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A Validated Stability-Indicating Densitometric Method For Simultaneous Quantitative Estimation Of Telmisartan And Hydrochlorothiazide In Bulk Drug And In Pharmaceutical Tablet Dosage Form

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Abstract: The present study deals with development of a simple, rapid, sensitive and economic stability indicating high performance thin layer chromatography (HPTLC) method for simultaneous quantitative estimation of telmisartan and hydrochlorothiazide in bulk and tablet dosage form. The chromatographic separation was achieved by using TLC plates precoated with Silica gel 60 F_{254} as stationary phase and the mobile phase consisting of toluene:ethyl acetate:acetic acid in the ratio of 1:5:1 v/v. Telmisartan and hydrochlorothiazide were well resolved with R_f 0.22 ± 0.03 and 0.57 ± 0.03 respectively. Densitometry scanning was carried out for the detection of spots at 280nm. ICH recommended stress degradation studies were performed on telmisartan, hydrochlorothiazide standard bulk drugs and further stressed samples were analyzed by the proposed method. Major degradation of telmisartan and hydrochlorothiazide were observed under acidic, alkali, hydrolytic and oxidative conditions. The described HPTLC method was validated as per ICH guideline and validation parameter such as linearity, accuracy, precision, specificity and robustness results were within acceptable limits.The developed method was found to be simple, specific, precise and stability indicating.

Key words: Forced Degradation, Hydrochlorothiazide, High Performance Thin Layer Chromatography, Method Validation. Simultaneous estimation,Telmisartan.

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I. INTRODUCTION

Telmisartan, chemically described as 4′-{[4-methyl-6-(1-methyl-1H-1,3-benzoimidiazol-2-yl)-2-propyl-1H-1,3-benzoimidazole-1-yl] methyl}-2-biphenyl carboxylic acid, molecular formula C_{33} H_{30} N_4 O_2 and molecular weight 514.6 is an angiotensin II receptor antagonist which helps to lower arterial hypertension.Hydrochlorothiazide is chemically 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide, molecular formula $C_7H_8ClN_3O_4S_2$ and molecular weight 297.7 is an antihypertensive drug belongs to thiazide class of diureticFig.1 and Fig.2[1,2].Telmisartan is a potent, highly selective antagonist of the angiotensin II type-1 (AT_1) receptor, which has high lipophilicity and long plasma half-life.Combination of an angiotensin II receptor antagonist and a thiazide diuretic is likely to become widely used in patients with moderatetosevere hypertension or with additional cardiovascular risk factors[3-8].

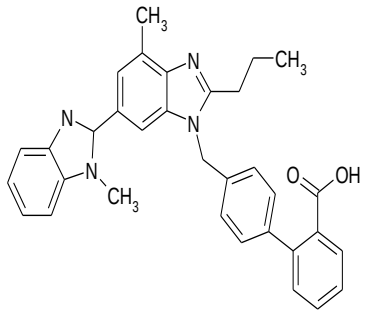

Fig. 1: Structure of Telmisartan

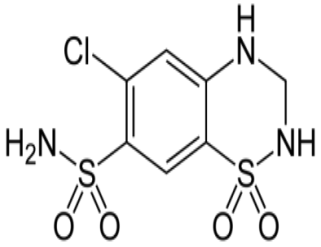

Fig. 2: Structure of Hydrochlorothiazide

A review of various literature and current ICH guideline tells about analysis of test samples for stability should be done by using a developed and validated stability indicating assay method which elucidates the inherent stability characteristics of the active substance after subjecting them to a variety of stress conditions, such as acid, base, hydrolytic, oxidation, photolytic and thermal degradation. An ideal stability-indicating method is one that quantifies the standard drug alone and also resolves its degradation products.Stress testing or forced decomposition studies are undertaken to elucidate the intrinsic stability of the drug substance and are carried out under more severe stress conditions of acid and base hydrolysis, oxidative, hydrolytic, photolytic and dry heatthan those used for acceleratedstability testing[9-11].

Extensive literature survey reveals several analytical methods that have been reported for the estimation of telmisartan alone or in combination with other drugs.The reported method includes several HPLC and HPTLC methods[12-24], UV spectroscopy method [25-27] bioanalytical methods [28-29]. But there is one HPTLC method [30]was reported for the simultaneous estimation of telmisartan and hydrochlorothiazide in the presence of their degradation products. Hence there is a need for the development of newer, simpler, sensitive, rapid, accurate and reproducible stability indicating HPTLC method for the simultaneous estimation of titled ingredients in presence of degradation products.This paper describes a very selective and rapid HPTLC method, where the preparation of sample and mobile phase are made simpler requiring no pH adjustments.

II. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Pure drugs of telmisartan and hydrochlorothiazide were obtained as a gift sample from Micro Laboratories Limited, Husur, India, Drugs were used without any further purification.Telmisartan and hydrochlorothiazide tablet (Telma H) label claim 40 mg and 12.5 mg from Glenmark, Himachal Pradesh, India was purchased from the local market.Analytical reagent sodium hydroxide, hydrochloric acid, hydrogen peroxide, toluene, ethyl acetate and acetic acid were used in this method wasobtained from Fisher Scientific, India. Double distilled water was used throughout the experiment.

2.2 Instrumentation

A HPTLC system (Camag, Switzerland) comprising of semi-automatic sample applicator (Camang Linomat 5), hamilton syringe (100 µl), camag TLC scanner 3, camag WinCATS software, camag twin trough chambers (10×10 cm), (20×10 cm), UV cabinet with dual wavelength UV lamps,digital weighing balance AUX220(shimadzu), ultrasonicator (spincotech), digital hot air oven (S V Scientific),digital water bath(Grant Sub-aqua 12) were used during the study.

2.3 Method

2.3.1 Preparation of standard stock solutions

Preparation of Stock Solution of Telmisartan

A 25mg sample telmisartan was accurately weighed, transferred in to 25ml volumetric flask and dissolved and diluted to 25 ml with methanol to obtain the concentration of 1000 µg ml^{-1}.

Preparation of Stock Solution of Hydrochlorothiazide

An accurately weighed quantity of 25mg of standard hydrochlorothiazide was transferred in to 25ml volumetric flask. Dissolved and diluted to 25 ml with methanol to obtain the concentration of 1000 µg ml^{-1}.

2.3.2Preparation of mixed Standard solution

A binary mixture standard solution was prepared by pipetting out 4ml of telmisartan and 1.25 ml of hydrochlorothiazide from stock solution (1000 µg ml^{-1}), transferred to 10ml volumetric flask and the volume was made up to 10ml by using methanol. This solution contained 400 µg $ml^{-1}$of telmisartan and 125 µg $ml^{-1}$of hydrochlorothiazide.

2.3.3 Preparation of Calibration curve Standard Solutions

A series of six different concentrations of calibration curve binary mixture standard solutions of telmisartan and hydrochlorothiazide were prepared from the stock solutions which are in the range of 400 to 2800ng $spot^{-1}$for telmisartan, 125 to 875ng $spot^{-1}$ for hydrochlorothiazide.

2.3.4 Preparation of Sample Solution

20 tablets of the commercial sample [Newtel-H, 40 mg, 12.5 mg] were weighed accurately and crushed to fine powder. The tablets powder equivalent 40 mg of telmisartan and 12.5 mg of hydrochlorothiazide was weighed and transferred in to a 100 volumetric flask. To this flask, 50 ml of methanol

was added and solution was sonicated for 30 minutes. The solution was cooled to ambient temperature. Then the volume was made up to 100ml with methanol.The prepared solution was filtered through what man filter paper**.**

Determination of Detection Wavelength

For the development of the method, UV spectrum of telmisartan, hydrochlorothiazide were obtained separately by scanning theanalytes at concentration levels of 10 µg ml^{-1} in the range of 400 nm to 200 nm against blank as methanol. After thorough examination of the spectra, wavelength of 280 nm was selected as symmetric peaks were found.

2.3.5 Densitometry Conditions

The binary mixture standard solution was spotted in the form of bands of width 8mm with a CAMAG 100µl sample syringe on precoated silica gel aluminum plate 60 F_{254} (10cm × 10cm with 200µm layer thickness) using a CAMAG Linomat 5 sample applicator, space between two bands were 8mm. The slit dimension was kept at 6mm×0.3mm and 20mms^{-1} scanning speed was employed. The mobile phase composed of toluene:ethyl acetate:acetic acid in the ratio of 1:5:1(v/v/v). Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase. The optimum chamber saturation time for mobile phase was 30minutes at room temperature (25±2 ^{0}C). The length of chromatogram run was 80mm. Subsequent to the development; TLC plates were dried with help of an air-dryer. Densitometry scanning was performed on CAMAG TLC scanner III in the absorbance mode at 280nm. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum in the range of 190-400nm.

2.3.6 Procedure for forced degradation studies of standard drugs

Forced degradation studies of standard drugs and tablet formulation were carried out under thermolytic, photolytic, acid, base hydrolytic, water hydrolysis and oxidative stress conditions.For acid, alkali, hydrolytic and oxidative degradation, pipetting out 4 ml of telmisartan and 1.25 ml of hydrochlorothiazide from standard stock solutions and transferring these solutions in to a 10ml volumetric flask, in to it 3ml of 0.1N HCl (for acid stress condition), 3ml of 0.1N NaOH (for alkali stress condition), 3ml of distilled water (for hydrolytic stress condition) and for stress under oxidative condition 1 ml of 3% v/v H_2O_2 solutions were added and diluted up to 10ml with methanol. The solutions were kept at 80 ^{0}C for 6 hours (for acid, alkali and hydrolytic stress condition), 60 ^{0}C (for oxidative stress condition) for 1 hour in a heating water bath. For degradation under dry heat the powdered telmisartan, hydrochlorothiazide standard drugs were taken in three different petri dishes (about 100mg) and heated in an oven at 80 ^{0}C for 24 hours and for photo degradation studies the powdered standard drugs were taken in three different petri dishes (about 100mg) exposed to UV radiation at 254nm and 326nm for 24 hours in a CAMAG UV chamber. The methanolic stock solutions of dry heat and the UV exposed drugs were prepared. From the stock solutions pipetting out 4 ml of telmisartan standard solution, 1.25 ml of hydrochlorothiazide and transferred to a 10ml volumetric flask, diluting up to 10ml with methanol. Appropriate volume of resultant solution for each stress condition of telmisartan, hydrochlorothiazide were applied on six different TLC plates. The same concentrations of standard telmisartan, hydrochlorothiazide solutions were also applied on TLC plates and densitograms were developed which are shown Table-1. The amounts of drug degraded were calculated from calibration curve.

Table-1: Forced Degradation Conditions of Telmisartan and hydrochlorothiazide

Sl. No	Conditions (Stress Induced)	Concentration TEL (ng $spot^{-1}$)	Concentration HCZ(ng $spot^{-1}$)	Temp. (^{0}C)	Time (Hours)
1.	Acid (0.1 N HCl)	400,1200, 2000, 2800	125,375,625,875	80	6
2.	Base (0.1N NaOH)	400,1200, 2000, 2800	125,375,625, 875	80	6
3.	Water	1200 ,2800	375, 875	80	6
4.	Hydrogen Peroxide (3% v/v)	1200 ,2800	375, 875	60	1
5.	Dry Heat	400, 2000, 2800	125,625 and 875	80	24
6.	UV light (254nm and 326nm	1200 ,2800	375, 875	---	24

III. RESULTS

3.1 Optimization of Mobile Phase

Mixed standard solution of telmisartan and hydrochlorothiazide was applied on Silica Gel 60 F_{254}TLC plates. Various pure solvent with varying polarity and their mixtures were tried for optimum movement of the drugs with sharp symmetrical peak. After trying several permutations and combinations, the solvent system containingtoluene: ethyl acetate: acetic acid in the ratio of 1:5:1 (v/v/v) with the chamber saturation time of 30 minutes at room temperaturewas found to be most satisfactory as it gave dense, compact and well separated spots of the drugs from the mixture. Developed mobile phase resulted in resolution for two drugs with R_f values of 0.22 ± 0.03 and 0.57 ± 0.03 for telmisartan and hydrochlorothiazide respectively which is shown in Fig.3.1 and Fig.3.2.In these selected optimized mobile phase conditions the drugs were separated adequately which also separates the degradants from telmisartan and hydrochlorothiazide. Densitometry analysis was carried out at 280 nm.

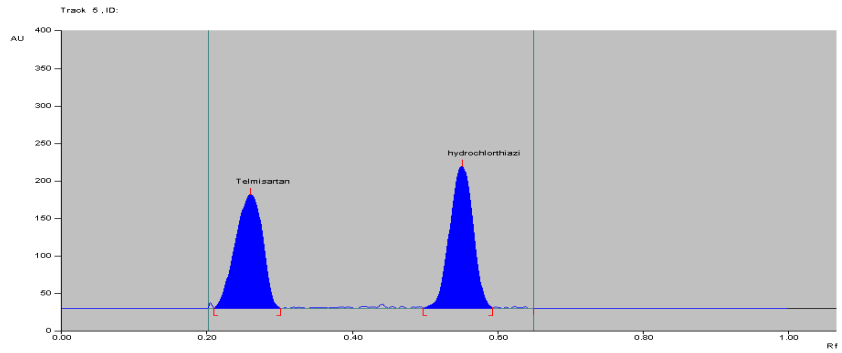

Fig.3.1 Densitotogram of Standard telmisartan and hydrochlorothiazide

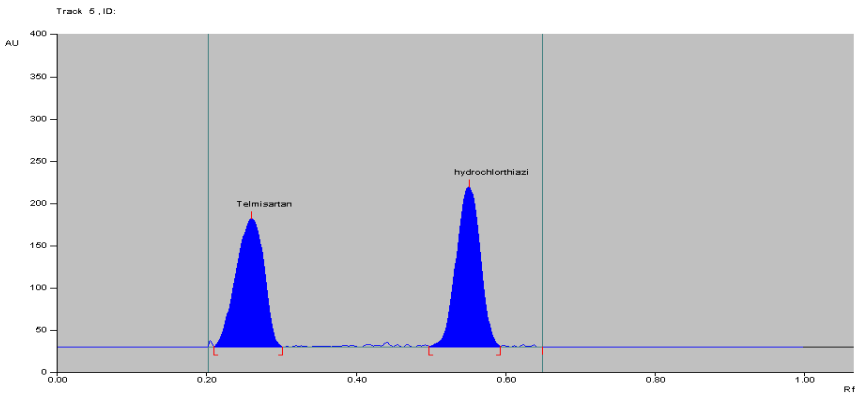

Fig.3.2 Densitotogram of telmisartan and hydrochlorothiazide in Newtel-H tablet

3.2. Degradation Observed

The densitograms of the forced degraded samples of standard solution showed well separated bands of pure telmisartan and hydrochlorothiazide as well as some additional bands at different R_f values when degraded with acid, alkali, neutral, hydrogen peroxide, dry heat and UV light conditions, The bands of the degraded products were well resolved from the drug bands. The degradants identification was based on the comparison of the densitogram of "stressed samples "with that of the "standard solution". Blessy and Ruchi[11], in their article on stress testing suggested a target degradation of 5-20% has been accepted as reasonable for validation of chromatography assay. Similarly Singh and Bakshi[10], in their article on stress testing suggested a target degradation of 20-80% for establishing stability-indicating studies, and also intermediate degradation products should not interfere with any stage of drug analysis. In this study, conditions used for forced degradation were attenuated to achieve degradation in the range of 5-80% for telmisartan and hydrochlorothiazide drug substances. The numbers of degradation products with their retention time and percentage degradation of telmisartan and hydrochlorothiazide are listed in Table-2 and shown in Fig.4.1 to Fig.4.6.

Table-2: Summary of Results for Forced Degradation Studies of Telmisartan and Hydrochlorothiazide

Conditions (Stress induced)	Telmisartan		Hydrochlorothiazide	
	% Telmisartan degradation	R_f Value of Degradants	% Hydrochlorothiazide Degradation	R_f Value of Degradants
Acid	21.2 %	----	7.3 %	0.72
Base	2.8 %	0.31	29.9 %	0.73
Hydrolytic	20.9 %	0.03	28.8 %	0.80
Hydrogen Peroxide (3% v/v)	12.9 %	------	6.9%	0.80
Dry Heat	9.5%	----	3.4 %	------
UV light (254 nm and 326nm	1.9%	----	1.5%	----

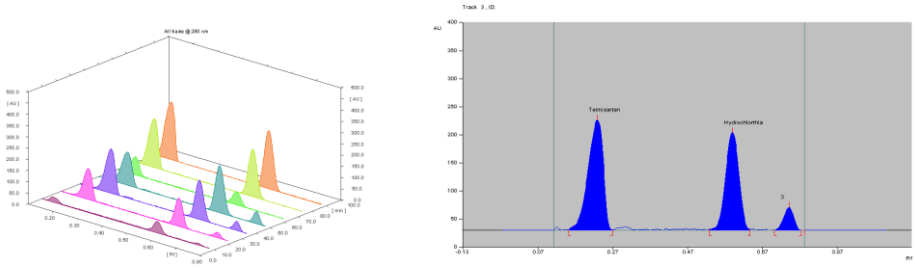

Fig.4.1: 3D HPTLC Densitogram of 0.1N HCl Degradation

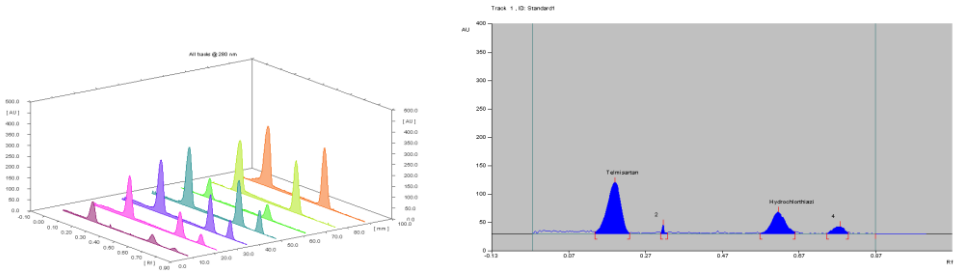

Fig.4.2:3D HPTLC Densitogram of 0.1N NaOH Degradation

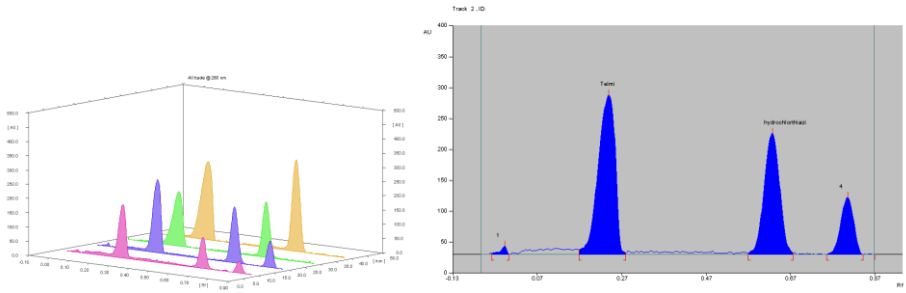

Fig.4.3: 3D HPTLC Densitogram of Neutral Hydrolytic Degradation

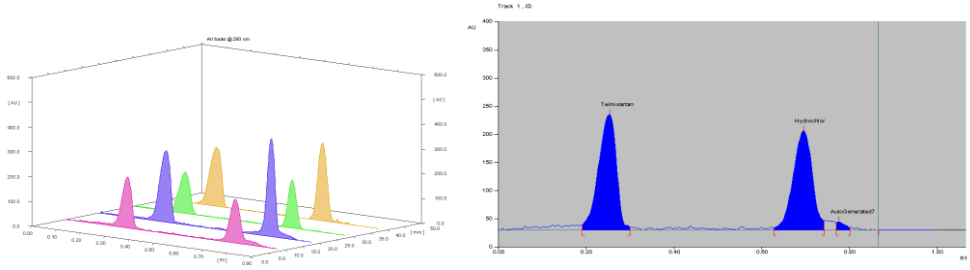

Fig.4.4:3D HPTLC Densitogram of Oxidative Degradation

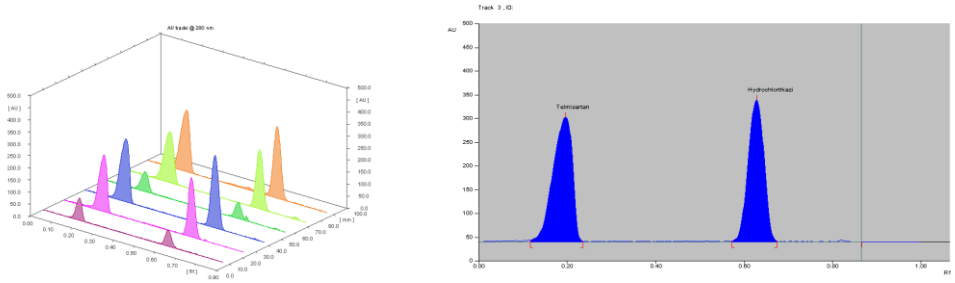

Fig.4.5: 3D HPTLC Densitogram of Dry Heat Degradation

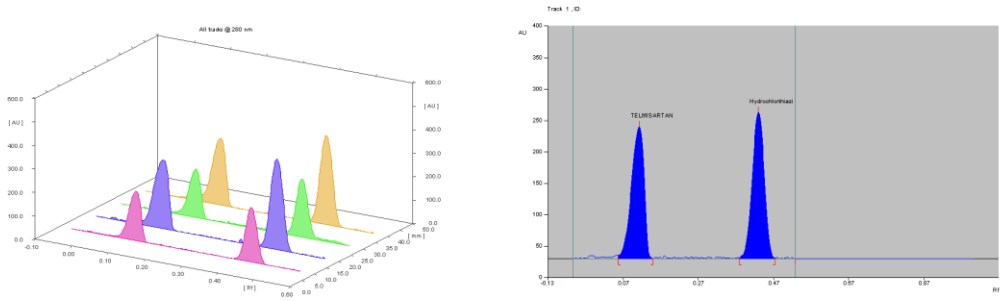

Fig.4.6:3D HPTLC Densitogram of Photolytic Degradation

3.3. Method Validation

The validation of the current method has been performed according to the ICH guideline [31]. The following validation parameters were considered for the newly developed method such as linearity, precision/reproducibility, accuracy, limit of detection and limit of quantification specificity, robustness.

3.3.1 Linearity

The linearity of the proposed HPTLC method for determination of telmisartan and hydrochlorothiazide was evaluated by analyzing a series of different concentrations of standard drug soutions. In this study, different volumes of binary mixture standard solutions 1µl, 2µl,3 µl,4 µl,5 µl,6 µl and 7µlwere spotted in the TLC plate to obtain the concentrations of 400,800,1200,1600,2000,2400 and 2800 ngband^{-1} for telmisartan and 125,250,375,500,625,750,875 ngband^{-1} for hydrochlorothiazide respectively. Each concentration was repeated three times and obtained information on the variation in peak area response**.** From the densitotograms, linearity plots were drawn by taking concentration on X-axis and area of peaks on Y-axis. The regression equations obtained for telmisartan and hydrochlorothiazide were 3.388x+2154.4 and13.414x+643.3 which is shown in Fig.5.1 to Fig.5.3. The linear regression coefficient values for telmisartan and hydrochlorothiazide were found to be 0.9985 and 0.9996 respectively indicating a high degree of linearity which are shown in Table-3.

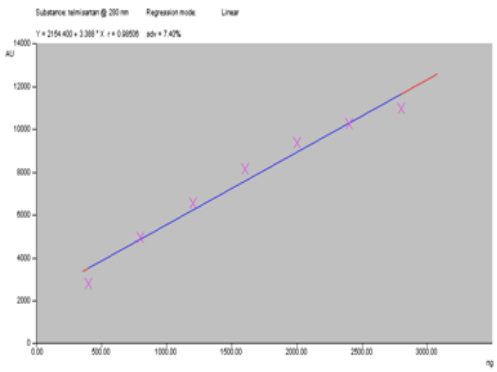

Fig.5.1: Linearity of Telmisartan

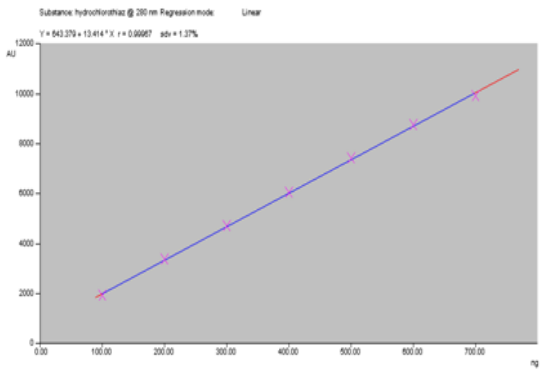

Fig.5.2 Linearity of Hydrochlorothiazide

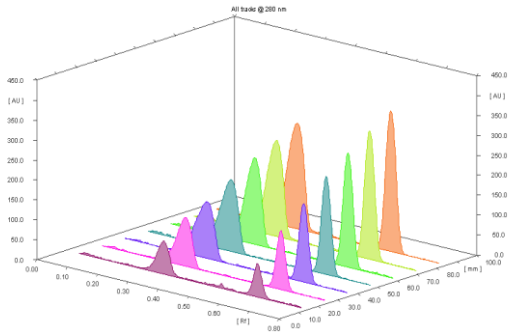

Fig.5.3:3D Densitogram of Linearity for Telmisartan and Hydrochlorothiazide

Table-3: Linearity data of Telmisartan and Hydrochlorothiazide

Sl. No	Telmisartan		Hydrochlorothiazide	
	Concentration (ng band^{-1})	Peak area	Concentration (ng band^{-1})	Peak area
1	400	3490.9	125	1990.3
2	800	4848.1	250	3365.2
3	1200	6094.1	375	4630.1
4	1600	7443.3	500	6089.2
5	2000	8756.5	625	7313.1
6	2400	10074.7	750	8553.2
7	2800	11423.2	875	9787.3
Regression coefficient		0.9985		0.9996
Slop		3.388		13.414
Intercept		2154.4		643.3

3.3.2 Precision

Reproducibility of the method was demonstrated by intraday and inter-day precision measurements of peak area for each title ingredient. The intraday (within-day in three replicates) and inter-day precision (for 3 days) was carried out using three different concentrations 400, 2000, 2800 ng band^{-1} of telmisartan and 125, 625, 875 ng band^{-1} of hydrochlorothiazide for minimum three times. The result expressed in terms of percent relative standard deviation (% R.S.D). The obtained results within and between days were in acceptable range (less than 2%) indicating good precision of the proposed method and are shown in Fig.6 and Table-4.

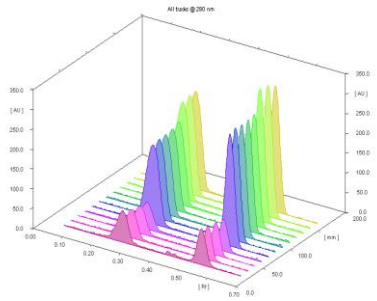

Fig.6:3D Densitogram of Precision Study

Table-4: Precision study data of Telmisartan and Hydrochlorothiazide

Precision study	Concentration (ng band^{-1}) Telmisartan	Concentration (ng band^{-1}) Hydrochlorothiazide	Peak area Telmisartan	Peak area Hydrochlorothiazide	% R.S.D Telmisartan	%R.S.D Hydrochlorothiazide
Intra day Precision	400	125	3398.88	2014.22	1.42	1.62
	2000	625	8242.02	7630.62	1.49	1.26
	2800	875	10178.68	9765.86	1.76	0.27
Inter day precision	400	125	3292.88	2261.22	1.14	1.02
	2000	625	8222.02	7461.62	0.94	1.26
	2800	875	10138.68	9494.06	1.53	0.28

3.3.3 Accuracy

Accuracy of the method was determined by performing recovery study in triplicate by standard addition method at three different levels. Sample solution containing 800 ng band^{-1} of telmisartan, 250 ng band^{-1} of hydrochlorothiazide was prepared from tablet formulation and spiked with pure telmisartan and hydrochlorothiazide standard solution, amount equivalent to 50,100, and 150 % in the original solution and these solutions were analyzed. The method showed the average % recovery for pure telmisartan and hydrochlorothiazide were 98.77 % and 99.73 % respectively and shown in Table-5 and Table-6. The results indicate that the developed method is accurate.

Table-5: Results of Recovery Study of Telmisartan

Level of Recovery (%)	Conc. taken (ng band^{-1})	Amount added (ng band^{-1})	% Recovery	Mean % Recovery
50	800	400	98.16	98.72
50	800	400	99.31	
50	800	400	98.70	
100	800	800	98.59	98.46
100	800	800	98.77	
100	800	800	98.02	
150	800	1200	98.04	98.54
150	800	1200	98.74	
150	800	1200	98.86	
Across of all level % recovery				98.57

Table-6: Results of Recovery Study of Hydrochlorothiazide

Level of Recovery(%)	Conc.taken (ng band^{-1})	Amount Added (ng band^{-1})	% Recovery	Mean % Recovery
50	250	125	102.87	100.72
50	250	125	100.19	
50	250	125	99.10	
100	250	250	98.60	98.58
100	250	250	97.81	
100	250	250	99.35	
150	250	375	99.08	98.56
150	250	375	98.90	
150	250	375	97.72	
Across of all level % recovery				99.73

3.3.4 Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantitation (LOQ) for telmisartan and hydrochlorothiazide were calculated from the linearity data and slope of the calibration curve. The LOD of a compound is defined as the lowest concentration that can be detected and LOQ is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy.Limit of detection and limit of quantification was calculated using following equation as per ICH guidelines. Detection limit and quantification limit were calculated by (3.3×S.D)/band was calculated by (10×S.D)*/b.* The results of LOD and LOQ for telmisartan and hydrochlorothiazide were4.09,12.41 and0.71,2.17ng spot^{-1} respectively.

Where "b" corresponds to the slope of the regression equation.

" S.D"corresponds to the standard deviation y intercept of calibration curve

3.3.5 Specificity

The specificity of the method was ascertained by analyzing standard drugs and tablet formulation. The bands of telmisartan andhydrochlorothiazide in the tablet formulation were confirmed by comparing the R_f values and overlaying peak purity spectrawith that of the standard drugs. The peak purity of the of telmisartan and hydrochlorothiazide in tablet formulationwas evaluated by comparing the peak spectra acquired at three different levels, that is, peak start (S), peak apex (M) and peak end (E) positions of the band which are shown in Fig.7.1 and Fig.7.2. The densitograms of the tablet formulation showed R_f values of 0.22 ± 0.03 and 0.57 ± 0.03 for telmisartan and hydrochlorothiazide respectively which were same as standard drugs.The peak purity of

telmisartan and hydrochlorothiazide in tablet formulation showed good correlation, which are r(S,M) =0.9996 and r(M,E) = 0.9992, which indicates specificity of the method in the presence of excipients.

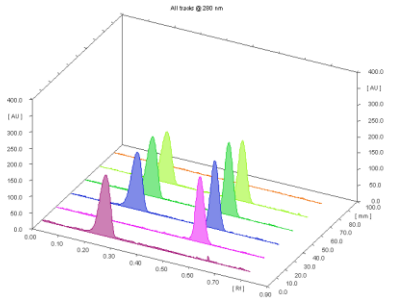

Fig.7.1:3D Densitogram of Specificity

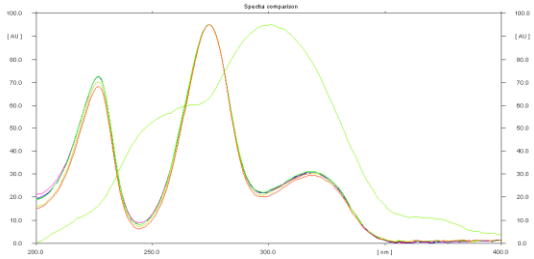

Fig.7.2: Overlying Spectra of Telmisartan and Hydrochlorothiazide in tablet formulation

3.3.6 Robustness

The robustness of the method was evaluated by slightly varying the optimized mobile phase composition, changing in saturation time of binary mixed standard solution at concentration level of 5 µl of mixed standard solution of telmisartan and hydrochlorothiazide(2000 ng band^{-1} and 625 ng band^{-1}) for six times and the % R.S.D of the peak areas were calculated. The low values of % R.S.D (less than 2%) obtained after introducing small deliberate changes in the developed HPTLC method indicated the robustness of the method which are shown in Table-7.

Table-7: Results of Robustness Study for Telmisartan and Hydrochlorothiazide

Robustness		**Saturation Time (in Minutes)**			**Mobile Phase (toluene: ethyl acetate : acetic acid)**	
		20	30	40	1:6:1 (v/v/v)	1:4:1(v/v/v)
Telmisartan	**R_f**	0.17	0.22	0.20	0.16	0.16
	Area	8245.6	8270.2	8198.6	7436.5	7645.2
	%R.S.D	1.89	1.90	1.88	1.71	1.75
Hydrochloro thiazide	**R_f**	0.52	0.57	0.55	0.41	0.42
	Area	7586.8	7667.3	7616.4	6945.3	7146.5
	%R.S.D	0.959	0.969	0.962	0.878	0.903

3.3.6 Analysis of tablet formulation

The developed method is applied for the analysis of marketed tablet formulation by applying1 µl sample solution on TLC plate.The standard and tablet sample solutions peak areas were compared to calculate the content of telmisartan and hydrochlorothiazide which is shown in Table-8.

Table-8: Assay Results of Telmisartan and Hydrochlorothiazide

Tablet formulation solution	Label claimed (mg)	Assay(% of label claim) Mean ± SD, n=3)
Telmisartan	40	99.68± 0.57
Hydrochlorothiazide	12.5	100.42± 0.83

IV. DISCUSSION

The bands of the degradants were well resolved from the telmisartan and hydrochlorothiazide standard drugs band.The densitogram of the acid degraded samples showed 01 additional band at R_f values of 0.72. The densitogram of the alkali degraded sample, showed 02 additional bands at R_f values of 0.31 and 0.73. The densitogram of the neutral degraded samples,showed 02 additional bands at R_f values of 0.03 and 0.80. The densitogram of the oxidative degraded samples, showed 01 additional band at R_f value of 0.80. No additional bands were developed in dry heat and photolytic degradation studies. Major degradations for telmisartan and hydrochlorothiazide were observed under acidic, alkali, neutral and oxidative degradation conditions. Very less degradation were observed under dry heat and photolytic degradation conditions.

The response for the both drugs were found to be linear in the concentration ranges of 400-2800 ngband^{-1} for telmisartan and 125-875 ngband^{-1} for hydrochlorothiazide with respect to the peak areas. The % R.S.D values for precision studies were found to be less than 2%, thus confirming about the method is precise. The accuracy of the method was determined and the mean recovery of telmisartan and hydrochlorothiazide were

found to be 98.57% and 99.73% respectively. The specificity of the method was ascertained by comparing the bandsR_f values as well as peak purity for both standard with that of tablet formulation.Further there is no interference of excipients were observed in densitogram of tablet formulationwhich indicates about the specificity of the method. The low values of % R.S.D were obtained after introducing small deliberate changes in the developed HPTLC method indicating about the robustness of the method.

V. CONCLUSIONS

The stability indicating HPTLC method was developed as per ICH guidelines for the simultaneous quantitative estimation of telmisartan and hydrochlorothiazide in bulk and in tablet formulation. The developed HPTLC method was found to be simple,rapid,accurate,specific and reproducible for the estimation of telmisartan andhydrochlorothiazide.This method was validated as per ICH guidelines. The developed method can be employed to isolate degradation products and for routine quality control analysis without interference of commonly encountered excipients of dosage form. The developed HPTLC method has several advantages like low cost, short duration of analysis, minimum mobile phase requirement, and no prior requirement of degassing and filtration of mobile phasein comparision with HPLC method.Forced degradation study showed that all degradation products were well separated from standard telmisartan and hydrochlorothiazide drugs under various stressed conditions, thus confirming the method as stability-indicating and that it can be employed for the determination of telmisartan and hydrochlorothiazidein stability studies.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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REFERENCES

[1]. M.J.Oneil, P.E .Heckelman,C.B.Koch, K..J. Roman,C.M. Kenny, M.R. Darecca, *TheMerck Index*: *an encyclopedia of chemicals, drugs and biological*(Merck Research Laboratories,Whitehouse Station,2006).

[2]. *Indianpharmacopoeia.*Indian Pharmacopoeia Commission, Ghaziabad, 2014)

[3]. M.Bergovac, D.Plavec, A. knezevic, V.Trkulja, Telmisartan in daily clinical practice: Factors affecting efficacy in treatment of primary arterial hypertension,*J Postgrad Med,55(1),*2009, 27-32.

[4]. W.Wienen, M.Entzeroth, J. Stangier, U.Busch, T.Ebner, J. Schmid, A review on telmisartan: a novel, long- acting angiotensin II-receptor antagonist,*Cardiovascular drug reviews,18(2),* 2000,127-154.

[5]. P .Patil, D.V.Nagarale, B.R .Chaudhari, Recent advances and applications of telmisartan,*Indo American J PharmaSci, 4(10),* 2017,3935-3945.

[6]. S.G.D.Vinuesa, M.Goicoechea, J.Kanter, P.Marta,V. Cachofeiro, V.Lahera,Insulin resistance, inflammatory biomarkers, and adipokines in patients with chronic kidney disease: Effects of Angiotensin II Blockade,*J Am Soc Nephrol,17,*2006, S206–S212.

[7]. M.Kubik,J. Chudek, M .Adamczak, A.Wiecek, Telmisartan improves cardio metabolic profile in obese patients with arterial hypertension,*Kidney Blood Press Res,35,*2012,281–289.

[8]. R.A.Benndorf, D.Appel, R.Maas, E.Schwedhelm, U.O.Wenzel,R.H.Boger,Telmisartan improves endothelial function in patients with essential hypertension,JCardiovascPharmacol,50(4)2007,367–371.

[9]. ICH, Q1A (R2)Stability testing of new drug substances and products, International conference on harmonization, Geneva 2003.

[10]. M.Bakshi, S.Singh. Development of validated stability-indicating assay methods-criticalreview. *J Pharm Biomed Anal, 28,* 2002,1011-1040.

[11]. M.Blessy, D.Ruchi Patel, N.PrajeshPrajapati, Y.K.Agrawal, Development of forced degradation and stability indicating studies of drugs-A review, *J Pharm Anal,4(3),* 2014, 159-165.

[12]. S.B.Wankhede, M.R.Tajne, K.R.Guptha, S.G.Wadodkar, RP-HPLC method for simultaneous estimation n of telmisartan & hydrochlorothiazide in tablet dosage form,*Ind J Pharma sci, 69(2),*2007,298-300.

[13]. N.J.Shah, B.N.Suhagia, R.R.Shah, P.B.Shah, Development and validation of HPTLC methodfor the simultaneous estimation of telmisartan & hydrochlorothiazide in tablet dosage form, *Ind J Pharma sci, 69(2),*2007,202-205.

[14]. D.Varma, A.L.Rao and S.C.Dinda, Stability indicating RP-HPLC method for simultaneousdetermination of telmisartan and hydrochlorothiazide in pharmaceutical dosage form, *Int JPharm chem Bio Sci,2(3),2012,*382- 391.

[15]. R.M.Reddy, A.P.Kumar, V.K.Reddy, S.W.Haque,Stability-indicating HPLC method forsimultaneous estimation of low level impurities of telmisartan and hydrochlorothiazide intablet dosage forms,*Int J Pharm Pharmceutical Sci,4(1),* 2012,497-504.
[16]. T.G.Swamya, K.N. Agarajub, A.L.Rao, RP-HPLC method for the simultaneous estimation oftelmisartan and hydrochlorothiazide in pharmaceutical dosage form,*Int J Drug DevRes,3(4),* 2011,362-368.
[17]. R.Nakum, A.K.Seth, D.B.Sen, A.K.Sen, Quantitative estimation of telmisartan andhydrochlorothiazide by RP-HPLC method,*Int J drug Dis Med Res,1(2),* 2012;45-48.
[18]. V.P.Rane, J.N.Sangshetti, D.B.Shinde, Simultaneous high-performance liquidchromatographic determination of telmisartan and hydrochlorothiazide in pharmaceuticalpreparation,*J Chrom Sci, 46(10),* 2008,887-891.
[19]. S.Bala, O.P.Mahatma, M.S.Azim, RP-HPLC method development and validation oftelmisartan and hydrochlorozide in combination dosage form (tablets). *Am J Pharm HealthRes,1(7),* 2013,60-77.
[20]. T.Vasuki, K. R. N.Dhanalakshmi, D Jotheiswari, Analytical method development andvalidation of telmisartan and hydrochlorothiazide in dissolution by RP-HPLC,*Int J BioPharm Res, 4(3),* 2013, 200-211.
[21]. J.Kavitha, J.S.K.Nagarajan, S.Muralidharan and B.Suresh, Development and validation ofRP-HPLC method for simultaneous estimation of telmisartan and hydrochlorothiazide intablets: its application to routine quality control analysis,*Int J Pharmacy Pharm Sci,3(4),* 2011,113-115.
[22]. K.R.Patil, D.B.Shinde, Stability-indicating LC method for the simultaneous determination oftelmisartan and hydrochlorothiazide in dosage form,*J Chil Chem Soc,57(1),* 2012,1017-1021.
[23]. S.Mukhopadhyay, K.Kadam, L.Sawant, D.Nachane, N.Pandita, Simultaneous determinationof related substances of telmisartan and hydrochlorothiazide in tablet dosageform by usingreversed phase high performance liquid chromatographic method,*Pharm Bioallied Sci,3(3),*2011,375-383.
[24]. T.G.Swamy, K .A.Nagaraju, AL Rao, RP-HPLC method for the simultaneous estimation oftelmisartan and hydrochlorothiazide in pharmaceutical dosage form. *Int J Drug Dev &Res 3(4),* 2011,362-368.
[25]. N.M Bhatia,H.V.Shinde, M.S.Bhatia, P.B. Choudhari, K.B.Ingale, Development and validation of spectrophotometric and ion pair chromatographic techniques for estimation of telmisartan and hydrochlorothiazide, Ars Pharmaceutica,51(3),2010,145-154.
[26]. R.Gangola, S.Kaushik, P.Sharma, Spectrophotometric simultaneous determination ofhydrochlorothiazide and telmisartan in combined dosage form,*J Applied Pharm Sci,1(1),*2011, 46-49.
[27]. K.Ilango, P.S.Shiji kumar, Simultaneous estimation of telmisartan and hydrochlorothiazidein pharmaceutical dosage form,*Asian J Pharm Health Sci,1(1),*2011, 12-15.
[28]. T.Yan, H.Li, L.Deng, Y.Guo, W.Yu, D.Zhang . Liquid chromatographic-tandem massspectrometric method for the simultaneous quantitation of telmisartan andhydrochlorothiazide in human plasma,*J Pharm Biomed Analysis, 48(4),*2008,1225-1229.
[29]. R.R.Ambadas, R.S.Poonam, Development and validation of bioanalytical method for determination of telmisartan and hydrochlorothiazide using HPTLC in human plasma,*American J Anal Chem, 3,*2012,774-778.
[30]. S. Rahul, N.Vijay, Shweta,Telmisartan and hydrochlorothiazide simultaneous quantitative estimation in bulk and marketed tablet formulation study by Hptlc-by stability indicating method,*DerPharma Chemica,10(8),*2018, 87-95.
[31]. *Validation of Analytical Procedures Text and Methodology*(ICH Harmonized TripartieGuidline Q2 (R1), November 2005)

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