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Research Article

Development and validation of stability indicating HPLC method for the estimation of Telmisartan related substances in tablets formulation

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ABSTRACT

A sensitive HPLC method was developed and validated for the estimation of Telmisartan related impurities in tablets formulation. The highly polar molecule requires aqueous mobile phase for the elution and separation of Telmisartan and its impurities (Impurity A, B, E and F official in EP).The developed method is found to be specific,reproducible,and stability indicating. The X-Bridge C18 150x4.6mm 3.5µ column was used and mobile phase consisted of 25mM potassium dihydrogen phosphate (KH_2PO_4) and 10mM of 1-hexaneusulphonic acid, sodium salt monohydrate buffer to achieve good resolution and retention of the analyte and its impurities. The detector linearity was established from concentrations ranging from 0.08µg/mL to 500µg/mL for Telmisartan and from 0.017 to 3.0 µg/mL for related impurities with a correlation co-efficient of 0.997.The relative response factor (RRF) values of impurityA, impurityE, impurityF, impurityB, TEL2, Dimer acid and Chloro analogue determined from linearity plots were 1.27,0.43,0.83,1.02,0.81,0.80 and 0.84 respectively. The limit of detection (LOD) and limit of quantification (LOQ) found to be in the range of 0.023µg/mL to 0.190 µg/mL for Telmisartan and impurities respectively. The molecule is forced to all stress conditions such as acid, base, oxidation, heat and photolysis as per the recommendations of ICH guidelines. All degradants are well separated from the main analyte.The method is proved to be robust with respect to change in flow rate, pH, organic phase composition and column temperature. The proposed method is found to be sensitive, precise, rapid, reproducible, and offers good column life.

Keywords: Telmisartan; HPLC method; Validation; Stability indicating; tablets formulation.

1. INTRODUCTION

Telmisartan is an angiotension receptor blocker that shows high affinity for the angiotension II type 1 receptors, has a long duration of action, and has the longest half-life of an ARB. In addition to blocking the Renin-Angiotensin System (RAS), telmisartan acts as a selective modulator of Peroxisome proliferator-activated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism. In the present study Telmisartan in a tablet formulation was used to evaluate the chromatographic separation of Telmisartan and its related impurities.

Literature reveals few RP-HPLC methods (Kiran R. Patil et al., 2008) and is not capable of producing proper resolution between impurity F and impurity E. The main objective is to develop and validate a simple, effective and reproducible HPLC method for the determination of Telmisartan related impurities in tablet formulation. Aqueous solution containing potassium dihydrogen phosphate and 1-hexaneusulphonic acid, sodium salt monohydrate was used as mobile phase .The X-Bridge C18 150x4.6mm 3.5µ column was selected to enhance retention capacity, sensitivity and specificity of the analyte and its related substances. Gradient flow was used to separate the all impurities with proper separation.

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2. MATERIALS & METHODS

2.1. Materials reagents

Telmisartan (purity-99.2%) and impurities A, B, C, D, E and F are official in European Pharmacopoeia. impurity C is a process related impurity and impurity D is unspecified impurity. Chloro analogue, dimer acid impurity and TEL2 are in-house impurities. Impurity E and impurity F are obtained from synpure laboratories. Telmisartan, impurity A, impurity B, Chloro analogue, dimer acid impurity and TEL2 are obtained from Dr.Reddy's laboratories Ltd . Potassium dihydrogen phosphate (AR grade-Merck (India) limited, 1-hexanesulphonic acid, sodium salt monohydrate (AR grade-Merck (India) limited. All other chemicals and solvents used were of analytical grade or HPLC grade.

2.2. Apparatus

The analysis was carried out on waters Alliance HPLC systems 2695 separation module connected to 2996

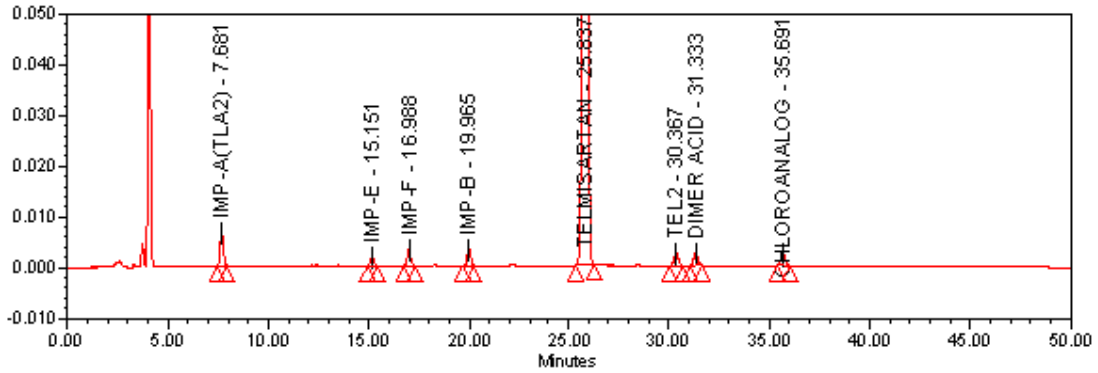

Figure 1: Inertsil ODS 3V, 250x4.6mm, 5µ

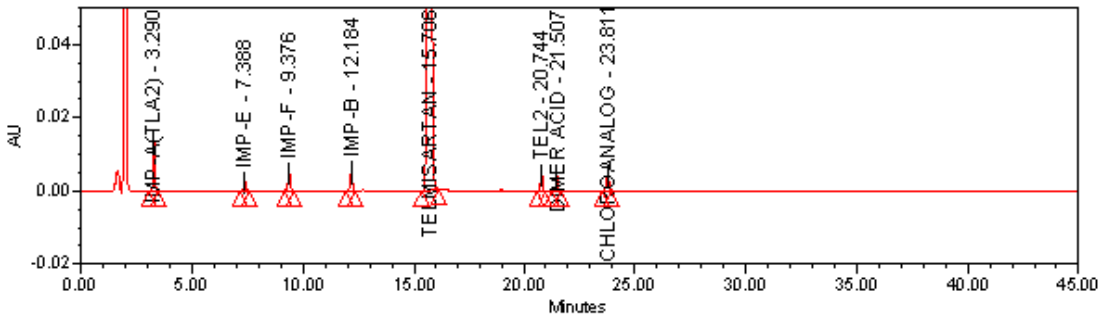

Figure 2: X-Bridge C18 150x4.6mm 3.5µ

Photo diode array detector. Data acquisition was carried out using Empower software. Different chromatographic column used during trials were

1. Inertsil ODS 3V, 250x4.6mm, 5µ (make-GL Sciences)
2. X-Bridge C18 150x4.6mm 3.5µ (make-Waters)

2.3 Chromatographic conditions

The separation of Telmisartan and related substances were achieved using 25mM Potassium dihydrogen phosphate and 10mM 1-hexanesulphonic acid, sodium salt monohydrate buffer ,pH adjusted to 3.5 using 1% ortho phosphoric acid solution as a mobile phase-A. Water and acetonitrile in the ratio of 10:90 as mobile phase –B at a flow rate of 1.0mL /minute (gradient).Detection and purity establishment of the main drug and impurities were achieved using photo diode array (PDA) detector at 290nm.The drug samples and formulation samples were prepared in 0.1N HCl: Methanol (80:20) which is used as a diluent to achieve a concentration of 500µg/mL and 20µL of the sample were injected. The run time optimized was found to be 45 minutes.

Table 1: Gradient programme

Time(min)	%A	%B
0	65	35
3	65	35
15	50	50
25	30	70
40	30	70
41	65	35
45	65	35

2.4 Standard preparation

Standard stock solution (1000µg/mL) was prepared in methanol. About 50mg of the working standard was transferred into 50mL volumetric flask, dissolved in methanol with sonication and diluted to volume, 2.0mL of the stock solution was pipette to 100mL volumetric flask and diluted to volume with diluents.5.0 mL the above solution pipette to 100mL and diluted to volume with diluent to achieve a concentration of 1µg/mL. The system suitability test was performed by injecting sample solution spiked with impurities at 1µg/mL level.

2.5 Sample preparation

The drug was extracted from tablet formulation of 80mg label claim using the diluent. About 25mg equivalent of the Telmisartan was taken into 50 mL volumetric flask, 30mL diluent was added and sonicated for 20minutes and cooled to room temperature. Diluted to volume with diluents to achieve a target concentration of 500µg/mL.

2.6 Spiked sample preparation

The drug was extracted from tablet formulation of 80mg label claim using the diluent. About 25mg equivalent of Telmisartan was taken into 50 mL volumetric flask, 30mL diluent was added and sonicated for 20minutes and cooled to room temperature.1mL of each impurity stock solution(50 µg/mL)added to the above solution. Diluted to volume with diluent to achieve a target concentration of 500µg/mL for Telmisartan and 1 µg/mL for impurities.

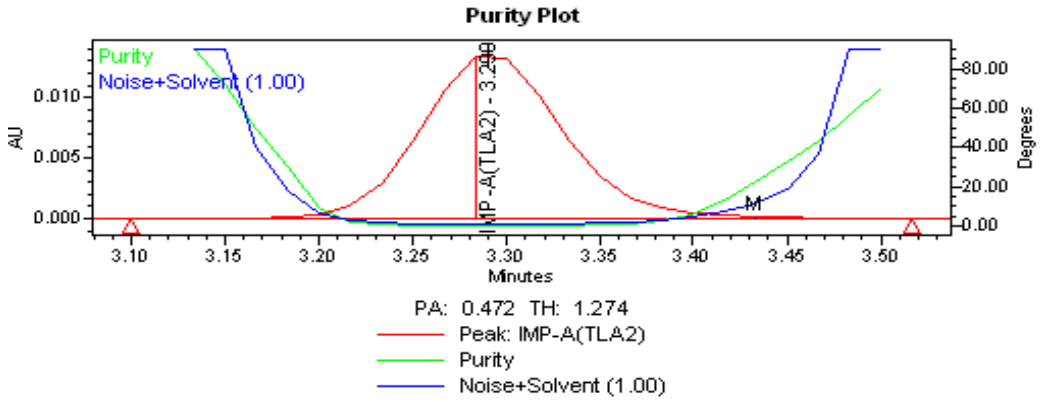

Figure 3: Peak purity of Impurity A

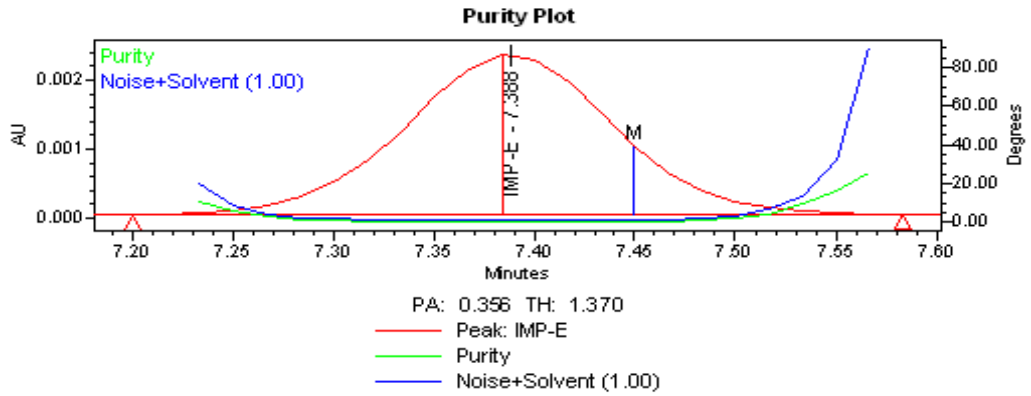

Figure 4: Peak purity of Impurity E

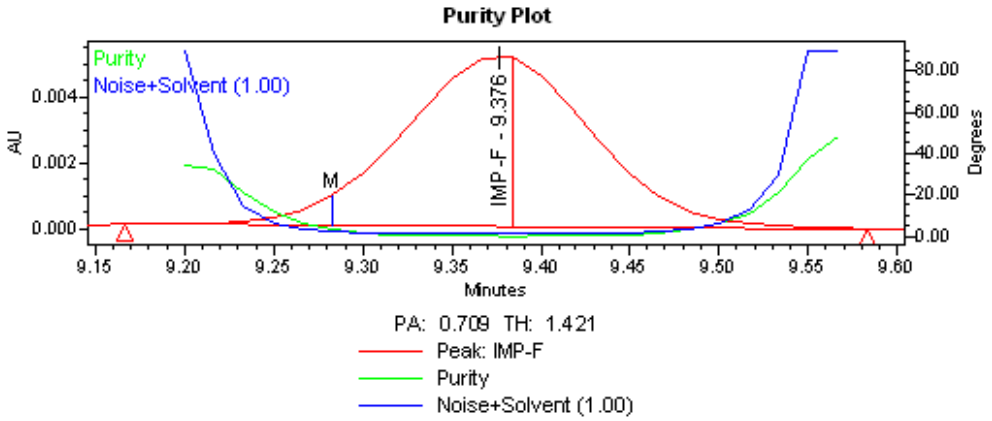

Figure 5: Peak purity of Impurity F

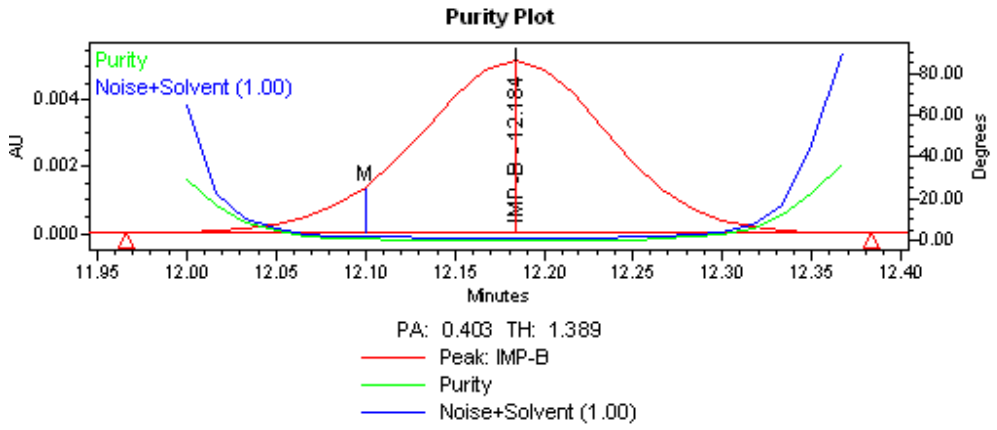

Figure 6: Peak purity of Impurity B

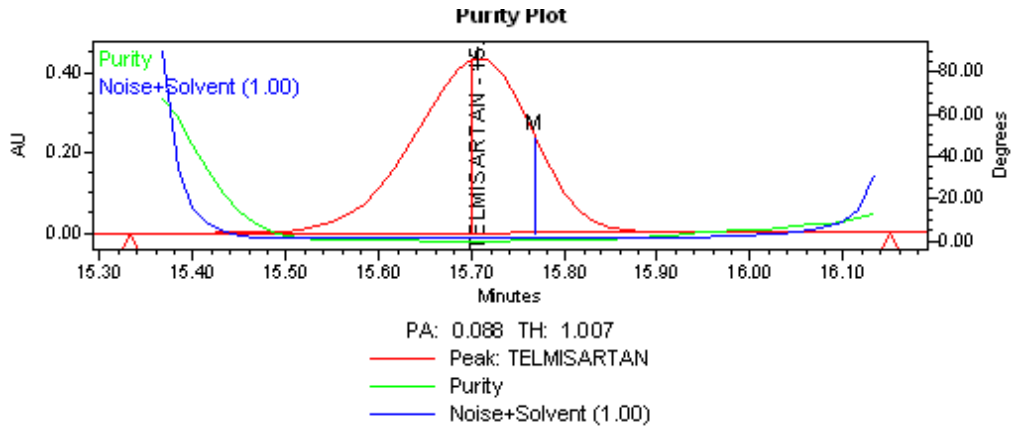

Figure 7: Peak purity of Telmisartan

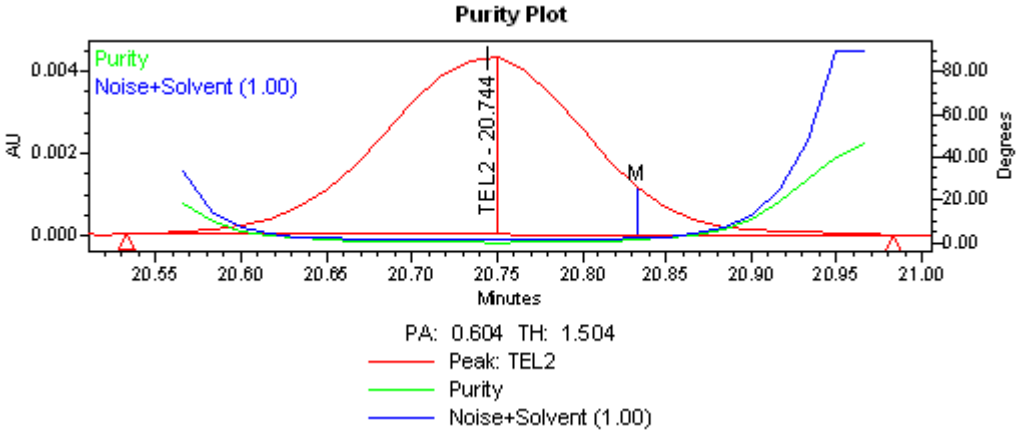

Figure 8: Peak purity of TEL2

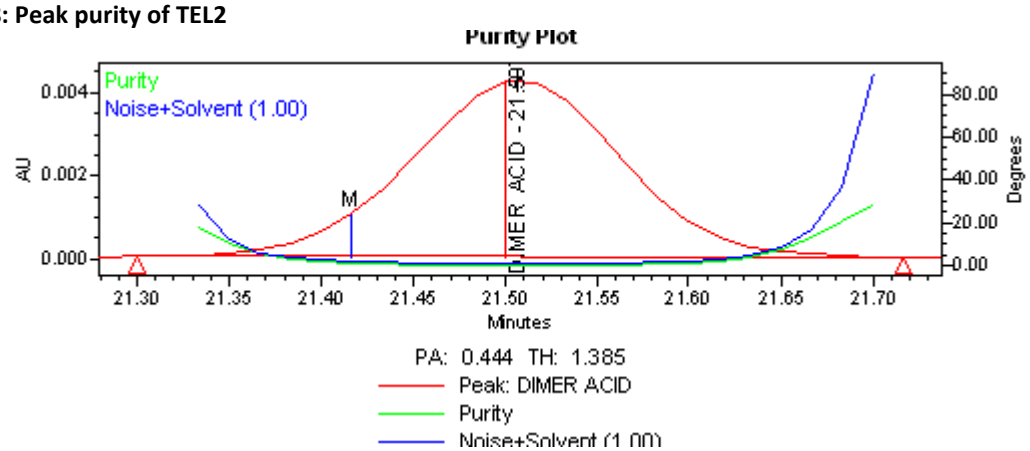

Figure 9: Peak purity of Dimer acid

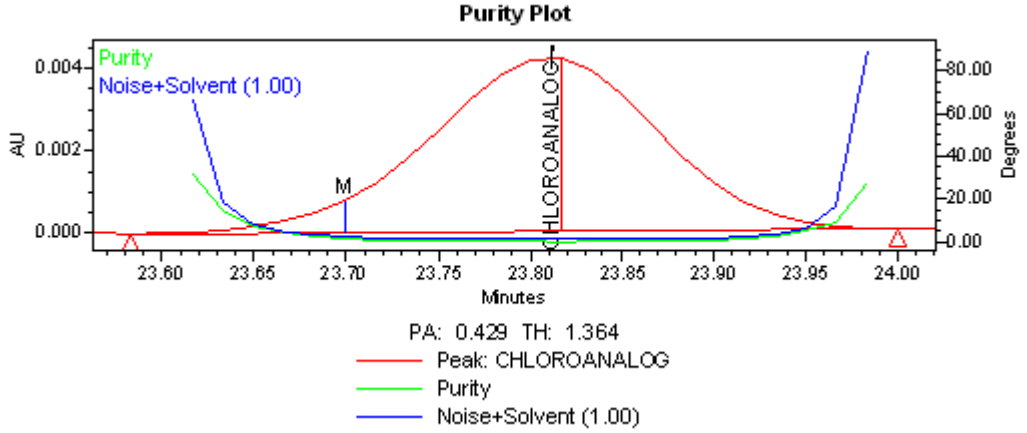

Figure 10: Peak purity of Chloro analog

3. RESULTS AND DISCUSSION

3.1 Optimization of Chromatographic conditions

Several columns were used for optimizing the chromatographic condition (SB Wankhede, et al., 2007). The parameters being focused were improvisation of resolution between all impurities. To elute highly non polar impurities gradient programme was selected. Different gradient programmes were used to obtain good resolution between the impurities. In Inertsil ODS 3V all impurities are separated. To achieve some more resolution between impurity E and impurity F, and to reduce the run time X Bridge 150x4.6mm, 3.5µ column was selected. The sensitivity of the method also increased with this column in comparison with Inertsil ODS 3V.Chromatograms in these two columns was shown in Figure1 and 2.

3.1.1. Buffer Selection.

Different buffers such as potassium phosphate, sodium per chlorate, ammonium acetate were evaluated for system suitability parameters and overall chromatographic performance. In the sequential trials potassium dihydrogen phosphate and 1-hexanesulphonic acid, sodium salt monohydrate were found to be suitable for effective separation of parent peak and impurities. potassium dihydrogen phosphate buffer ranging from 10 mM to 50mM were tried .It was observed that change in the buffer concentration did not offer significant changes in the elution pattern and resolution ,but 25mM concentration increased the sensitivity of the method.

Table 2: Peak purity of Telmisartan in stressed condition

Stress condition	%Degradation	Purity angle	Purity threshold	Purity flag
Acid degradation	0.46	4.645	42.780	No
Base degradation	0.48	1.489	35.279	No
Peroxide degradation	1.52	0.372	90.000	No
Photo light degradation	NA	6.403	14.999	No
UV light degradation	NA	5.813	12.011	No
Heat degradation	0.15	5.870	76.070	No
Water degradation	0.14	4.414	72.711	No
Sample as such	NA	5.814	53.439	No

Table 3: System suitability parameters

S.No	Retention time	USP Tailing	USP Resolution	Purity angle	Purity threshold
Impurity A	3.290	1.1	NA	0.472	1.274
Impurity E	7.388	1.0	24.2	0.356	1.370
Impurity F	9.376	1.0	9.9	0.709	1.421
Impurity B	12.184	1.0	13.8	0.403	1.389
Telmisartan	15.706	0.9	15.7	0.088	1.007
TEL2	20.774	1.0	21.4	0.604	1.504
Dimer acid	21.507	1.0	3.4	0.444	1.385
Chloro analogue	23.811	0.9	10.3	0.429	1.364

Table 4: Linearity of Telmisartan from LOQ level to 100% of target sample concentration

Concentration in %	Linearity of Telmisartan	
	Concentration in µg/mL	Area response
0.0172% (LOQ)	0.0858	6805
0.1%	0.5010	6805
0.15%	0.7514	34377
0.2%	1.0019	45808
0.25%	1.2524	60155
0.3%	1.5029	73272
10%	50.096	2737762
100%	500.96	23400012

3.1.2. Effect of pH

The pH had an effect on the retention times of the Telmisartan and its related compounds. Resolutions and peak symmetry are found good at pH 3.5.

3.1.3. Effect of ion pair reagent.

The usage of ion pair agent like 1-hexanesulphonic acid, sodium salt monohydrate gave a better resolution between main peak and related substances.

Table 5: Linearity, LOD, LOQ, RRF, RRT of Telmisartan and impurities

Desired concentration	Imp A		Imp E		Imp F		Imp B	
	Conc in µg/mL	Area response	Conc in µg/mL	Area response	Conc in µg/mL	Area response	Conc in µg/mL	Area response
LOQ	0.038	14618	0.019	2032	0.0172	2402	0.017	6979
0.5	0.49783	39442	0.50194	11622	0.50792	23400	0.4209	24572
0.75	0.74674	59262	0.75291	17171	0.76188	36290	0.63135	35452
1.0	0.99566	78202	1.00388	23415	1.01584	47441	0.8418	46336
1.25	1.24457	93321	1.25486	28688	1.2698	59092	1.05225	59432
1.5	1.49349	109328	1.50583	35697	1.52376	68164	1.2627	71713
Slope	0.9972		0.9991		0.9984		0.9992	
R^2	0.9790		0.9959		0.9986		0.9933	
LOD (µg/mL	0.0135%		0.005%		0.007%		0.007%	
LOQ (µg/mL	0.038%		0.016%		0.016%		0.020%	
RRF	1.27		0.43		0.83		1.02	
RRT	0.20		0.49		0.60		0.80	
Desired concentration	Telmisartan		TEL2		Dimer acid		Chloro analogue	
	Conc in µg/mL	Area response	Conc in µg/mL	Area response	Conc in µg/mL	Area response	Conc in µg/mL	Area response
LOQ	0.085	6805	0.093	4381	0.123	8967	0.186	10734
0.5	0.5010	34377	0.50467	21552	0.49674	21758	0.49215	24540
0.75	0.7514	45808	0.75700	30406	0.74511	34658	0.73822	35135
1.0	1.0019	60155	1.00933	43788	0.99348	43952	0.9943	48168
1.25	1.2524	73272	1.26167	54649	1.24185	54833	1.23037	57602
1.5	1.5029	89355	1.51399	67989	1.49022	65448	1.47645	71656
Slope	0.9990		0.9980		0.9989		0.9985	
R^2	0.9991		0.9903		0.9888		0.9840	
LOD (µg/ml)	0.010%		0.007%		0.009%		0.012%	
LOQ (µg/ml)	0.029%		0.019%		0.025%		0.037%	
RRF	1.00		0.81		0.80		0.84	
RRT	1.00		1.27		1.35		1.48	

3.2. Optimized method

The chromatographic condition optimized were X-Bridge C18 150x4.6mm 3.5µ with 25mM Potassium dihydrogen phosphate and 10mM 1-hexanesulphonic acid, sodium salt monohydrate buffer pH 3.5.The retention times of Telmisartan, impurity A, impurity E, impurity F, impurity B, TEL2, Dimer acid and Chloro analogue were found to be15.706, 3.290, 7.388, 9.376, 12.184, 20.774, 21.507 and 23.811minutes respectively. The chromatogram is shown in (figure2). The relative retention time (RRT) of impurity A, impurity E, impurity F, impurity B, TEL2, Dimer acid and Chloro analogue were found to be 0.21, 0.47, 0.60, 0.78.1.32, 1.37 and 1.52 respectively with respect to analyte peak. The method is capable of separating the impurities and the main drug with resolution not less than 3.0.The tailing factor for main peak and impurities was found to be 1.0.The peak purity of all impurities was passed and no flag in purity was observed. The purity curves for Telmisartan and all impurities are given in (figure 3 to figure10).System suitability parameters are given in (tab3).

3.3. Drug extraction from formulations

The extraction of the drug from formulation tried with different solvents such as methanol, methanol with water, 0.1 N HCl with methanol. The complete extraction of drug was achieved with 0.1 N HCl with methanol. Telmisartan has solubility in 0.1 N HCl and methanol.

3.4. Validation of method

3.4.1. Specificity

The Forced degradation of placebo and formulation was carried out as per ICH guidelines (ICH Q_2B) and photolysis. The acid, base, and oxidation stress conditions were studied out by refluxing API for 6hrs with 10mL of 1N HCl, 1N NaOH and 3% hydrogen peroxide respectively. The thermal degradation was carried out by heating the drug powder at 105◦ Cfor about 24hrs and the photo degradation was performed exposing the drug material to 1.2 million lux hours and 200 watt hours/M^2.All the stress conditions with purity angle and purity threshold are reported in (Table 2).

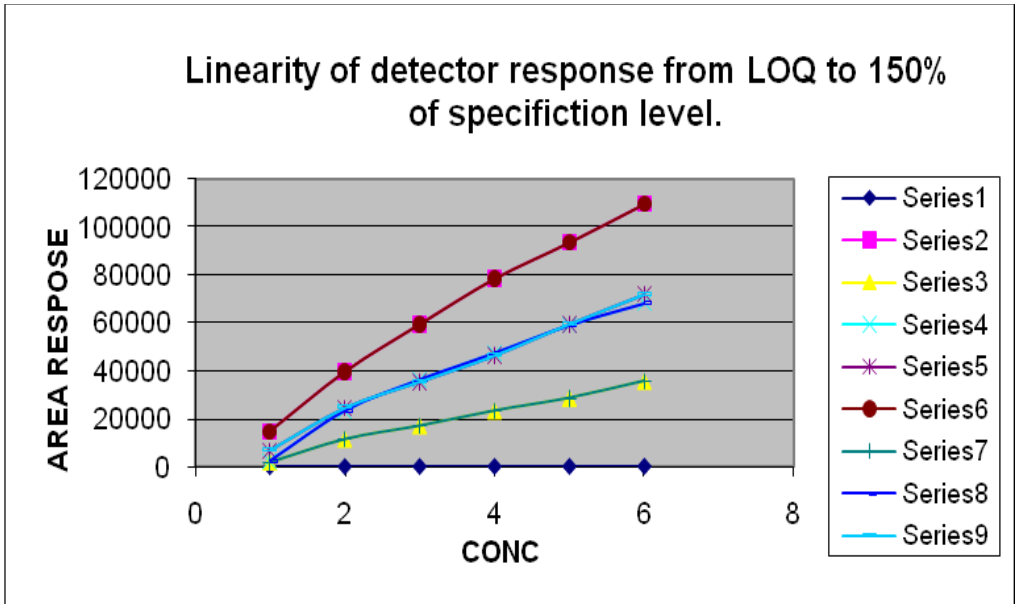

Figure 11: Linearity of Telmisartan and impurities

Table 6: Precision for impurities at specification level

Impurity	Average	%RSD
Impurity-A	0.209	0.0
Impurity-E	0.185	0.2
Impurity-F	0.195	0.3
Impurity-B	0.154	0.3
TEL2	0.195	1.2
Dimer acid	0.187	0.3
Chloro analogue	0.201	0.5

Table 7: Recovery data of Telmisartan impurities from LOQ to 150%

	Impurity A		Impurity E		Impurity F		Impurity B	
	Mean	%RSD	Mean	%RSD	Mean	%RSD	Mean	%RSD
LOQ	112.0	1.79	102.4	3.67	105.8	3.72	95.5	2.15
50%	110.8	0.78	92.9	1.52	95.9	1.88	95.6	1.08
75%	107.7	1.18	93.7	1.13	94.5	0.07	92.6	1.26
100%	107.4	0.34	92.8	0.32	94.5	0.24	92.8	0.13
125%	102.5	0.36	94.9	0.32	94.1	0.13	94.6	0.07
150%	101.3	0.35	94.8	0.06	95.5	0.15	95.2	0.15

TEL2		Dimer acid		Chloro analogue	
Mean	%RSD	Mean	%RSD	Mean	%RSD
103.2	1.72	104.5	3.37	104.8	2.39
94.5	0.98	95.1	0.36	94.4	1.05
93.1	1.23	93.7	0.23	94.1	0.42
94.7	0.59	94.0	0.92	94.1	0.42
94.0	0.07	94.0	0.06	93.0	0.17
96.1	0.29	96.0	0.37	95.7	0.07

3.4.2. System suitability

The system suitability was checked by making the injection of test sample spiked with all impurities The system is deemed to be suitable as the tailing factor ≤1.5,and the resolution between closely eluting impurity>2.0 (Figure 2).

3.4.3. Linearity, LOD & LOQ

The linearity solutions were prepared in diluent. Analyte solution has shown linearity response for concentration levels ranging from 0.0858 µg/mL to 500 µg/mL. The correlation co-efficient value was found to be 0.9994.The relative response factor (RRF) was determined by slope method.LOD and LOQ for all impuri-

Table 8: Robustness

Parameters	Flow rate(ml/min)			pH		
Changes in parameters	**0.8**	**1.0**	**1.2**	**3.3**	**3.5**	**3.7**
The tailing factor for telmisartan	**NMT 2.0**	**NMT 2.0**	**NMT 2.0**	**NMT 2.0**	**NMT 2.0**	**NMT 2.0**
RT of ImpA	4.432	3.561	2.947	2.952	3.561	3.656
RT of ImpF	12.106	10.413	9.073	8.254	10.413	10.514
RT of ImpE	10.001	8.374	7.038	7.383	8.374	7.011
RT of ImpB	15.385	13.618	12.095	11.844	13.618	12.491
RT of TEL2	23.838	22.010	20.437	19.026	22.010	21.182
RT of Dimer acid	24.608	22.876	21.374	20.885	22.876	21.868
RT of Chloro analogue	26.915	24.985	23.401	22.572	24.985	23.626
	Column temperature			**Organic phase variation**		
	40◦C	**45◦C**	**50◦C**	**90%**	**100%**	**110%**
	NMT 2.0	**NMT 2.0**	**NMT 2.0**	**NMT 2.0**	**NMT 2.0**	**NMT 2.0**
	3.511	3.561	3.597	3.302	3.561	3.496
	9.926	10.413	10.109	9.430	10.413	10.114
	8.207	8.374	8.024	7.436	8.374	8.010
	12.999	13.618	12.797	12.279	13.618	13.029
	20.806	22.010	20.998	20.710	22.010	21.438
	22.033	22.876	21.676	21.401	22.876	22.103
	24.373	24.985	23.876	23.501	24.985	24.188

ties determined by STYEX method. The %RSD of LOD and LOQ samples were well within the limits (Table 5).The linearity plot is shown in (Figure 11).

3.4.4. Precision

The method was found to be precise with six sample preparation for the estimation of impurities. Impurity solution spiked to the sample preparation containing Telmisartan. The %RSD of all impurities in six sample preparation was found to be less than 2.0 (Table 6).

3.4.5. Accuracy

The recovery of impurities and Telmisartan were determined by spiking each impurity and main peak at six different levels starting from LOQ to 150% of the specification level of the impurities. The recovery range for all impurities and telmisartan was found to be between 90% and 115% with RSD between 0.06% and 3.72 % (Table 7)

3.4.6. Solution stability

The solution stability of the standard and impurities prepared in diluents was studied for about 2 days at bench top. The solution under study was compared with freshly prepared standard solution, the samples were found to be stable for a period of 24 hours.

3.4.7. Robustness

The robustness was investigated by varying the conditions W.R.T. change in the flow rate, pH, column temperature and organic phase composition. The study was conducted at different flow rates of 0.8ml/min, and 1.2ml/min. The mobile phase pH was modified to 3.3 and 3.7 and column temperature was adjusted to 40°C and 50°C to study the effect of pH and column temperature respectively. Organic phase composition was varied to 90% and 110% in mobile phase-B to study the effect of organic phase composition variation. The method was found to be robust with respect to flow rate, pH, column temperature and organic phase composition without any changes in system suitability parameters such as tailing factor and resolution (table8).

CONCLUSION

The method provides selective quantification of Telmisartan impurities without interference of blank, placebo, thereby affirming stability-indicating nature of the method. The proposed method is highly selective, reproducible, specific and rapid. The developed method was robust in the separation and quantification of Telmisartan related impurities. This method can be used in the routine analysis of production samples. The information presented herein could be very useful for quality monitoring of bulk samples and as well employed to check the quality during stability studies.

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