IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
August 2019 Vol.:16, Issue:1

A New RP-HPLC Method Development and Validation of Tolcapone in Bulk and Tablets

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

V.Asha Ranjani*, S.Bhavani, Y.Aditya, Sreenivas Reddy, B. Bhargavi, N. Sai Pratyusha

MLR Institute of Pharmacy, Dundigal, Hyderabad, Telangana 500043

Submission: 23 July 2019
Accepted: 28 July 2019
Published: 30 August 2019

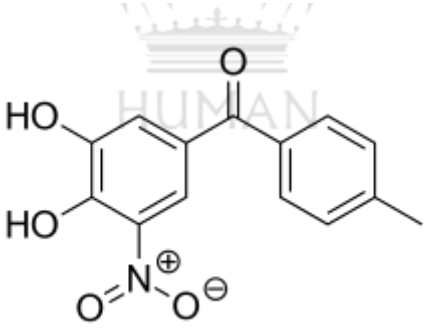
Keywords: Method development, System suitability, Validation, RP-HPLC, Dosage forms.

ABSTRACT

A reverse-phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of tolcapone in bulk and tablet dosage forms. The separation was achieved on Inertsil ODS C18, 250 x 4.6 mm, 5µm i.d. column using 40 volumes of Mixed Phosphate buffer (KH_2PO_4 + K_2HPO_4) pH 4.0, 20 volumes of acetonitrile, 40 volumes of methanol as mobile phase and at a flow rate of 1.0 mL/min. Detection was carried out using a PDA detector at 264 nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The total chromatographic analysis time per sample was about 5 min with tolcapone eluting at retention time of about 2.450 min. The method was validated as per ICH guidelines. Validation studies demonstrated that the proposed HPLC method is simple, specific, rapid, reliable and reproducible. The standard curves were linear over the concentration range of 60-140µg/mL. The LOD and LOQ values for tolcapone were 1.56 and 4.73 µg/mL, respectively. The percentage recovery was found to be 99.14 to 100.74 and the % RSD for precision was found to be 0.7. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the determination of tolcapone in bulk and tablet dosage forms.

INTRODUCTION

Tolcapone is a drug used for the treatment of parkinson's disease. It is selective, potent and reversible nitrocatechol type inhibitor of enzyme catechol-O-methyltransferase. Dose of tolcapone is 100 or 200 mg. It is administered orally. Half-life of tolcapone is 2-3 hrs, volume of distribution is 0.3 L/kg and bioavailability is 60 %. Tolcapone has the ability to cross the blood–brain barrier and thus exerts its COMT inhibitory effects in the central nervous system (CNS) as well as in the periphery. Tolcapone is administered adjunct with levodopa and AADC inhibitor because it increases the bioavailability of levodopa. Since tolcapone has a greater affinity for COMT, it inhibits the enzyme from methylating levodopa. This results in greater levels of levodopa and increases the time period before clearance. Tolcapone helps alleviates the issues with levodopa 50% of PD patients have experienced. Without administration of tolcapone, the beneficial effects of levodopa have worn off resulting in motor fluctuations. In comparison with entacapone, another nitrocatechol COMT inhibitor, tolcapone has longer half life (2.9 hours vs 0.8 hours), and can better penetrate the blood–brain barrier in that it acts both centrally and peripherally. However, entacapone is less toxic in regard to liver function.



Structure of Tolcapone

MATERIALS AND METHODS

Materials, Chemicals and reagents

Tolcapone standard (B. No. 0651BG/01) was provided by Glenmark Pharmaceuticals, tolcapone tablets (TASMAR) containing 100 mg of tolcapone were procured from the local market. Analytical grade sodium dihydrogen orthophosphate, potassium dihydrogen orthophosphate, ammonium acetate was purchased from S.S. fine chemicals, Hyderabad.

HPLC grade tetrahydrofuran, methanol, acetonitrile and water were obtained from Merck, Mumbai.

Instrumentation

The chromatographic system used to perform development and validation of this assay method was comprised of a LC-10STvp binary pump, a SPD - M10 Avp photo diode array detector and a rheodyne manual injector model 7725i with 20µl loop connected to a multi–instrument data acquisition and data processing system, spinchrome software. (Class-VP 6.13SP2, Shimadzu)

Mobile phase preparation

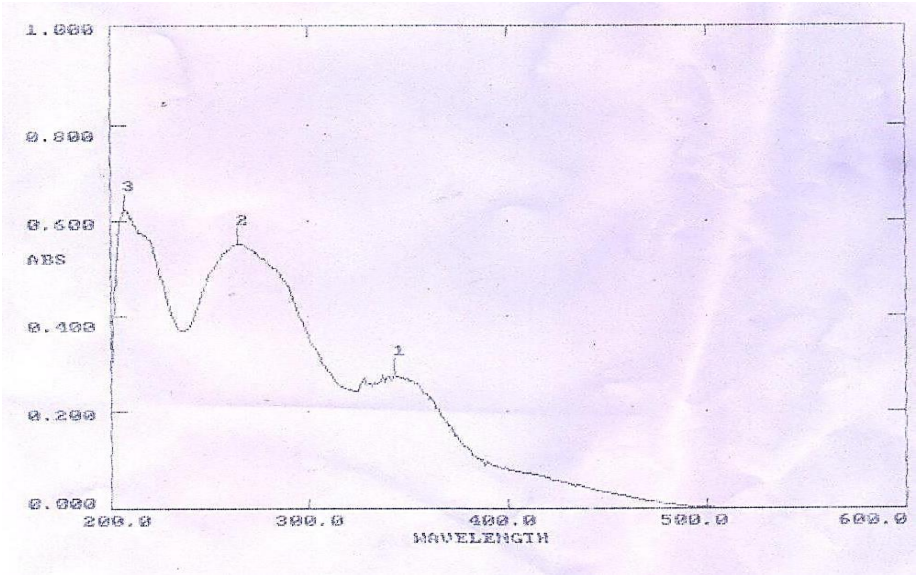
The mobile phase consisted of a mixture of 40 volumes of mixed phosphate buffer (KH_2PO_4+ K_2HPO_4) pH 4.0, 20volumes of acetonitrile, 40 volumes of methanol. To prepare buffer 1.625 gm of potassium dihydrogen phosphate (KH_2PO_4) and 0.3 g of dipotassium hydrogen phosphate was weighed and dissolved in 100 ml of water and volume was made up to 550 ml with water. Adjust the pH to 4.0 using orthophosphoric acid. The buffer was filtered through 0.45 µ filters to remove all fine particles.

Wavelength detection

An accurately weighed amount of 10 mg of tolcapone was transferred into 10 ml volumetric flask and dissolved in mobile phase and then makeup to the mark with mobile phase and prepare 10 µg/ml of solution by diluting 0.1 ml to 10 ml with mobile phase. The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig. no. 3.7.1 and the absorption curve shows characteristic absorption maxima at 264 nm for tolcapone.

Chromatographic conditions

Chromatographic analysis was performed on a Inertsil ODS C18, 250x4.6mm, 5µm column. The mobile consisted of 40 volumes of Mixed Phosphate buffer (KH_2PO_4+K_2HPO_4) pH 4.0, 20 volumes of acetonitrile, 40 volumes of methanol. The flow rate of the mobile phase was adjusted to 1.0 ml/min and the injection volume was 20 µl. Detection was performed at 264 nm.



Preparation of standard solution

An accurately weighed amount of 10 mg of tolcapone was transferred to 10 ml of volumetric flask and dissolved in mobile phase and make up the volume with mobile phase. From above stock solution pipette out 1 ml and make it up to 10 ml to get 100 µg/ml of tolcapone.

METHOD VALIDATION

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Specificity

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Linearity

The linearity was plotted for five concentration levels 60, 80, 100, 120 and 140 µg/ml of tolcapone. The peak area versus concentration data were evaluated by linear regression analysis. 1000 µg/ml of standard stock solution of tolcapone was prepared and further diluted to attain concentration of about 60, 80, 100, 120 and 140 µg/ml of standard solution

concentration. From standard stock solution of 1000 µg/ml accurately pipette out exact 6, 8, 10, 12 and 14 ml and dilute it up to 10 ml each with diluents to achieve 60-140 µg/ml concentration range. Coefficient of determination of the linearity study was found to R^2=0.996 with linear regression equation Y=6.416x+16.91, which proves the method is highly linear over the working range.

Limit of detection and Limit of quantitation study

LOD is the lowest amount of the drug content which can be detected by the proposed method while LOQ is the lowest amount which can be quantified by the method. The guideline suggest minimum signal to noise ratio (S/N) more than 3.3 for LOD and more than 10 for LOQ. On the basis of linearity data theoretically it can also be calculated by the given formula.

$$LOD = 3.3\ \sigma/S \qquad LOQ = 10\ \sigma/S$$

Where σ= residual standard deviation of regression line

S= slope of regression line.

Precision study:

Precision study was established by evaluating method precision and system precision study. Method precision of the analytical method was determined by analyzing six sets of the sample preparation. Assay of all six replicate sample preparations was determined and mean % assay value, standard deviation and % RSD for the same was calculated. System precision of the analytical method was carried out to ensure that the analytical system was working properly. Standard solution was injected six times into system and chromatograms were recorded.

Accuracy study

This experiment can be performed by the recovery test. Recovery of the method was evaluated at three different concentration levels by addition of known amounts of standard placebo preparation. For each concentration level, three sets were prepared and injected as duplicate. Accuracy of the method was determined by recovery studies. To the formulation (Preanalyzed sample), the reference standards of the drugs were added at the level of 100 %,

120 %, 140 %. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table 3.7.8

Robustness study

Robustness of the method was evaluated by assaying test solution under slight but deliberate changes in analytical conditions such as change in flow rate, change in wavelength, change in proportions of mobile phase.

a) Flow rate change: In this experiment, the test samples were analyzed at the flow rate of 0.8 ml/min and 1.2 ml/min each and the results were observed in terms of assay value and chromatographic compatibility. Blank, standard and sample solutions were prepared as per the assay procedure. The result shown that during all variance conditions assay value of the test preparation solution was not affected and it was the accordance with that of actual system suitability parameters were also found satisfactory. Hence the analytical method would be concluded as robust.

b) Wavelength change: In this experiment the test samples were analyzed at the wavelength of 262 and 266 nm each and the results were observed in terms of assay value and chromatographic compatibility. Blank, standard and sample solutions were prepared as per the assay procedure.

Ruggedness study

The ruggedness of the method was studied by determining the system to system variation and the analyst to analyst variation. The present ruggedness study was performed by two different HPLC systems.

Assay

Standard preparation

An accurately weighed amount of 10 mg of tolcapone was transferred to 10 ml of volumetric flask and dissolved in mobile phase and made up the volume with mobile phase. From above stock solution, pipette out 1ml and make up to 10 ml to get 100 µg/ml of tolcapone.

Sample preparation

Twenty tablets (each Tab contains 100 mg of tolcapone) were weighed and taken into a mortar uniformly mixed. Test stock solutions of tolcapone (1000 µg/ml) was prepared by dissolving weight equivalent to 100 mg of tolcapone and dissolved in sufficient mobile phase. After that filter the solution using 0.45-micron syringe filter and sonicated for 5 min and dilute to 100 ml with mobile phase. Further dilutions are prepared to get 100 µg/ml of tolcapone by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of Tolcapone present in the formulation by using the formula given below, and results shown in above table:

$$\%\ \textbf{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

RESULTS & DISCUSSION:

Optimization of chromatographic conditions

Chromatographic analysis was performed on a Inertsil ODS C18, 250 x 4.6 mm, 5µm column. The mobile phase consisted of 40 volumes of mixed Phosphate buffer (KH_2PO_4+ K_2HPO_4) pH 4.0, 20 volumes of Acetonitrile, 40 volumes of methanol. The flow rate of the mobile phase was adjusted to 1.0 ml/min and the injection volume was 20 µl. Detection was performed at 264 nm.

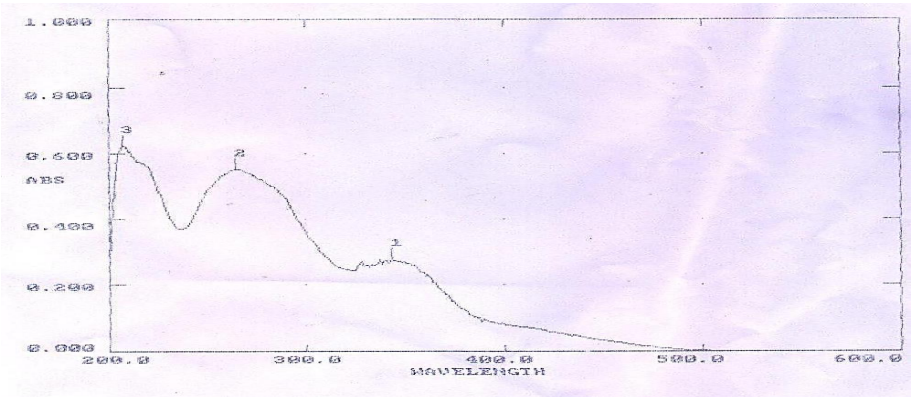


Fig 1: UV Spectrum of Tolcapone

Validation

Specificity

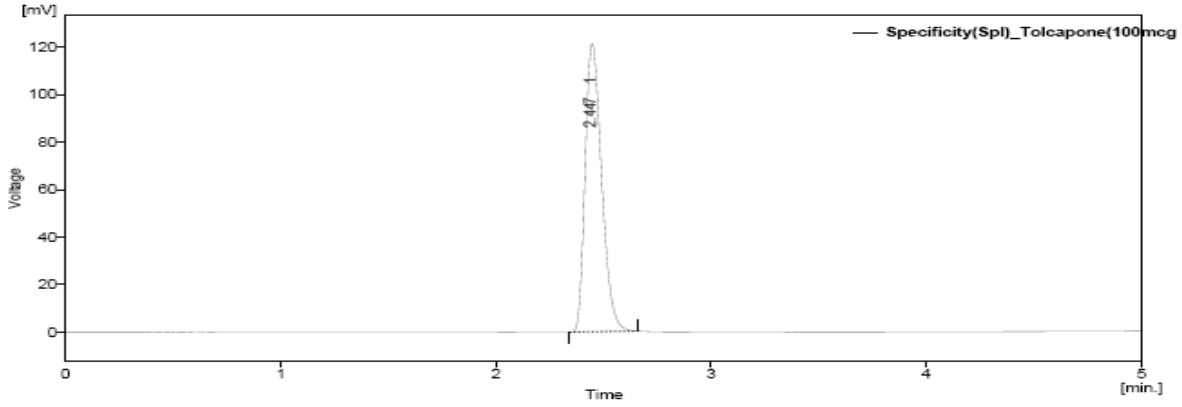


Fig 2: Chromatogram of specificity of Tolcapone

Precision

Results for method precision of tolcapone

Sample No	% Assay
1	98.1
2	99.5
3	99.7
4	100.1
5	98.4
6	99.7
Mean (N=6)	99.2
% RSD(N=6)	0.7

Linearity

Table 1: Linearity preparations of Tolcapone

Sr. No.	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution (µg /ml)	Area
1	6	10	60	421.501
2	8	10	80	513.420
3	10	10	100	680.922
4	12	10	120	795.121
5	14	10	140	931.452

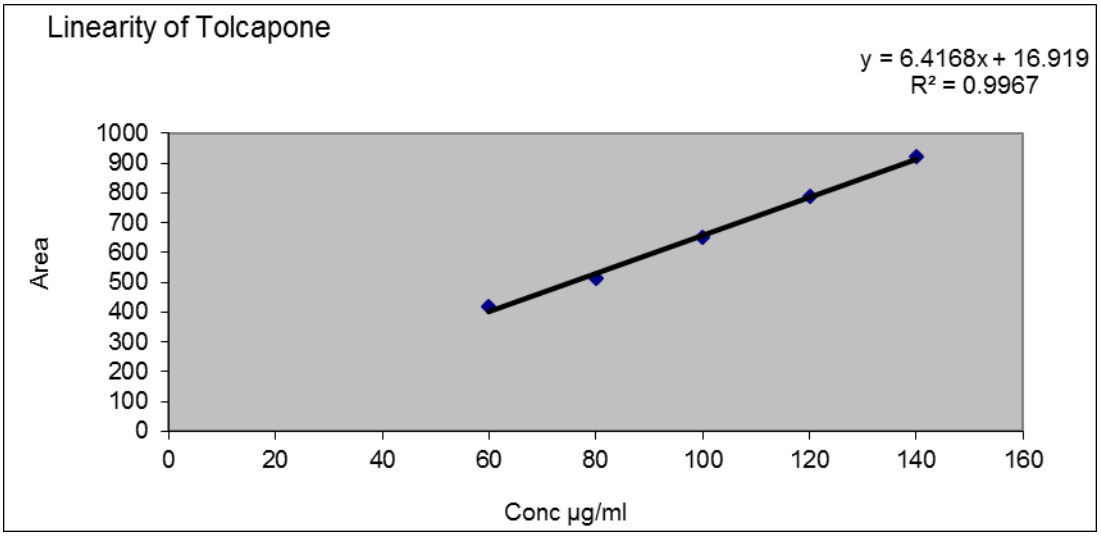


Fig 3: Linearity of Tolcapone

Accuracy

The % recovery is within the limit of 100 to 140 % this is in the limit of acceptance criteria and % RSD value of % recovery of replicate set is below 2%. Hence this suggests that proposed method is highly accurate.

Table 2: Results for recovery

Recovery level	Accuracy			
	Amount taken(µg/ml)	Amount recovered (µg/ml)	% Recovery	Average % Recovery
100%	100	99.14	99.14	99.92%
	100			
	100			
120%	120	120.89	100.74	
	120			
	120			
140%	140	139.85	99.90	
	140			
	140			

Robustness

The result of robustness of the developed assay method was established in table. The result shown that during all variance conditions assay value of the test preparation solution was not affected and it was the accordance with that of actual system suitability parameters were also found satisfactory. Hence the analytical method would be concluded as robust.

Table 3: Results for robustness

Parameter	Tolcapone	
	Retention time (min)	Tailing factor
Flow		
0.8 ml/min	2.633	1.632
1.2 ml/min	2.300	1.647
Wavelength		
262 nm	2.143	1.474
266 nm	2.433	1.611

LOD & LOQ

The LOD for this method was found to be 1.56 µg/ml. The LOQ for this method was found to be 4.73 µg/ml for tolcapone.

System suitability

The % RSD for the retention times and peak area of tolcapone were found to be less than 2 %. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit.

Table 4: Results for system suitability of tolcapone

Injection	Retention time (min)	Peak area
1	2.460	681.12
2	2.453	680.78
3	2.453	680.15
4	2.447	679.89
5	2.450	682.12
6	2.450	681.58
Mean	2.4522	680.94
SD	0.0044	0.846
% RSD	0.18	0.71

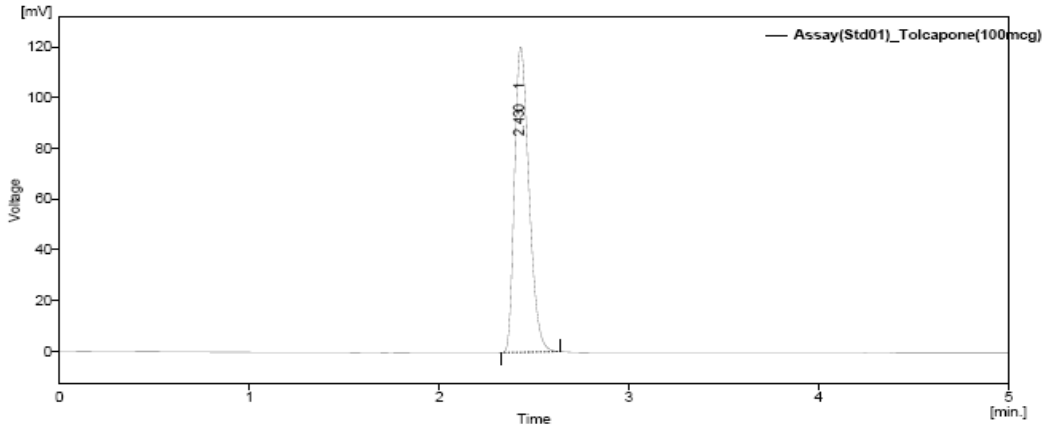


Fig 4: Chromatogram of standard preparation of Tolcapone

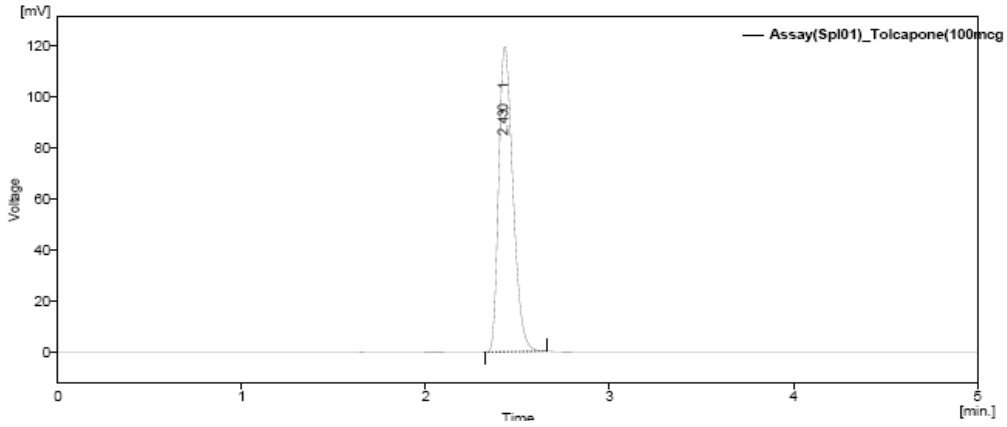


Fig 5: Chromatogram of sample preparation of Tolcapone

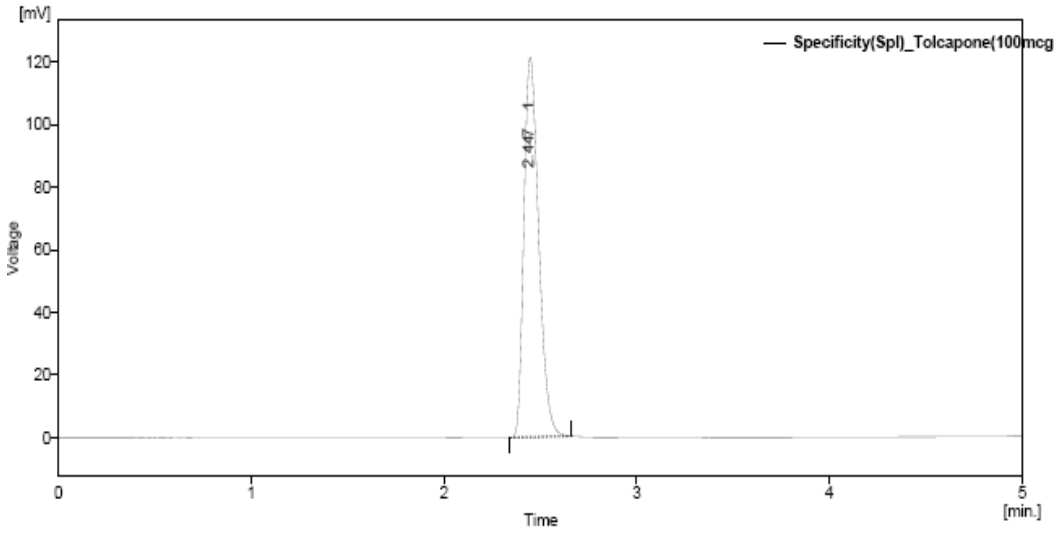


Fig 6: Chromatogram of specificity of Tolcapone

SUMMARY & CONCLUSION

Development and validation of RP-HPLC method for the estimation of tolcapone in bulk and pharmaceutical dosage forms with the facilities and the results are incorporated in this thesis.

In conclusion, a validated RP-HPLC method has been developed for determination of tolcapone in the bulk and combined tablet dosage forms. The results show that the method was found to be specific, simple, accurate, precise and sensitive. The method was successfully applied for the determination of both drugs in combined tablet dosage form. In the future, this method may be applied for routine analysis of both the drugs in API and in tablet formulation.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing % RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

REFERENCES

1. Zahi Mohammad Turabi, O'hood A. Khatatbeh, Dalal N. Al-Abed Zahi Mohammad Turabi, O'hood A., and Khatatbeh, Dalal N. Al-Abed International Journal of Pharmaceutical Sciences and Drug Research 2014; 6(1): 70-74.
2. Kumar S, Mathkar S, Romero C, and Rustum A.M. Development and validation of a single RP-HPLC assay method for analysis of bulk raw material batches of four parabens that are widely used as preservatives in pharmaceutical and cosmetic products. J. of Chromatographic Sciences. 2011; l: 405.
3. Kuchekar, B.S., Badhan, A.C., Mahajan, H.S., Mouth Dissolving Tablets: A Novel Drug Delivery System, Pharma Times. 2003, 35, 789. 2.
4. Seager, H., Drug & delivery product and the zydis fast dissolving form, J. Pharm. Pharmacol. 1998, 50(4), 375882. 3.
5. Yutaka, M., Yuki, T., Masanobu, Y., Ryoji, T., Junko, A., Kozo, T., Evaluation of the disintegration time of rapidly disintegrating tablets via a novel method utilizing a CCD camera, Chem. Pharm. Bull. 2002,50(9), 118181186. 4.
6. Shu, T., Suzuki, H., Hironaka, K., Ito, K., Studies of rapidly disintegrating tablets in the oral cavity using cogrinding mixtures of mannitol with crospovidone, Chem. Pharm. Bull., 2002, 50, 19381985.
7. Prakash S.R., Gothoskar A.V., A Review: Pharmaceutical technology 2003, 928-98.