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LIQUID CHROMATOGRAPHIC SEPARATION AND ANALYSIS OF TELMISARTAN AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT: An accurate, simple and precise RP-HPLC method for the simultaneous determination of telmisartan and hydrochlorothiazide in pharmaceutical formulations was developed and validated. Chromatographic separation of two drugs was achieved on a Kromasil C18, (250x4.6nm, 5µm) column by using the mobile phase consisted of methanol, tetrahydrofuran and water in the ratio 55:05:40 at a flow rate of 1mL/min and the wavelength of detection was at 234 nm. The retention time for telmisartan and hydrochlorothiazide were found to be 2.3 and 3.8 min respectively. The linearity of the developed method was tested over a concentration range of 50-100 µg/mL and the correlation coefficient (r^2) of regression was 0.999 which is almost equal to 1. The limit of quantification was 0.5µg/mL for telmisartan and 0.4µg/mL for hydrochlorothiazide and the limit of detection was 0.1µg/mL for both telmisartan and hydrochlorothiazide. The mean percentage recoveries were 99.2% for telmisartan and 99.8% for hydrochlorothiazide respectively.

INTRODUCTION: Telmisartan (TEL), (fig1) a non-peptide molecule and its IUPAC name is 4′-[(1,4′-dimethyl-2′-propyl [2,6′-bi-1H-benzimidazol]-1′-yl) methyl] [1,1′-biphenyl]-2-carboxylic acid [1]. It is an angiotensin II, type I blocker and is used as an antihypertensive along with hydrochlorithiazide.

It is a thiazide diuretic which reduces the reabsorption of electrolytes from the renal tubules, thereby increasing the excretion of sodium and chloride ions and consequently water.

Hydrochlorothiazide (HCTZ), (**fig. 1**) is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide-1, 1-dioxide.

The combination is useful in the treatment of mild to moderate hypertension, well tolerated with a lower incidence of cough than ACE inhibitors [2].
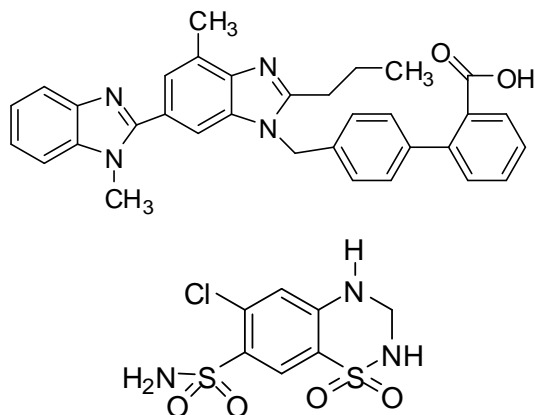
FIG.1: TELMISARTAN & HYDROCHLOROTHIAZIDE

A literature survey revealed that there are some liquid chromatographic [3-9] methods reported for the determination of simultaneous estimation of telmisartan and hydrochlorothiazide in pharmaceutical dosage forms. In the present work an attempt was made to develop a simple, precise and accurate RP-HPLC method for the simultaneous estimation of telmisartan and hydrochlorothiazide in pharmaceutical dosage forms.

MATERIALS AND METHODS:

Chemicals and reagents: THF, methanol and water of HPLC grade were produced from Merck specialties Pvt. Ltd. Mumbai. A working standard of telmisartan and hydrochlorothiazide was provided by V.V.Med laboratories, Hyderabad.

Equipment: Analysis of the samples was carried out using PEAK 7000 isocratic HPLC with rheodyne manual sample injector with switch (77251) and the column used was analytical column Kromasil 100-5 C18(250X4.6nm). Electronic balance; ELB 300, DIGISUN for pH measurements.

Chromatographic conditions: Kromasil C18 (250X4.6mm) column was used for the separation; a mobile phase of methanol, THF and water in the ratio 55:05:40 was filtered through 0.45μm membrane filter paper and delivered at a flow rate of 1mL/min and the wavelength of detection was at 234 nm. The injection volume was 20μL, and the total run time 8min. The resulting chromatogram was shown in **Fig. 2**.
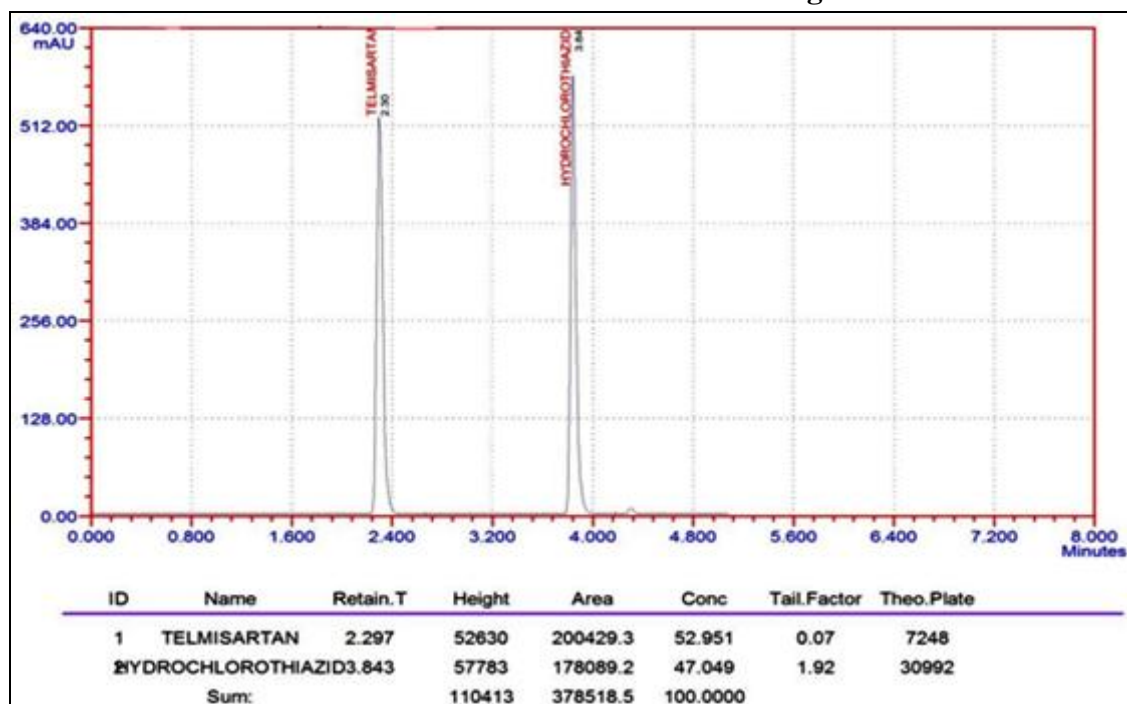

ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	TELMISARTAN	2.297	52630	200429.3	52.951	0.07	7248
2	HYDROCHLOROTHIAZID	3.843	57783	178089.2	47.049	1.92	30992
	Sum:		110413	378518.5	100.0000		

FIG. 2: STANDARD CHROMATOGRAM OF TELMISARTAN (t_R 2.3min) AND HYDROCHLOROTHIAZIDE (t_R 3.8min)

Preparation of standard solutions: Standard stock solutions of 1.0 mg/mL telmisartan and hydrochlorothiazide were prepared separately using methanol as solvent. In order to get the required ratio (3.2:1) of both the drugs telmisartan and hydrochlorothiazide appropriate quantities of

respective solutions of each drug were mixed and diluted with the mobile phase.

Preparation of sample solution: Twenty tablets were weighed and finely powdered. A quantity of powder equivalent to 40 mg of telmisartan and

12.5 mg of hydrochlorothiazide were weighed and transferred to a 50 mL volumetric flask; methanol was added to the same flask and shaken for some time and the volume was made up to the mark. The solution was filtered using Whatman filter paper. From the filtrate appropriate dilution was done to get a solution of 80 µg/mL of telmisartan and 50 µg/mL of hydrochlorothazide.

System suitability studies: The resolution, numbers of theoretical plates were calculated for the working standard solutions and are shown in **table 1**. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination.

TABLE 1: SYSTEM SUITABILITY STUDIES

S. no.	Parameters	Telmisartan	Hydrochlorothiazide
1	Theoretical plates/meter	7248	30992
2	Tailing factor	0.07	1.92
3	L.O.Q	0.5ppm	0.4ppm
4	L.O.D	0.1ppm	0.1ppm

Method development: Different mobile phases containing of methanol, THF and water in different proportions were tried, and finally, methanol: THF: H_2O in the ratio 55:05:40 was found to be an appropriate mobile phase, which gave acceptable peak parameters for both telmisartan and hydrochlorothiazide.

Method validation: The validation of the method was carried out in terms of parameters like linearity, precision, accuracy, limit of detection, limit of quantification, specificity and robustness.

Specificity: The Specificity of the RP-HPLC method was determined by the complete separation of telmisartan and hydrochlorothiazide as shown in fig. 2 with parameters like retention time (R_t= 2.3 min for TEL and R_t=3.8 min for HCTZ) and tailing factor (0.07 for TEL and 1.92 for HCTZ). Moreover, the clear baseline separation of the drugs and sharp peaks also supports the specificity of the method.

Linearity: The linearity of the method was tested over a concentration range of 50 to 100µg/mL of TEL and HCTZ. 20µL of each concentration was injected in duplicate into the HPLC system.

The response was read at 234nm and the corresponding chromatograms were recorded. The regression value of the plots was computed by least square regression method. Peak area (A) and concentration (c) of each drug substance was subjected to regression analysis to calculate the correlation coefficients. A correlation coefficient r^2=0.999 for both TEL and HCTZ of the regression was found to be almost equal to 1. Linearity results were presented in **Table 2** and graph in **Fig. 3**.
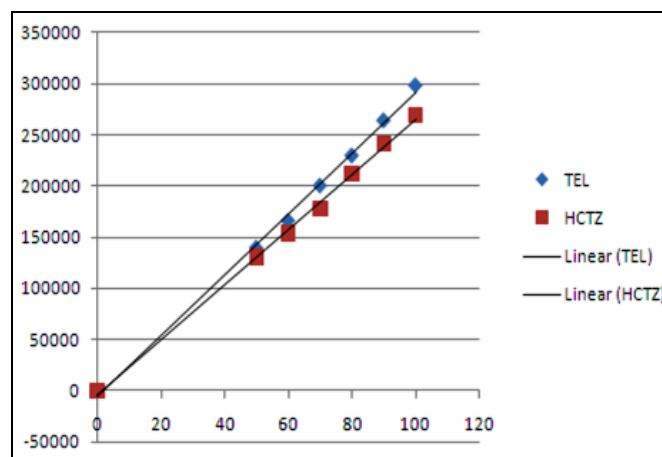

FIG. 3: LINEARITY OF TELMISARTAN AND HYDROCHLOROTHIAZIDE

TABLE 2: RESULTS OF LINEARITY

	Linearity	areas		PEAK 1(TEL)	PEAK 2 (HCTZ)
S. No.	Conc. in ppm	Peak-1	Peak 2		
1	100	298430	269558	INTERCEPT = -23024.80952	INTERCEPT = - 15004.4761
2	90	264186	241219		
3	80	229861	212130		SLOPE = 2833.7885
4	70	200429	178089	SLOPE = 3192.868	
5	60	166022	153624		C.C = 0.999
6	50	139714	130558	C.C = 0.999	

Precision: The precision of the method was determined by inter day and intraday studies, solutions of standard and sample were repeated thrice in a day and percentage relative standard deviation (%RSD) was calculated. Results of system precision studies were shown in **Table 3 and 4**. The %RSD found were less than 1%.

The intraday %RSD of telmisartan and hydrochlorothiazide were found to be 0.135% and 0.44%, respectively. The inter day %RSD of telmisartan and hydrochlorothiazide were found to be 0.9% and 0.51% respectively. The values of %RSD within a day, day to day variation <1% proves that the method is precise.

TABLE 3: RESULTS OF PRECISION STUDY (INTRA DAY)

	INJECTION	Area of Peak -1 (TEL)	Area of Peak -2 (HCTZ)	RSD
	1	200803	177316	
	2	198625	178976	TEL
CONC= 70	3	198873	177930	R.S.D 1 =0.135
µg/mL	4	198485	178084	
	5	198295	179507	HCTZ
	6	200429	178089	R.S.D 1 = 0.44

TABLE 4: RESULTS OF PRECISION STUDY (INTER DAY)

	INJECTION	Area of Peak -1 (TEL)	Area of Peak -2 (HCTZ)	RSD
	1	198945	179856	
	2	201274	180536	TEL
CONC=70	3	199900	178516	R.S.D 2 =0.9
µg/mL	4	199914	178241	
	5	196006	178688	HCTZ
	6	199722	178516	R.S.D 2 = 0.51

Accuracy: The accuracy of the method was determined by recovery experiments. The recovery studies were performed by the regular addition method. At 50%, 75%, 100% level, the percentage recovery was calculated and presented in **table 5**. Recovery was found to be within the range of 99.2% to 99.8%, which indicates the accuracy of the method. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. The results of recover studies were found to be satisfactory.

TABLE 5: ACCURACY DATA OF THE DEVELOPED METHOD

Recovery	Conc. of sample	Recovery -1	Recovery -2	% of recovery-1	% of recovery-2
50%	50ppm	49.06	50.07	98.12	100.14
75%	75ppm	74.92	74.85	99.89	99.8
100 %	100ppm	99.6	99.60	99.6	99.6

Robustness: Robustness of the method was studied by making slight changes in chromatographic conditions, such as mobile phase ratio, pH and wavelength of detection (**table 6**). It was observed that there were no marked changes in the chromatograms obtained. So the developed RP-HPLC method was robust.

TABLE 6: RESULTS OF ROBUSTNESS

Parameter	Modification	Peak 1-Area	Peak 2-Area	Peak 1-% of change	Peak 2 -% of change
MP	45:05:50	199162	178850	0.99	1.00
pH	5.1	198112	177190	0.98	0.99
Wavelength	230nm	201619	176996	1.00	0.99

Limit of Detection and Limit of Quantification: The limit of detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula:

$$LOD = \frac{3.3 \text{ X Standard Deviation}}{\text{Slope of calibration curve}}$$

The LOD for telmisartan and hydrochlorothiazide was found to be 0.1ppm for both the drugs (**Table 1**).

The limit of quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the formula:

$$LOQ = \frac{10 \text{ X Standard Deviation}}{\text{Slope of calibration curve}}$$

The LOQ was 0.5ppm and 0.4ppm for telmisartan and hydrochlorothiazide, respectively (**Table 1**).

RESULTS AND DISCUSSION: The method was developed using the mobile phase MeOH: THF: H_2O in the ratio 55:05:40, and the standard chromatogram obtained showed the elution of telmisartan at t_R =2.3 min, tailing factor 0.07 and elution of hydrochlorothiazide at t_R =3.8 min, tailing factor 1.92. The separation of two drugs for the selected mobile phase is much prominent. Moreover, the order of elution of the two drugs is in good agreement with the earlier reported method [4], which was developed, with the mobile phase MeOH: CH_3CN in the ratio 70:30.

However these two methods in respect of order of elution of drugs were contradictory to other methods [3, 5, 6, 7, and 8] which were developed using 1% ortho phosphoric acid as one of the solvents in the mobile phases with either methanol or acetonitrile. This order of elution of telmisartan (at t_R =2.3 min) and hydrochlorothiazide (at t_R =3.8 min) may be because of greater affinity of telmisartan towards the mobile phase compared to hydrochlorothiazide i.e. the change is determined by the polarity of the mobile phase selected in the present study.

A system suitability test was applied to representative chromatograms for various parameters. A graph was constructed covering a concentration range 50 to 100ppm. The calibration curve was obtained for a series of concentrations in the range of 50 to 100ppm and it was found to be linear. The data of regression analysis of the calibration curves are shown in table 2. The method was found to be accurate and precise, as indicated by the recovery studies and %RSD calculated is less than 1.

Moreover, the LOD and LOQ for telmisartan were found to be 0.1µg/mL and 0.5µg/mL, respectively, and for hydrochloro-thiazide the values are 0.1µg/mL and 0.4µg/mL, respectively (**Table 1**).

CONCLUSION: The developed RP-HPLC method for the simultaneous estimation of telmisartan and hydrochlorothiazide in combined dosage form is more precise, robust, and accurate in respect of validation and the quality of separation achieved of the two drugs is also good in terms of retention times and tailing factor.

Hence, the proposed method is applicable to the determination of telmisartan and hydrochloro-thiazide in pharmaceutical formulations.

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