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DEVELOPMENT AND VALIDATION OF RP -HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF BISOPROLOL FUMARATE AND TELMISARTAN IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

New, simple and sensitive RP-HPLC method was developed for simultaneous estimation of Bisoprolol Fumarate and Telmisartan in bulk and tablet formulation. The present study was undertaken to develop and validate an accurate, robust, precise and cost – effective RP-HPLC method for the simultaneous estimation of Bisoprolol fumarate and Telmisartan in bulk and tablet pharmaceutical formulation. Separation was achieved on C_8 Column (150mmx4.6mm,5µm) using Ammonium formate, Acetonitrile :Methanol (50:50 v/v) as a mobile phase with flow rate of 1.0ml/min. The detection carried out at wavelength 231 nm. The retention times of Bisoprolol fumarate and Telmisartan were found that 6.7 min and 9.0 min respectively. The method was validated in terms of linearity, precision, Robustness, calibration as per ICH guidelines. linearity was carried out by using concentration range 5-25 µg.mL^{-1} for Bisoprolol fumarate and 40-200 µg.mL^{-1} for Telmisartan. The method shows linearity in the mentioned concentration having correlation coefficient R^2 0.999 and 0.999respectively. The mean recoveries of bisoprolol fumarate and telmisartan were found to be 99-101% respectively. The %RSD of the Bisoprolol fumarate and Telmisartan were found to be 0.38-0.54 and 0.01-0.51 respectively. The development RP-HPLC method was found to be highly specific. The method could be applicable for routine determination in bulk drug as well as dosage formulation containing Bisoprolol and Telmisartan combination.

KEY WORDS **:** RP-HPLC; Bisoprolol fumarate; Telmisartan; Validation; Simultaneous estimation; etc

INTRODUCTION:

The many numbers of drugs introduced in market is increasing every year. These drugs are either new entities or partial structural modification of the existing one. very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties and introduction of better drugs by competitors. Under these conditions, standard and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary to develop newer analytical method for such drugs.

Safety and efficacy of medical products can only be achieved by analytical monitoring of quality and by validated analytical procedure as per ICH guidelines.[1-4] In the modern pharmaceutical industry, high – performance liquid chromatography is the major and integral analytical tool applied in all stages of drug discovery, development and production .[5-6]

HPLC is the method of choice for checking purity of new chemical entities, monitoring reaction changes is in synthetic procedure or scale up, evaluating new formulation and carrying out quality control/assurance of

the final drug products.[7] the goal of HPLC method is to separate, quantify the main drug, reaction impurities ,all available synthetic intermediates and any degradants.[8]

Method validation

Validation of an analytical method is the " A documented program ,which provide a high degree of assurance that a specific process will consistency produce, a product meeting its pre – determined specification and quality attribute". Validation is concerned with assuring a measurement process produces valid measurement. Results from method validation can be used to judge the quality, reliability and consistency of analytical results.[9,10]

Aim and Objective of Present Work

Present work is an attempt have been made to developed simple, accurate, precise and rapid RP-HPLC methods for determination of Bisoprolol fumarate and Telmisartan.

Analysis is an important in every product but it is vital in medicine as it involves life. The assurance of quality is achieved through analysis of product. The objective of this work is as follows:

- To developed simple, rapid and sensitive, stable and highly effective simple RP-HPLC method for determination of Bisoprolol fumarate and Telmisartan.
- To validate methods as per ICH Guidelines.
- To analyzed Bisoprolol fumarate and Telmisartan using validated methods quantitatively and qualitatively.

Introduction of Bisoprolol fumarate

Chemically Bisoprolol fumarate is (E)-but-2-enedioicacid;(propan-2-ylamino)-3-[4-(2-propan-2-yloxyethoxymethyl)phenoxy]propan-2-ol. Bisoprolol Fumarate competitively blocks the activation of this cascade, so decreases the adrenergic tone/stimulation of the heart muscle and pacemaker cells. Decreased adrenergic tone shows less contractility of heart muscle and lowered heart rate of pacemakers. Structure of Bisoprolol fumarate Shows in **fig.1.**

Introduction of Telmisartan

Chemically Telmisartan is 2-[4 -[[4-methyl-6-(1-methylbenzimidazol-2-yl) -2-propylbenzimidazol–1-yl]methyl]phenyl]benzoate. Telmisartan blocks the vasoconstriction and aldosterone secreting effect of angiotensin – 2 by selectively blocking the binding of angiotensin 2 to the AT1 receptor in many tissues such as vascular smooth and the adrenal gland. Structure of Telmisartan Shows in **fig.2.**

EXPERIMENTS

Material and Methods-

Bisoprolol fumarate was provided by Ajanta Pharma Pvt. Ltd Besicor T Tablets containing Bisoprolol fumarate (5mg) and Telmisartan (40 mg) was obtained from market. HPLC grade methanol obtained from merck Ltd., India.

Diluent -

The mobile phase was used as a diluent.

Instrumentation-

Instrumentation is showing in **Table 1.**

Preparation of standard stock solution:

Bisoprolol Fumarate Standard Solution:

Accurately weighed Bisoprolol Fumarate 5 mg was dissolved in Methanol and volume was make upto 100 ml Methanol. The stock Solution was diluted further with mobile phase to get final concentration of about 5µg/ml of Bisoprolol Fumarate.

Telmisartan Standard Solution:

Accurately weighed Telmisartan 40 mg was dissolved in Methanol and volume was make upto 100 ml Methanol. The stock Solution was diluted further with mobile phase to get final concentration of about 40.0 µg/ml of Telmisartan.

Procedure:

Filtered mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. A 20µl std. drug solution was injected which was made in five replicates and system suitability parameters were recorded.

Preparation of laboratory mixture (sample):

Five different laboratory mixtures of BISO and TEL are prepared by appropriately weighing the quantities of drug samples so as to get the concentration of 5 µg/ml of BISO and 40µg/ml of TEL .The peak area of standard laboratory mixture and sample laboratory mixture compared to obtained the concentration. **Table 2.**

The amount of each drug estimated in laboratory mixture was calculated using following formula:-

$$\% \text{ Estimation} = \frac{At}{As} \times \frac{Ds}{Dt} \times \frac{Ws}{Wt} \times 100$$

At = Area count for sample solution.

As = Area count for standard solution.

Ds =Dilution factor for standard.

Dt = Dilution factor for sample.

Ws =Weight of standard.

Wt = Weight of sample.

Selection of Analytical Wavelength

Accurately, 5 mg of Bisoprolol Fumarate and 40 mg Telmisartan reference standard was weighed and transferred to a 100 ml of volumetric flask and make up the volume with diluents i.e. methanol. The solution is further diluted to get final concentration of (5.0 µg/mL of BISO & 40.0 µg/mL of TEL) respectively. The solutions were scanned separately in the range of 400-200 nm against methanol as blank. The BISO & TEL shows their maximum absorbance at 230.0 & 234.0 nm respectively. While 231.0 nm was the point of equal absorbance i.e. Iso-bestic point. The resulting overlain spectrum is shown in **Fig.3.**

METHOD VALIDATION

Validation Parameters:

a) Accuracy:-

Recovery studies were performed to validate the accuracy of developed method. To pre analysed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed **Table 3**. Statistical validation of recovery studies shown in **Table 4**.

b) Precision:-

The method was established by analyzing various replicates standards of BISO and TEL and all the solution were analyzed thrice in order to record any intra-day & inter-day variation in the result. The result obtained for intraday are shown in **Table 5.** the result obtained for interday variation are shown in the **Table 6.** respectively.

c) Specificity:

Specificity was measured as ability of the proposed method to obtained well separated peaks for TEL & BISO without any interference from component of matrix.

Mean retention time for-

BISOPROLOL FUMARATE : 6.5min

TELMISARTAN : 8.9min

Chromatogram obtained by tablet formulation of BISO & TEL is shows in the **Fig 4.**

d) Linearity:

According to USP tablet powder equivalent to 80, 90,100, 110, 120% of label claim was taken and dissolved & diluted appropriately with mobile phase to obtain concentration in the range of 80-120% of the test concentration. The chromatograms of the resulting solution were recorded. BISO and TEL in marketed formulation was found to be linear ± 20% of the test concentration of the respective drug. The plot showing linearity and range study for Bisoprolol Fumarate and Telmisartan is shown in the **Fig 5.** and **Fig 6.** and **Table 7.** and **Table 8.**

e) Robustness:

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and tailing factor of drug peak was studied.

The wavelength was changed in ± 1 nm and the flow rate was varied by ± 0.1 ml min^{-1}, of optimized chromatographic condition. The results of robustness studies are shown in **Table 9.**System suitability parameters were also found satisfactory; hence the analytical method would be concluded.

RESULT AND DISCUSSION

RP-HPLC method was developed for simultaneous estimation of Bisoprolol Fumarate and Telmisartan in tablet dosage form. The separation was achieved by C-8 (Zodiac 100) column of (4.6×150 mm) with particle size packing 5 µm and 35% 20M ammonium Formate + 65 % Methanol-acetonitrile (50:50 v/v) as mobile phase at a flow rate of 1.0 ml/min. The detection was carried out at 231nm. The retention time of was found to be 6.7 min and 9.0 min respectively.

After establishing the chromatographic conditions, analysis of tablet formulation was done. The results are given in **Table 10.**

Table 1. Instrumentation

Sr. No.	Instrument	Make	Model
1.	**UV-Visible Spectrophotometer**	Shimadzu	Double beam carry- 07 Bio UV 1600
2.	**HPLC**	Shimadzu SCL-10AVP	UV Detector
3.	**Balance**	Wensar	CY 104 (Micro-Analytical Balance)
4.	**PH Meter**	Equip-tronich	EQ-614A
5.	**Ultrasonicator**	Meta-lab	5 L

Table 2. Result and Statistical dada for estimation of BISO & TEL in Laboratory Mixture

Sr. no	Weight of Standard(µg/ ml)		Wt of sample (µg/ml)		Peak area of standard		Peak area of sample		% Label claim	
	BISO	TEL	BISO	TEL	BISO	TEL	BISO	TEL	BISO	TEL
1	5	40	5	40	4601718	12230177	4596390	12196828	99.88	99.72
2	5	40	5	40	4602098	12191009	4597561	12185993	99.90	99.95
Mean									99.89	99.83
SD									0.014	0.1626
%RSD									0.01	0.162

Table 3. Recovery study of BISO and TEL

Level of Recovery (%)	80		100		120	
	BISO	**TEL**	**BISO**	**TEL**	**BISO**	**TEL**
Amount Present (mg)	**5**	**40**	**5**	**40**	**5**	**40**
	5	**40**	**5**	**40**	**5**	**40**
Amount of Std added	**4**	**32**	**5**	**40**	**6**	**48**
	4	**32**	**5**	**40**	**6**	**48**
Amount Recovered	**9.11**	**72.10**	**10.12**	**80.25**	**11.27**	**88.17**
	9.05	**72.12**	**10.06**	**80.15**	**11.17**	**88.24**
(%) Recovery	**101.22**	**100.14**	**101.20**	**101.56**	**102.45**	**100.19**
	100.56	**100.17**	**100.60**	**100.27**	**101.55**	**100.27**

Table No 4 Statistical Validation of Recovery Studies

Level of Recovery (%)	Drug	Mean (%) Recovery	Standard Deviation	% RSD
80	BISO	101	0.38	0.38
	TEL	100	0.01	0.01
100	BISO	100.80	0.35	0.34
	TEL	101	0.78	0.78
120	BISO	102.55	0.55	0.54
	TEL	100	0.51	0.51

Table 5. Intra-day Precision study of BISO & TEL

Samples	Amount Taken [µg/mL]		Area of Peaks		Amount Found [µg/mL]		% Amount Found	
	BISO	TEL	BISO	TEL	BISO	TEL	BISO	TEL
Precision 1	5	40	4599602	11968207	5.005	40.012	100.10	100.03
Precision 2	5	40	4597561	11915032	5.009	40.008	100.18	100.02
Precision 3	5	40	4596391	11929363	4.999	40.12	99.98	100.03
	Mean		4597851	11898690	5.004	40.04	100.08	100.02
	SD		1625	27513	0.005	0.063	0.10	0.005
	%RSD		0.03	0.23	0.100	0.158	0.10	0.005

Table 6. Inter-day Precision study of BISO & TEL:

Samples	Amount Taken [µg/mL]		Area of Peaks		Amount Found [µg/mL]		% Amount Found	
	BISO	TEL	BISO	TEL	BISO	TEL	BISO	TEL
Precision (Day 1)	5	40	4469439	12381011	4.999	39.99	99.98	99.99
Precision (Day 2)	5	40	4604059	12323800	5.006	39.97	100.12	99.97
Precision (Day 3)	5	40	4546561	12381011	5.002	39.99	100.04	99.99
	Mean		4573351	21361971	5.002	99.98	100.04	99.98
	SD		28948	33030	0.03	0.01	0.07	0.01
	%RSD		0.63	0.26	0.07	0.01	0.07	0.01

Table 7 . Linearity studies of BISO

Sr. No.	CONC.	Average Area
1	5	219867
2	10	449734
3	15	661601
4	20	880228
5	B25	1109335

Table 8. Linearity studies of TELMI

Sr. No.	CONC.	Average Area
1	40	497633
2	80	989471
3	120	1517963
4	160	2027134
5	200	2469351

Table 9. Result of Robustness Study of BISO & TEL

Robustness Studies							
Samples	Condition	Amount Taken [µg/mL]		Retention Time		% Amount Found	
		BISO	TEL	BISO	TEL	BISO	TEL
Robustness 1	Flow rate (< 0.1 mL/min)	5	40	7.14	9.23	99.84	99.82
Robustness 2	Flow rate (> 0.1 mL/min)	5	40	6.61	8.98	99.76	100.02
Robustness 3	Wavelength (< 2.0 nm)	5	40	6.6	8.9	100.21	100.13
Robustness 4	Wavelength (> 2.0 nm)	5	40	6.52	9.0	100.12	100.27

Table 10 Results and statistical data for estimation of BISO and TEL in marketed formulation.

Sr. no	Weight of Standard(µg/ml)		Wt of sample (µg/ml)		Peak area of standard		Peak area of sample		% Label claim	
	BISO	TEL	BISO	TEL	BISO	TEL	BISO	TEL	BISO	TEL
1	5	40	5	40	4601718	12230177	4590130	12215912	99.74	99.88
2	5	40	5	40	4602098	12191009	4597030	1220596	99.88	100.10
Mean									99.81	99.99
SD									0.098	0.155
%RSD									0.099	0.155

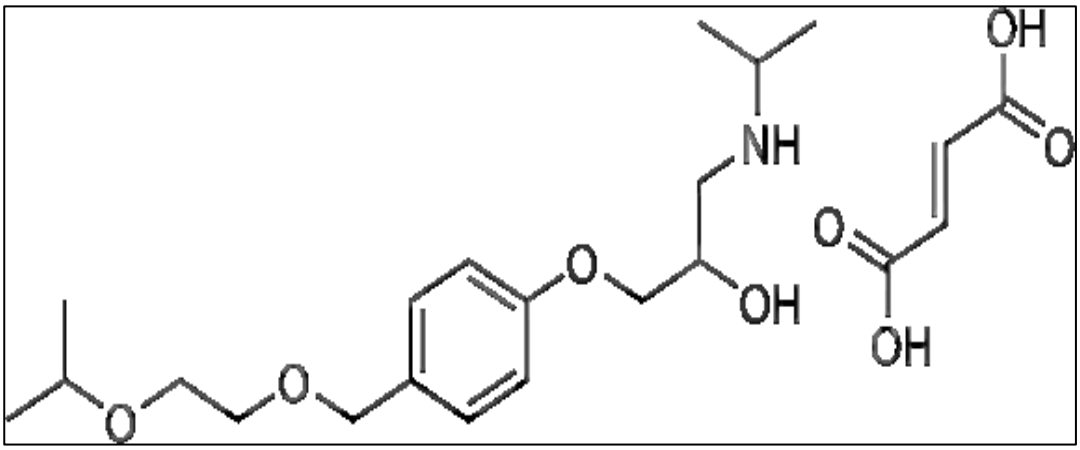

Fig 1. Structure of Bisoprolol fumarate

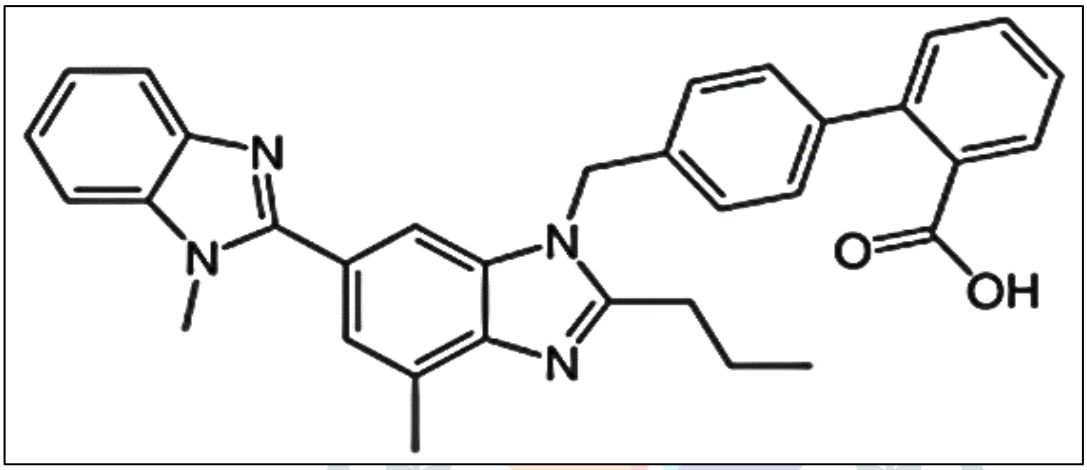

Fig 2. Structure of Telmisartan

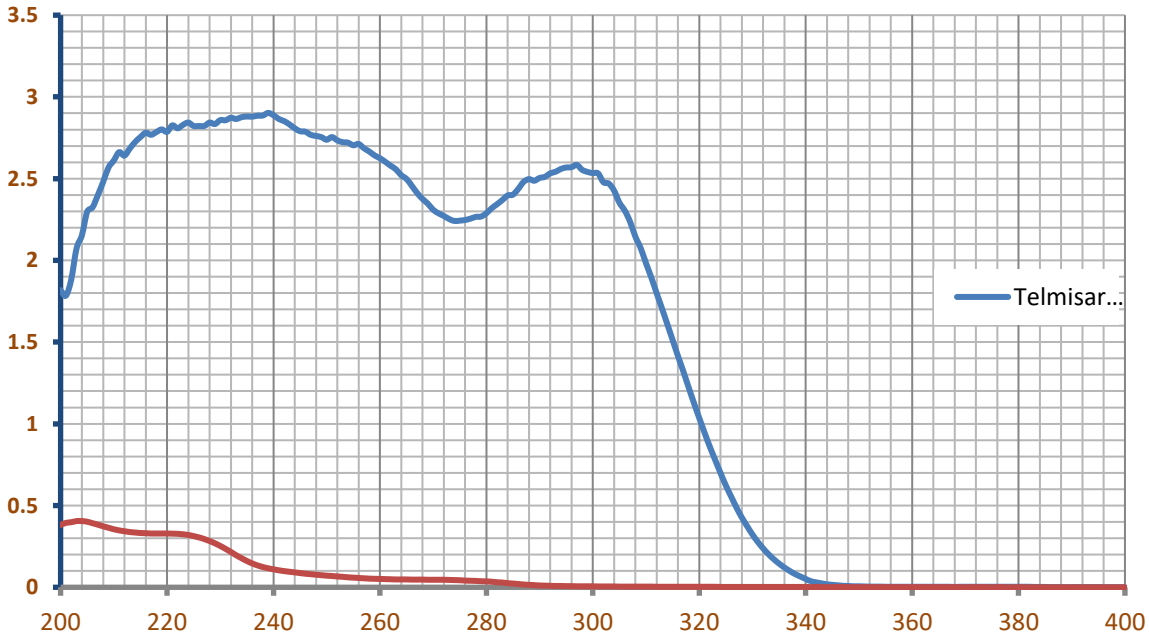

Fig No 3: UV Graph of Bisoprolol and Telmisartan

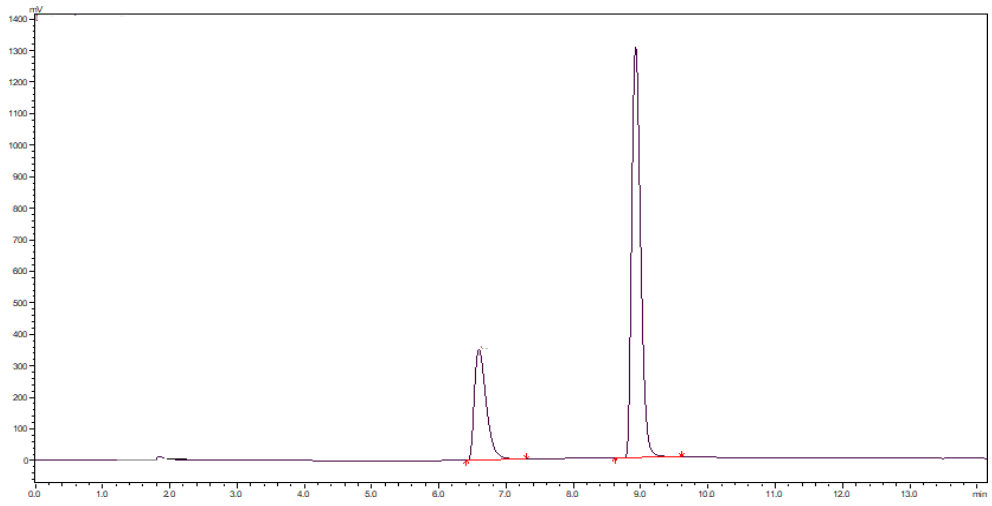

Fig 4 .Chromatogram obtained by tablet formulation of BISO & TEL

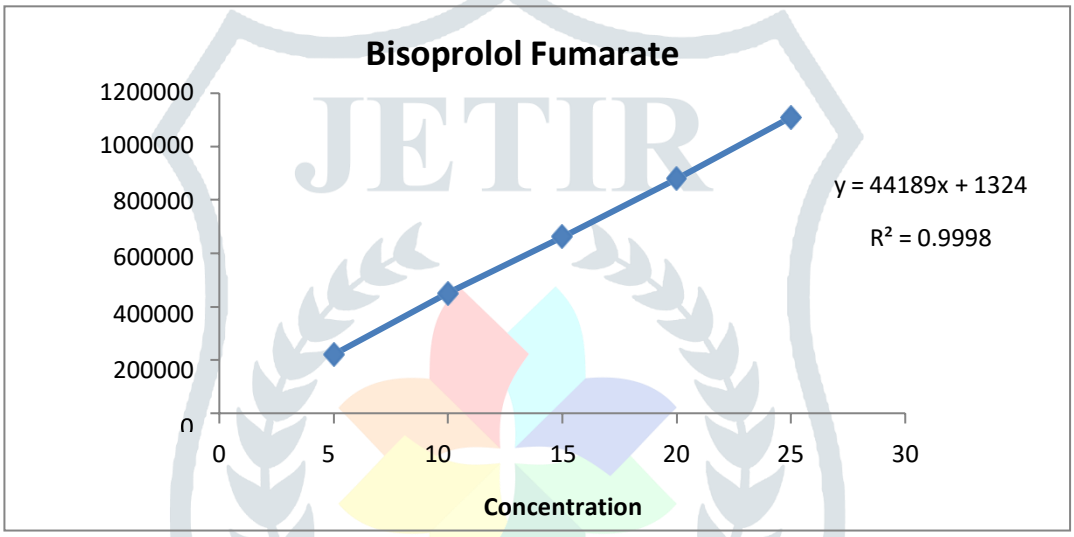

Fig 5. Observations of Standard Calibration Curve of Bisoprolol Fumarate

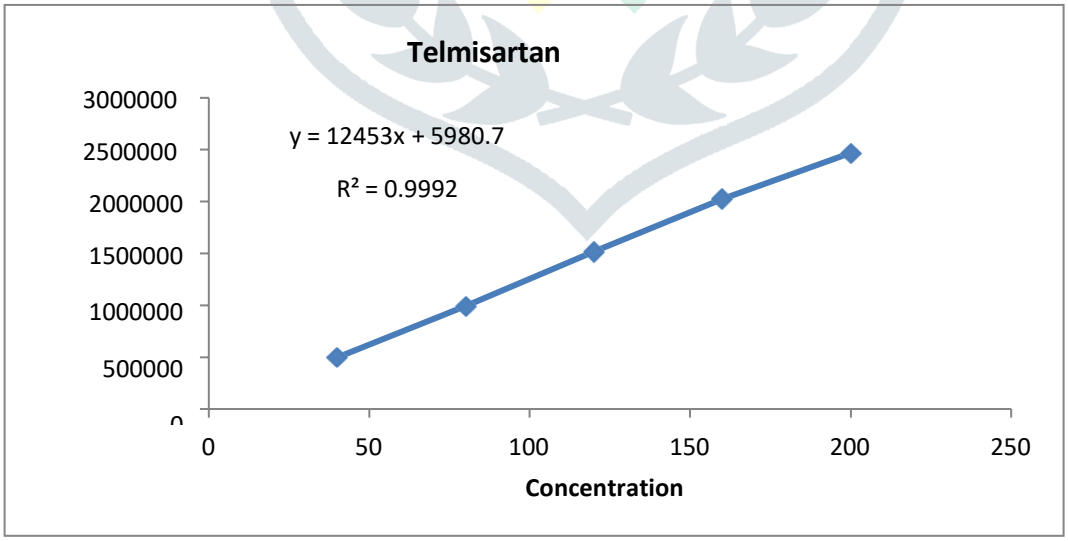

Fig 6. Observations of Standard Calibration Curve of Telmisartan

Discussion

Analysis of bulk and tablet formulation was done and the results obtained within acceptable limits. The results obtained for validation study were within the limit specified by the ICH guidelines and hence the method was found to be linear, precise. The results of recovery study were within ICH limits, thus indicating the accuracy of method.

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