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New Analytical Method Development And Validation For The Simultaneous Estimation Of Telmisartan And Hydrochlorothiazide In Bulk And Tablet Dosage Form Using RP-HPLC

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ABSTRACT

A simple, sensitive, precise and specific reverse phase high performance liquid Chromatographic method was developed and validated for the determination of Telmisartan and Hydrochlorothiazide in combined tablet dosage form. The method was validated as per ICH guidelines. The separation was carried out by using a mobile phase consisting of methanol: acetonitrile: water in the ratio (40:40:20%v/v). The column used was Zodiac C18, 250 × 4.5 mm with flow rate of 1.0ml/min using UV detection at 270 nm. The described method was linear over a concentration range of 40-280µg/ml and 12.5-87.5µg/ml for Telmisartan and Hydrochlorothiazide respectively. The retention times of Telmisartan and Hydrochlorothiazide were found to be 4.63 and 6.14 min respectively. Results of analysis were validated statistically and by recovery studies (recovery range of Telmisartan was found to be 98.19-101.32% and for Hydrochlorothiazide it was obtained as 98.67-100.99%. The limit of detection (LOD) of Telmisartan was found to be 0.75µg/ml and for Hydrochlorothiazide it was 2.5µg/ml. The limit of quantification (LOQ) for Telmisartan and Hydrochlorothiazide were found to be 2.5µg/ml and 8.25µg/ml respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Telmisartan and Hydrochlorothiazide in its pharmaceutical dosage form.

1. INTRODUCTION

Telmisartan is an angiotensin II receptor antagonist (angiotensin receptor blocker, ARB) effectively used in the treatment of hypertension. Telmisartan is indicated in the treatment of essential hypertension [1,2]. It is also used to reduce the risk of heart attack, stroke, or death due to heart problems in certain patients. Telmisartan is also used sometimes to treat congestive heart failure (condition in which the heart is unable to pump enough blood to the rest of the body) and diabetic nephropathy (kidney disease in people with diabetes and high blood pressure).

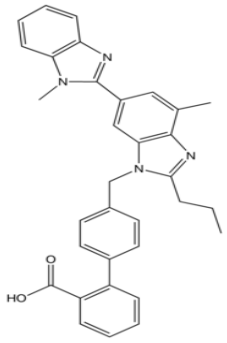

Figure: 1.A: Structure of Telmisartan

It works by relaxing blood vessels, which helps to lower blood pressure. It is an angiotensin II receptor blocker that shows high affinity for the angiotensin II receptor type 1 (AT_1), with a binding affinity 3000 times greater for AT_1 than AT_2. It has the longest half-life of any ARB (24 hours) [1,3] and the largest volume of distribution. In addition to blocking the RAs, telmisartan acts as a selective modulator of peroxisome proliferator-activated receptor gamma (PPAR-γ), 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) [3]. Telmisartan's activity at the PPAR-γ receptor has prompted speculation around its potential as a sport doping agent as an alternative to GW 501516[4]. Telmisartan activates PPARδ receptors in several tissues [5-8]. Side effects with Telmisartan are similar to other angiotensin II receptor antagonists and include tachycardia and bradycardia (fast or slow heartbeat), hypotension (low blood pressure), edema (swelling of arms, legs, lips, tongue, or throat, the latter leading to breathing problems), and allergic reactions [9].

Hydrochlorothiazide is a diuretic drug of the thiazide class that acts by inhibiting the kidneys ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance [10]. It is used for the treatment of fluid retention (edema) in people with congestive heart failure, cirrhosis of the liver, or kidney disorders, or edema caused by taking steroids or estrogen.

It is also sometimes used for treatment of hypoparathyroidis [11] hypercalciuria, Dent's disease and Meniere's disease. For diabetes insipidus, the effect of thiazide diuretics is presumably mediated by a hypovolemia-induced increase in proximal sodium and water reabsorption, thereby diminishing water delivery to the ADH-sensitive sites in the collecting tubules and reducing the urine output. Thiazides are also used in the treatment of osteoporosis. Thiazides decrease mineral bone loss by promoting calcium retention in the kidney, and by directly stimulating osteoblast differentiation and bone mineral formation [12]. It is frequently given together with other antihypertensive agents in fixed combination preparations, such as losartan (an angiotensin II receptor antagonist) as hydrochlorothiazide.

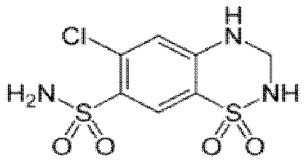

Figure 1.B: structure of Hydrochlorothiazide

Hydrochlorothiazide belongs to thiazide class of diuretics. It reduces blood volume by acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule. The major site of action in the nephron appears on an electroneutral Na^+-Cl^- co-transporter by competing for the chloride site on the transporter. By impairing Na transport in the distal convoluted tubule, hydrochlorothiazide induces a natriuresis and concomitant water loss. Thiazides increase the reabsorption of calcium in this segment in a manner unrelated to sodium transport [13, 14] Additionally, by other mechanisms, HCTZ is believed to lower peripheral vascular resistance [15].

Side effects with the Hydrochlorothiazide includes Hypokalemia, an occasional side effect, can be usually prevented by potassium supplements or by combining hydrochlorothiazide with a potassium-sparing diuretic, Hypomagnesemia, Hyponatremia, Hyperuricemia, High blood sugar, Hyperlipidemia, Hypercalcemia, Headache, Nausea/vomiting, Photosensitivity, Weight gain, Gout, Pancreatitis. World Anti-Doping Agency classified Hydrochlorothiazide as a "specified substance" While Hydrochlorothiazide is not itself a performance-enhancing drug, it may be used to mask the use of performance-enhancing drugs [16-19].

1.2 Materials and methods

1.2.1 Materials

The pharmaceutical grade Active pharmaceutical ingredients of Telmisartan and Hydrochlorothiazide (99.9% pure) were gifted by Hetero Labs, Hyderabad, India. All solvents used in the present study i.e. Methanol, Acetonitrile and Water were of HPLC grade and other chemicals used for the analysis were of analytical grade were procured from Merck Specialties Pvt. Lmt. Mumbai, India.

1.2.2 Instrumentation

To develop a High Pressure Liquid Chromatographic method for simultaneous estimation of Telmisartan and Hydrochlorothiazide isocratic PEAK HPLC instrument with Chromosil C18 column (250 mm x 4.6 mm, 5µ) and Electronic balance-DENVER (SI234) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

1.2.3 Chromatographic conditions:

The mobile phase was selected as Methanol: Acetonitrile: Water in the ratio (40:40:20%v/v). The flow rate of the mobile phase was adjusted as 1.0ml/min at ambient temperature of 25 ± 2ºC with binary isocratic mode. The UV detection wavelength was set as 270nm using UV absorption spectrum from spectrophotometer. The spectrum of diluted solutions of the Telmisartan and Hydrochlorothiazide in Acetonitrile was recorded separately on UV-2301 spectrophotometer and suitable wavelength was selected prior to selecting it for UV detection in RP-HPLC.

1.2.4 Preparation of standard solution

For analysis 1000µg/ml standard Telmisartan and Hydrochlorothiazide solutions were prepared by dissolving 10mg of pure drug in to 10ml of Methanol and required concentrations were obtained from 1000µg/ml solution by proper dilution. Seven sets of the drug solution were prepared in the mobile phase containing Telmisartan and Hydrochlorothiazide at a concentration of 40-280µg /ml of former and 12.5-87.5µg/ml in case of latter. Each of these drug solutions (20µl) was injected into the column, the peak area and retention times were recorded in triplicates.

1.2.5 Procedure for pharmaceutical formulation

Twenty tablets of TELISTA-H (consisting of 40mg Telmisartan and 12.5mg of Hydrochlorothiazide) were weighed and average weight of a single tablet was calculated. Then the tablets were crushed and grounded to fine powder using mortar and pestle. From the tablet powdered form 10mg was accurately weighed and transferred to 10ml of volumetric flak containing 10ml of Methanol. The solutions were sonicated for proper mixing for about 5min and then filtered through 0.45µm nylon membrane.

1.3 Method development

Many trails were carried out in order to develop the suitable chromatographic conditions to estimate the Telmisartan and Hydrochlorothiazide in combined tablet formulation. The suitable chromatographic conditions were developed only after carrying out various trails. Method development was considered to be one of the crucial steps in the analytical method. The essential chromatographic conditions like column, flow rate, mobile phase, wavelength and other system suitability parameters were verified. Basing on the property of solubility of both the drugs the mobile phase was selected as Methanol: Acetonotrile and water in the ratio 50:30:20%v/v. The peak shape was not upto the mark due to its broadness and poor resolution. So, the mobile phase was changed as Methanol: Acetonitrile and water in the ratio (40:40:20% v/v). It was observed that the peak was obtained as sharp and meeting the acceptable conditions of resolution. Thus the mobile phase ratio was set as Methanol: Acetonitrile: Water (40:40:20 v/v).The flow was maintained and set as 1.0ml/min, after checking the flow rate in the range of 0.9-1.5ml/min. The suitable wavelength for analysis and estimation of both the drugs was set as 270nm, after observing the UV absorption spectrum on UV-2301 spectrophotometer.

1.4 Method validation

Validation of the proposed method plays crucial role, whether the method can be used for the routine estimation of both the drugs in pharmaceutical formulation. Validation parameters such as system suitability parameters, linearity, precision, accuracy, robustness, ruggedness, LOD, LOQ and selectivity were verified for both of the drugs in two different linearity ranges. The validation of developed method was clear indication of whether the proposed method can be used for the intended purpose or not as in stated in ICH guidelines.

The system suitability parameters were resolution, tailing factor, theoretical plates and retention time. When all the values of parameters were obtained within the acceptable range then that method was said to meet the system suitability parameters. The acceptable range of resolution was below 2, tailing factor should be less than 2, theoretical plates should be more than 2000, these were some of the acceptable values.

Linearity of the method was determined by taking six to seven concentrations of the drug and peak areas of the corresponding concentrations were noted. The calibration curve of concentration on x-axis and peak area values on y-axis were taken, regression equation and co-relation coefficient were obtained. The regression equation passing through origin was in the form of y=mx+c and the co-relation coefficient very close to 1 i.e. above 0.998 were most acceptable.

The precision of analytical procedure express the closeness of agreement (Degree of scatter) between series of measurement obtained from multiple sampling of the same homogeneous sample under prescribed condition. It was evaluated by carrying out analysis on the same day of preparation of drug solution and on the next consecutive days.

To ensure the reliability and accuracy of the method, the recovery studies were carried out by adding a known quantity of drug with pre analyzed sample and contents were reanalyzed by the proposed method. Accuracy was evaluated at six different concentrations equivalent to 50,100 and150% of the active ingredient, by adding a known amount of both of the standard drug sample of known concentration and calculating the recovery of both the drugs with RSD (%) and % recovery for each concentration.

The ruggedness of the method can be studied in different versions, such as changing the instrument, changing the analyst performing the analysis. As per ICH norms, small, but deliberate variations, by altering the pH or concentration of the mobile phase were made to check the method's capacity to remain unaffected.

The lowest detectable and quantitable concentration of the method plays vital role in stating the method can be used for the further analysis or not. So, the sensitivity is judged by using two parameters LOD and LOQ. Mostly it is calculated by Signal to noise level ratio, stated in ICH guidelines.

1.5. RESULTS AND DISCUSSIONS

1.5.1 Method development

After carrying out number of trials suitable chromatographic conditions were developed. During this step of development in our study mobile phase composition which is considered as the most essential chromatographic condition and it was set as Methanol: Acetonitrile: water in the ratio (40:40:20 %v/v). The pH was maintained as 5.2. The flow rate was set as 1.2ml/min. The wavelength was set as 270nm, which was suitable for analysis for both the drugs Telmisartan and Hydrochlorothiazide depicted from UV absorption.

Figure 1.A: Optimized chromatographic conditions for Telmisartan and Hydrochlorothiazide

S.NO	Parameter	Results
1	MP	Methanol:Acetonitrile:water(40:40:20%v/v)
2	Wavelength	270nm
3	Stationary Phase	Zodiac C18 Column
4	pH of MP	5.2
5	Flow Rate	1.0ml/min
6	Pump Mode	Isocratic
7	Pump Pressure	12.5±5MPa

S.NO	Parameter	Results
1	Api Concentration	Telmisartan – 40µg/ml
		Hydrochlorothiazide – 12.5µg/ml
2	RT	Telmisartan – 80µg/ml
		Hydrochlorothiazide - 100µg/ml
3	Resolution	Telmisartan – 4.63
		Hydrochlorothiazide – 6.14
4	Area	Telmisartan – 3750445.0
		Hydrochlorothiazide – 457173.90
5	Theoretical Plates	Telmisartan – 5782
		Hydrochlorothiazide - 10733
6	Tailing Factor	Telmisartan – 1.63
		Hydrochlorothiazide - 0.87

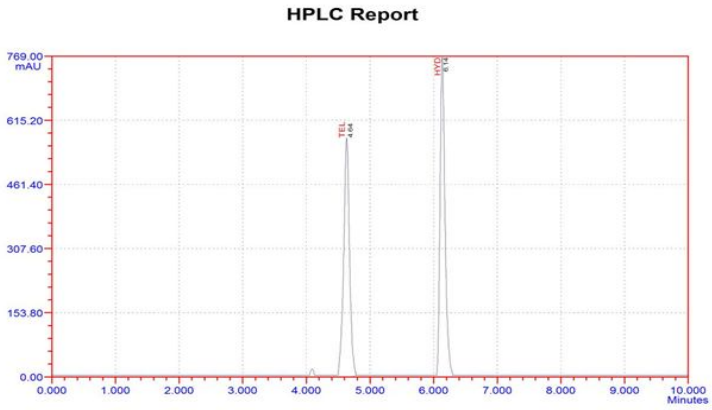

Figure 1.D: Standard chromatogram of Telmisartan and Hydrochlorothiazide

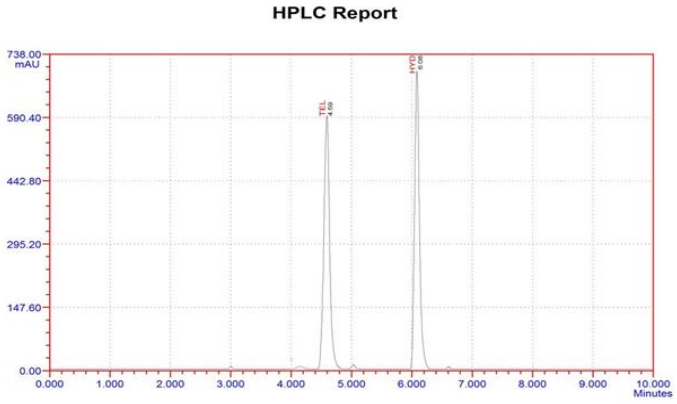

Figure 1.E: Formulation chromatogram of Telmisartan and Hydrochlorothiazide

1.5.2 Method validation

The proposed method was validated according to ICH guidelines. Different validation parameters such as linearity, precision, accuracy, robustness, ruggedness and sensitivity were verified for the developed method. All the parameters were validated and drugs showed values in the acceptable range. The results of different validation parameters were discussed below:

1.5.2.1 Linearity

The standard solutions of both the drugs were diluted to get different concentrations; different concentrations from standard Telmisartan solution were prepared, concentrations in the range of 40 to 280gμ/ml were prepared. In case of Hydrochlorothiazide different concentrations ranging from 12.5-87.5μg/ml were prepared. The calibration curves for both the drugs were plotted separately and regression equation, co-relation coefficients were obtained. It has been reported that the co-relation coefficient was obtained as 0.999, which was highly acceptable and suitable for analysis. The regression equation of standard Telmisartan was obtained as y=14293.14x+27288.58 and that of Hydrochlorothiazide was y=16739.42x+28110.42.

Table 1.B: Linearity results of Telmisartan and Hydrochlorothiazide

S.NO	Telmisartan		Hydrochlorothiazide	
	Concentration in µg/ml	Peak Area	Concentration in µg/ml	Peak Area
1	40	228045	12.5	228045
2	80	375044	25	457173
3	120	579908	37.5	694331
4	160	745442	50	878692
5	200	934721	62.5	1076590
6	240	1100389	75	1283970
7	280	1257359	87.5	1464879
		Slope:14293.14 Intercept:27288.58 Cc:0.999		**Slope:16739.42 Intercept:28110.42 Cc:0.999**

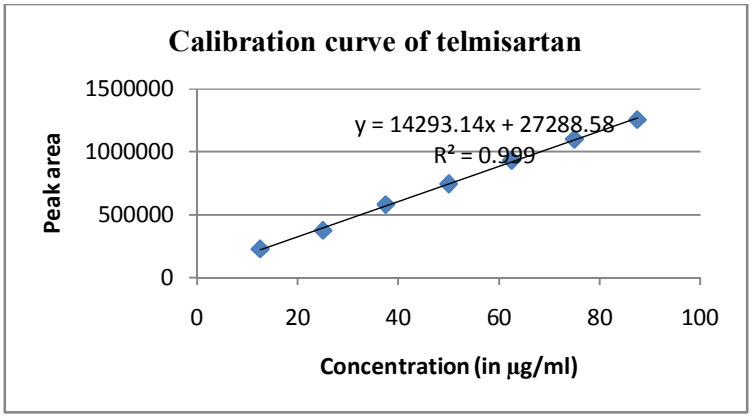

Figure 1.F: Calibration curve of Telmisartan

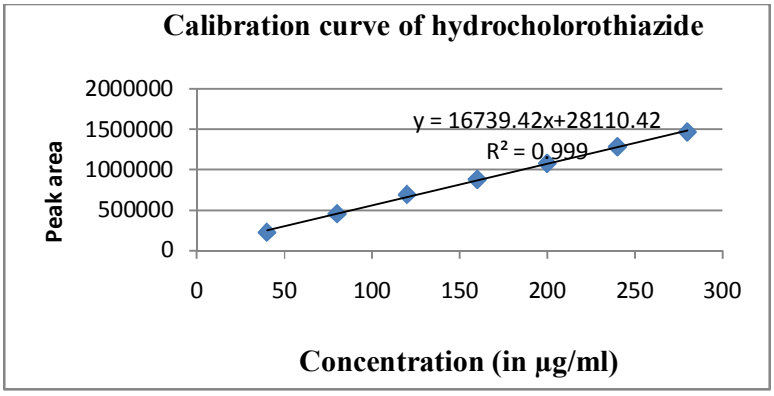

Figure 1.G: Calibration curve of Hydrochlorothiazide

1.5.2.2 Precision

The precision of the method was verified by repeatability and intermediate precision studies. The precision studies were carried out on the same day and next consecutive days. The intraday precision was carried out on the same day and the value of %RSD states the acceptability of the method. The interday precision was done on next consecutive days or within one week from preparation of solutions for analysis. The result showed that the %RSD of both the drugs was obtained within the acceptable criteria, which were below 2; 0.55 %RSD was obtained for Telmisartan and %RSD of 0.72 was calculated Hydrochlorothiazide in case of intraday precision. The interday precision of both the drugs were tabulated in table 1.F.

Table 1.C: Table showing results of intraday precision

Drug	Telmisartan		Hydrochlorothiazide	
S.NO	Concentration in (µg/ml)	Peak Area	Concentration in (µg/ml)	Peak Area
1	120	557710	37.5	682572
2	120	559163	37.5	694819
3	120	565938	37.5	689476
4	120	561906	37.5	691261
5	120	564381	37.5	686388
6	120	558185	37.5	697729
		RSD:0.55		**RSD:0.72**

Table 1.D: Table showing results of intraday precision

Drug	Telmisartan		Hydrochlorothiazide	
S.NO	Concentration in (µg/ml)	Peak Area	Concentration in (µg/ml)	Peak Area
1	120	563824	37.5	694322
2	120	565703	37.5	694644
3	120	570006	37.5	698304
4	120	576776	37.5	685227
5	120	565464	37.5	688309
6	120	560750	37.5	686862
		RSD:0.90		**RSD:0.68**

1.5.2.3 Accuracy

The accuracy of the method was determined by calculating recovery of Telmisartan and Hydrochlorothiazide 50%, 100% and 150% was added to a pre-quantified sample solution. The recovery studies were carried out three times over the specified concentration range and the percentage recovery of Telmisartan and Hydrochlorothiazide was found to be in the range of 98.19% to 101.32%, 98.67 to 100.99% and the results are presented in the table 1.G and 1.H.

Table 1.E: Table showing recovery results of Telmisartan

% of Recovery	Telmisartan					
	Target Conc., (µg/ml)	Spiked conc., (µg/ml)	Final Conc., (µg/ml)	Conc., Obtained	% of Assay	%RSD
50%	80	40	120	118.68	98.90	0.460
	80	40	120	118.29	98.57	
	80	40	120	119.38	99.48	
100%	80	80	160	160.45	100.28	1.59
	80	80	160	162.11	101.32	
	80	80	160	157.11	98.19	

150%	80	120	200	200.69	100.34	0.843
	80	120	200	197.84	98.92	
	80	120	200	197.69	98.84	

Table 1.F: Table showing recovery results of Hydrochlorothiazide

% of Recovery	**Hydrochlorothiazide**					
	Target Conc., (µg/ml)	**Spiked conc., (µg/ml)**	**Final Conc., (µg/ml)**	**Conc., Obtained**	**% of Assay**	%RSD
50%	25	12.5	37.5	37.87	100.99	0.89
	25	12.5	37.5	37.29	99.45	
	25	12.5	37.5	37.28	99.433	
100%	25	25	50	49.33	98.67	1.15
	25	25	50	49.76	99.52	
	25	25	50	50.47	100.95	
150%	25	37.5	62.5	63.09	100.95	0.90
	25	37.5	62.5	62.75	100.40	
	25	37.5	62.5	61.98	99.18	

1.5.2.4 Ruggedness

The ruggedness of the method is evaluated by calculating %RSD of six replicate injections. The %RSD of Telmisartan was found to be 1.17 and that of Hydrochlorothiazide was 0.77. Both the values were obtained within acceptable range below 2, which illustrate the ruggedness of the method.

Table 1.G: Table showing ruggedness results

Drug	**Telmisartan**		**Hydrochlorothiazide**	
S.NO	**Concentration in µg/ml**	**Peak Area**	**Concentration in µg/ml**	**Peak Area**
1	120	598548	37.5	683799
2	120	581025	37.5	684273
3	120	585154	37.5	691386
4	120	595466	37.5	688011
5	120	587866	37.5	685037
6	120	599281	37.5	697530
		RSD:1.17	**RSD:0.77**	

1.5.2.5 Robustness

The robustness of the method was studied by varying the method parameters such as change in mobile phase composition, Wavelength of the UV detector and flow rate of the mobile phase. The results showed that the retention time and peak area of Telmisartan and Hydrochlorothiazide were remained almost unchanged and no significant degradation was observed.

Table 1.H: Table showing robustness results

	Telmisartan		**Hydrochlorothiazide**	
Condition	**Mean area**	**% difference**	**Mean area**	**% difference**
Standard	579908	**0.0**	694331	0.0
Mp Changes-1 v/v M: A: W (35:45:20%) Mp Changes-2 M: A: W (45:35:20%)	 572268 577278	-1.31 -0.45	700315 699648	0.86 0.76
WL Changes-272 WL Changes- 268	586362 576776	1.11 -0.54	683941 693624	-1.49 -0.10
PH -5.0	580856	0.16	694312	-0.002
PH -5.4	584587	0.80	686681	-1.10

1.6 Analysis of a marketed formulation:

To determine the content of Telmisartan and Hydrochlorothiazide in conventional tablet (Brand name: TELISTA-H) twenty tablets were weighed, their mean weight was determined and finely powdered. The weight of the tablet triturate equivalent to 10mg of mixture of both the drugs Telmisartan and Hydrochlorothiazide was transferred into a 10 ml volumetric flask containing 10ml of acetonitrile, sonicated for 5min. The resulting solution filtered using 0.45-micron filter (Millipore, Milford, MA). The above stock solution was further diluted to get sample solution of 120 and 37.5 µg/ml for Telmisartan and Hydrochlorothiazide respectively. A 20 µL volume of sample solution was injected into HPLC, six times, under the conditions described in method development. The peak areas were measured at 270 nm and concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation.

Table 1.I: Table showing formulation results

Brand name	**Available form**	**Label claim**	**Concentration µg /ml**	**Amount found µg /ml**	**% Assay**
TELISTA-H	Tablet	Telmisartan(Tel) - 40mg Hydrochlorothiazide(Hyd)-12.5mg	Tel -120 Hyd-37.5	Tel -118.59 Hyd-37.14	Tel-98.82 Hyd-99.04

1.7 CONCLUSION

HPLC method was developed and validated as per ICH guidelines. UV detection allowed an accurate quantitation of chromophoric compounds, the drugs Telmisartan and Hydrochlorothiazide were effectively estimated in combination tablet formulation. The proposed method for the assay of Telmisartan and Hydrochlorothiazide tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the ease of mobile phase facilitated better elution of the drugs. The method was validated for specificity, linearity, precision, accuracy and robustness. The present developed can be effectively used for the routine analysis of Telmisartan and Hydrochlorothiazide in pharmaceutical formulations.

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