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Original Article

Stability indicating RP-HPLC method development and validation of Etizolam and Propranolol hydrochloride in pharmaceutical dosage form

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ABSTRACT

A stability indicating RP-HPLC method was developed and validated for Etizolam and Propranolol Hydrochloride in pharmaceutical dosage form. The chromatographic separation was carried out by using PURITUS C_{18} column (250 mm x 4.6 mm, 5 µm) with gradient mobile phase Ammonium phosphate buffer pH 3 and Acetonitrile by PDA detector. Drug and degradant were monitored at 245nm, the flow rate was 1 ml/min, injection volume was 20 µl and temperature of column was 25 °C. Retention time of Propranolol Hydrochloride and Etizolam was about 7.4 min and 14.4 min respectively. Stability indicating method was performed by using 1N HCl, 1N NaOH, 3 % H_2O_2. Thermal degradation was performed at 100 °C for 7 days. The photolytic degradation was performed for 7 days. The developed method was validated as per ICH guideline using various validation parameters. Linearity was observed in concentration range 5 to 15 µg/ml of Etizolam and 200 to 600 µg/ml of Propranolol Hydrochloride with correlation coefficient of 0.9995 and 0.9983 respectively. All the parameters were within specifications. So, the developed method was found to be stability indicating, specific, linear, accurate, precise and robust.
Key words: Etizolam, Propranolol Hydrochloride, RP-HPLC and Forced degradation

INTRODUCTION

Etizolam: Etizolam is chemically 7-(2-chlorophenyl-4-ethyl-13-methyl-3-thia-1, 8, 11, 12-tetraazatricyclo 8.3.0.02, 6) trideca -2(6), 4, 7, 10, 12 pentane. Etizolam is one of the most widely prescribed drugs for the treatment of anxiety and has strong muscle relaxing properties.

Propranolol Hydrochloride: Propranolol hydrochloride is chemically (2RS)-1-[(1-methylethyl) amino] -3-(naphthalen-1-yloxy) propan-2-ol. Propranolol Hydrochloride is a non-selective beta blocker, that is, it blocks the action of epinephrine on both β1 and β2- adrenergic receptors. Combination used in treatment of anxiety and hypertension.

HPLC is a separation technique in which sample is separated into its analyte by distributing between stationary phase and mobile phase and forced degradation study carried out to detect primary degradent of products. A tablet formulation containing Etizolam 0.5 mg and Propranolol Hydrochloride 20 mg. literature survey revealed that HPLC, and Spectroscopic methods are reported for determination of Etizolam and Propranolol Hydrochloride. The present work describes stability indicating RP-HPLC method for Etizolam and Propranolol Hydrochloride in pharmaceutical dosage form and it was validated as per ICH guideline.

EXPERIMENTAL WORK

Material: Etizolam was obtained as gift sample from Macleodes Pharmaceuticals, Mumbai and Propranolol Hydrochloride was obtained as gift sample from Sun Pharmaceuticals, Vadodara. Tablet dosage form was available from local market. Acetonitrile and Orthophosphoric acid obtained from Merk. All the chemicals were HPLC grade. Milli-Q-water was used throughout the experiment.

Equipment: The waters HPLC system (alliance) with photodiode array detector was used for method.
Chromatographic condition: The mobile phase used was mixture of phosphate buffer (pH 3 with

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0.5% OPA) and Acetonitrile in gradient elution at flow rate of 1.0 ml/min (table 1). The analytical column used PURITUS C_{18} column (250 mm x 4.6 mm, 5 µm). The detection was carried out at 245nm for run time of 20 min. Diluent used Acetonitrile: water (50:50 % v/v). Gradient programme is given in table 1.

Preparation of standard solution: Standard Stock solution of 100 µg/ml of Etizolam and 4000 µg/ml of Propranolol Hydrochloride was prepared. From this 1 ml was transfer into 10 ml volumetric flask and make up the volume up to mark with diluent to make standard working solution of 10 µg /ml and 400 µg/ml of Propranolol hydrochloride.

Assay of pharmaceutical dosage form (sample preparation): Powder equivalent to 0.5 mg of ETI and 20 mg of PROP was taken in 25 ml of volumetric flask and 20 ml of diluent was added, sonicated for 5 min and make up the volume up to mark with diluent to make sample stock solution of 100 µg/ml of Etizolam and 4000 µg/ml Propranolol Hydrochloride. From this 1 ml was transfer into 10 ml of volumetric flask and make up the volume up to mark with diluent to make sample working solution of 10 µg/ml of Etizolam and 400 µg/ml of Propranolol Hydrochloride.

RESULT AND DISCUSSION

Method development: Chromatographic parameters were preliminary optimized to develop HPLC method for estimation of Etizolam and Propranolol hydrochloride. 245 nm was selected as detection wavelength as both drugs shows good absorbance at this wavelength**.** Column was PURITUS C_{18} column (250 mm x 4.6 mm, 5 µm). Mobile phase was mixture of phosphate buffer (pH 3 with 0.05% OPA) and Acetonitrile in gradient elution at flow rate of 1.0 ml/min. chromatogram is shown in figure no.1

System suitability: System suitability parameters were calculated for standard solution. Chromatogram is shown in figure no 1 and data shown in table 2.

Forced degradation studies:

% Degradation = Area of TEST/ Area of STD x 100

Acid degradation: From sample stock solution, 1 ml was transfer to 10 ml of volumetric flask and then 1 ml of 1 N HCl was added and kept on reflux at 60°C for 2 hrs. After 2 hrs add 1 ml of 1 N NaOH and make up the volume up to mark with diluent. This solution was filtered and sonicated for degassing. Chromatogram is shown in figure no 6.

Alkali degradation : From sample stock solution, 1 ml was transfer to 10 ml of volumetric flask and then 1 ml of 1 N NaOH was added and kept on reflux at 60°C for 3 hrs. After 3 hrs add 1 ml of 1 N HCl and make up the volume up to mark with diluent. This solution was filtered and sonicated for degassing. Chromatogram is shown in figure no 8.

Oxidative degradation: From sample stock solution, 1 ml was transfer to 10 ml of volumetric flask and then 1 ml of 3 % H_2O_2 was added and kept at room temperature for 7 days. After 3 days, diluent was added up to mark in 10 ml volumetric flask. This solution was filtered and sonicated for degassing. Chromatogram is shown in figure no 10.

Thermal degradation: Powder equivalent to 0.5 mg of Etizolam and 20 mg of Propranolol Hydrochloride were taken in Petri dish and exposed to 100°C for 7 days. After 7 days, transfer pure samples and powder of formulation in three different 25 ml of volumetric flask respectively and 20 ml of diluent was added in each volumetric flask, sonicated for 5 min and make up the volume up to mark with diluent to make 100 ppm (ETI) and 4000 ppm (PROP) and 100 ppm and 4000 ppm (ETI and PROP) of formulation in 25 ml volumetric flask. From this solution 1ml was taken in 10 ml volumetric flask and makeup the volume up to mark with diluent. This solution was filtered and sonicated for degassing. Chromatogram is shown in figure no 12.

Photo degradation: Powder of formulation were irradiated with a UV lamp (254 nm) (Camag, Muttenz, Switzerland) for 7 days. 2.5 mg of ETI , 100 mg of PROP and average amount of formulation were weighed, transferred to separate volumetric flasks (25 ml), dissolved in 20 ml of diluent and volume was made up to the mark with diluent. From the above each flask, 1 ml of each solution was transferred into three different 10 ml of volumetric flask and volume was made up to the mark with diluent to make 10 µg/ml ETI and 400 µg/ml of PROP and mixed. This solution was filtered and sonicated for degassing. Chromatogram is shown in figure no 14.

Blanks for all degradation were prepared without addition of drug solution which are shown in figure 5,7.9 and 11.Results of forced degradation have been shown in table 3.

Method validation:

Specificity: Specificity was performed by injecting blank, standard and sample solutions. Chromatograms are shown in figure no 1, 2 and 3.

Linearity and Range: The linearity of analytical procedure is its ability to obtained test results which are directly proportional to the concentration of analyte in the sample. Five Concentration ranges of 5 µg/ml -15 µg/ml of Etizolam were prepared

and analyzed. Five Concentration ranges of 200 µg/ml -600 µg/ml of Propranolol hydrochloride prepared and analyzed. Results have been shown in table 3 and calibration curve has been shown in figure no 15 and 16 for Propranolol hydrochloride and Etizolam respectively.

Precision: For repeatability six replicates of concentration 10 µg /ml of standard solution of Etizolam and 400 µg/ml of Propranolol Hydrochloride was analyzed. For intraday precision three replicates of three concentrations 7.5 µg/ml, 10µg/ml and 12.5 µg/ml of standard solution of Etizolam and 300µg/ml, 400µg/ml, and 500 µg/ml of Propranolol Hydrochloride was analyzed at three different time intervals. For Interday precision three replicates of three concentrations of 7.5 µg/ml, 10µg/ml and 12.5 µg/ml of standard solution of Etizolam and 300 µg/ml, 400 µg/ml, 500 µg/ml of Propranolol Hydrochloride was analyzed at three consecutive days. Result has been shown in table 4

Accuracy: Known amount of working standard was added to the fixed concentration of pre-analyzed tablet sample. For both the drugs recovery of was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 80%, 100% and 120% level and percentage recovery was calculated by subtracting the total area from pre-analyzed sample area. Results have been shown in table 5.

Robustness : Robustness of method was done by making slight deliberate change in chromatographic condtions like change in flow rate (± 0.1 ml/min) and change in wavelength ((± 0.1). Results have been shown in table 4.

Assay of pharmaceutical dosage form: The proposed validated method was successfully applied to determine Etizolam and Propranolol Hydrochloride in tablet dosage form. The result obtained for Etizolam and Propranolol Hydrochloride was comparable with corresponding labeled amounts. Results have been shown in table 6.

Conclusion: The developed method is accurate, precise and specific and has ability to separate the drugs Etizolam and Propranolol Hydrochloride and their degradants with each other in tablet dosage form. Developed stability indicting method was validated as per ICH guidelines and thus indicating general applicability of method for analysis of marketed formulation. The simplicity of method is allows its application in the laboratory for routine quality check as well as stability studies for formulation.

ACKNOWLEDGEMENT:
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Table 1: Gradient programme

Time (min)	Flow Rate	% A	% B
0	1 ml/min	60	40
5	1 ml/min	60	40
10	1 ml/min	50	50
17	1 ml/min	60	40
20	1 ml/min	60	40

Table 2: System Suitability Report

DRUGS	Retention time	Tailing factor	Area	Plate count
Propranolol Hydrochloride	7.437	1.500	3903217	3232
Etizolam	14.456	1.301	1326810	19445

Table 3: Forced degradation result of proposed RP-HPLC method

Type of degradation	Degradation condition	% Degradation	
		PROP	ETI
Initial	-	-	-
Acidic	2 hrs at 60 °C	19.60 %	12.24 %
Alkali	3 hrs at 60 °C	21.38%	NIL
Oxidative	3 % H_2O_2 for 7 days	22.49%	7.07%
Thermal	100 °C for 7 days	NIL	NIL
Photo	UV 254 nm for 7 days	NIL	NIL

Table 4: Result of validation parameter

Sr.No	Parameter	Result	
		Propranolol Hydrochloride	Etizolam
1.	**Correlation coefficient**	0.9985	0.9995
2.	**Precision (%RSD)**	**% RSD**	**% RSD**
	i. Repeatability	0.9 %	1.36
	ii. Intraday precision	0.8-1.2%	0.715-0.999%
	iii. Interday precision	0.665-0.1004%	0.611-0.928
3.	**Accuracy**	99% -100.41%	99.1%- 101.7%
4.	**Robustness(%RSD)**	**% RSD**	**% RSD**
	Variation in flow rate	1.348-1.423%	1.141-1.487%
	Variation in wavelength	1.004-1.497 %	0.945-1.386%

Table 5: Data of accuracy

Drug	Level	Amount of sample (ppm)	Amount of standard spiked(ppm)	Total amount (ppm)	Amount Recover (ppm)	%Recovery
PROP	**80%**	200	160	360	356.4	99
	100%	200	200	400	400.6	100.15
	120%	200	240	440	441.86	100.41
ETI	**80%**	5	4	9	8.92	99.11
	100%	5	5	10	10.07	100.70
	120%	5	6	11	11.19	101.7

Table 6: Data of assay of pharmaceutical dosage form

Parameter	**Value**	
	Propranolol Hydrochloride	**Etizolam**
% Assay	100.16	100.83
% RSD	0.924	1.14

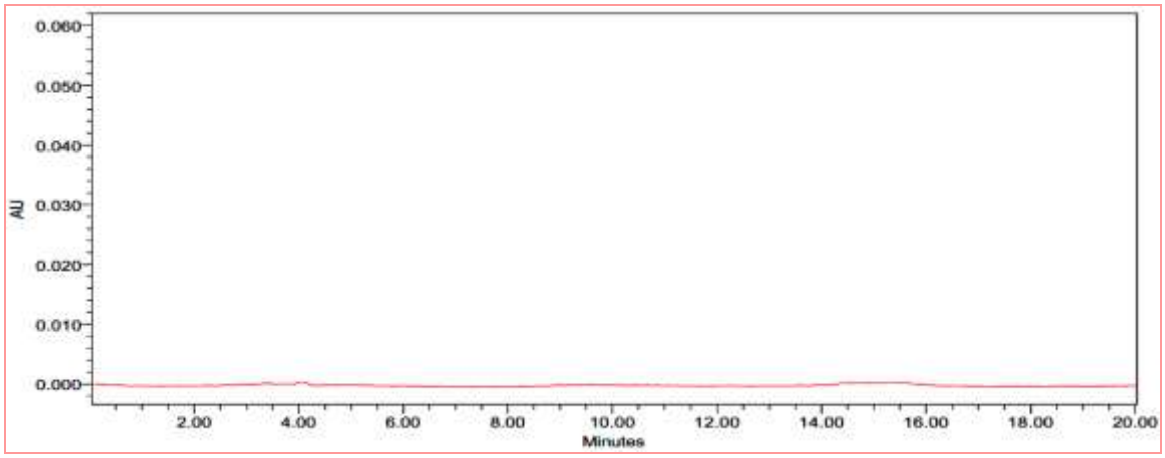

Fig No: 1 RP-HPLC chromatogram of Blank

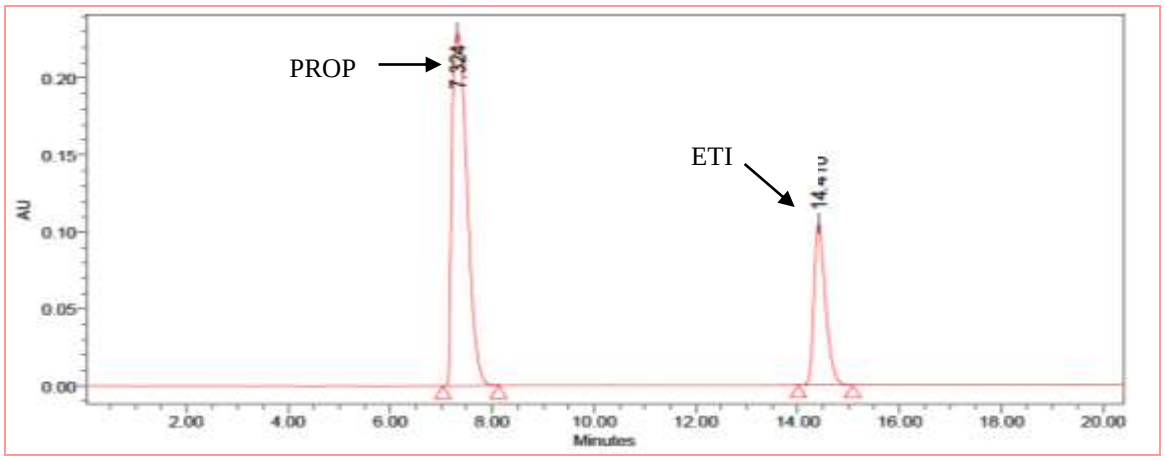

Fig No: 2 RP-HPLC chromatogram of Standard solution

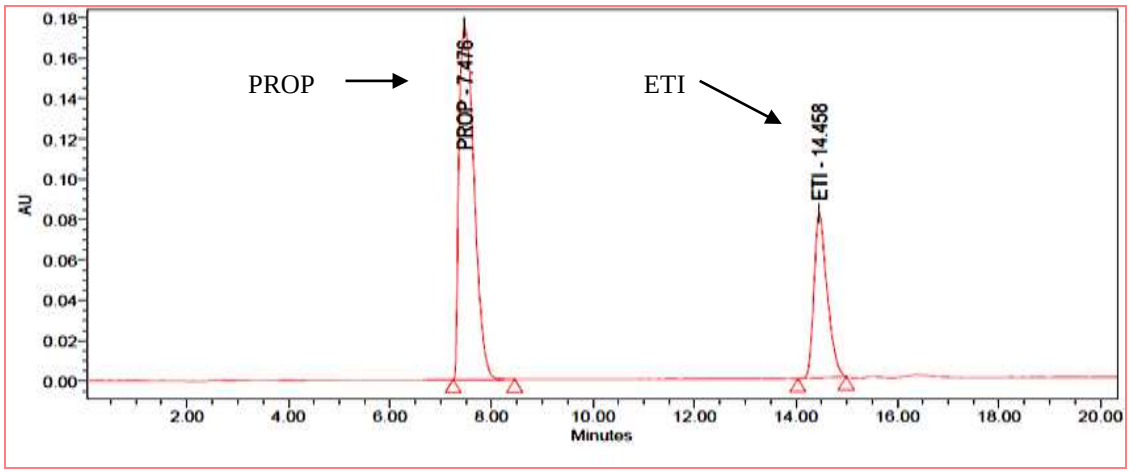

Fig No: 3 RP-HPLC chromatogram of Sample solution (Formulation)

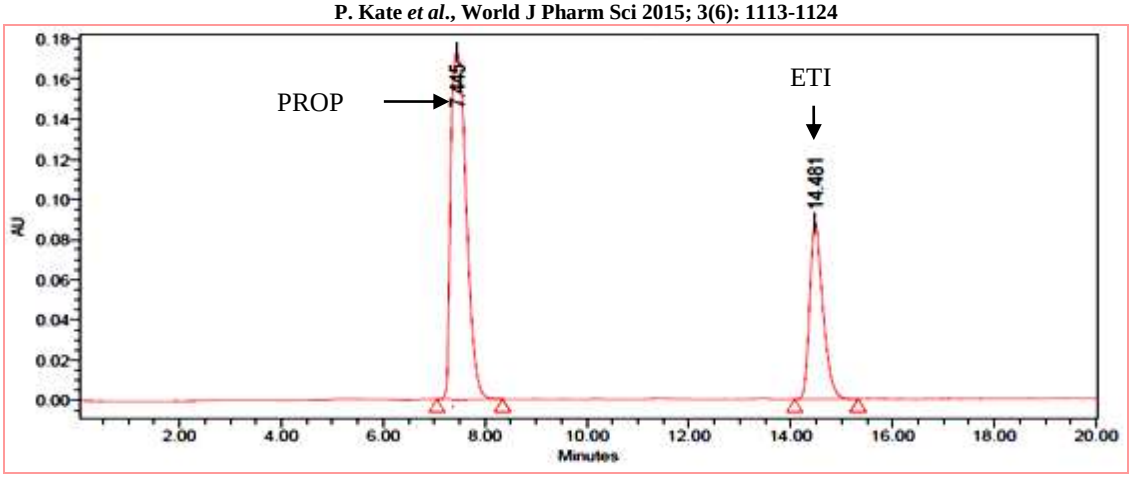

Fig No: 4 Chromatogram of Sample solution (Formulation) at initial condition

Drug	Retention Time	Area	Theoretical plate	Tailing factor	Resolution
PROP	7.476	3903217	3260	1.5	
ETI	14.458	1326810	13969	1.4	13.6

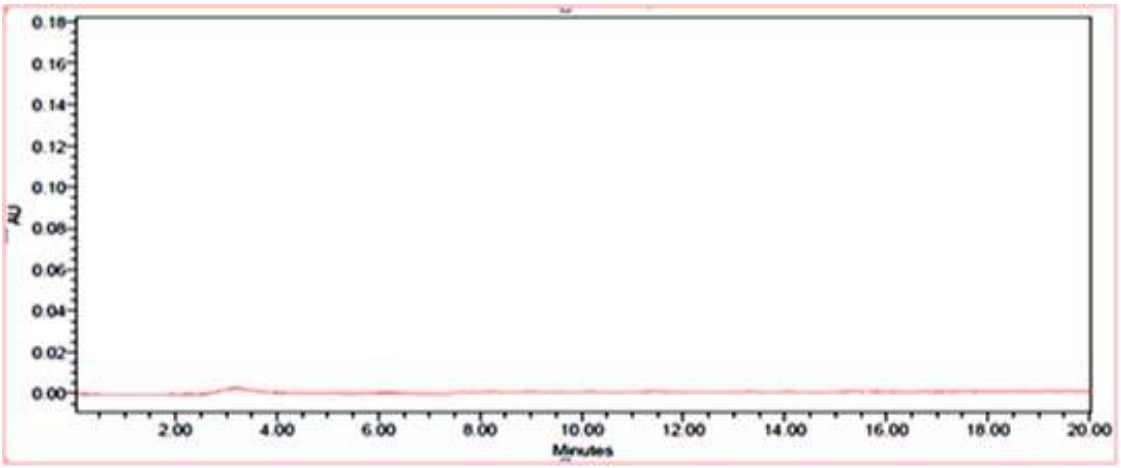

Fig No: 5 Chromatogram of blank in acidic condition

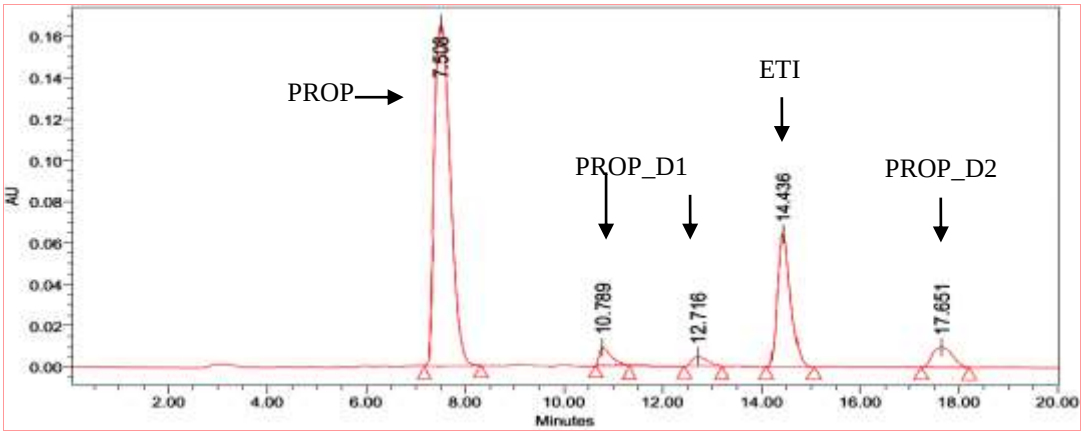

Fig No: 6.a Chromatogram of Sample solution (Formulation) in acidic condition

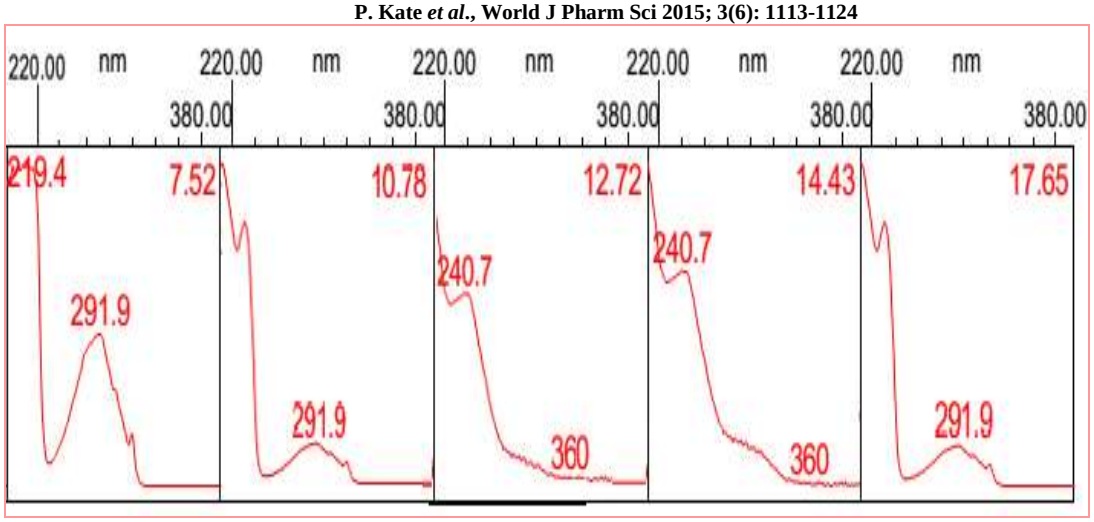

Fig No: 6.b PDA spectra in acidic condition

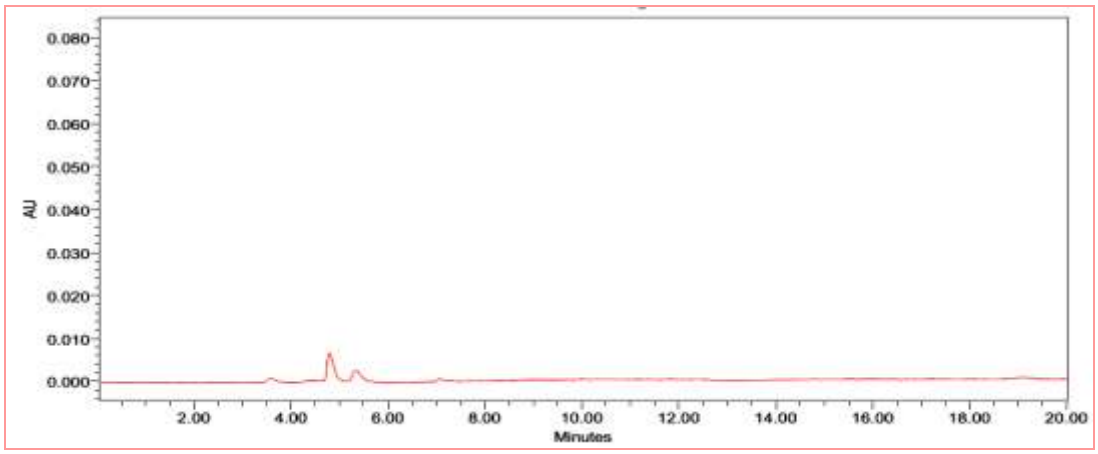

Fig No: 7 Chromatogram of blank in alkali condition

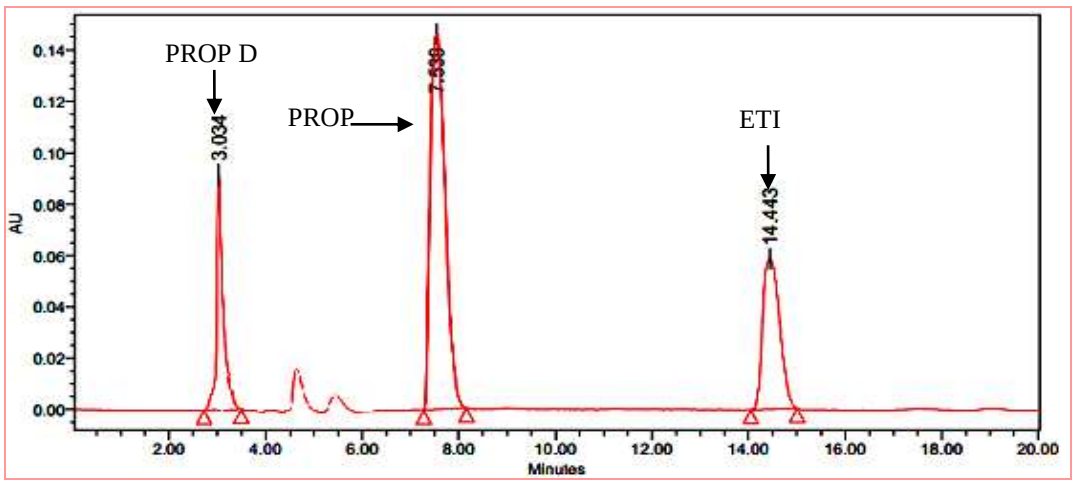

Fig No: 8.a Chromatogram of Sample solution (Formulation) in alkali condition

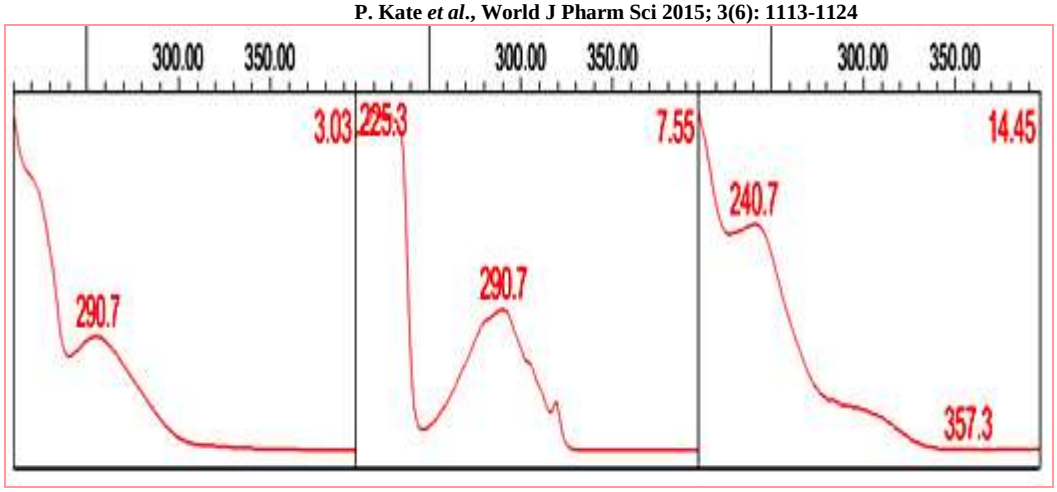

Fig No: 8.b PDA spectra in alkali condition

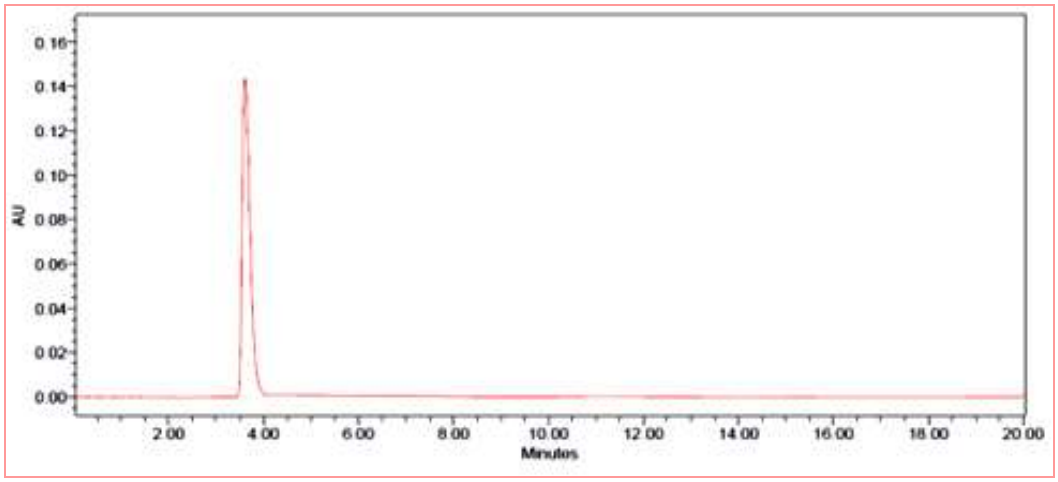

Fig No: 9 Chromatogram of blank in oxidative condition

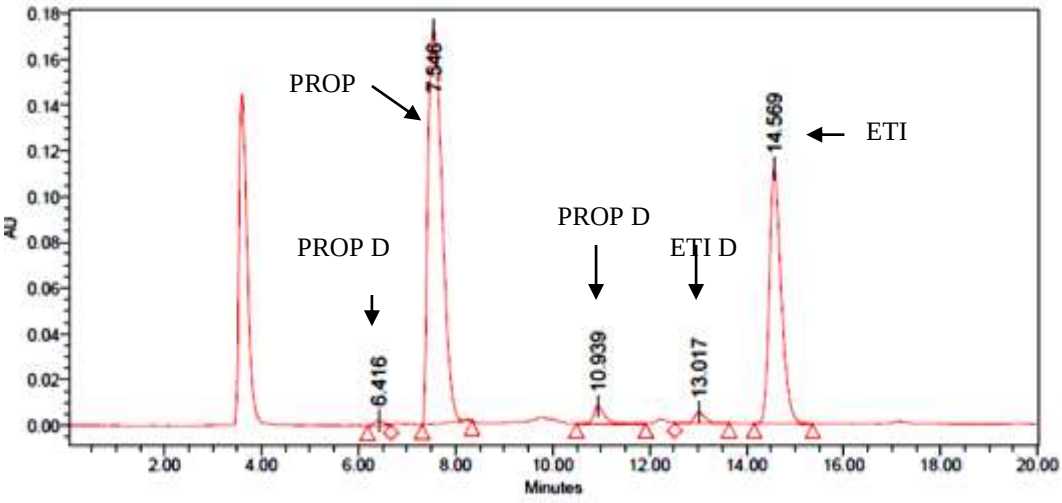

Fig No: 10.a Chromatogram of Sample solution (Formulation) in oxidative condition

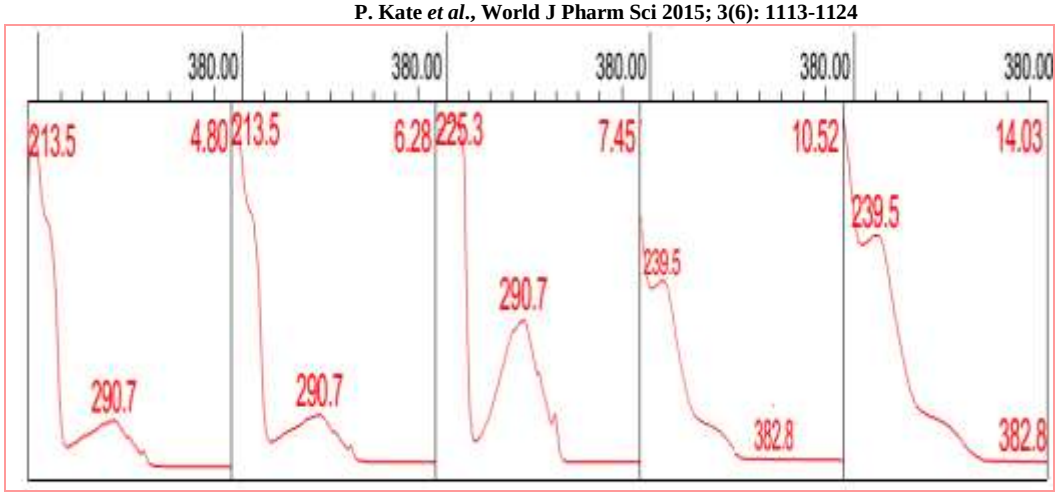

Fig No: 10.a PDA spectra in oxidative condition

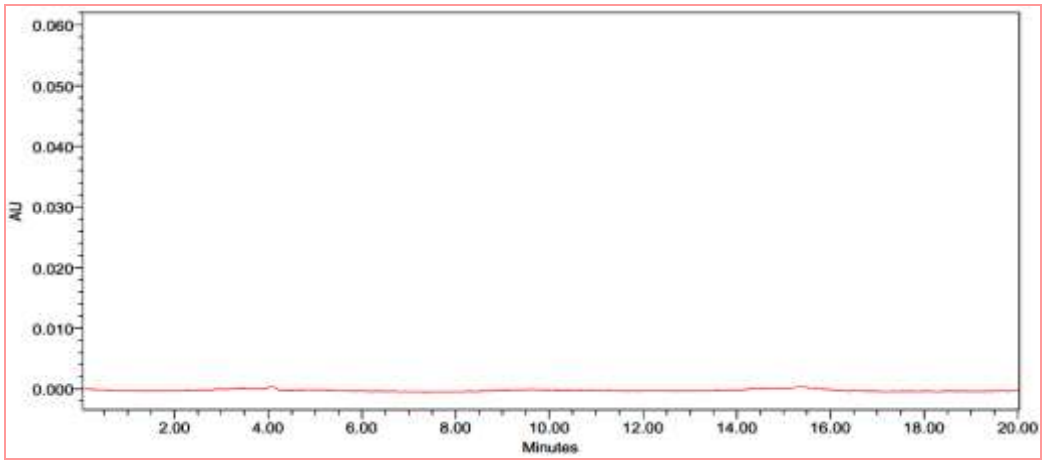

Fig No: 11 Chromatogram of blank in Thermal condition

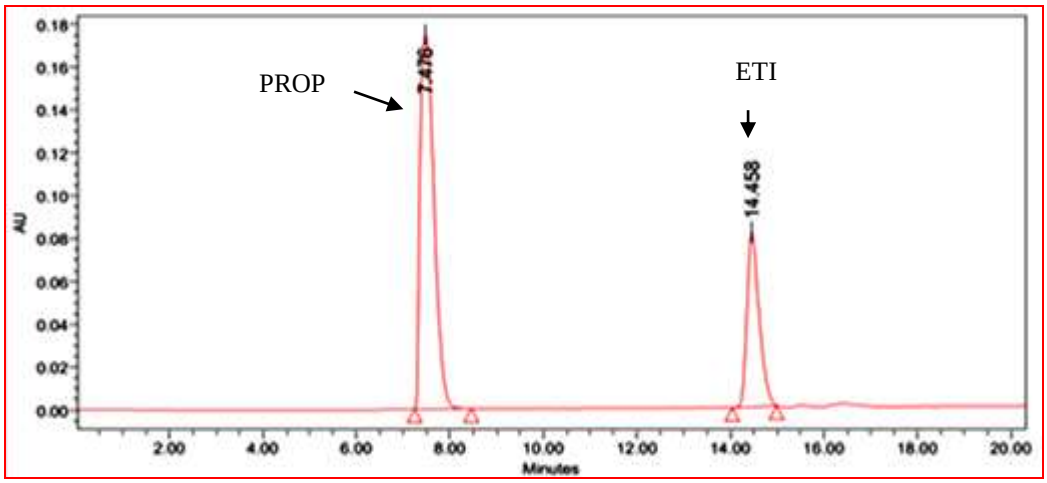

Fig No: 12.a Chromatogram of Sample solution (Formulation) in thermal condition

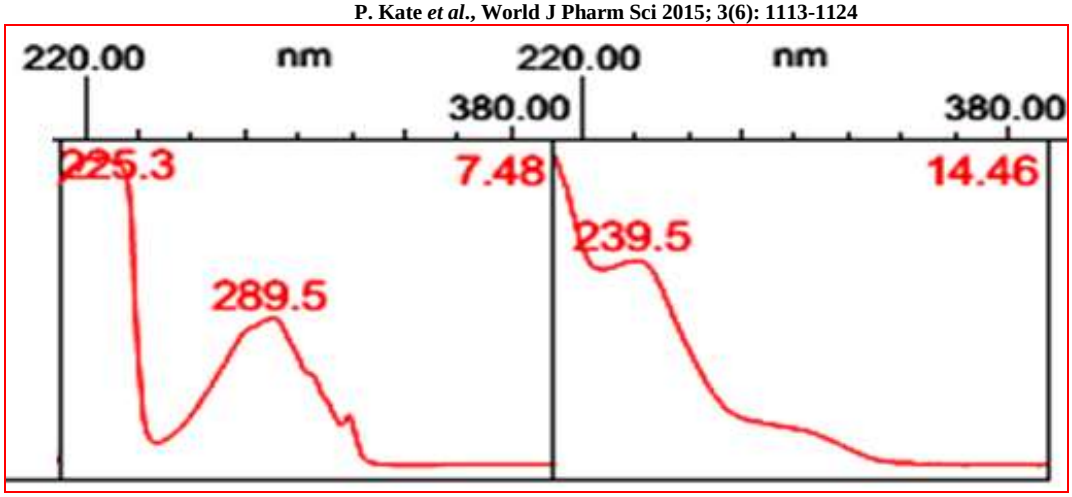

Fig No: 12.b PDA spectra in thermal condition

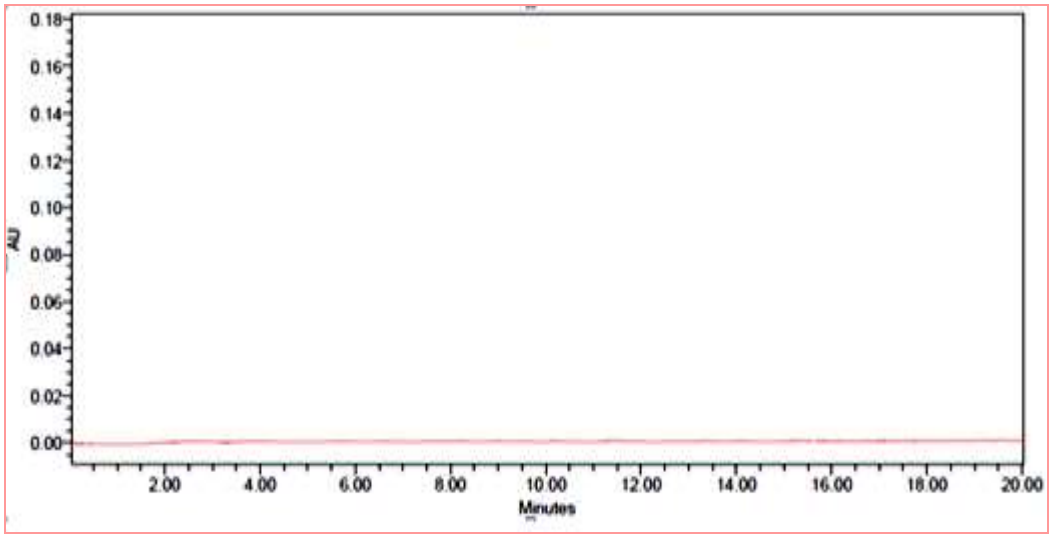

Fig No: 13 Chromatogram of blank in photolytic condition

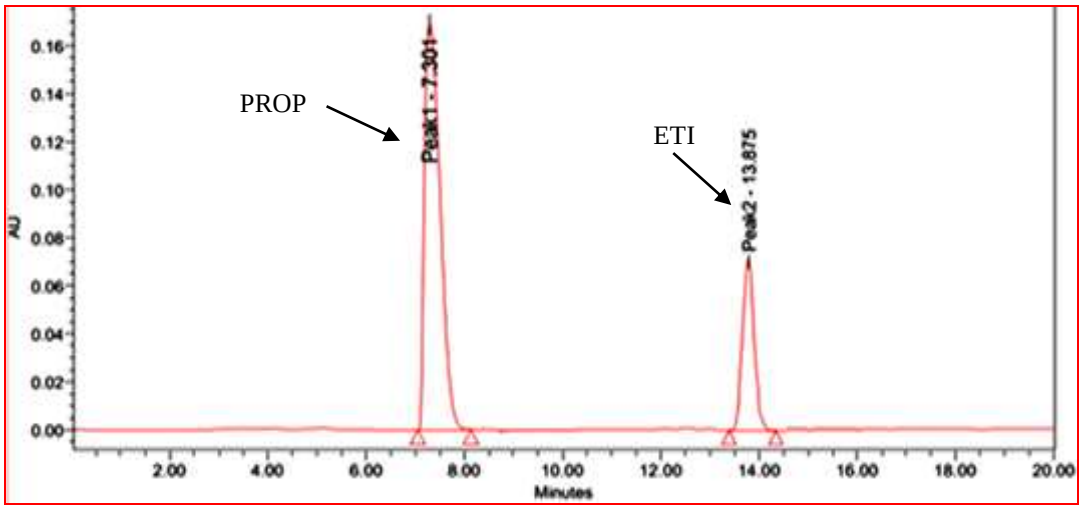

Fig No: 14.a Chromatogram of Sample solution (Formulation) in photolytic condition

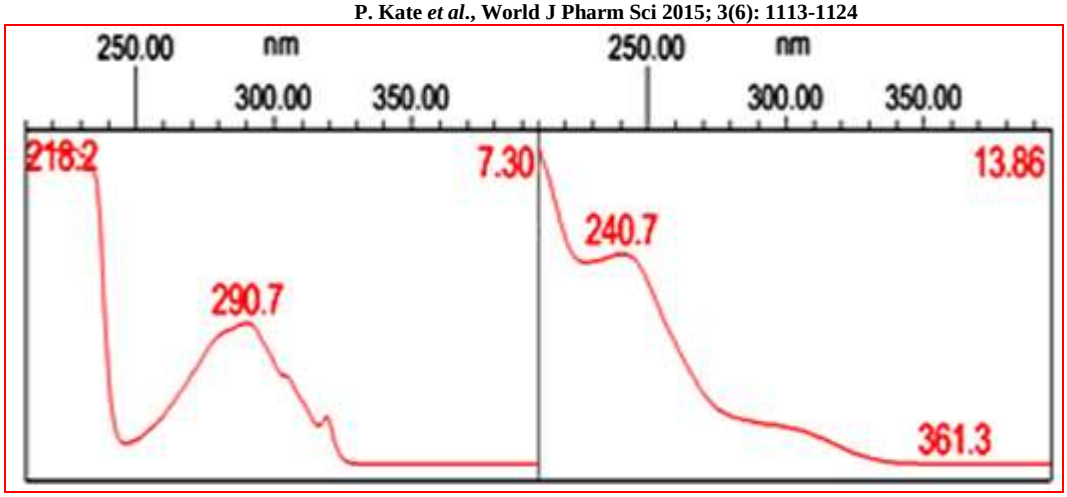

Fig No: 14.b PDA spectra in photolytic condition

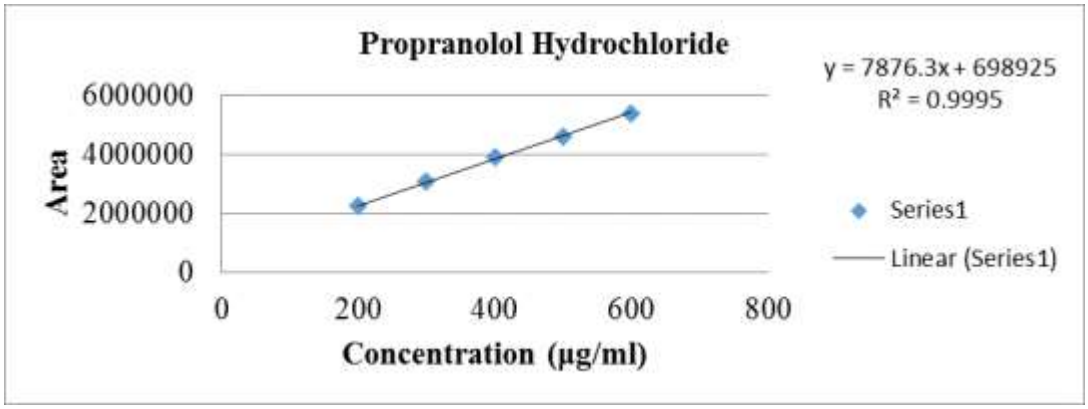

Fig No: 15 Calibration curve of Propranolol Hydrochloride

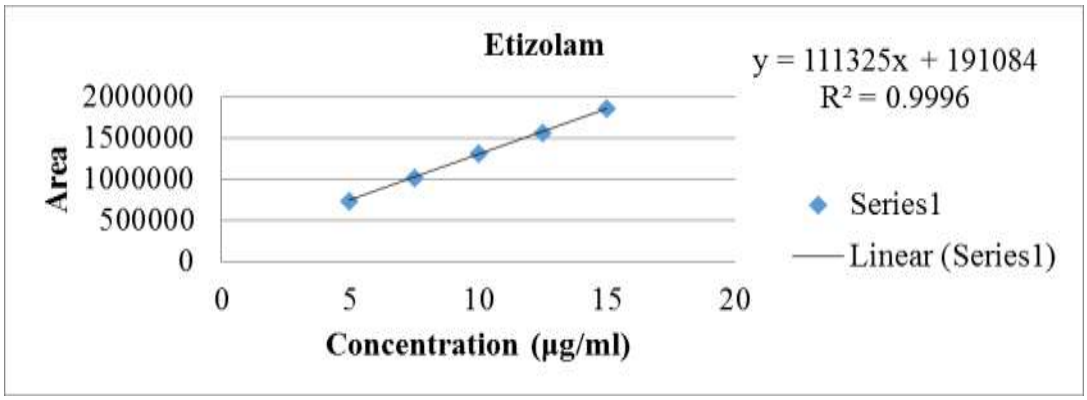

Fig No: 16 Calibration curve of Etizolam

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