An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TELMISARTAN AND HYDROCHLORTHIAZIDE IN TABLETS BY RP-HPLC

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ARTICLE INFO

Key Words

Telmisartan,
Hydrochlorthiazide,
RP-HPLC,
Validation

ABSTRACT

A very simple, rapid and sensitive RP-HPLC method has been developed and validated for the analysis of Telmisartan and Hydrochlorthiazide in Tablet formulation. Best chromatographic resolution was achieved on a Reverse-phase C_{18} column using Phosphate buffer (pH 4): Acetonitrile in the ratio of 55:45 as mobile phase with a flow rate of 1ml/min and isocratic elution with a total run time of 6 minutes. The retention time of Telmisartan and Hydrochlorthiazide was found to be 4.09, 2.85 respectively. Detection of compounds was carried out at 222 nm. The percentage mean recoveries were 99.85 and 99.99 for Telmisartan and Hydrochlorthiazide respectively which shows the accuracy of the method. The present method was found to be accurate, precise and can be used for routine analysis.

INTRODUCTION:

Telmisartan is an angiotensin II receptor antagonist used in the management of hypertension. Telmisartan chemically is 2-(4-{[4-methyl-6-(1-methyl-1 H-1, 3-benzodiazol-2-yl) -2-propyl-1H-1, 3-benzodiazol-1-yl] methyl} phenyl) benzoic acid. Telmisartan blocks the vasoconstrictor and aldosterone secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland. Its action is therefore independent of the pathways for angiotensin II synthesis. There is also an AT2 receptor found in many tissues, but AT2 is not known to be associated with cardiovascular homeostasis. Telmisartan has much greater affinity (>3,000 fold) for the AT1 receptor than for the AT2 receptor. *Hydrochlorothiazide* is a diuretic medication often used to treat high blood pressure and swelling due to fluid buildup. Hydrochlorthiazide. Hydrochlorthiazide acts by inhibiting Na^+ reabsorption by kidney. It is used in the treatment of Hypertension, edema, diabetes insipidus and hypercalciuria. Based on the detailed review of the literature, there are few reported analytical methods for the estimation of Telmisartan and Hydrochlorothiazide in Tablet dosage forms. The objective of this work was to develop a simple sensitive and economical method for simultaneous estimation of Telmisartan and Hydrochlorothiazide in tablet dosage form.

MATERIALS AND METHODS [4-9]

Chemicals:

Water HPLC grade, Methanol HPLC grade, Acetonitrile HPLC grade, Potassium dihydrogen Phosphate are obtained from from Merck,pvt.Ltd. Mumbai. Telmisartan, Hydrochlorthiazide and Telvas-H were obtained from Bridge Pharma. Ltd.

Instruments

Schimazdu-make HPLC, model no-SPD was used for the experiment. All weights are taken on Analytical balance(Citizen). p^H of the mobile phase was adjusted by using p^H meter(Sisco).

Phosphate buffer preparation: Dissolve 13.61 gm. of Potassium di hydrogen Phosphate in sufficient water to produce 1000ml.

Mobile phase preparation: Filtered degassed mixture of Phosphate buffer, Acetonitrile in the ratio of 55:45.

Preparation of standard stock solutions: Telmisartan powder (10 mg) was accurately weighed and transferred to a 10 ml volumetric flask. It was dissolved and diluted to 10 ml with the mobile phase to obtain a final concentration of 1mg/ml. Hydrochlorthiazide powder (10 mg) was accurately weighed and transferred to a 10 ml volumetric flask. It was dissolved and diluted to 10 ml with the mobile phase to obtain a final concentration of 1mg/ml.

Preparation of working standard solutions: Standard solution of Telmisartan (1ml) from the stock was transferred to a 10ml volumetric flask and diluted to the mark to obtain 100µg/ml.Standard solution of Hydrochlorthiazide (1ml) from the stock was transferred to a 10ml volumetric flask and diluted to the mark to obtain 100µg/ml.Using a 100µl syringe, 20µl volumes of each solution were injected into the liquid chromatography under the previously mentioned chromatographic conditions.

Preparation of Sample Solution (ASSAY) Weigh about 10 tablets and determine the average weight of tablet. Transfer the average weight of one tablet into 100 ml volumetric flask and sonicate for 5 minutes and then filter through 0.45 micron filter paper . Further pipette 1 ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with mobile phase. Mix well and filter through 0.45µm filter.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions: A rapid, simple HPLC method was developed and validated for the simultaneous estimation of Telmisartan and Hydrochlorthiazide. Mobile Phase consists of Phosphate buffer P^H 4: Acetonitrile (55:45) with a run time of 6 minutes. Chromatographic conditions are optimized for mobile phase using Inertsil ODS Column at a flow rate of 1 ml/min. Effluents are detected at 222 nm using UV detector.

Method validation: The method was validated as per ICH guide lines for the parameters like System suitability, Linearity, Accuracy, Precision, Robustness, Ruggedness and LOD &LOQ.

SYSTEM SUITABILITY: The standard solution was injected for six times and measured the area for all six injections in HPLC. Theoretical plate count and tailing factors are within the limits The results were presented in Table 1.

LINEARITY: Linearity study was performed for the concentration of 48-112µg/ml for Telmisartan and 15-35µg/ml for Hydrochlorthiazide. Area of each level was used for calculation of correlation coefficient. The results were presented in Table 2 and figure 3& 4.

ACCURACY: Accuracy studies were conducted using triplicate determinations at each level of 80%, 100% and 120% of test concentration of as per test method. The percentage recovery at all the levels of concentration is calculated. The results were presented in Table 3.

Table No: 1 Values showing the results of System suitability

S.no	Peak name	Retention time	Area height	USP plate Count	USP tailing	Resolution
1	Telmisartan	4.405	3498.032	4275	1.47	
2	Hydrochlorthiazide	2.9567	356.050	4793	1.3	6.314

Observation: The theoretical plates and tailing factor are within the limits

Table 2: Values showing the results of linearity

S.no	**Telmisartan**		**Hydrochlorthiazide**	
	µg/ml	**Area**	**µg/ml**	**Area**
1	48	2124.641	15	212.082
2	64	2734.196	20	274.081
3	80	3614.224	25	351.469
4	96	4205.076	30	423.469
5	112	4959.097	35	492.695
slope	44.62		14.21	
Co-relation Coefficient	0.996		0.999	

Observation: The method is linear in the concentration range of 48-112 µg/ml for Telmisartan and 15-35 µg/ml for Hydrochlorthiazide. The Co-relation Coefficient was found to be 0.996 for Telmisartan and 0.999 for Hydrochlorthiazide

Table. No. 3 Accuracy study of Telmisartan and Hydrochlorthiazide

Sample name	%concentration (at specification level)	Average area	% Recovery	Mean Recovery
Telmisartan	80	3473.492	101.63%	99.85
	100	4276.872	98.61%	
	120	4872.03	99.31%	
Hydrochlorthiazide	80	344.385	100.52%	99.99
	100	424.709	100.73%	
	120	487.747	98.72	

Observation: The % recovery of each concentration level is obtained between 98 102%.The mean recovery for Telmisartan and Hydrochlorthiazide was found to be 99.85% & 99.99% respectively which indicates that the method is accurate and satisfies its predetermined specifications.

Table. No. 4. Values showing the results of Precision

sample	Injection number	Peak area	% RSD (acceptance criteria < 2)
Telmisartan	1	3515.269	
	2	3513.118	0.41
	3	3484.753	
	4	3501.980	
	5	3490.006	
	6	3483.064	
Hydrochlorothiazide	1	358.060	1.09
	2	360.049	
	3	354.429	
	4	355.992	
	5	358.513	
	6	349.258	

Observation : The %RSD for the area of six standard injections results should not be more than 2% .From the above experiment the % RSD for the area of six standard injections results is 0.41,1.09 for Telmisartan and Hydrochlorthiazide respectively which indicates precision of the method.

Table 5: Robustness results for change in flow rate and wavelength

Changed Parameter	Telmisartan		Hydrochlorthiazide	
	Theoretical Plate Count	Tailing factor	Theoretical Plate Count	Tailing factor
Flow rate (±0.2ml/min)	4088	1.519	5391	1.162
0.8ml/min 1.2ml/min	4500	1.414	5384	1.389
Wavelength (±2nm)	4207	1.47	5336	1.333
220nm and 224nm	4032	1.44	5349	1.292

Observation: On evaluation of the above results, it can be concluded that the variation in flow rate ±0.2%. and change in wavelength ±2nm do not affect the method significantly. Hence the method was found to be robust.

Table 6: Values showing the results of Ruggedness

Telmisartan	% purity	%RSD	Hydrochlorthiazide	% purity	%RSD
Analyst 1	99.40%	0.25	Analyst 1	100.43%	0.28
Analyst 2	99.81	0.52	Analyst 2	100.89%	0.52

Observation: The percentage RSD between two analysts assay values not greater than 2%. Hence the method was rugged.

Table 7: Values showing the results of Assay

S.no	Peak Name	Average standard area	Average sample area	Labelled amount	Tablet average weight	Standard weight	Sample weight	%purity
1	Telmisartan	3440.367	3443.883	40mg	125mg	80mg	250mg	99.40
2	Hydrochlorthiazide	348.648	352.972	12.5mg	125mg	25mg	250mg	100.43

Observation: The percentage purity of Telmisartan was found to be 99.4% and Hydrochlorthiazide was found to be 100.43%.

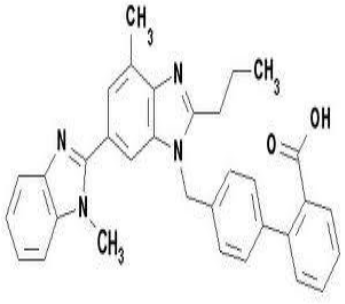


Fig.1 Telmisartan

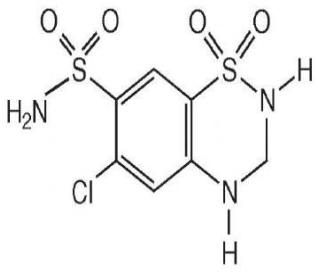


Fig.2 Hydrochlorthiazide

Fig 3: Calibration curve for Telmisartan

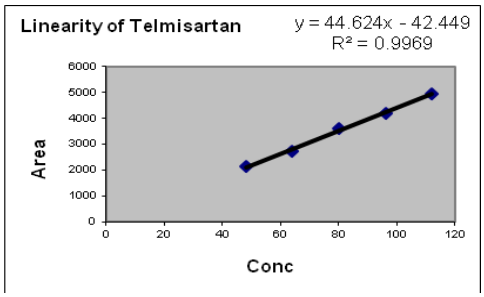
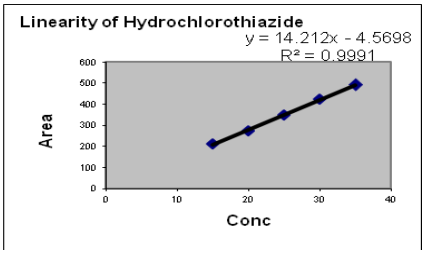


Fig 4: Calibration curve for Hydrochlorthiazide



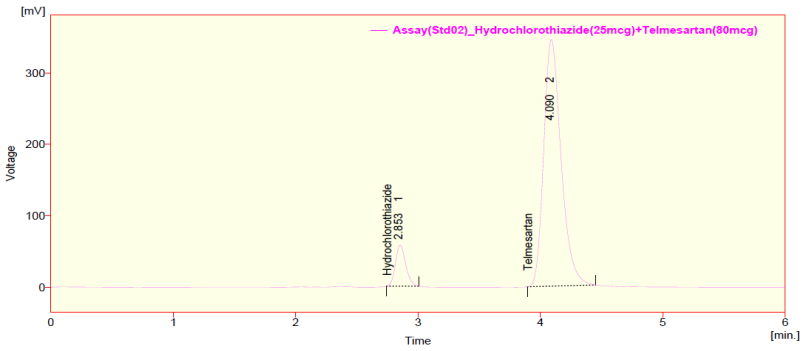


Fig.5 standard chromatogram of Telmisartan and Hydrochlorthiazide

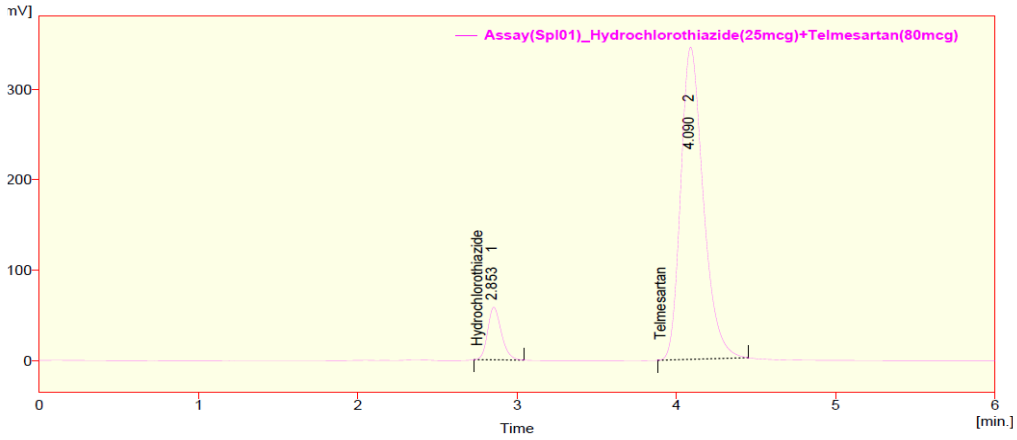


Fig.6 Sample chromatogram of Telmisartan and Hydrochlorthiazide

PRECISION: Standard solutions of Telmisartan and Hydrochlorthiazide were injected for six times on the same day and measured the area for all six injections in HPLC. The results were presented in Table 4.

ROBUSTNESS: Robustness of the method was performed by preparing solution as per test method and injected at different variable conditions like using different conditions like flow rate 0.8 ml/min to1.2ml/min and wavelength 220 nm to 224 nm. The results were presented in Table 5.

RUGGEDNESS: Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst. The ruggedness of the method was determined by carrying out the experiment by different analysts. The results are tabulated in Table 6.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

LOD and LOQ of Telmisartan and Hydrochlorthiazide were determined by calibration curve method. Solutions of both Telmisartan and Hydrochlorthiazide were prepared in the range of 48-112 µg/ml and 15-30 µg/ml, respectively. Where, σ = the standard deviation of the response, S = the slope of the calibration curve. The LOD for this method was found to be 1.870 µg/ml for Telmisartan and 1.834 µg/ml for Hydrochlorthiazide. The LOQ for this method was found to be 5.667 µg/ml for Telmisartan and 5.559 µg/ml for Hydrochlorthiazide.

Assay: The results are tabulated in table 7 and figure 5& 6.

CONCLUSION:

The present proposed method is simple, precise and accurate comparison to the reported methods in the literature. Telmisartan and Hydrochlorthiazide in tablets were completely separated on C_{18} column by using the isocratic elution of Phosphate buffer pH 4 and Acetonitrile (55:45) as mobile phase. UV detection was carried out at 222 nm. Linearity was observed in the range of 48-112µg/ml for Telmisartan (R^2 =0.9969) and 15-35µg/ml for Hydrochlorthiazide (R^2 =0.9991).The percentage mean recoveries were 99.85 and 99.99 for Telmisartan and Hydrochlorthiazide respectively which shows the accuracy of the method. The LOD for this method was found to be 1.870µg/ml for Telmisartan and 1.834µg/ml for Hydrochlorthiazide. The LOQ for this method was found to be 5.667µg/ml for Telmisartan and 5.559 µg/ml for Hydrochlorthiazide. The present developed method is more sensitive and can be used in a wide concentration range for the determination of Telmisartan and Hydrochlorthiazide in Pharmaceutical formulations. The mobile phase is simple to prepare and economical. Hence it can be applied for routine analysis.

Acknowledgement

I Wish to express my sincere gratitude to Principal Dr. Vijaya Kuchana, HOD Mr. C.V.S Raghu Kiran and the management of TKR Educational society for giving me better opportunity to carry out the research work and for their support throughout the work.

REFERENCES

1. http://www.drugbank.ca/drugs/DB01220
2. http://en.wikipedia.org/wiki/TELVAS
3. http://www.medindia.net/doctors/druginformation
4. J. Kavitha, J.S.K. Nagarajan, S. Muralidharan and B.Suresh, International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(4):113-115.
5. M. Rama Mohana Reddy, A. Praveen Kumar, V. Krishna Reddy, S. Wasimul Haque , International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4(1):497–504.
6. Dwarakanadha Reddy. P, Swarnalatha., Design, Development and Characterization of Telmisartan Controlled Release Matrix Tablets by Using Natural Polymers, J. Pharm. Sci. & Res. Vol. 8(8), 2016, 710-714
7. Rekha Gangola, Narendra Singh, AnandGaurav, Mukesh Maithani and Ranjit Singh,Pharmacie Globale International Journal of Comprehensive Pharmacy. 2011; 2 (4):1-3.
8. T.Gopala Swamy, K. Nagaraju and A. Lakshmana Rao. International Journal of Drug Development and Research 2011; 3(4):362-368.
9. Thirupathaiah sanikommu, International Journal of Advances in Pharmaceutical Research. 2013; 4 (3):1540-1556.
10. Santaji Nalwade, Sceintia Pharmaceutica. 2011; 79(1): 69–84