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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TELMISARTAN AND HYDROCHLOROTHIAZIDE IN BULK AND PHARMACEUTICAL FORMULATIONS

Vatchavai Bhaskara Raju*, Bonthu Mohan Gandhi,

Kamatham Srinivas Sumanth, Kolli Srinivas and Yalla Nageswari

Sri Vasavi Institute of Pharmaceutical Sciences, Pedatadepalli,
Tadepalligudem-534 101, Andhra Pradesh, India.

ABSTRACT

The main objective of the present work was to develop and validate a simple, fast, reliable, rapid, selective and accurate RP-HPLC method for the simultaneous determination of Telmisartan (TEL) and Hydrochlorothiazide (HCTZ) in Pharmaceutical formulations & to perform forced degradation studies. The separation of these two drugs was achieved on an Enable C18, G column 250×4.6mm, 5 micron size column with a mobile phase consisting of acetonitrile and phosphate buffer (60:40 v/v) at p^H 3.0 and flow rate of 1.0 ml/min and UV detection at 282 nm. The retention times were observed to be 5.728 and 3.193 minutes for TEL and HCTZ respectively. Linearity was found to be 6-18 µg/ml and 6-18 µg/ml for TEL and HCTZ respectively. The method was statistically validated for linearity, recovery, limit of detection, limit of quantification, accuracy and precision. The stress testing of the drugs individually and their mixture is carried out under acidic, alkaline, oxidation, photo-stability and thermal degradation conditions and its degradation products are well resolved from the analyte peaks. The method was successfully validated for accuracy, precision, linearity, limit of detection, limit of quantification & robustness. Forced degradation studies were also performed successfully for HPLC method.

Keywords: Telmisartan, Hydrochlorothiazide, RP-HPLC, Validation.

INTRODUCTION

Telmisartan (fig.1) is chemically 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. It is an angiotensin II receptor antagonist (ARB) used in the management of hypertension. Generally, angiotensin II receptor blockers (ARBs) such as telmisartan bind to the angiotensin II type 1 (AT1) receptors with high affinity, causing inhibition of the action of angiotensin II on vascular smooth muscle, ultimately leading to a reduction in arterial blood pressure[1].

Hydrochlorothiazide (fig.2) is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1λ^6,2,4-benzothiadiazine-7-sulfonamide. It is A thiazide diuretic often considered the prototypical member of this class. It reduces the reabsorption of electrolytes from the renal tubules. This results in increased excretion of water and electrolytes, including sodium, potassium, chloride, and magnesium. It has been used in the treatment of several disorders including edema, hypertension, diabetes insipidus, and hypoparathyroidism[2].

Detailed literature survey revealed analytical methods like spectrophotometric[3-5], LC-MS[6], HPTLC[7-14], HPLC[15-19] are available for the estimation of these drugs individually or in combination with other drugs. But very few methods are available for the simultaneous estimation of these drugs. Hence, we tried to develop new and simple spectrophotometric and RP-HPLC methods for the simultaneous

estimation of these drugs. The developed methods were validated as per the guidelines of ICH[20]. To establish Stability Indicating[21] natures of the RP-HPLC method, forced degradation of drug substances was performed under stress conditions (peroxide, acid, base, thermal, ultraviolet and neutral hydrolysis).

MATERIALS AND METHODS

Instrumentation and analytical conditions

The UV method was performed on a double-beam LABINDIA UV-Visible Spectrophotometer, 3092, with spectral bandwidth of 2nm. Wavelength accuracy 0.5nm and a pair of 1cm matched quartz cells were used to measure absorbance of solution. RP-HPLC method was performed on the HPLC system (Shimadzu) consisting of binary gradient pump and UV detector (LC-20AD) was employed for analysis and Rheodyne injector with 20µl fixed loop was used for the present study.

CHEMICALS AND REAGENTS

TEL and HCTZ working standards were procured from Hetero Pharma and Aurobindo Pharma Ltd., Hyderabad, Telangana. HPLC grade acetonitrile was purchased from Merck Specialities Pvt. Ltd., Mumbai. HPLC grade water and methanol were purchased from Fisher Scientifics Ltd., Mumbai. Orthophosphoric acid, hydrochloric acid, sodium hydroxide pellets purified and hydrogen peroxide 30% of AR grade, potassium di-hydrogen phosphate were procured from Merck Specialties Pvt. Ltd., Mumbai.

Preparation of solutions

Preparation of standard solutions

Standard stock solution of TEL and HCTZ were prepared by transferring accurately weighed TEL (25 mg) and HCTZ (25 mg) to a 25 ml volumetric flask separately, dissolved and diluted to a mark with methanol, to obtain a standard solution of TEL (1000 µg/ml) and HCTZ (1000 µg/ml). From these solutions, standard stock solutions were prepared in 10 ml volumetric flasks and made up the volume of the mobile phase to get the concentration of 12 µg/ml of TEL and 12 µg/ml of HCTZ.

Preparation of the sample solutions

20 tablets (Telmo-H) were taken and their average weight was calculated. Tablets were crushed to a fine powder and dose equivalent to 25 mg of TEL 25 mg of HCTZ were transferred to a 25 ml vol. flask, dissolved & made upto final vol. with methanol to get 1000µg/ml. From the above solution, 10 ml was pipette out into 100ml vol. flask and made upto final vol. with methanol. From this solution pipette out 1.2 ml into 10 ml vol. flask and make up the vol. with mobile phase to get the required concentration of 12 µg/ml of TEL and 12 µg/ml of HCTZ . Injected 20µL of this sample preparation into HPLC system as per proposed optimized conditions and chromatogram was recorded.

Optimized analytical method

Enable C18, G column 250 × 4.6mm, 5 micron size column was used as stationary phase. TEL and HCTZ were eluted isocratically with a flow rate of 1.0 ml/min using a mobile phase consisting of phosphate buffer and acetonitrile in a proportion of 40:60 v/v respectively. The wavelength of the UV detector was set at 282 nm. The prepared mobile phase was filtered through 0.45 µm membrane filter (Millipore) and sonicated before use.

Validation of HPLC method

System suitability

12 µg/ml of TEL & 12 µg/ml of HCTZ were prepared from 1000 µg/ml stock solution and injected 6 times at an interval of 8mins.

Specificity

HPLC chromatograms were recorded for blank and sample under optimized analytical conditions, compared them with that of standard solution, and found no additional peaks. The two peaks were completely separated in HPLC chromatogram and the resolution was found to be more than 2. Even in presence of excepients of the sample, no interfering peaks were found in HPLC chromatogram.

Linearity

For HPLC method, the calibration curves for TEL and HCTZ were constructed in the concentration range of 6-18 µg/ml and 6-18 µg/ml of TEL and HCTZ respectively and the correlation coefficient for both the drugs was found to be nearer to 1.

Accuracy

It was determined by performing triplicate by standard addition method at 50%, 100% & 150% These levels were prepared by taking 6µg/ml, 12 µg/ml & 18µg/ml for TEL and 6µg/ml, 12µg/ml & 18µg/ml for HCTZ from 1000 µg/ml standard solution and injected into HPLC 3 times at an interval of 8 min.

Precision

Precision of RP-HPLC method was checked by analyzing the samples at different time intervals of the same day (intra day precision) as well as on different days (inter day precision) at 50%, 100% & 150% intervals. The concentrations

were prepared by taking dilutions of 6 µg/ ml (50%), 12 µg/ml (100%) and 18 µg/ml (150%) for TEL and 75µg/ml (50%), 150µg/ml(100%) & 225µg/ml(150%) for HCTZ from 1000 µg/ml std solution. These concentrations were injected 3 times at an interval of 8 min.

Limit of detection (LOD) and Limit of quantitation (LOQ)

For both methods, The LOD and LOQ were calculated using the values of slopes and intercepts of the calibration curves for both the drugs.

Robustness

For robustness studies, conditions like flow rate, concentration of organic phase, and p^H and wavelength were changed and method was performed. It was performed by altering the flow rates from 0.9 ml/min to 1.0 ml/min & 1.1 ml/min and p^H from 3.0 to 2.5 & 3.5 and organic phase concentration was changed from 60% to 55 & 65 %.

Assay

20 tablets were taken and their average weight was calculated. Tablets were crushed to a fine powder and dose equivalent to 25mg of TEL, 25mg of HCTZ were transferred to a 25ml volumetric flask, dissolved & made up to final vol. with methanol to get 1000 µg/ml. From the above solution, 10ml was pipette out into 100ml vol. flask and made up to final vol. with methanol. From this solution pipette out 1.2 ml into 10ml vol. flask and make up the vol. with mobile phase to get the required concentration and injected 20µL of this sample preparation into HPLC system. The peak areas of the standard and sample were read and compared.

Stability Studies

25 mg of TEL and HCTZ were accurately weighed and transferred in to 25 ml volumetric flask and few ml of methanol was added to dissolve the drugs. The following procedures were followed for the prepared solution under the forced degradation studies,

Acidic degradation

2 ml of 1N HCl was added to the solution and reluxed at 60ºC for 4 hrs. It was then diluted up to 25 ml with methanol. From this solution 1 ml was taken and diluted to 10 ml using mobile phase to obtain a concentration of 100 µg/ml. The resulting solution was injected thrice under optimized conditions and the chromatograms were studied.

Alkaline degradation

2 ml of 1N NaOH was added to the solution and reluxed at 60ºC for 4 hrs. It was then diluted up to 25 ml with methanol. From this solution 1 ml was taken and diluted to 10 ml using mobile phase to obtain a concentration of 100 µg/ml. The resulting solution was injected thrice under optimized conditions and the chromatograms were studied.

Oxidative degradation

2 ml of 3%, H_2O_2 were added to the solution and reluxed at 60ºC for 4 hrs. It was then diluted up to 25 ml with methanol. From this solution 1 ml was taken and diluted to 10 ml using mobile phase to obtain a concentration of 100 µg/ml. The resulting solution was injected thrice under optimized conditions and the chromatograms were studied.

Photolytic degradation

The prepared solution was kept in photo stability chamber and the diluted sample of .V light for 6 hrs.

The rmal degradation

The final drug solution solution was kept at a temp of 60°c for 6 hrs.

RESULTS AND DISCUSSION

System suitability

Twenty micro liters of working standard solution was prepared and injected into the system under optimized chromatographic conditions. Chromatograms were recorded and studied for different system suitability parameters like tailing factor, theoretical plates, resolution and peak area, peak heights were also studies. Six different working standard solutions were injected to study this parameter and all the suitability parameters were found to be within the limits. The system suitability parameters were shown in table 1.

Specificity

HPLC chromatograms were recorded for blank and sample under optimized analytical conditions, compared them with that of standard solution, and found no additional peaks. The two peaks were completely separated in HPLC chromatogram and the resolution was found to be more than 2. Even in presence of excipients of the sample no interfering peaks were found in HPLC chromatogram.

Linearity

The chromatograms were recorded for the concentration range of 6-18 µg/ml and 6-18 µg/ml of TEL & HCTZ respectively. The

chromatograms were shown in fig: and graphs were shown in fig:. The correlation coefficient for both the drugs was found to be nearer to 1 (Table 2).

Accuracy
The accuracy for developed method was determined for both the drugs, recovery studies were performed in mentioned levels and recorded (Table 3). Obtained results were found to be within the limits of 98-102%.

Precision
Precision was calculated as intra-day and inter-day variations for the drugs. Percent relative standard deviations for estimation of TEL and HCTZ under intra-day and inter-day variations were found to be less than 2 (Table 4).

Limit of detection (LOD) and Limit of quantitation (LOQ)
For the developed method, the LOD and LOQ were calculated using the values of slopes and intercepts of the calibration curves for both the drugs (Table 5).

Robustness
For robustness studies, conditions like flow rate, concentration of organic phase, wavelength and p^H were changed and method was performed. In all deliberately varied conditions, percent relative standard deviations for peak areas, retention times, theoretical plates and tailing factor were found to be less than 2% (Table:6).

Assay
The percent of assay was calculated using peak areas of standard and sample for HPLC method. The experimental values obtained for the determination of TEL and HCTZ in formulation were within the claimed limits.

Stability Studies
The following degradation results were found when TEL and HCTZ were subjected to

Acidic degradation
TEL and HCTZ were found to be 6.24% and 8.98% degraded at 4th hour at 60˚C.

Alkaline degradation
TEL and HCTZ were found to be 11.69% and 4.39% degraded at 4th hour at 60˚C.

Oxidative degradation
TEL and HCTZ were found to be 14.51% and 36.13% degraded at 4th hour at 60˚C.

Photolytic degradation
TEL and HCTZ were found to be 14.77% and 13.48% for 48hrs at 60˚C.

Thermal degradation
TEL and HCTZ were found to be 14.90% and 14.21% degraded for 48hrs at 60˚C.
The percent amount of drug degraded after degradation studies is given in Table :(8b).

SUMMARY
The developed HPLC method, is carried out using different proportions of acetonitrile and ortho phosphoric acid (OPA), acetonitrile and Phosphate buffer, methanol and phosphate buffer, acetonitrile were tried for selection of the mobile phase. Ultimately, phosphate buffer (pH-3.0) and acetonitrile in a proportion of 40:60, v/v respectively was finalized as the mobile phase. The elution order was TEL (Rt=3.150 min) and HCTZ (Rt=5.660 min), at a flow rate of 1.0 ml/min. The chromatogram was recorded at 282 nm. The developed method was validated as per ICH guidelines. Parameters like linearity, precision, accuracy, specificity, robustness were done and found to be within the acceptance criteria. LOD and LOQ were determined and the developed method was applied for determination of assay of TEL & HCTZ. The stability of the drugs was examined under different stress conditions such as acidic, basic, peroxide, thermal, and photolytic conditions.

CONCLUSION
The method based on the RP-HPLC was developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the method developed are low, indicating a high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. The RP-HPLC method could selectively quantifies TEL and HCTZ in presence of its degradation products hence; it can be employed as a stability indicating method. From the found experimental data it can be concluded that the developed stability indicating chromatographic method is accurate, precise and selective and can be employed successfully for the estimation of TEL and HCTZ in pharmaceutical dosage forms.

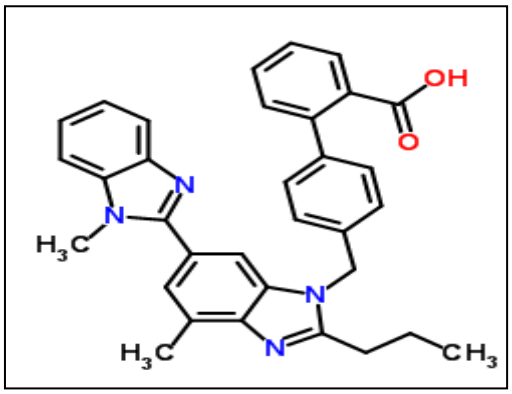

Fig. 1: Chemical structure of Telmisartan

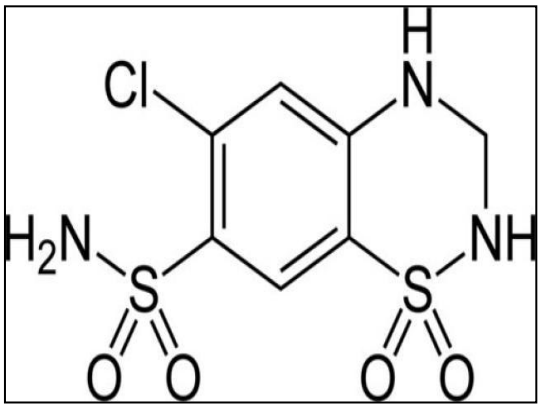

Fig. 2: Chemical structure of Hydrochlorothiazide

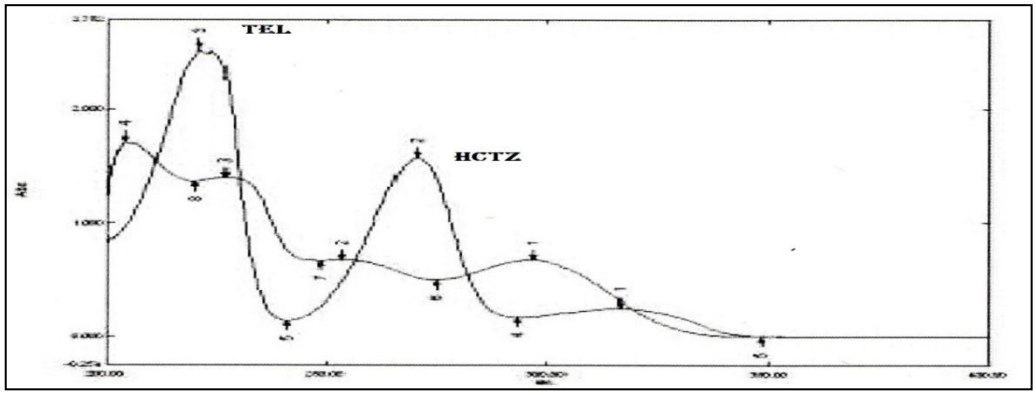

Fig. 3: UV overlap spectrum of Telmisartan & Hydrochlorothiazide

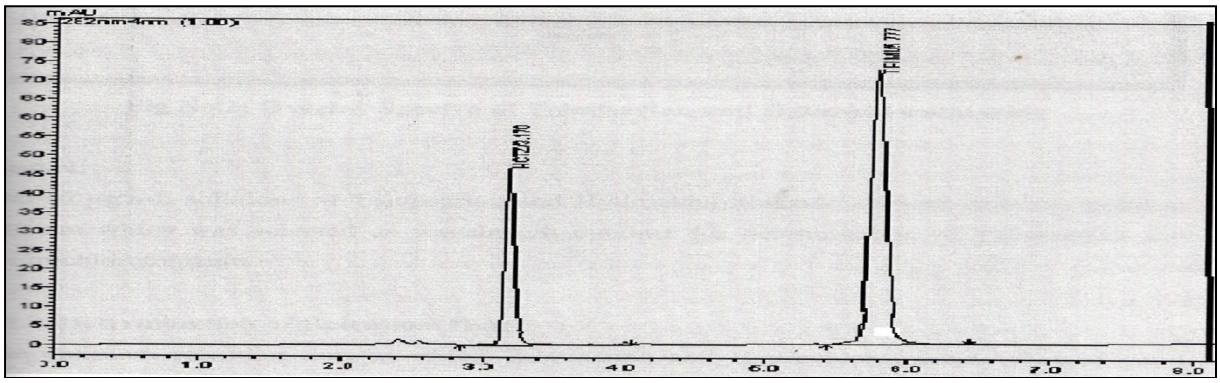

Fig. 4: Chromatogram showing well resolved peaks of TEL and HCTZ

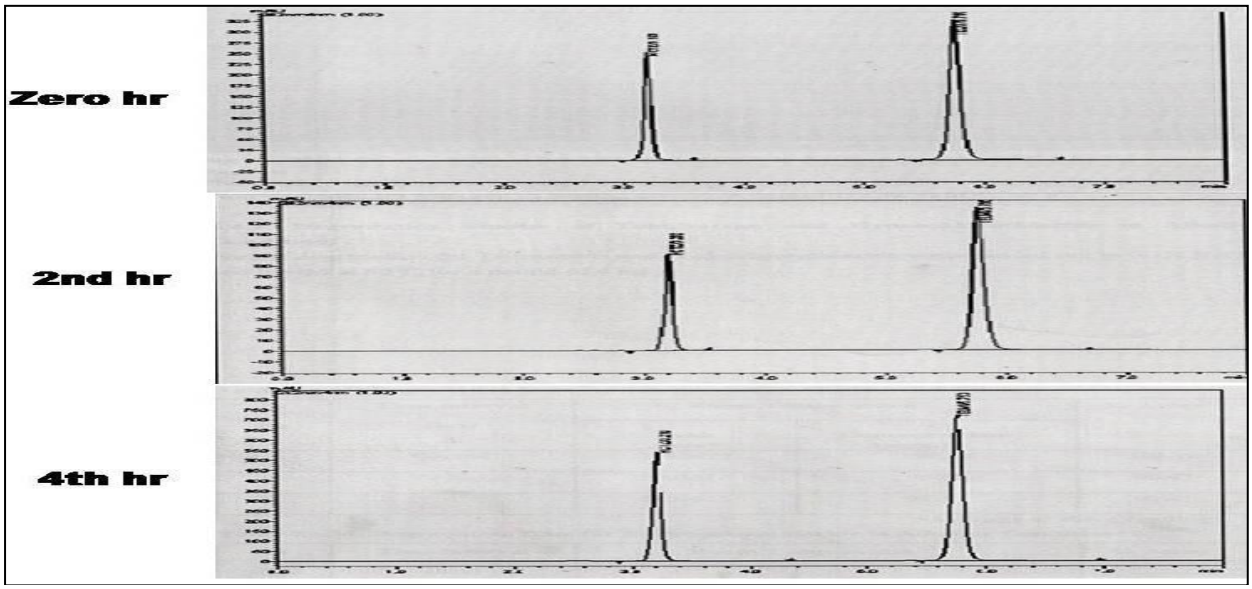

Fig. 5: Chromatogram of TEL & HCTZ in acid stress condition

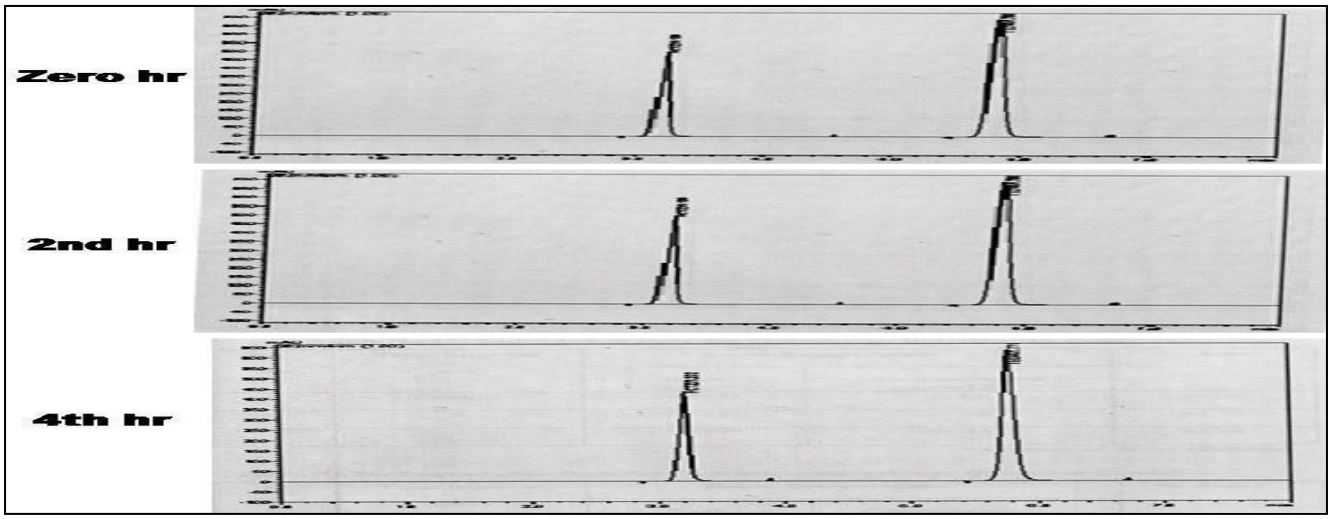

Fig. 6: Chromatogram of TEL & HCTZ in basic stress condition

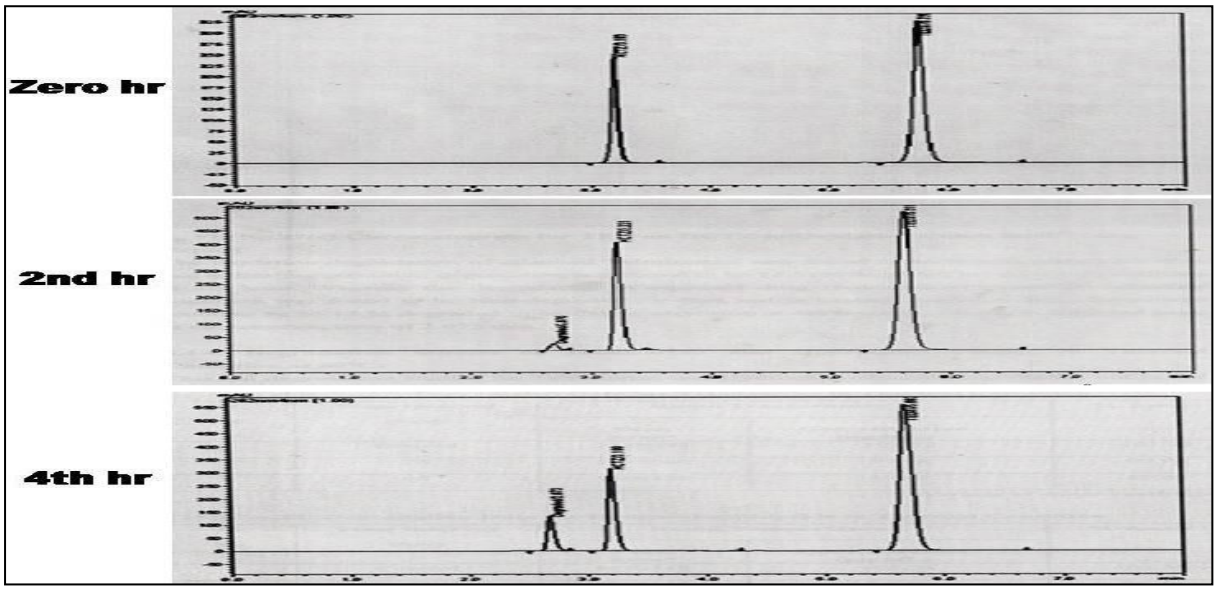

Fig. 7: Chromatogram of TEL & HCTZ in peroxide stress condition

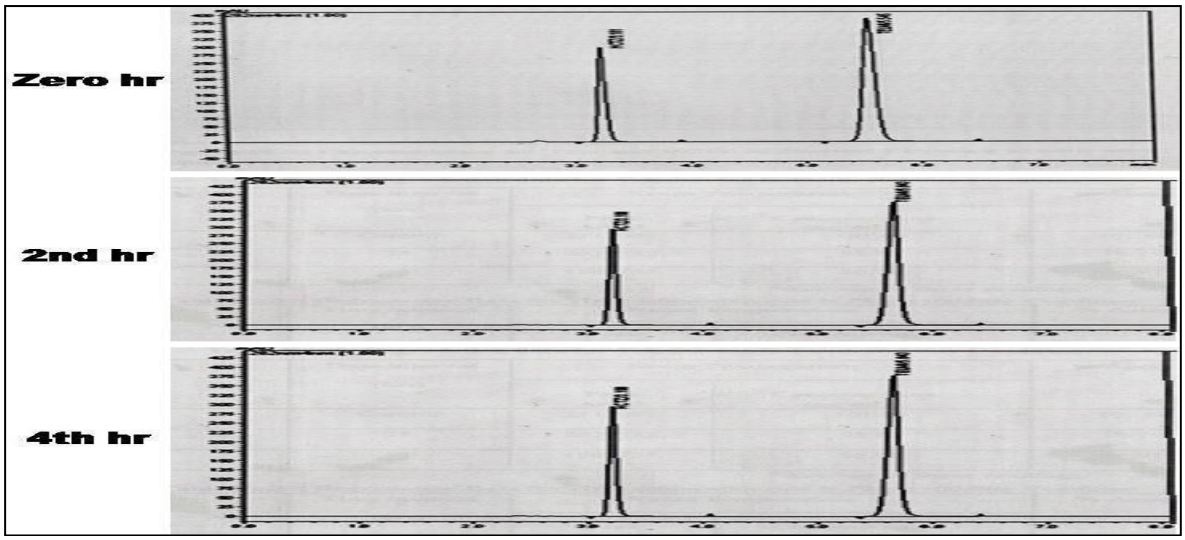

Fig. 8: Chromatogram of TEL & HCTZ in photolytic stress conditions

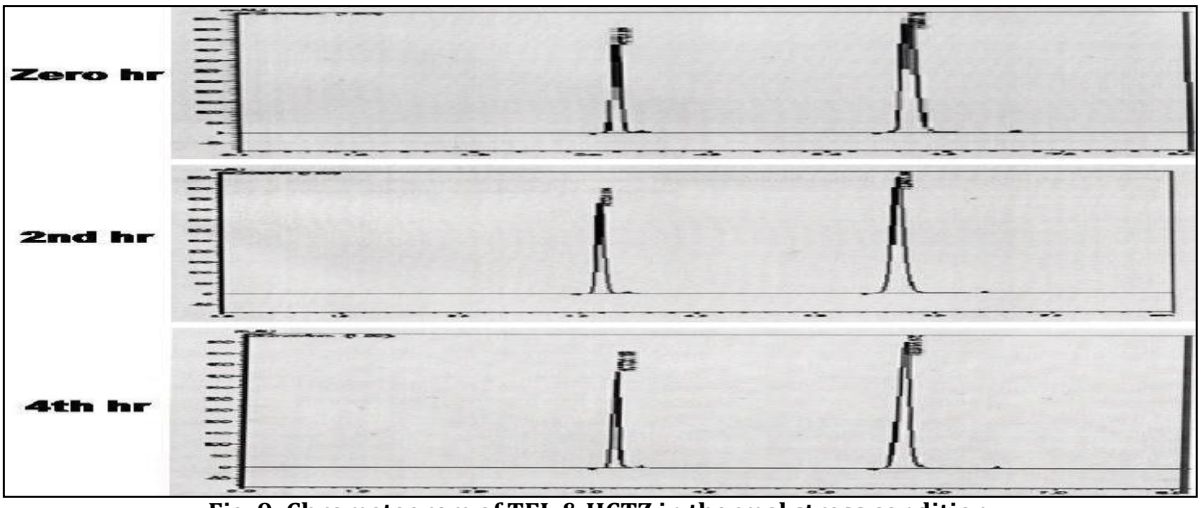

Fig. 9: Chromatogram of TEL & HCTZ in thermal stress condition

Table 1: RP-HPLC System suitability parameters

Parameter	Observation*	
	TEL	HCTZ
Retention time (min.)	**5.728**	**3.193**
No. of Theoretical plates	11256	8570
Tailing Factor	1.146	1.150
Resolution	-	14.608

*Average of six readings

Table 2: Linearity values of TEL and HCTZ

Parameter	TEL	HCTZ
Regression equation	Y=21073X+29786	Y=13427X+42452
Linearity(µg/ml)	6-18	6-18
Correlation coefficient	0.9992	0.9991

Table 3: Recovery values of TEL and HCTZ

Drug	% Recovery			% RSD		
	50%	100%	150%	50%	100%	150%
TEL	101.75	98.81	100.49	0.54	0.20	0.28
HCTZ	99.83	101.85	101.42	0.54	0.17	1.25

Table 4: Precision values of TEL and HCTZ

Drug	Concentration (µg/ml)	Intraday (% RSD)	Inter day (% RSD)
TEL	6	0.31	1.62
	12	0.78	1.21
	18	0.15	0.29
HCTZ	6	0.78	0.39
	12	0.72	0.54
	18	1.07	0.67

Table 5: LOD and LOQ of TEL and HCTZ

Drug	LOD (ng/ml)	LOQ (ng/ml)
TEL	0.99	3.00
HCTZ	1.55	4.70

Table 6: Robustness parameters of TEL and HCTZ

S. No.	Parameter	TEL	HCTZ	TEL	HCTZ
		Peak area*	Peak area*	% recovery	% recovery
1	Optimized conditions	240786	175982	100.00	100.00
2	Flow 0.9 ml/min	240916	178675	100.05	101.53
3	Flow 1.1 ml/min	243764	179382	101.23	100.93
4	Organic phase, 5 % more (65 %)	241123	176132	100.13	100.08
5	Organic phase, 5 % less (55 %)	240917	175790	100.05	99.89
6	At pH 2.5	241917	176147	100.46	100.09
7	At pH 3.5	242012	175824	100.50	99.91
8	At 284 nm	240676	177463	99.95	100.84
9	At 280 nm	242768	177485	100.82	100.85

Table 7: Assay data of marketed formulation

Drug	Amount labeled	Amount found	% Label claim	% RSD
TEL	40 mg	39.66	99.16	0.39
HCTZ	40 mg	39.33	98.33	0.95

Table 8: Degradation data* of stress studies

S. No	Sample ID	Peak area*	Conc. found (µg/ml)	% degradation	Peak area*	Conc. found (µg/ml)	% degradation
Acid degradation studies							
		TEL			**HCTZ**		
1	Control	2104288	100.00	-------	1418961	100.00	-------
2	At 2nd hr	2015039	95.75	4.25	1400834	98.72	1.28
3	At 4th hr	1991829	94.65	5.35	1298366	91.50	8.50
Base degradation studies							
		TEL			**HCTZ**		
1	Control	2142977	100.00	-------	1430285	100.00	-------
2	At 2nd hr	2003315	93.48	6.52	1403345	98.11	1.89
3	At 4th hr	1896615	88.50	11.50	1371331	95.87	4.13
Oxidative degradation studies							
		TEL			**HCTZ**		
1	Control	2098671	100.00	-------	1491840	100.00	-------
2	At 2nd hr	2021429	96.31	3.69	1217964	81.64	18.36
3	At 4th hr	1792875	85.42	14.58	1006735	67.48	32.52
Photolytic degradation studies							
		TEL			**HCTZ**		
1	Control	2113087	100.00	-------	1424683	100.00	-------
2	At 2nd hr	2039582	96.52	3.48	1378421	96.75	3.25
3	At 4th hr	1801735	85.26	14.74	1243726	87.29	12.71
Thermal degradation studies							
		TEL			**HCTZ**		
1	Control	2118092	100.00	-------	1430348	100.00	-------
2	At 2nd hr	2035040	96.07	3.93	1342115	93.83	6.17
3	At 4th hr	1804188	85.17	14.83	1239373	86.64	13.36

*Average of three determinations (each condition), DEG: Degradation, SD: Standard deviation

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