Research Article

DEVELOPMENT AND VALIDATION OF RP - HPLC METHOD FOR THE ESTIMATION OF TELMISARTAN IN BULK DRUG USING INTERNAL STANDARD

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ABSTRACT
The aim of this work is to develop & validate a simple, precise, rapid and accurate reverse phase HPLC method for the estimation of Telmisartan using eprosartan mesylate as an internal standard (IS) in bulk and tablet dosage form. Separation was achieved under optimized chromatographic conditions on a phenomenex C-18 column (250 X 4.6 mm, particle size 5µ) with mobile phase consisting of 10mM potassium di hydrogen phosphate buffer:methanol in the ratio 20: 80 v/v, PH adjusted to 5.8 with 10%v/v ortho phosphoric acid at a flow rate of 0.8 ml/ min at ambient temperature. The detection was carried out at 296 nm using waters UV Visible detector. The calibration curve was linear in the concentration range of 1–10mg/ ml (r2= 0.9979).The limit of detection and the limit of quantification were found to be 50 ng/ml and 80 ng/ml respectively. The amount of Telmisartan present in the formulation was found to be 99.95. The method was validated statistically using the SD and %RSD and the values are found to be within the limits and the recovery studies were performed. The percentage recoveries were found to be 99.55± 0.7211 %. So, the proposed method was found to be simple, specific, linear, and rugged. Hence it can be applied for routine analysis of Telmisartan in bulk and Pharmaceutical formulations.

Keywords: Telmisartan, Eprosartan mesylate, RP-HPLC, Bulk Drug, Internal Standard, validation.

INTRODUCTON
Telmisartan,4-[(2-n-propyl-4-methyl-6-(1-methylbenzimidazole-2-yl)-benzimidazole-1-yl)methyl]-biphenyl-2-carboxylicacid, is a selective angiotensin II type 1 receptor (AT1R) blocker, which belongs to the group of angiotensin II receptor antagonists[1]. It inhibits the angiotensin II receptor in a way that the effect of angiotensin II is blocked resulting in a decrease of blood pressure. There are different mechanisms: increasing the activity of the sympathetic nervous system, causing a boosted sodium revertive resorption in the kidneys and promotion of the secretion of aldosterone in the adrenal glands. The most recent clinical trials demonstrated that telmisartan also has preventive roles against ischemic heart diseases in diabetic patients with a similar potency to angiotensin converting enzyme inhibitor. Several studies recently suggest that the effects of telmisartan are mediated via not only blockade of AT1R but also activation of peroxisome proliferators-activated receptor (PPAR) a central regulator of insulin and glucose metabolism. It is

believed that telmisartan's dual mode of action may provide protective benefits against the vascular and renal damage caused by diabetes and cardiovascular disease (CVD). Telmisartan has binding affinity 3000 times with AT-2 receptor than AT-1 receptor. Telmisartan is also having maximum half-life in sartans - 24 hrs[2-6]. Structure is shown in figure-1.

M. Laxmi surekha et.al reported a RP-HPLC method for determination of telmisartan in bulk &tablet dosage form but the retention time was long (6.269). M.S.Charde et al developed method for determination of telmisartan and forced degradation behavior by RP-HPLC in tablet dosage form, in this method the mobile phase used was not economical and retention time was more. N.R.Vekaria et al also reported a RP-HPLC method for telmisartan with acetonitrile-phosphate buffer (60:40) with a retention time of 7.02 min[6-8]. A few analytical methods were developed for telmisartan in human plasma[9-16].

The aim of the present work was to develop and validate a simple, fast and reliable is RP-HPLC method with UV detection for the determination of telmisartan in bulk and in tablet dosage forms with low retention time using eprosartan mesylate(IS) as an internal standard. The important features and novelty of the proposed method included simple sample treatment with sonication of small amount of powder sample at ambient temperature, short elution time (less than 5 min) TST, good precision (R.S.D.less than 2%) and high recovery (greater than 98%). Confirmation of the applicability of the developed method validated according to the International Conference on Harmonization (ICH) for the determination of telmisartan[17].

MATERIALS AND METHODS
Reagents and chemicals
Telmisartan (99.4%) was obtained from local manufacturer, Hyderabad, India, as gift sample. Methanol (HPLC Grade), Potassium di hydrogen phosphate (AR Grade), ortho-phosphoric acid (AR Grade) were purchased from E. Merck (India) Ltd. Mili-Q water was used throughout the experiment.

Instrumentation and chromatographic conditions
The HPLC system consisted of a Shimadzu LC-20AT liquid chromatographic pump, Rheodyne injection port(Rheodyne, Cotati, CA,USA) with a 20µl sample loop and SPD-M20A Photo diode array(PDA) detector (Shimadzu, Kyoto,

Japan).Data collection, integration and calibration were accomplished using LC Solutions chromatography Data system.

Chromatography was performed on a SYMMETRY C18 packed column (5µm, 250×4.6mm particle size, WATERS, Ireland) maintained at 40^0 c. The HPLC system consists of Shimadzu LC-20 AT liquid chromatographic pump, Rheodyne injection port (Rheodyne, cotati, CA, USA) with a 25µl sample loop and SPD-M20A photo diode array (PDA) detector (Shimadzu, Kyoto, Japan). During method development various mobile phases and different solvent systems were used.The mobile phase with Potassium di hydrogen phosphate (10mM, pH 7.0 adjusted with ortho-phosphoric acid) and methanol in ratio of 20:80 (v/v) at a flow rate of 0.8ml/min was found to be optimum, which gave chromatogram with a sharp peak and good retention time. The mobile phase was filtered through 0.45µm filter and degassed for 10 minutes by sonication.The detection was carried out using a UV spectrophotometric detector at 296 nm and the run time was 7 min. The peaks were identified by retention time; a typical chromatogram is shown in fig-2.

Preparation of standard drug solution
Stock solutions of telmisartan with a concentration of 1000µg/ml was prepared by dissolving 25mg of telmisartan into 25ml volumetric flask and add about 25ml of diluent (methanol) and sonicate to dissolve completely, make volume up to the mark with the same diluent. The stock solution is further diluted to obtain standard solutions of 1, 3, 5, 7, 9 and 10µg/ml of telmisartan.

System suitability
The HPLC system was stabilized for forty min. One blank followed by six replicates of a single calibration standard solution of Telmisartan was injected to check the system suitability. To ascertain the systems suitability for the proposed method, a number of parameters such as theoretical plates, peak asymmetry, retention time and parameters were taken and results were presented in Table 1.

RECOMMENDED PROCEDURE
Construction of calibration curve
Replicates of each calibration standard solutions (1, 3, 5, 7, 9, 11 µg/mL) were injected into the column, the retention times and average peak areas were recorded. Calibration graph was plotted by taking concentration of telmisartan on

X-axis and peak area on Y-axis and results and relevant data were furnished in Table 2.

Validation procedure
An integral part of analytical method development is validation. Once the method has been developed, it is necessary to evaluate under the expected conditions for real samples before being used for the specific purpose. The proposed method of analysis was validated as per the ICH guidelines for the parameters like specificity, precision, accuracy, linearity, robustness, system suitability, limit of detection (LOD) and limit of quantitation (LOQ).

Precision
Intraday and interday precision studies are carried out by estimating corresponding responses of telmisartan and IS, five times on the same day and on two different days for the concentration of 10µg/mL. The repeatability of sample and IS application and measurements for peak area at 296nm are expressed in terms of % RSD. The percent relative standard deviation (% RSD) is calculated which is within the acceptable criteria.

Linearity
The linearity graphs for the proposed assay methods are obtained over the concentration range of 1-11 µg/ml telmisartan and IS. The representative chromatograms indicating the telmisartan and IS were shown in Fig. 2 to 8. A calibration curve was plotted between concentration and area response and statistical analysis of the calibration curve was shown in Fig. 9. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient, regression data values, and the results of regression statistics were represented in Table 2.

Specificity
The effect of wide range of excipients and other additives usually present in the formulations of telmisartan in the determinations under optimum conditions was investigated. The specificity of the RP-HPLC method was established by injecting the mobile phase and placebo solution in triplicate and recording the chromatograms. Marketed formulations are analyzed to determine the specificity of the optimized method in presence of common tablet excipients.

Accuracy (Recovery studies)
The accuracy of the method is determined by calculating recovery of telmisartan and IS by the method of addition. Known amount of telmisartan and IS at 1µg/ml, 5µg/ml and 10µg/ml. The recovery studies are carried out and the mean percentage recovery of telmisartan and IS at each level is not less than 95% and not more than 105%.

Stress Analysis
Stability testing studies of Telmisartan were carried out according to ICH guidelines by subjecting it to different stress conditions. Photostability was carried out in photostability chamber containing 40^0C 75% RH, UV light for six hours according to ICH Guidelines.

Under acidic condition: 1ml of 5µg/ml of drug solution and internal standard (Eprosartan) were subjected to 0.1N HCl for six hours. The peak area of internal standard was degraded to 50% was observed, but there was no degradation observed in drug's peak area.

In Basic Condition: 1ml of 5µg/ml of drug solution and internal standard (Eprosartan) were subjected to 0.1N NaOH for six hours. Retention time of drug gradually decreased as well as peak area of drug also decreased.

UV Exposure: No significant changes were observed in UV light.

Oxidative Stress Analysis: when subjected to oxidative stress analysis both the drug and internal standard were also degraded to 50%.

Temperature Effect: There was no significant change, upon changing temperature of the drug and internal standard.

RESULTS AND DISCUSSION
The mobile phase consists of methanol buffer pH 7.0 in the ratio of 80:20 v/v. Isocratic elution was carried out with an optimized flow rate of 0.8mL/min which gave sharp peak, and a minimum tailing factor with runtime for telmisartan and IS. The retention time for telmisartan and IS was found to be 4.804min 3.175min respectively. UV spectra of telmisartan and IS showed that the drug absorbed maximum at 296nm, so this wavelength was selected as the detection wavelength. System suitability parameters & optimized chromatographic conditions are shown in Table 1. The calibration

curve for telmisartan and IS was found to be linear over the range of 1-11 µg/mL. The data of regression analysis of the calibration curve was shown in Table 2. The representative standard chromatogram of telmisartan and IS were shown in Fig. 2, correlation coefficient, r^2=0.9979 which indicate this method has good linearity. The representative chromatograms indicating the telmisartan and IS were shown in Fig.2 to 8. The calibration curve was shown in Fig. 9. Precision was studied to find out intra and inter day variations in the test methods of telmisartan and IS for three times on the same day and different days. The intra-day and inter-day precision with % RSD < 2% would indicate that the proposed method was quite precise and reproducible and results were shown in Table 3 to 5. Recovery studies of the drug were carried out for the accuracy parameter at three different concentrations levels i.e. multiple level recovery studies. Known amounts of telmisartan and IS standards were added into pre-analyzed sample and subjected them to the proposed HPLC method. The % recovery was found to be within the limits as listed in Table 6. In this case percentage recovery of telmisartan and IS was found to be 102.3% and 95.68%. The method precision was done and the %RSD (<2) would indicate that the proposed method was in good agreement with precision. Robustness was done by small changes in the chromatographic conditions like mobile phase flow rate, temperature, mobile phase composition, detection wavelength (± 5nm) etc., It was observed that there were no marked changes in the chromatograms. In fact the parameters were within the limit, which would indicate that the method was robust and suitable for routine use. The Robustness results were presented in Table 7. The tailing factor of the peak was found to be less than 1.2 and the results were shown in Table 7. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the standard deviation of the response and the slope of the calibration curve at levels approximating the LOD and LOQ. The LOD and LOQ were found to be 50ng/mL, and 80ng/mL respectively, which would show that this method was very sensitive. The results were presented in Table 10.

CONCLUSION
A validated RP-HPLC method has been developed for determination of telmisartan and IS (Eprosartan) in bulk and tablet dosage forms. Statistical analysis of the results shows that the proposed procedure has good precision and accuracy. This method is simple, reliable, accurate, linear, sensitive economical and reproducible. Hence this method can be suitable for routine quality control analysis of telmisartan and IS (Eprosartan) in active pharmaceutical ingredient (API) and pharmaceutical preparations.

Table 1: Optimized chromatographic conditions and system suitability parameters for proposed method

Parameter	Chromatographic conditions
Column	Phenomenex C-18,250*4.6mm,5µm
Mobile phase	Methanol:buffer [80:20][pH 7]
Flow rate	0.8ml/min
Detection wave length	296nm
Run time	7min
Temperature	40^0C
Volume of injection loop	25µL
Retention time of drug	4.804min
Retention time of IS	3.175min
Theoretical plates[th.pl] (Efficiency)	23400
Peak area & height	351121 & 27939

Table 2: Linearity and statistical analysis data for telmisartan and IS (Eprosartan)

S. No.	Concentration (µg/ml)	Peak area		Slope	Correlation coefficient(r^2)
		drug	IS		
1	1	32550	122810		
2	3	96555	118376		
3	5	157070	126502	0.2736	0.998
4	7	210253	119668		
5	9	263288	118355		
6	11	326493	116543		

Table 3: Results of Intraday precision study

Conc.[ug/ml]	1		10	
	Peak area(API)	Peak area(IS)	Peak area(API)	Peak area(IS)
Injection 1	124504	32547	116611	328456
Injection 2	121631	32485	114615	326418
Injection 3	123342	32258	118623	330007
Injection 4	121717	32473	115808	321629
Injection 5	122860	32989	117060	325959
Average	122810.8	32550.4	116543.4	326493.8
Std dev	1197.899	268.3967	1487.928	3168.234
% RSD	**0.975402**	**0.824557**	**1.276716**	**0.970381**

Table 4: Results of Interday precision study: day 1, Analyst 1

Conc.[ug/ml]	1		10	
	Peak area(API)	Peak area(IS)	Peak area(API)	Peak area(IS)
Injection 1	123564	32589	116589	328569
Injection 2	123458	32685	114325	326152
Injection 3	122584	32458	118546	330124
Injection 4	124586	32894	115602	321896
Injection 5	121956	32985	116235	326124
Average	123229.6	32722.2	116259.4	326573
Std dev	1005.319	216.5103	1542.064	3116.36
% RSD	**0.81581**	**0.661662**	**1.3264**	**0.954261**

Table 5: Results of Interday precision study: Day 2, Analyst 2

Conc.[ug/ml]	1		10	
	Peak area(API)	Peak area(IS)	Peak area(API)	Peak area(IS)
Injection 1	124504	32547	116611	328456
Injection 2	121631	32485	114615	326418
Injection 3	123342	32258	118623	330007
Injection 4	121717	32473	115808	321629
Injection 5	122860	32989	117060	325959
Average	122810.8	32550.4	116543.4	326493.8
Std dev	1197.899	268.3967	1487.928	3168.234
% RSD	**0.975402**	**0.824557**	**1.276716**	**0.970381**

Table 6: Recovery data of telmisartan and IS for the proposed RP-HPLC method

Accuracy	Peak Area			Peak Area			Peak Area	
	IS	API		IS	API		IS	API
1µg/mL	124504	32547	5µg/mL	126502	170269	10µg/mL	116611	328456
	121631	32485		119357	167258		114615	326418
	123242	32258		117400	163684		118623	330007
Average	123125.7	32430	Average	121086.3	167070.3	Average	116616.3	328293.7
Recovery	112917	36469	Recovery	115867	170917	Recovery	120338	351121
	94.70	102.45		95.68	102.30		103.19	106.95

Table 7: Robustness results of telmisartan and IS

| S. No.	Parameters	Optimized	Used	Peak Area		Retention Time		Plate Count
				D	IS	D	IS	D & IS
1	Flow rate (±0.2ml/min)	0.8ml/min	0.6ml/min	429196	152576	6.4	4.2	24264
			0.8 ml/min	326493	116543	4.8	3.1	24768
			1.0 ml/min	271455	97471	3.8	2.5	19895
2	Temperature (±1°C)	40°C	39 °C	329031	117010	4.9	3.2	25607
			40 °C	326493	116543	4.8	3.1	24768
			41 °C	329090	118413	4.9	3.2	25607
3	Wavelength (±2nm)	296nm	294	324313	116565	4.83	3.1	23210
			296	326493	116543	4.81	3.1	24768
			298	318499	115189	4.81	3.1	22843
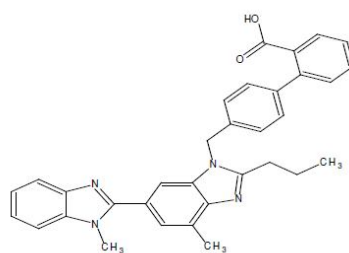
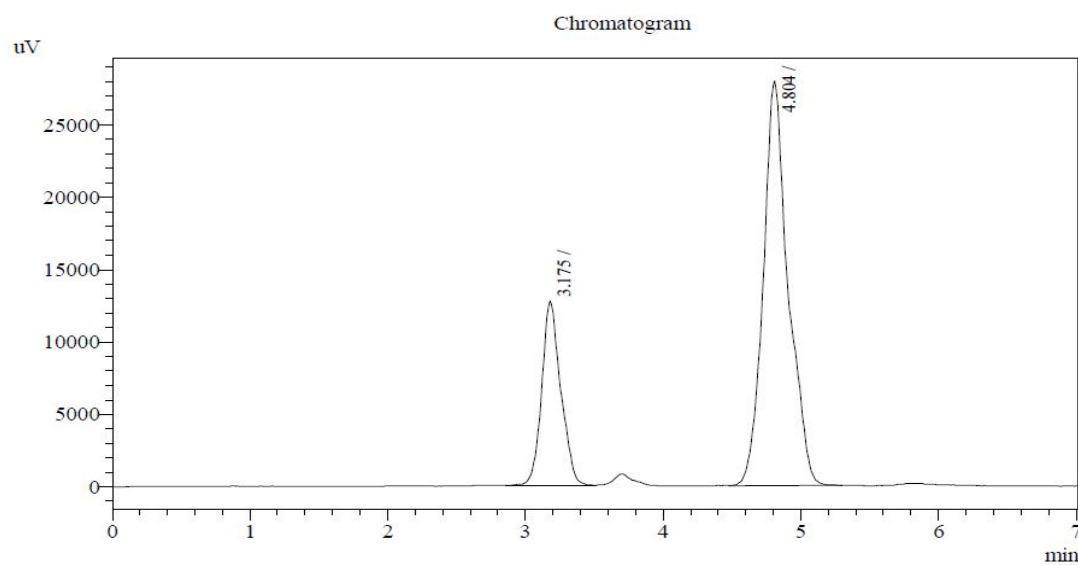**Fig. 1: chemical structure of telmisartan**

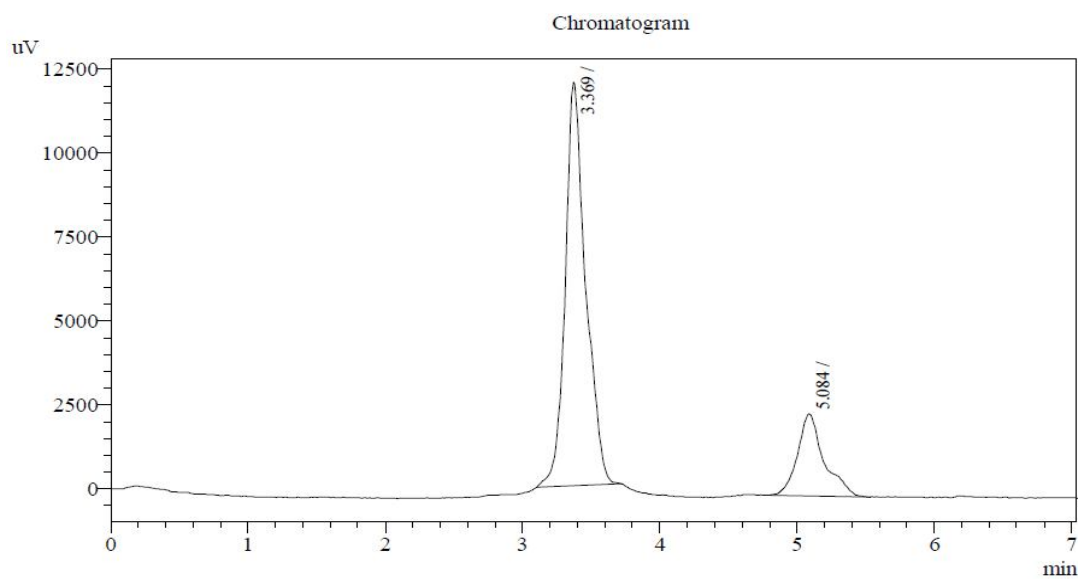**Fig. 2: Standard chromatogram of telmisartan and IS (10µg/mL)**

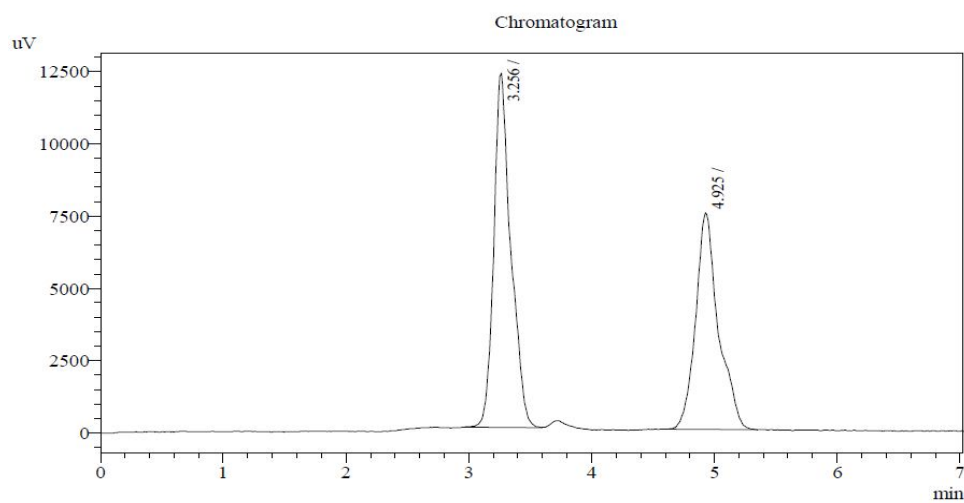**Fig. 3: Standard chromatogram of telmisartan and IS (1 µg/mL)**

Fig. 4: Standard chromatogram of telmisartan and IS (3 µg/mL)
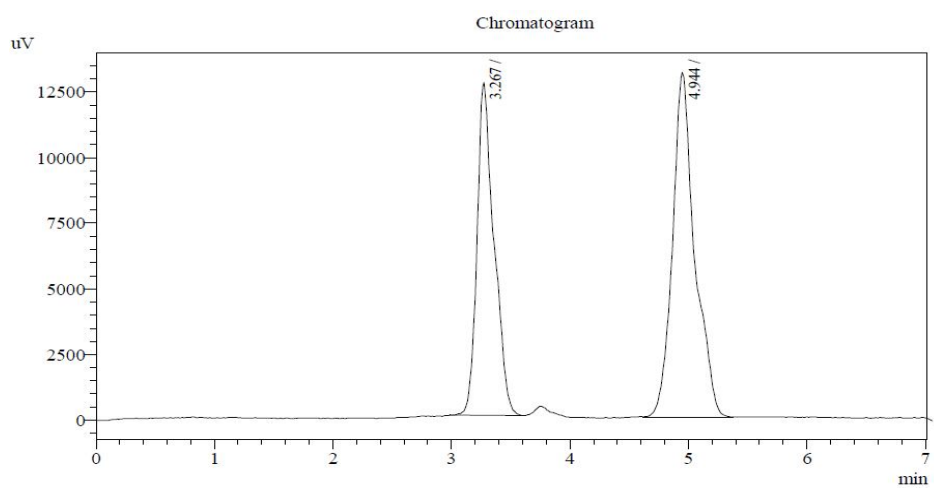

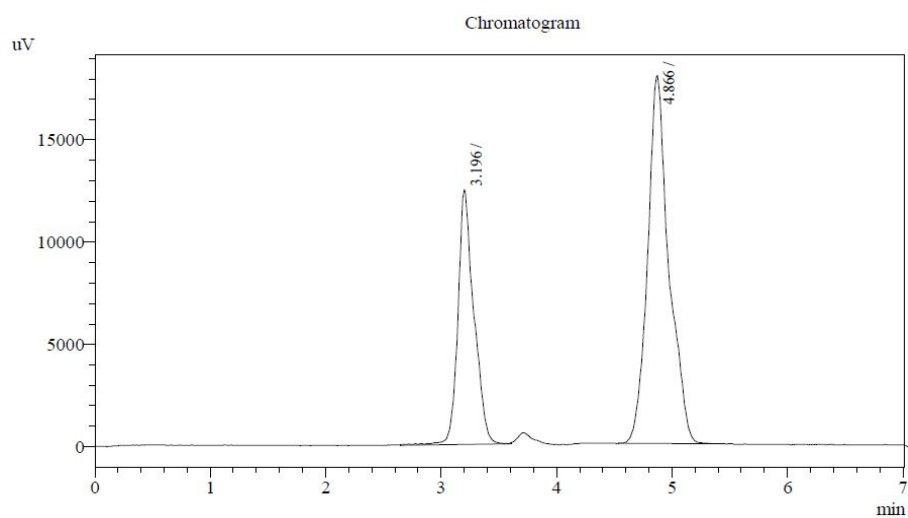**Fig. 5: Standard chromatogram of telmisartan and IS (5 µg/mL)**

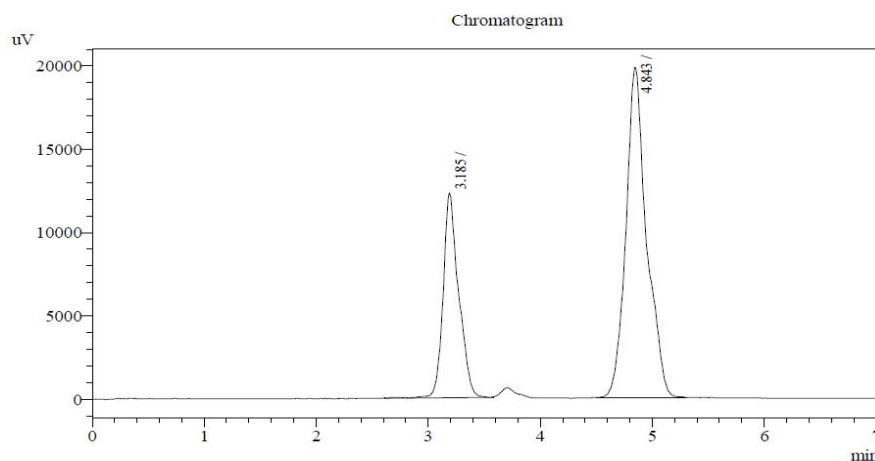**Fig. 6: Standard chromatogram of telmisartan and IS (7 µg/mL)**

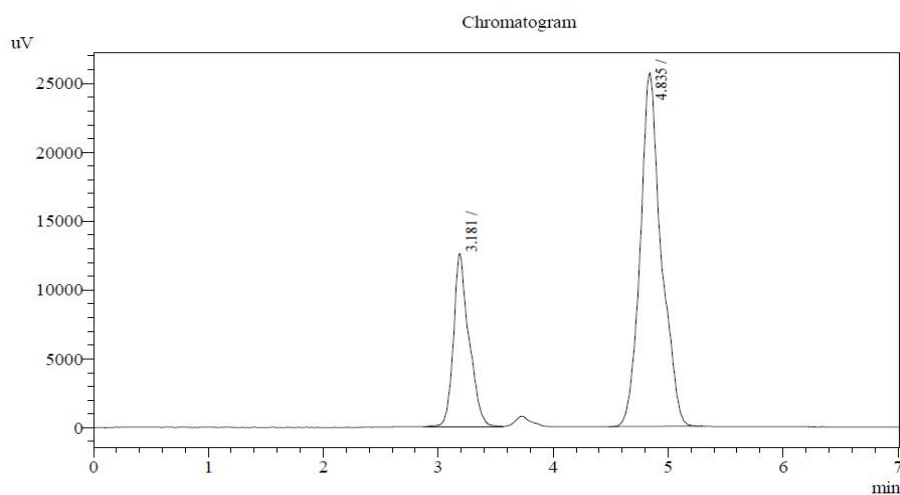**Fig. 7: Standard chromatogram of telmisartan and IS (9 µg/mL)**

Fig. 8: Standard chromatogram of telmisartan and IS (11 µg/mL)
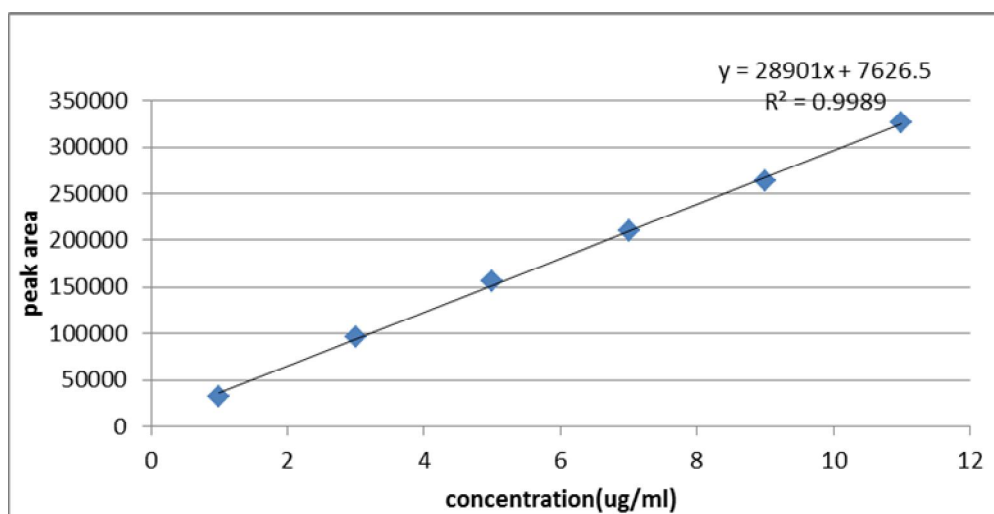

Fig. 9: Calibration plot of telmisartan and IS

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