



INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TELMISARTAN HCL AND HYDROCHLOROTHIAZIDE AS API AND IN COMBINATION IN TABLET DOSAGE FORM

MANISH K, AJAY G, SINGH MP

Lachoo Memorial College of Science and Technology (Autonomous), Pharmacy Wing, Jodhpur,
Rajasthan.

Accepted Date: 25/04/2014; Published Date: 27/06/2014

Abstract: A simple, accurate, precise, rapid and economical UV spectrophotometric method was developed for simultaneous estimation of Telmisartan Hydrochloride and Hydrochlorothiazide in a combined tablet dosage form. This method was based on simultaneous equations for analysis of both drugs using methanol as solvent. Telmisartan Hydrochloride has absorbance maxima at 234.0 nm and Hydrochlorothiazide has absorbance maxima at 273.0 nm in methanol. Linearity range was observed in the concentration range of 4-24 µg/ml for Telmisartan Hydrochloride and 2-8 µg/ml for Hydrochlorothiazide with correlation coefficient within range of 0.997 - 0.998 for both drugs. The method was validated according to ICH guidelines. The accuracy and precision of the method was determined and validated statically. The method showed good reproducibility and recovery with % RSD less than 2. The proposed method was successfully applied to commercial combined tablet dosage form of both drugs.

Keywords: Telmisartan Hydrochloride, Hydrochlorothiazide (HCTZ), Simultaneous equation, recovery



PAPER-QR CODE

Corresponding Author: MR. MANISH KUMAR

Access Online On:

www.ijprbs.com

How to Cite This Article:

Manish K, Ajay G, Singh MP; IJPRBS, 2014; Volume 3(3): 73-86

INTRODUCTION

Telmisartan Hydrochloride is chemically 4'[(1,4'-Dimethyl-2'-propyl[2,6'-bi-1H-benzimidazol]-1'-yl)methyl][1,1'-biphenyl]-2-carboxylic acid (Fig. no. 1), is an angiotensin II receptor blocker that shows high affinity for the angiotensin II receptor type 1. It is used in the treatment hypertension and heart failure[1-4]. It is official in Indian Pharmacopoeia 2010[5]

Hydrochlorothiazide is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide(Fig.no.2),Hydrochlorothiazide, is thiazide diuretic and also used as antihypertensive agent.[6,7] It is official in British Pharmacopoeia 2012, United States Pharmacopoeia 2011 and Indian Pharmacopoeia 2010[8,9]

Literature survey reveals spectrophotometric, HPLC, HPTLC and other methods for the estimation of Telmisartan Hydrochloride and Hydrochlorothiazide individually and in combination with other drugs[10-18] However, no method has been reported in any literature so far for simultaneous estimation of these two drugs in combined dosage form. Hence the specific aim of the research was to develop and validate simple, accurate, precise and specific UV spectrophotometric method for simultaneous estimation of Telmisartan Hydrochloride and Hydrochlorothiazide in pharmaceutical tablet dosage form.

MATERIALS AND METHODS

Apparatus & Software

A double beam UV-VIS Spectrophotometer (UV 1800, Shimadzu, Japan) Spectral bandwidth of 1 nm and wavelength accuracy of ± 0.5 nm with a pair of 1 cm matched quartz cells was used to measure the absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (UV Probe version 2.31). All weights were taken on Digital electronic balance Sartorius, CP225D.

Reagents and Chemicals

All the chemicals used were of analytical grade. Methanol A.R. grade was procured from Loba Chem. Ltd., Mumbai. Telmisartan Hydrochloride was kindly supplied by Lupin Healthcare Ltd., Goa and Hydrochlorothiazide was kindly supplied by Glanmark Pharmaceuticals Ltd., Noida (New Delhi) as a gift sample.

Marketed Formulation

The commercial fixed dose combination tablet Telista-H[19] (Telmisartan Hydrochloride 40mg and Hydrochlorothiazide 12.5 mg) was procured from local market.

Preparation of standard stock solutions

Accurately weighed Telmisartan Hydrochloride (10 mg) was transferred to 100 ml volumetric flask, dissolved and sonicated in methanol and made-up the volume to 100 ml with methanol as solvent. The final solution contained 100 µg per ml of Telmisartan Hydrochloride.

Determination of wavelength of maximum absorbance for Telmisartan Hydrochloride

Standard solution of Telmisartan Hydrochloride (1ml) was transferred to a 10 ml volumetric flask. The volume was adjusted to 10 ml with methanol as solvent. The absorbance of the final solution (25 µg/ml) was scanned in the range 400-200 nm against methanol as blank and λ max was found to be 234.0 nm.

Determination of wavelength of maximum absorbance for Hydrochlorothiazide

Standard solution of Hydrochlorothiazide (0.4 ml) was transferred to 10 ml volumetric flask. The volume was adjusted to 10 ml with methanol as solvent. The absorbance of the final solution (10 µg/ml) was scanned in the range 400 to 200 nm against methanol as blank and λ max was found to be 273.0 nm. (The overlay spectra of Telmisartan Hydrochloride and Hydrochlorothiazide) (Fig.no. 3)

Preparation of calibration curve of Telmisartan Hydrochloride and Hydrochlorothiazide

Standard solutions of Telmisartan Hydrochloride in the concentration range of 4 µg/ml to 24 µg/ml were obtained by transferring 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 ml of Telmisartan Hydrochloride stock solution (100 ppm) to a series of 6 volumetric flasks of 10 ml and standard solutions of Hydrochlorothiazide in the concentration range of 2 µg/ml to 8 µg/ml were obtained by transferring 0.2, 0.3, 0.4, 0.4, 0.6 and 0.7 , 0.8 ml of Hydrochlorothiazide stock solution (100 ppm) to a series of 8 volumetric flasks of 10 ml. The volume in each volumetric flask was made up with methanol as solvent. the absorbances of the solutions were measured at both the wavelength 234.0 nm and 273.0 nm against the methanol as blank and calibration curves were plotted. (Fig.no.4&5)

Preparation of synthetic API mixture of Telmisartan Hydrochloride and Hydrochlorothiazide

The synthetic API mixture of Telmisartan Hydrochloride and Hydrochlorothiazide was prepared in ratio of 40:12.5. Accurately weighed 100 mg of Telmisartan Hydrochloride and 31 mg of Hydrochlorothiazide were transferred to 100 ml volumetric flask, 100 ml of methanol as solvent was added to it up to quantity sufficient. Further 10 ml of prepared solution (1000 ppm) was transferred to a 100 ml volumetric flask. The volume was adjusted to 100 ml with methanol as solvent. The final standard stock solution contains 100 µg /ml of Telmisartan Hydrochloride .

Estimation of Telmisartan Hydrochloride and Hydrochlorothiazide in synthetic mixture

The API synthetic mixture (0.8, 1.1, 1.4, 1.7, 2.0 and 2.3 ml) was transferred to a series of six volumetric flask of 10 ml and volume was made up to mark using methanol as solvent. The absorbances of these solutions were measured at 234.0nm and 273.0 nm wavelength.

At 234.0 nm and 273.0 nm two simultaneous equations were formed using absorptivity coefficient values [20,21]

$$CX = \frac{(A2 ay1 - A1 ay2)}{(ax2ay1 - ax1 ay2)} \quad (1)$$

and

$$CY = \frac{(A1 ax2 - A2 ax1)}{(ax2 ay1 - ax1 ay)} \quad (2)$$

Where,

Cx = Concentration of Telmisartan Hydrochloride in g/L

Cy = Concentration of Hydrochlorothiazide in g/L

A1= Absorbance of mixture at 234.0 nm

A2 = Absorbance of mixture at 273.0 nm

ax1 = Absorptivity of Telmisartan Hydrochloride at 234 nm

ax2 = Absorptivity of Telmisartan Hydrochloride at 273.0 nm

ay1 = Absorptivity of Hydrochlorothiazide at 234.0 nm

ay2 = Absorptivity of Hydrochlorothiazide at 273.0 nm

Validation of proposed method

The proposed method was validated according to International Conference on Harmonization (ICH) guidelines[22]

Specificity

The synthetic mixture of Telmisartan Hydrochloride and Hydrochlorothiazide was prepared in ratio of 40:12.5. Common excipients used in the tablet formulation were added into this mixture. The absorbance was measured before addition of excipients and after addition of excipients at 234.0 nm and 273.0 nm.

Linearity and Range

Aliquots of standard stock solutions of Telmisartan Hydrochloride and Hydrochlorothiazide were taken in 10 ml volumetric flasks and diluted with methanol to get final concentration in the range of 4-24 µg/ml for Telmisartan Hydrochloride and 2-8 µg/ml for Hydrochlorothiazide. This calibration range was prepared six times and absorbances were measured at 234.0 nm and 273.0 nm for each drug separately.

Accuracy

The accuracy of the method was determined by carrying out recovery studies using standard addition method. Known amounts of standard solutions of TEL and HCTZ were added to prequantified sample solutions of TEL and HCTZ (14 µg/ml TEL and 4.60 µg/ml HCTZ). The amounts of TEL and HCTZ were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for three times

Method Precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for TEL (14 µg/ml) and HCTZ (4.60 µg/ml) without changing the parameter of the proposed spectrophotometry method.

Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day at 2 hours interval and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of TEL and HCTZ (11.1,14.4,16.43 µg/ml for TEL and 3.3,4.2,4.0 µg/ml for HCTZ). The result was reported in terms of relative standard deviation (% RSD).

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations designated by International Conference on Harmonization (ICH) guidelines:

$$\text{LOD} = 3.3 \times \text{SD}/S$$

$$\text{LOQ} = 10 \times \text{SD}/S$$

Where, SD = the standard deviation of the response

and S = slope of the calibration curve

LOD and LOQ were determined from the standard deviations of the responses for six replicate determinations.

Robustness

Solution containing mixture of 14.0 µg/ml TEL and 4.6 µg/ml HCTZ was prepared from the standard stock solution (100 µg/ml of TEL and 30 µg/ml of HCTZ) through dilution. Prepared solution was analyzed as per proposed method with small but deliberate change in λ_{max} ($\lambda_{\text{max}} \pm 1 \text{ nm}$) and scanning speed (slow, medium and fast).

Estimation of Telmisartan Hydrochloride and Hydrochlorothiazide in combined tablet dosage form

Twenty tablets were weighed and finally powdered and triturated well. A quantity of powder equivalent to 10 mg of Telmisartan Hydrochloride and 3.124 mg of Hydrochlorothiazide was transferred to 100 ml volumetric flask and mixed with 70 ml of methanol as a solvent and solution was sonicated for 20 minutes there after volume was made up to 100 ml with same solvent to produce the resultant solution of 100 µg/ml and 31.24 µg/ml of Telmisartan Hydrochloride and Hydrochlorothiazide respectively. The solution was filtered through Whatmann filter paper 42. From the filtrate, 1.4 ml was transferred to five different 10 ml volumetric flasks and volume in each was made up to 10 ml with methanol as a solvent. Absorbances of these solutions were measured at 234.0 nm and 273.0 nm using methanol as blank Percent tablet claim for Telmisartan Hydrochlorothiazide and Hydrochlorothiazide tablets was determined by using equation 4 and 5 values of the A1 and A2 were substituted in Eqn. 1 and 2 to obtain the concentration of TEL and HCTZ respectively

RESULTS AND DISCUSSION

Validation Specificity

The developed method was found to be specific as percent interference obtained was 0.068% and 0.207% for TEL and HCTZ respectively, which were less than prescribed limit (0.4%) as per ICH guidelines. Thus it was concluded that the addition of excipients had a very negligible change in the concentration of TEL and HCTZ

Linearity and Range

Linearity range was found to be 4.0 - 24.0 µg/ml for TEL at 234.0 nm and 273.0 nm. The correlation coefficient was found to be 0.998 & 0.998 which showed good linearity between ranges. The slope was found to be 0.0342 & 0.00464 and intercept was found to be -0.0117 and -0.0006.

For HCTZ at 234.0 nm and 273.0 nm, linearity range was found to be 2.0 – 8.0 µg/ml. The correlation coefficient was found to be 0.997 & 0.998 which showed good linearity between ranges. The slope was found to be 0.0204 and 0.01164 and intercept was found to be 0.0024 and 0.01189. (Table no. 1)

Accuracy

The results obtained for the accuracy study (recovery method) from three sample studies (n = 3) for each level indicated that the mean of the % recovery was 99.619% and 99.944% and % RSD was 0.0327 % and 0.471 % for TEL and HCTZ respectively in synthetic mixture. The method was found to be accurate. (Table no. 2)

Method Precision (Repeatability)

Repeatability study showed a RSD of 0.437% for TEL and 0.1.23% for HCTZ, which was less than 2%.

Intermediate Precision (Reproducibility)

The method was found to be precise as intra-day precision study showed a RSD of 0.164 % for TEL and a RSD of 0.433% for IND and inter-day precision study showed a RSD of 0.323% for NEB and a RSD of 0.0.346% for IND. The % RSD values were less than 2%. (Table no. 3)

Limit of Detection and Limit of Quantification

The LOD was found to be 0.431mg/ml and 0.1.30mg/ml and LOQ was found to be 0.260 mg/ml and 0.789mg/ml for TEL and HCTZ respectively which represents that sensitivity of the method was high.

Robustness

The robustness study for change in scanning speed showed a RSD of 0.457% and 0.624% for NEB and IND respectively. The robustness study for change in λ_{max} showed a RSD of 0.110% and 0.151% for NEB and IND respectively. The method was found to be robust at small variations in scanning speed and λ_{max} .(Table no.4)

Summary of all validation parameters (Table no. 5)

Estimation of TEL and HCTZ in combined tablet dosage form. When Telista-H tablets were analyzed by simultaneous equation method, the % purity was found to be 99.36% for TEL and 98.04% for HCTZ. (Table no.6)

CONCLUSION

The results of the analysis of pharmaceutical tablet formulation by the proposed UV spectrophotometric method were highly reproducible and reliable and were in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of Telmisartan HCl and Hydrochlorothiazide. The observations and results obtained from this study including linearity, accuracy and precision (method precision as repeatability and intermediate precision as intra and inter day precision) are lie well within acceptable limits. From the experimental studies it was concluded that proposed method was simple, sensitive, economic, precise, accurate and specific and can be adopted for the routine quality control analysis of both drugs in combined tablet formulation without interference of excipient.

ACKNOWLEDGEMENT

The authors are thankful to Lupin Healthcare Ltd., and Glanmark Pharmaceuticals Ltd., Noida (New Delhi) for providing gift sample of TEL and HCTZ, respectively to carry out the research work. The authors are highly thankful to Lachoo Memorial College of Science and Technology (Autonomous), Jodhpur, Pharmacy Wing for providing all the facilities to carry out the research work.

Table 1: Optical parameters & regression characteristics for Telmisartan Hydrochloride and Hydrochlorothiazide

Parameters	Telmisartan Hydrochloride		Hydrochlorothiazide	
	234.0 nm	273.0 nm	234.0 nm	273.0 nm
Beer' law limit (µg/ml)	4-24	4-24	2-8	2-8
Sandell's sensitivity (mg/cm ² /.001 absorbance unit)	0.0321	0.2543	0.00435	0.01157
Regression equation (y= mx + c)				
slope (m)	0.0342	0.005	0.022	0.091
intercept (c)	-0.0117	-0.0006	0.0025	-0.01189
Correlation coefficient (r ²)	0.998	0.998	0.997	0.998

Table 2: Data showing recovery study

S.No.	WL. (nm)	Conc.of tablet sol ⁿ (ppm)	Std. Added (ppm)	Amt. Found (mg)	Mean Recovery ± SD	% RSD
1.	234.0	14.032	4.60	19.628	TEL	TEL
	273.0	4.378	1.74	6.124	99.619 ± 0.326	0.327
2.	234.0	14.063	7.00	21.060		
	273.0	4.383	2.18	6.461	HCTZ	HCTZ
3.	234.0	14.064	8.40	22.460	99.944 ± 0.470	0.471

Table 3: Data showing Intra and inter day precision analysis

Intra day precision	11.127 ± 0.023	0.206	3.340 ± 0.028	0.838
Inter day precision	14.213 ± 0.071	0.499	4.241 ± 0.014	0.344

Table 4: Data showing robustness study for change in λ_{max}

Variation and Level	Conc. ($\mu\text{g/ml}$)	Abs.	Conc. Found ($\mu\text{g/ml}$)	Mean ± SD	% RSD	
Change in λ_{max} ($\lambda_{max} \pm 1 \text{ nm}$)	233.0	Telmisartan:HCTZ	0.578	17.66	TEL	TEL
	234.0	(18.0+5.5)	0.593	17.78	17.697	0.406
	235.0		0.574	17.65	± 0.072	
	272.0		0.647	5.39	HCTZ	HCTZ
	273.0		0.655	5.42	5.403	0.555
				± 0.030		

Table 5: Summary of validation parameters by UV method

Validation parameters	Telmisartan HCl	Hydrochlorothiazide
Specificity	% interferences <0.5%	
Range ($\mu\text{g/ml}$)	Linear range	4.0 – 24
	Working range	2.0 – 8.0
	Target range	0.43-24
		0.26-8.0
		11.2,14.0
		4.0,5.0,6.0

	Target concentration	14.0	5.0
Accuracy (% recovery)		99.67%	99.53%
Precision (% RSD)		0.537	1.23
		0.165	0.533
		0.323	0.356
LOD ($\mu\text{g/ml}$)		0.431	1.30
LOQ ($\mu\text{g/ml}$)		0.260	0.789
Robustness (% RSD)	Change in scanning speed	0.231	0.555
	Change in λ_{max}	0.406	0.305

Table 6: Estimation of Telmisartan Hydrochloride and Hydrochlorothiazide in combined tablet dosage form by proposed UV spectroscopic method

S.No.	Brand Name	Label claim (mg)	Amt. found in mg/tablet	
			NEB	IND
1	Telista-H	TEL	39.482	12.068
2	Tablet	(40 mg)	39.737	12.331
3		+	40.346	12.264
4		HCTZ	39.443	12.264
5		(12.5 mg)	39.420	12.317
Mean			39.744	12.244

Figure Legends

Figure 1 : Chemical Structure of Telmisartan Hydrochloride

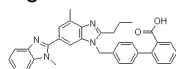


Figure 2 : Chemical Structure of Hydrochlorothiazide

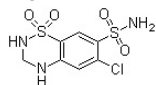


Figure 3: Overlain Spectra of Telmisartan Hydrochloride (10 µg/ml) and Hydrochlorothiazide (25µg/ml)

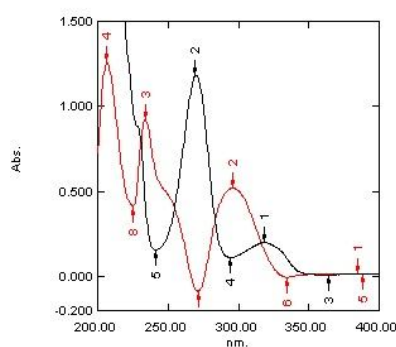


Figure 4: Calibration curve for Telmisartan HCl at 234.0 nm

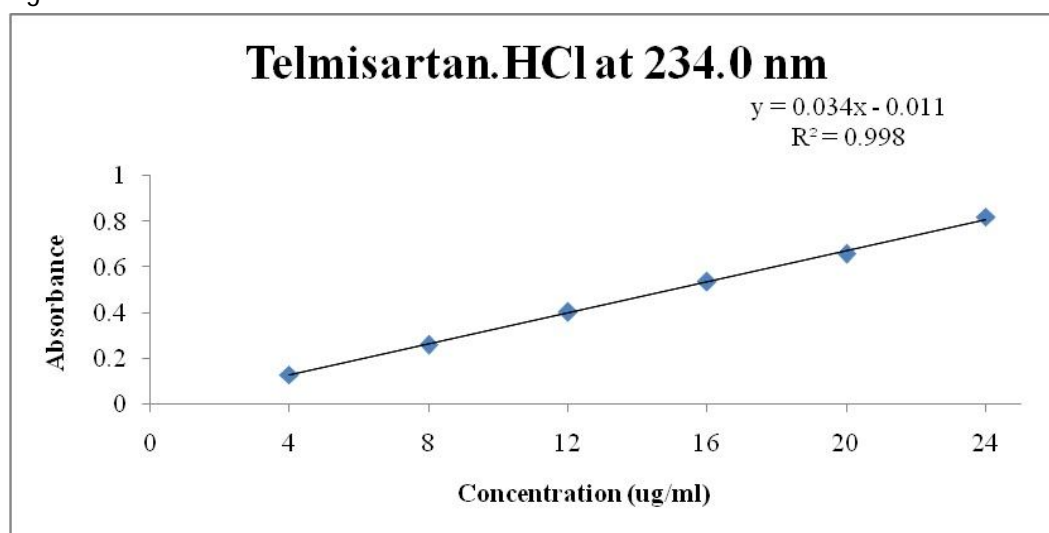
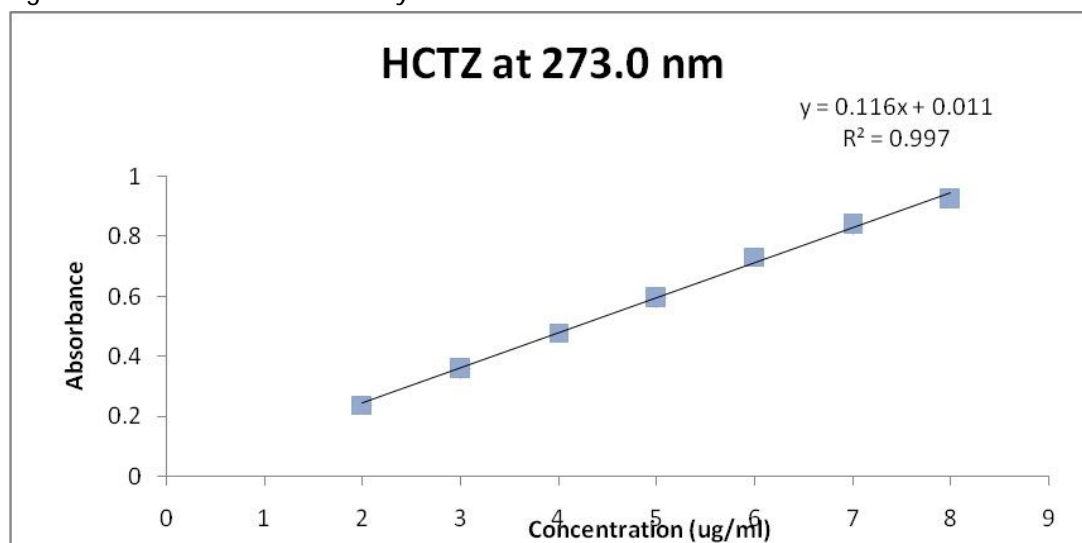


Figure 5. Calibration curve of Hydrochlorothiazide 273.0 nm



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