area
1	62.5	2774.562
2	93.75	4032.779
3	125	5007.437
4	156.25	6609.492
5	187.5	7528.872

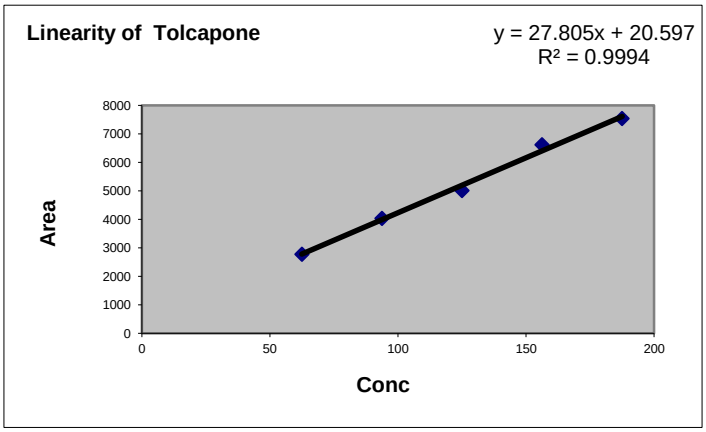

Fig. 2: Linearity graph of Tolcapone

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% & 150%. The percentage mean recovery of Tolcapone is 99.55% respectively. The recovery results are shown in the table 3.

Table 3 : Recovery results for Tolcapone

Recovery level	**Accuracy** Tolcapone					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
50	62.5	3473.134	3439.427	61.98	99.17	
	62.5	3598.073				
	62.5	3247.073				
100	125	5997.437	6111.610	124.54	99.63	
	125	6338.818				
	125	5998.575				
150	187.5	9428.872	9397.869	187.24	99.86	**99.55%**
	187.5	9338.19				
	187.5	9426.545				

Observation

The percentage mean recovery of Tolcapone is 99.55% respectively.

Precision

Method precision

Prepared sample preparation of Tolcapone as per test method and injected 6 times in to the column. The results of recovery table are shown in the table 4.

Table 4 : Results for Method precision of Tolcapone

Tolcapone		
S.No.	Rt	Area
1	2.813	5005.745
2	2.760	5044.357
3	2.760	4993.823
4	2.753	4890.628
5	2.813	4976.613
6	2.767	4997.266
avg	2.778	4982.233
stdev	0.028	56.942
%RSD	**1.00**	**1.56**

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision. The system suitability parameters were within limit at all variable conditions. The results are shown in the table 5.

Table 5: Result of Robustness study

Parameter	Tolcapone	
	Retention time(min)	Tailing factor
Flow		
0.8ml/min	3.433	1.902
1.0 ml/min	2.780	1.882
1.2ml/min	2.337	1.857
Wavelength		
237nm	2.753	1.824
239nm	2.780	1.882
241nm	2.763	1.909
		2.763

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the assay by two different analysts. The %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged. The results are shown in the below in the table 6.

Table 6: Results for Ruggedness

Tolcapone	%Assay
Analyst 01	99.96
Analyst 02	97.59
%RSD	1.69

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