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Research Article

STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF TELMISARTAN AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL DOSAGE FORM

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A simple, fast and precise reverse phase, isocratic HPLC method was developed for the separation and quantification of telmisartan and hydrochlorothiazide in pharmaceutical dosage form. The quantification was carried out using ProntoSIL C18-EPS 4.6X150mm, 3µm enhanced polar selectivity column and mobile phase comprised of potassium dihydrogen phosphate buffer pH adjusted to 3.2 ± 0.5 with orthophosphoric acid and acetonitrile in proportion of ratio 55:45 and degassed under ultrasonication. The flow rate was 0.8mL/min and the effluent was monitored at 271nm. The retention time of telmisartan and hydrochlorothiazide were 5.01±0.5 and 2.94±0.5 respectively. The method was validated in terms of linearity, precision, accuracy, specificity, limit of detection and limit of quantitation. Linearity of telmisartan and hydrochlorothiazide were in the range of 15.01 to 75.05µg/mL and 5.02 to 25.10µg/mL respectively. The percentage recoveries of both the drugs were 100.8% and 99.5% for telmisartan and hydrochlorothiazide respectively from the tablet formulation. The proposed method is suitable for simultaneous determination of telmisartan and hydrochlorothiazide in pharmaceutical dosage form.

Key words: Telmisartan, Hydrochlorothiazide, RP-HPLC, Validation.

INTRODUCTION

Telmisartan (TEL) is chemically described as 4'-[(1,4'-dimethyl-2'-propyl [2,6'-bi-1*H*-benzimidazol]-1'-yl)methyl]-[1,1'-biphenyl]-2-carboxylic acid (Fig. 1). Telmisartan is a non-peptide angiotensin II receptor antagonist, indicated for the treatment of essential hypertension. It may be used alone or in combination with other antihypertensive agents like hydrochlorothiazide. The combination of telmisartan and hydrochlorothiazide is useful in the treatment of mild to moderate hypertension. It selectively inhibits angiotensin II AT_1 receptor subtype without affecting other systems involved in cardiovascular regulation. Hydrochlorothiazide (HCT) is chemically described as 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Fig. 2). Hydrochlorothiazide is a widely used thiazide diuretic which blocks the reabsorption of sodium and chloride ions, and it thereby increases the quantity of sodium traversing the distal tubule and the volume of water excreted. The combination of TEL and HCT is available as tablet dosage form.

Several analytical methods have been reported for the determination of TEL and

HCT alone in biological fluids and in pharmaceutical formulations. Literature survey revealed few analytical methods have been reported in combination of TEL and HCT like, spectrophotometry[1-3], spectrofluorimetry[4], capillary electrophoresis[5], HPTLC[6], HPLC[7-13], and LC-MS[14] methods. To the best of our knowledge there is no stability indicating HPLC method reported for the simultaneous estimation of two components TEL and HCT in a single dosage form. Hence an attempt has been made to develop a simple, precise, reliable, sensitive and selective stability indicating HPLC method for the analysis of TEL and HCT in bulk samples and in combined tablet dosage form. The proposed method was validated according to ICH guidelines[15-16].

EXPERIMENTAL

Materials, reagents and chemicals

Telmisartan and hydrochlorothiazide were obtained as gift samples from Aurobindo Pharma Ltd, Hyderabad. Telmisartan and Hydrochlorothiazide combined dosage form tablets were purchased from local market. HPLC grade acetonitrile, methanol and analytical grade potassium dihydrogen phosphate, orthophosphoric acid was obtained from Qualigens Fine Chemicals Ltd, Mumbai. Hydrochloric acid, sodium hydroxide, hydrogen peroxide of analytical grade was obtained from Merck Chemicals Ltd, Mumbai. Milli-Q water was used throughout the experiment dispensed through 0.22µ filter of the Milli-Q water purification system from Merck Millipore.

Chromatographic conditions

Waters Alliance HPLC, integrated with Auto Sampler and UV detector was used. The output of signal was monitored and integrated using waters Empower 2 software. ProntoSIL C18-EPS 4.6X150mm, 3µm particle size enhanced polar selectivity column was used as stationery phase. Mobile phase comprised of potassium dihydrogen phosphate buffer (3.5grams of KH_2PO_4 transferred into a 1000mL volumetric flask, add 500mL Milli-Q water, dissolve, sonicate for five minutes, make volume up to the mark with Milli-Q water and adjusted the pH to 3.2±0.5 with orthophosphoric acid) and acetonitrile in proportion of ratio 55:45. The mobile phase was mixed, filtered through 0.45µ membrane filter and degassed under ultrasonication. The mobile phase was used as diluent. Injection volume was 20µL and flow rate was 0.8mL/min and run time was 6.5min.The column was maintained at ambient temperature and the eluent was monitored at 271nm.

Preparation of standard solution

Accurately weigh and transfer 30mg of telmisartan and 10mg of hydrochlorothiazide working standard into a 10mL clean dry volumetric flask add about 5mL of methanol and sonicate to dissolve it completely, cool the solution to room temperature and dilute to volume with methanol and used as standard stock solution. Pipette 1mL of standard stock solution into a 10mL volumetric flask and dilute to volume with methanol and used as working standard solution. Further pipette 1mL of the above working standard solution into a 10mL volumetric flask and dilute to volume with diluent of mobile phase. Standard stock solution was diluted in mobile phase to contain a mixture of telmisartan and hydrochlorothiazide in over the linearity range from 15.01 to 75.05µg/mL and 5.02 to 25.10µg/mL respectively.

Preparation of sample solution

Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 30mg of telmisartan and 10mg of hydrochlorothiazide into a 10mL clean dry volumetric flask add about 5mL of methanol and sonicate to dissolve it completely, cool the solution to room temperature and dilute to volume with methanol. Filter about 5mL of the above sample solution through 0.45µ membrane filter. Pipette 1mL of the above filtered sample solution into a 10mL volumetric flask and dilute to volume with methanol. Finally pipette 1mL of the above sample solution into a 10mL volumetric flask and dilute to volume with diluent of mobile phase. 20µL of the sample solution was injected in to the HPLC system.

RESULTS AND DISCUSSION

Method development

To develop a simple and robust method for the simultaneous determination of TEL and HCT in combined tablet dosage form using HPLC. Solubility of standard drug was checked and methanol was chosen as the solvent. Different mobile phase compositions were pumped in binary mode to achieve the resolution of drug peaks, initial experimental conditions were column with C18 stationery phase, potassium dihydrogen phosphate as buffer, methanol/acetonitrile as organic solvent at a flow rate of 1.0mL/min was chosen and respective injections shown considerable resolution of drug peaks with a run time of 10min. For better resolution the mobile phase composition was altered slightly between buffer and organic solvent acetonitrile, finally a premixed composition (55:45, v/v) of buffer and acetonitrile, pH of potassium dihydrogen phosphate buffer adjusted to 3.2±0.5 with orthophosphoric acid was chosen as mobile phase, and the ProntoSIL C18-EPS stationery phase with enhanced polar selectivity of particle size 3µm, 4.6X150mm was used and the runtime of the method was got minimized to 7.5min with better resolution, better peak shape was found with mobile phase as diluent in samples injected into chromatographic system. Flow rate of 1.0mL/min selected initially was changed to 0.8mL/min and column was maintained at ambient temperature to fine tune the method and the runtime was ended at 6.5min finally. Injections with UV detection at a wavelength of 271nm for both drug peaks in the trail results were observed to be specific, precise and fast. Further the proposed method validation was initiated. System suitability test was performed on each day prior to initiation of the validation run. The system suitability results of the method are presented in Table 1.

Validation of the proposed method

Specificity

A study conducted to establish specificity of the proposed method involved injecting diluent and placebo using the chromatographic conditions defined for the proposed method. The blank chromatogram showed no interference peaks at the retention time of telmisartan and hydrochlorothiazide respectively. This indicates that diluent solution used in sample preparation do not interfere in the estimation of telmisartan and hydrochlorothiazide. Similarly the placebo sample chromatogram showed no interference peaks at the retention time of telmisartan and hydrochlorothiazide respectively. Additional peaks were observed in the channel may be due to excipients present in the formulations. These peaks however did not interfere with the standard peak indicating that the placebo used in sample preparation do not interfere in estimation of telmisartan and hydrochlorothiazide in combination tablet, which demonstrates the specificity of the proposed method. The chromatogram of the blank and placebo using the proposed method for telmisartan and hydrochlorothiazide is shown in Fig. 3 and Fig. 4. The typical chromatogram of the sample using the proposed method for telmisartan and hydrochlorothiazide is shown in Fig. 5.

Linearity

Detector response for the proposed method determined to be linear over the range of five concentration levels prepared and injected, 15.01 to 75.05µg/mL for telmisartan and 5.02 to 25.10µg/mL for hydrochlorothiazide. The calibration curve was plotted as concentration of the respective drug versus the obtained peak area at each concentration level. The linearity of the method was evaluated by linear regression analysis. The linear regression equation of proposed method representing slope and intercept for telmisartan and hydrochlorothiazide were given in Fig. 6 and Fig. 7. The statistical data calculated for telmisartan and hydrochlorothiazide found to be accurate and was given in Table 2.

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on blend collected from

twenty tablets of telmisartan and hydrochlorothiazide and analyzed as per the proposed method. The percentage recoveries found are in the range of 99.3 to 102.4 and 98.5 to 101.5 for telmisartan and hydrochlorothiazide respectively. From the data obtained, the proposed method found to be accurate. The results are summarized in Table 3.

Precision

In the study of the instrumental system precision where, a RSD of 0.3% and 0.1% was obtained for the standard area obtained corresponding to the first day for TEL and HCT respectively, being 0.2% and 0.1% for the second day, respectively for TEL and HCT. The method precision study for six sample preparations in marketed samples showed a RSD of 0.5% and 0.7%, the assay range of 99.6-101.1 and 99.2-101.2, respectively for TEL and HCT. For the intermediate precision, a study carried out by the same analyst working on different day. The results calculated as inter-day RSD (For Standard) corresponded to 0.2% and 0.1% for TEL and HCT respectively. The same study was carried out for different analysts (n=6 number of samples per analyst) obtaining a RSD of 0.2% and 0.2% (Intermediate Precision) and the assay range of 100.5-101.1 and 100.6-101.1, respectively for TEL and HCT. The overall %RSD for n=12 is 0.4 for TEL and 0.5 for HCT. Both results together with the individual results are showing that the proposed analytical technique has a good intermediate precision. Results are summarized in Table 4. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

Quantification limit

The LOD is the lowest concentration of the analyte that can be detected and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy. The limit of detection (LOD) and limit of quantification (LOQ) for telmisartan were 2.45µg/mL and 7.43µg/mL respectively and for hydrochlorothiazide were 0.93µg/mL and 2.81µg/mL respectively by the proposed method.

Stability studies

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24hr at room temperature. The results show that for both solutions, the retention time and peak area of telmisartan and hydrochlorothiazide remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24hr, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of proposed method. The results of the degradation studies are presented in Table 5.

Control sample

Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 300mg of telmisartan and 100mg of hydrochlorothiazide into a 100mL clean dry volumetric flask add about 70mL of methanol and sonicate to dissolve it completely, cool the solution to room temperature and dilute to volume with methanol. Filter about 5mL of the above sample solution through 0.45µ membrane filter. Pipette 1mL of the above filtered sample solution into a 10mL volumetric flask and dilute to volume with methanol. Finally pipette 1mL of the above sample solution into a 10mL volumetric flask and dilute to volume with diluent of mobile phase.

Acid degradation sample

Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 300mg of telmisartan and 100mg of hydrochlorothiazide into a 100mL clean dry volumetric flask add about 70mL of methanol and sonicate to dissolve it for about 30minutes with intermittent shaking at controlled temperature. Then add 10mL

of 5N acid (Hydrochloric acid), refluxed for 60minutes at 60°C, then cooled to room temperature, neutralize with 5N base (Sodium hydroxide) and dilute to volume with methanol. Filter about 5mL of the above sample solution through 0.45µ membrane filter. Pipette 1mL of the above filtered sample solution into a 10mL volumetric flask and dilute to volume with methanol. Finally pipette 1mL of the above sample solution into a 10mL volumetric flask and dilute to volume with diluent of mobile phase.

Base degradation sample

Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 300mg of telmisartan and 100mg of hydrochlorothiazide into a 100mL clean dry volumetric flask, add about 70mL of methanol and sonicate to dissolve it for about 30minutes with intermittent shaking at controlled temperature. Then add 10mL of 5N base (Sodium hydroxide), refluxed for 60 minutes at 60°C, then cooled to room temperature, neutralize with 5N acid (Hydrochloric acid) and dilute to volume with methanol. Filter about 5mL of the above sample solution through 0.45µ membrane filter. Pipette 1mL of the above filtered sample solution into a 10mL volumetric flask and dilute to volume with methanol. Finally pipette 1mL of the above sample solution into a 10mL volumetric flask and dilute to volume with diluent of mobile phase.

Peroxide degradation sample

Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 300mg of telmisartan and 100mg of hydrochlorothiazide into a 100mL clean dry volumetric flask add about 70mL of methanol and sonicate to dissolve it for about 30minutes with intermittent shaking at controlled temperature. Then add 2mL of 30% peroxide, refluxed for 60minutes at 60°C, then cooled to room temperature and dilute to volume with methanol. Filter about 5mL of the above sample solution through 0.45µ membrane filter. Pipette 1mL of the above filtered sample solution into a 10mL volumetric flask and dilute to volume with methanol. Finally pipette 1mL of the above sample solution into a 10mL volumetric flask and dilute to volume with diluent of mobile phase.

Thermal degradation sample

Weigh and finely powder not fewer than 20 tablets, this powder is exposed to heat at 105°C for about 2 days. Accurately weigh and transfer a quantity of powder sample equivalent to 300mg of telmisartan and 100mg of hydrochlorothiazide into a 100mL clean dry volumetric flask add about 70mL of methanol and sonicate to dissolve it completely, cool the solution to room temperature and dilute to volume with methanol. Filter about 5mL of the above sample solution through 0.45µ membrane filter. Pipette 1mL of the above filtered sample solution into a 10mL volumetric flask and dilute to volume with methanol. Finally pipette 1mL of the above sample solution into a 10mL volumetric flask and dilute to volume with diluent of mobile phase.

Similarly UV-light exposure, sunlight exposure and water hydrolysis stress samples are prepared and checked for their purity by proposed method. From the above data of degradation profile it can be conclude that there is no interference found for telmisartan and hydrochlorothiazide peak.

CONCLUSION

Thus the proposed stability indicating RP-HPLC method for the simultaneous determination of telmisartan and hydrochlorothiazide in tablet dosage form was accurate, precise, linear, reliable, simple, economic and robust. The method has several advantages, including simple mobile phase, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The method can be used for routine analysis of marketed products of telmisartan and hydrochlorothiazide in combined tablet formulation.

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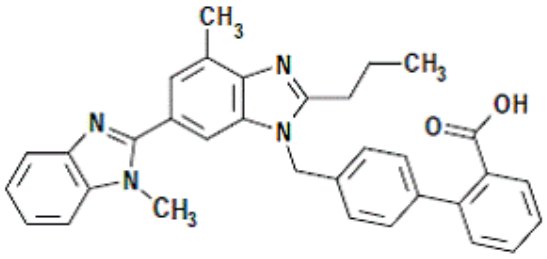

Fig. 1: Chemical structure of telmisartan

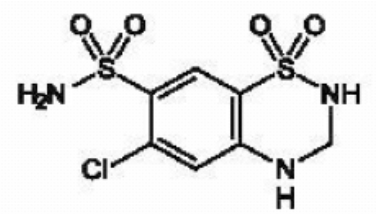

Fig. 2: Chemical structure of hydrochlorothiazide

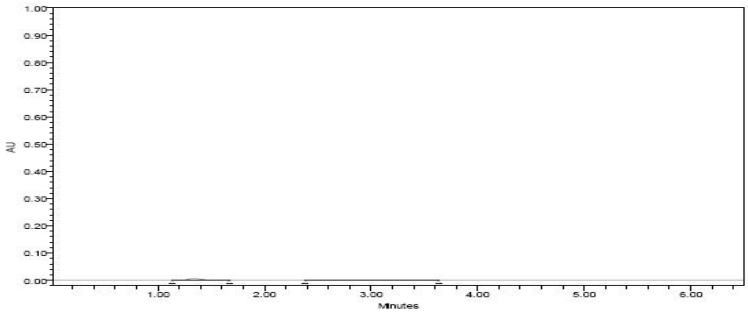

Fig. 3: Typical chromatogram showing no interference of blank for TEL and HCT

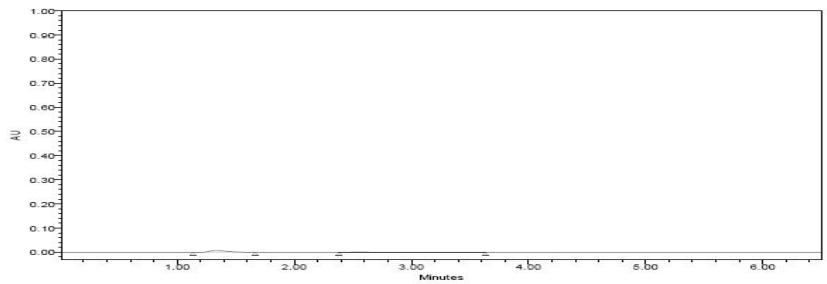

Fig. 4: Typical chromatogram showing no interference of placebo for TEL and HCT

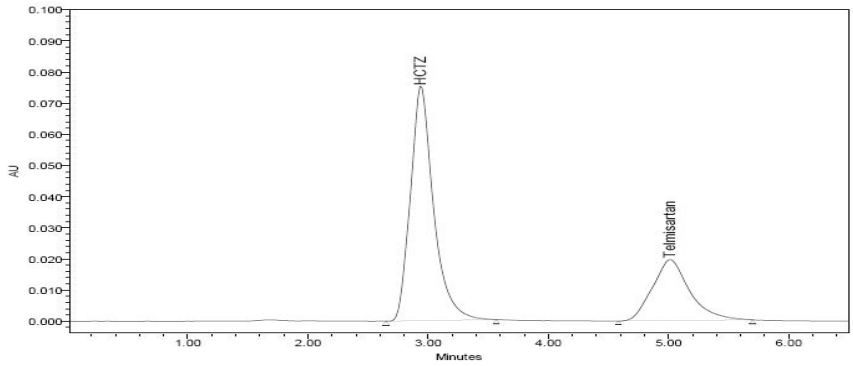

Fig. 5: Typical chromatogram of sample showing TEL and HCT

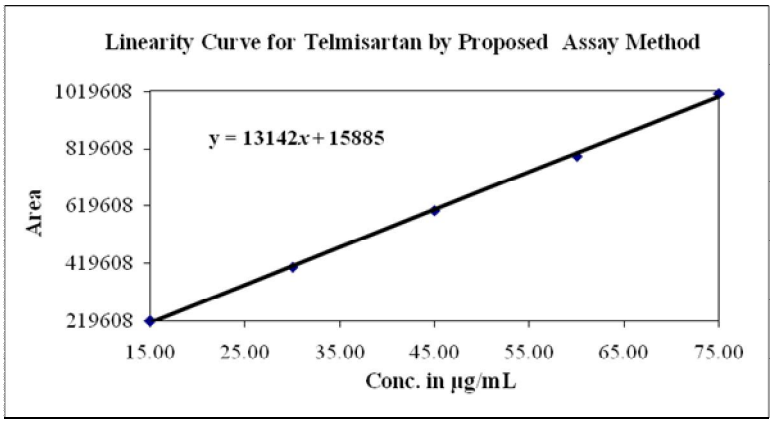

Fig. 6: Linearity curve of telmisartan

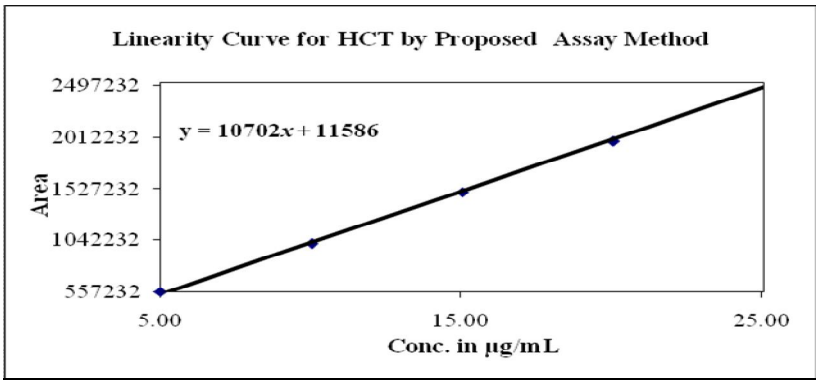

Fig. 7: Linearity curve of hydrochlorothiazide

Table 1: System suitability parameters for TEL and HCT by proposed method

Parameter	Telmisartan	Hydrochlorothiazide
Theoretical Plates	3749	3340
Tailing Factor	1.5	1.7
%RSD	0.19	0.12
Retention Time (min)	5.01±0.5	2.94±0.5

Table 2. Linearity study for TEL and HCT by proposed method

% Level (Approx.)	Telmisartan		Hydrochlorothiazide	
	Conc. (µg/mL)	Peak Area	Conc. (µg/mL)	Peak Area
50	15.01	219608	5.02	557232
100	30.02	407345	10.04	1008397
150	45.03	604779	15.06	1499229
200	60.04	794022	20.08	1972791
250	75.05	1012569	25.10	2513506
Slope		13142.0		97151.0
Intercept		15885.0		47148.0
% Y-Intercept		120.9		48.5
Residual Sum of Squares		9767.0		27274.0
CC(r)		0.9996		0.9995
RSQ(r^2)		0.9993		0.9991
LOD		2.45		0.93
LOQ		7.43		2.81

Table 3: Recovery studies for TEL and HCT by proposed method

% Level	Telmisartan			Hydrochlorothiazide		
	% Recovery	Mean % Recovery at each level	% RSD at each level	% Recovery	Mean % Recovery at each level	% RSD at each level
50	99.8	100.3	0.8	98.5	99.4	1.1
50	100.0			99.1		
50	101.2			100.7		
100	102.4	101.7	0.6	98.6	98.8	0.4
100	101.7			98.5		
100	101.1			99.3		
150	99.3	100.2	1.1	98.7	100.2	1.4
150	101.5			100.4		
150	99.8			101.5		
Mean Recovery	100.8			99.5		
SD	1.06			1.11		
% RSD	1.1			1.1		

Table 4: Inter-day and Intra-day precision summary for TEL and HCT by proposed method

Telmisartan		Hydrochlorothiazide	
Inter-day	Intra-day	Inter-day	Intra-day
100.9	100.7	100.8	100.9
100.5	101.0	100.6	101.2
100.9	101.1	101.0	101.2
100.8	99.6	100.6	99.2
100.9	100.4	100.9	100.6
101.1	100.8	101.1	100.8
Overall Avg.	100.7		100.7
Overage Std Dev.	0.41		0.53
Over all %RSD	0.40		0.50

Table 5: Forced degradation study results for TEL and HCT by proposed method

Stress Conditions	Degradation Time (hrs)	Telmisartan			Hydrochlorothiazide		
		Peak Area	% Degradation	% Active drug remained	Peak Area	% Degradation	% Active drug remained
Standard Drug		407029			1004676		
Acid	1	391256	3.9	96.1	981247	2.3	97.7
Base	1	347453	14.6	85.4	708364	29.5	70.5
Peroxide	1	317934	21.9	78.1	741266	26.2	73.8
Thermal	48	401983	1.2	98.8	986537	1.8	98.2

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