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RP-HPLC Method for the Simultaneous Estimation of Hydrochlorothiazide and Enalapril Maleate in Bulk and Tablet dosage form in Biorelevant Media (FaSSIF)

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ABSTRACT

A simple, rapid, and precise reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous analysis of Hydrochlorothiazide (HTZ) and Enalapril Maleate (ENL) in a tablet dosage form and in Biorelevant media has been developed and validated. This method was performed with a Inertsil C_{18} (4.6 x 150mm, 5µm) column with 75:25 (v/v) 50mM potassium dihydrogen orthophosphate buffer : methanol as mobile phase at a flow rate of 1.0 ml/min. UV detection at 252 nm; HTZ and ENL were eluted with retention times of 2.09 and 5.289min, respectively. The method was continued and validated accordance with ICH guidelines. Validation revealed the method is rapid, specific, accurate, precise, reliable, and reproducible. Calibration curve plots were linear over the concentration ranges 2.5-50µg/mL for HTZ, and 1-20µg/mL for ENL. Limits of detection (LOD) were 0.075 and 0.03µg/mL and limits of quantification (LOQ) were 0.25 and 0.1µg/mL for HTZ and ENL respectively. Statistical analysis was proves the method is suitable for the analysis of HTZ and ENL as a bulk, in tablet dosage form and in biorelevant media without any interference from the excipients. It was also proved study for degradation kinetics of three drugs. It may be extended for its estimation in plasma and other biological fluids.

Keywords: *Hydrochlorothiazide (HTZ), Enalapril Maleate (ENL), RP-HPLC, Validation, FaSSIF.*

INTRODUCTION

Hydrochlorothiazide (HTZ) chemically 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide (Figure 1). It is a diuretic drug and derivative of the thiazide class. It acts by inhibit the kidneys' to ability to retain water. This decrease the volume of the blood and decrease blood return to the heart and then cardiac output and, by the other mechanisms, it's believed to the inner peripheral vascular resistance [1]. Enalapril Maleate (ENL) chemically N-[(1S)-1-(Ethoxy carbon-yl)-3-Phenylpropyl]-L- Proline (Figure 1) [2]. It was Anangiotensin Converting Enzyme (ACE) inhibitor as used in treatment of the hypertension, diabetic nephropathy, and also some types of the chronic heart failures. ACE is converts the peptide hormone angiotensin I to the angiotensin II. One of action of the angiotensin II is vasoconstriction of the blood vessels resulting in the increase blood pressure. ACE inhibitors such as the ENL prevent this effect. ENL has been shown to the lower death rate in the systolic heart failure [3].

In the scientific literature, analysis of HTZ and ENL has been reported as individual ingredients and in combination with other compounds. Analytical methods have included estimation of HTZ [4, 5], ENL [6] individually. And in two component formulations of HTZ and ENL have been analyzed in combination [7-9]. And HTZ and ENL with other drugs individually have also been reported [10-13].

No other chromatographic methods are found for simultaneous analysis of HTZ and ENL in a combined dosage form and in biorelevant media. The method described is rapid, economical, precise, and accurate and can be used for routine analysis of tablets. It was validated as per ICH guidelines [14-16].

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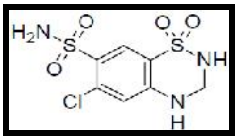

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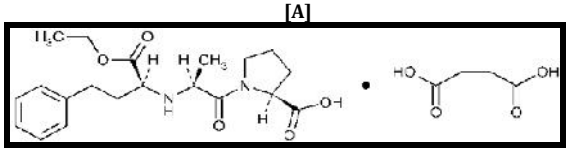


[B]

Fig. 1: Chemical structures of (A) Hydrochlorothiazide (HTZ) and (B) Enalapril Maleate (ENL)

MATERIALS AND METHODS

1. Experimental:

1.1. Materials and Methods:

Pharmaceutical grade working standards Hydrochlorothiazide (HTZ) and Enalapril Maleate (ENL) were obtained from Hetero Labs, Jedcharla, India. All chemicals and reagents were HPLC grade and were purchased from Merck Chemicals, Mumbai, India.

1.2. Instrumentation:

The analysis was performed using Waters-2695 (Modal Alliance) High Performance liquid chromatography, analytical balance (Mettler Toledo), PDA Detector (Standard cell) and data handling system (Empower 2), pH meter (lab India), Sonicator. The column used is Inertsil C_{18} (150×4.6mm, packed with 5µm) with the flow rate 1.0ml/min (isocratic).

1.3. Preparation of blank Fasted State Simulated Intestinal Fluid (FaSSIF):

Accurately weighed 1.74g of Sodium hydroxide pellets, 19.77g of Sodium dihydrogen orthophosphate, and 30.93g of

Sodium chloride dissolve in 5 L of purified water and adjust the pH 6.5 exactly by using 1N Hydrochloric acid [17].

1.4. Preparation of FaSSIF:

Accurately weighed 3.3g of sodium taurocholate dissolved in 500mL blank FaSSIF solution, added 11.8mL of a solution to 100mg/mL lecithin in methylene chloride, and forming an emulsion. The methylene chloride was eliminated under vacuum at 40°C. Then draw a vacuum for 15minutes at 250mbar and also followed by 15minutes at 100mbar. These results gave in a clear, micellar solution, having no perceptible odor for methylene chloride. After that it was cool to room temperature and adjusted the volume upto 2L with blank FaSSIF [17].

1.5. Preparation of Standard Stock solution:

Accurately weighed 10 mg of HTZ, and ENL working standard and separately transferred into a 10ml clean dry volumetric flasks, about 7mL of biorelevant media (FaSSIF) was added to each volumetric flask and sonicated to dissolve it completely and make volume up to the mark with the same solvent. Calibration standards at five levels were prepared by appropriately mixed and further diluted stock standard solutions in the concentration ranges from 2.5-50µg/mL for HTZ and 1-20µg/mL for ENL. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the Linearity graphs.

1.6. Preparation of Standard solution:

The above standard stock solution was containing 1000µg/mL of each HTZ and ENL in separate volumetric flasks. Then transferred the 0.1ml of HTZ and 0.25ml of ENL of prepared standard stock solution into a clean 10ml volumetric flask and made upto the mark with diluent. And finally the standard solution concentrations were 25µg/mL and 10µg/mL of HTZ and ENL respectively.

1.7. Preparation of Test solution:

For the analysis of a tablet dosage form, 20 tablets were weighed individually and their average mass was determined. Then, the tablets were crushed to a fine powder. The powder equivalent to 25mg of HTZ and 10mg of ENL were transferred to a 10mL volumetric flask and dissolved in 10mL of biorelevant media (FaSSIF), sonication was done for 15 min with swirling. After sonication, the solution was filtered through a membrane filter paper (#0.45µ). From the above stock solution 0.1mL was transferred in to 10mL volumetric flask and made volume upto the mark with diluent, the final concentrations were 25µg/mL and 10µg/mL of HTZ and ENL respectively, then injected into the chromatographic system, and analyzed quantitatively. The analysis was repeated six times and the possibility of excipient interference with the analysis was examined.

1.8. Optimization of HPLC Method:

The HPLC method was optimized and developed with a simultaneous method for HTZ and ENL. The mixed standard solution (25mg of HTZ and 10mg of ENL) injected in HPLC by the followed chromatographic conditions. The chromatographic separation was achieved on a Inertsil C_{18} (4.6 x 150mm, 5µm). The isocratic mobile phase consisting of 50mM potassium dihydrogen orthophosphate and methanol in the ratio of (75:25v/v) was used throughout the analysis and the pH 3.5 adjusted with orthophosphoric acid. The flow rate of the mobile phase was 1.0ml/min. Detection was monitored at wavelength of 252nm. The column temperature was kept at ambient and injection volume was 20µl (**Table. 1**).

1.9. Method validation:

The method validation was done according to the ICH guidelines. The following validation characteristic parameters are accuracy, precision, linearity, and specificity, LOD, LOQ and robustness.

1.9.1. Linearity and range:

Linearity of the method was studied by the injecting the mixed standard solutions with the concentration ranges from 2.5-50µg/mL for HTZ, and 1-20µg/mL for ENL levels of target concentrations were prepared and injected six times into the HPLC system keeping the constant injection volume. The peak areas were plotted against the concentrations to obtain the linearity graphs.

1.9.2. Precision:

The precision of the optimized method was evaluated by carrying out six independent assays of test sample. %RSD of six assay values was calculated. Intermediate precision was carried out the samples by using another instrument and with different analyst.

1.9.3. Limit of Detection and Quantification:

The LOD and LOQ procedures were performed on samples contain very lower concentrations of analytes under the ICH guidelines. By applying the visual evaluation method, LOD was expressed by establishing the lowest concentration at which the analyte can be detected. LOQ was considered as the lowest concentration of analytes that can be detected and quantified, with acceptable accuracy and precision.

1.9.4. Robustness:

Robustness was studied by evaluating the effect of small variations in the chromatographic conditions. The conditions studied were flow rate altered by ±0.1ml/min, mobile phase composition with methanol ±5ml. These chromatographic variations are evaluated for resolution between HTZ and ENL.

1.9.5. System suitability:

The system suitability parameters with respect of tailing factor, theoretical plates, repeatability and resolution between HTZ and ENL peaks were defined.

1.9.6. Specificity:

The specificity of the analytical method is the ability of the method to estimate the analyte response in the presence of additional components such as impurities, degradation products and matrix. The peak purity of HTZ and ENL were assessed by comparing the Retention time of standard HTZ and ENL good correlation was obtained between the Retention time of standard and sample of HTZ and ENL.

The specificity method was also evaluated to ensure that there were no interference products resulting from forced degradation studies.

1.9.6.1. Forced degradation study:

Forced degradation or Stress testing of a drug substance will help to identify the degradation products, which can help to establish the intrinsic stability of the molecule.

All stress decomposition studies were performed at an initial drug concentration 25µg/mL of HTZ and 10µg/mL of ENL.

The Stability indicating study of HTZ and ENL were undergoes acid, alkali and oxidation degradation, photolysis and heat condition.

Placebo Interference: The placebo (in the present of excipients in tablet) sample were prepared as per the test method and analyzed in the HPLC. It expressed there is no additional peaks at the retention time of HTZ and ENL in the chromatograph it indicates that there is no placebo interference.

Acid Degradation: Sample was treated with 3ml of 1N hydrochloric acid and kept for 10hrs. After 10hrs the solution was neutralized with 3ml of 1N sodium hydroxide, made the volume upto the mark with biorelevant media and analyzed using HPLC.

Alkali Degradation: Sample was treated with 3ml of 1N sodium hydroxide and kept for 10hr. After 10hr the solution was neutralized with 3ml of 1N hydrochloric acid, made the volume upto the mark with biorelevant media and analyzed using HPLC.

Oxidative Degradation: HTZ and ENL solutions of 25 and 10µg/ml were mixed with 3mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10hrs. After 10hrs made the volume upto the mark with biorelevant media and analyzed using HPLC.

Photolytic Degradation: The **s**amples were kept under UV light for different time intervals (15mins – 7days) and made the volume upto the mark with biorelevant media and analyzed using HPLC.

Thermal Degradation: Samples were heated at 80^0 C for 15mins - 60mins and 220^0 C for 2-5mins and analyzed.

1.9.7. Accuracy:

Accuracy was carried out by applying the method to drug sample (HTZ and ENL combination of tablets) to which known amounts of HTZ and ENL standard powder corresponding to 50, 100 and 150% of label claim was added, mixed and the powder was extracted and determined by the system in optimized mobile phase. The experiment was performed in triplicate and percentage recovery, % RSD was calculated.

1.9.8. Analysis of marketed formulation:

The marketed formulation was assayed by above description. The peak areas were monitored at 252nm, and determination of sample concentrations were using by multilevel calibration developed on the same HPLC system under the same conditions using linear regression analyzed for HTZ and ENL in the same way as described above.

RESULTS AND DISCUSSION

The simultaneous estimation of HTZ and ENL was done by RP-HPLC and in the optimized method the mobile phase consists of buffer (750 volumes of phosphate buffer and 250 volumes of Methanol and the pH was adjusted to be 3.5. Then finally filtered using 0.45µ membrane filter paper and degassed in sonicator for 15 minutes. The detection is carried out using PDA detector at 252nm. The solutions are following at the constant flow rate of 1.0 ml/min. The retention time for HTZ and ENL was 2.09 and 5.289minutes respectively. Linearity ranges for HTZ and ENL were 2.5-50µg/mL and 1-20µg/mL respectively and the results were found for in the acceptable as (R^2) = 0.9998 and 0.9992 for HTZ and ENL respectively. LOD was 0.075 and 0.03µg/mL and LOQ was 0.25 and 0.1µg/mL for HTZ and ENL respectively. The all parameters value of RSD is less than 2.0% indicating the accuracy and precision of the method. The percentage recoveries were found 99.28-100.12% and 100.15-100.8% for HTZ and ENL respectively.

1. Method Development and Optimization:

The HPLC procedure was optimized with a view to develop a suitable LC method for the analysis of HTZ and ENL in fixed dose for bulk and combined dosage form. It was found that 75:25 v/v (50mM) potassium dihydrogen orthophosphate buffer: methanol gave acceptable retention time (2.09 and 5.289min for HTZ and ENL), plates, and good resolution for HTZ and ENL at the flow rate of 1.0ml/min (**Table. 1**; **Fig. 2 & 3**).

Table No. 1: Optimized Chromatographic Conditions

Parameters	Method
Stationary phase (column)	Inertsil C_{18} (4.6 x 150mm, 5µm)
Mobile Phase	75:25v/v, (50mM Phosphate Buffer : Methanol)
pH	3.5 ± 0.02
Flow rate (ml/min)	1.0
Run time (minutes)	10.0
Column temperature (°C)	Ambient
Volume of injection loop («l)	20
Detection wavelength (nm)	252
Drugs RT (min)	2.09 & 5.289

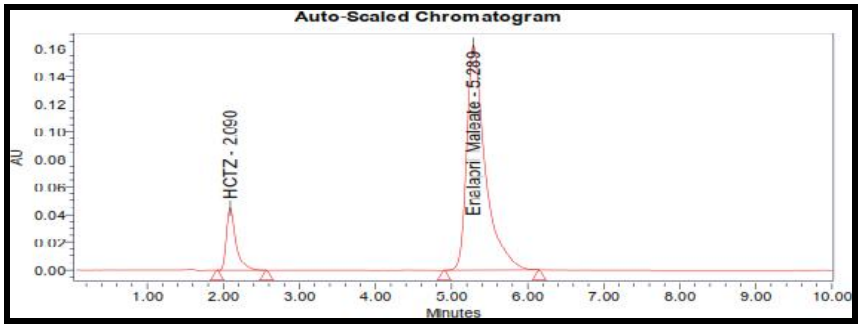


Fig. 2: Chromatogram of HTZ and ENL at 252nm from bulk drug

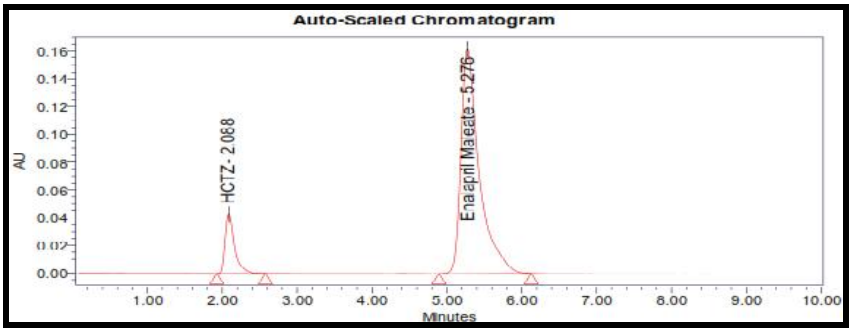


Fig. 3: Chromatogram of HTZ and ENL at 252nm from pharmaceutical formulation (Enapril-HT)

2. Validation of Developed method:

2.1. Linearity:

Linearity was evaluated by analysis of working standard solutions of HTZ and ENL of five different concentrations. The range of linearity ranges from 2.5-50µg/ml for HTZ and 1-20µg/ml for ENL (**Table. 2**). The result of correlation coefficients of HTZ and ENL (R^2) = 0.9998 & 0.9992 respectively (**Fig. 4-6**). There was an excellent correlation between peak areas and concentrations of each drug.

Table 2: Data for linearity

Analyte	Concentration range (µg/mL)	Correlation Coefficient (R^2)	Slope	Intercept
HTZ	2.5-50	0.9998	25859x	10636
ENL	1-20	0.9992	72803x	83486

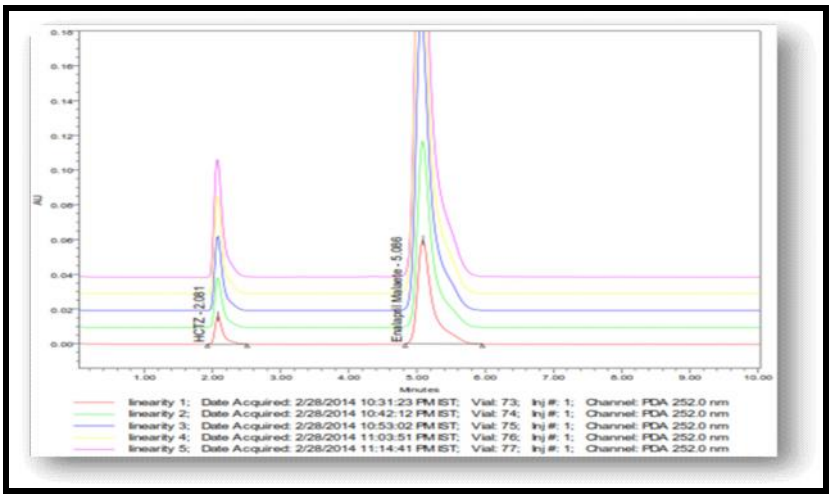


Fig. 4: Overlay linearity Chromatogram for HTZ and ENL

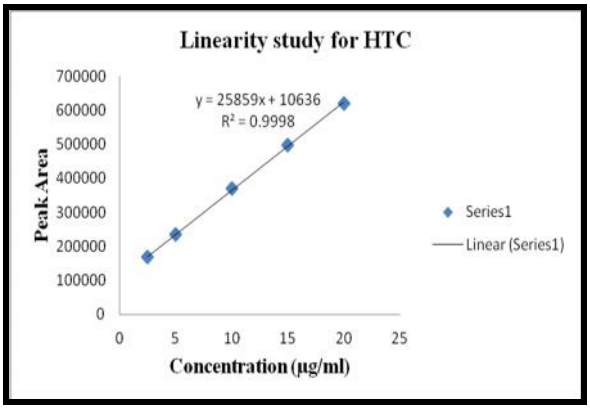


Fig. 5: Linearity Curve of Standard Cilnidipine (HTZ)

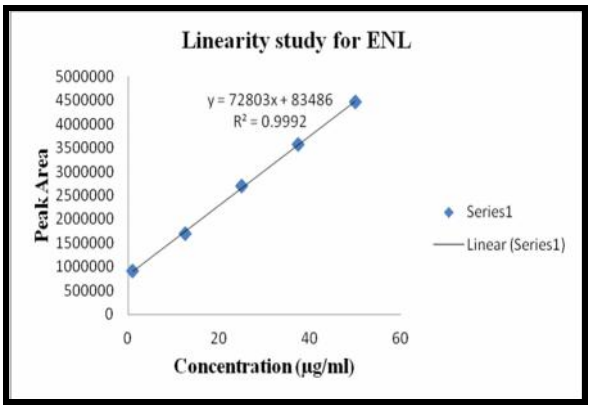


Fig. 6: Linearity Curve of Standard Metoprolol Succinate (ENL)

2.2. Precision:

The results of precision method were evaluated by carrying out six independent test samples of HTZ and ENL. The percentage of RSD of six sample peak area values was calculated. Different analyst from the same laboratory conditions analyzed the intermediate precision for the optimized method. The RSD values of intra-day and inter-day studies for HTZ and ENL confirming good precision of the optimized method (**Table. 3**).

Table No. 3: Intra-day and inter-day Precision results of HTZ and ENL from tablets

Replicate	HTZ		ENL	
	Intra-day precision	Inter-day precision	Intra-day precision	Inter-day precision
1	369212	361829	2731586	2747819
2	369988	365761	2732792	2718263
3	370020	359257	2733404	2698254
4	370868	363826	2734689	2702675
5	371366	367257	2735120	2721674
Mean	370290.8	363586	2733518.2	2717737
St. dev.	839.3516546	3165.902557	1432.6846	19539.4121
% RSD	**0.226673645**	**0.870743801**	**0.0524117**	**0.7189589**

2.3. LOD and LOQ:

The LOD and LOQ values were found to be 0.075 and 0.25µg/mL for HTZ and 0.03 and 0.1µg/mL for ENL (**Table. 5**).

2.4. Specificity:

Injected the extracted solutions commonly used excipients were performed to demonstrate for the absence of interaction with the drugs. These results are expressed that there was no interference from the other excipients in the tablet formulation; therefore, confirm the method was specific.

2.5. System suitability:

System suitability parameters such as the theoretical plates count, resolution, % RSD and peak tailing factors are determined (**Table. 5**).

Table No. 5: System suitability parameters for HTZ and ENL

System suitability parameters	HTZ	ENL
Retention time (min)	2.090	5.289
Repeatability of retention time; %R.S.D (n=5)	0.121	0.399
Repeatability of peak area; %R.S.D= (S.D./Mean)×100	0.226	0.052
Resolution (Rs)	-	9.82
Tailing factor (asymmetric factor)	1.53	1.61
USP plate count	6606	12627
LOD (µg/mL)	0.075	0.03
LOQ (µg/mL)	0.25	0.1

2.6. Robustness:

To ensure the insensitivity of the optimized RP-HPLC method to small alteration in the experimental conditions. The conditions studied were flow rate altered by ±0.1ml/min, mobile phase composition with methanol ±5ml. These chromatographic variations are evaluated for resolution between HTZ and ENL (**Table. 6**).

Table No. 6: Robustness study for analytical method validation of HTZ and ENL tablets

Parameters		Adjusted to	Mean Area [a]	Mean RT	SD	% RSD
HTZ	Flow Rate As per method 1.0ml/min	0.9 ml/min	496240	2.736	1489.69	0.3
		1.1ml/min	298452	1.673	1348.28	0.451
	Mobile Phase (75:25) (Buffer: methanol)	80:20	432274	2.68	824.87	0.19
		70:30	314729	2.049	657.58	0.208
ENL	Flow Rate As per method 1.0ml/min	0.9 ml/min	3252882	6.746	3927.16	0.12
		1.1ml/min	2299005	4.032	4102.88	0.178
	Mobile Phase (75:25) (Buffer: methanol)	80:20	3034167	6.692	10403.75	0.342
		70:30	2454520	2.847	8811.45	0.358

[a] = 5 *Replicates*

2.7. Solution stability studies:

Three different concentrations of HTZ (25µg/mL) and ENL (10µg/mL) were prepared from the sample solution and stored at room temperature for 24 hrs. Then injected into the HPLC system and the additional peaks were not found in the chromatograms so, it was indicating the stability of HTZ and ENL tablet in the solution (**Table. 7**).

Table No. 7: Solution stability study for analytical method validation of HTZ and ENL tablets

Name	*Replicate (n = 5)*	*Initial*	*After 3 hrs*	*After 6 hrs*	*After 12 hrs*	*After 24 hrs*
HTZ	Mean	370290.8	368611.4	365104.4	357729.2	349765.2
	SD	839.351	807.710	1124.763	1633.192	1462.386
	% RSD	0.226	0.219	0.308	0.456	0.418
ENL	Mean	2733518	2713897	2682260	2618510	2557387
	SD	1432.685	11144.57	9095.202	7962.3	12968.79
	% RSD	0.052	0.410	0.3390	0.304	0.507

2.8. Recovery studies:

Good recoveries of the HTZ and ENL were obtained at different added concentrations for the tablets (**Table. 8**).

Tablet No. 8: Accuracy Results of HTZ and ENL from tablets

Brand Name	Analyte	Recovery levels	Actual Conc. (µg/mL)	Added Conc. (µg/mL)	Theoretical Conc. (µg/mL)	Found Conc. (µg/mL)	% Recovery	% RSD	% Error [a]
Enapril-HT	HTZ	50 %	25	12.5	37.5	37.23	99.28	0.97	-0.72
		100 %	25	25	50	50.06	100.12	0.26	0.12
		150 %	25	37.5	62.5	62.48	99.96	0.41	-0.032
	ENL	50 %	10	5	15	15.12	100.8	0.17	0.8
		100 %	10	10	20	20.03	100.15	0.39	0.15
		150 %	10	15	25	25.07	100.28	1.02	0.28

[a][found conc. – theoretical conc./theoretical conc.] x 100.

2.9. Ruggedness: The ruggedness was studied by evaluating by different analysts but in the same chromatographic conditions. The result of ruggedness study of the developed method was established in **Table 9**. The result shown that during by different analysts but in the same chromatographic condition of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as rugged.

Table 9: Evaluation data of Ruggedness study of HTZ and ENL

ID Precisions	No. of Injections	HTZ		ENL	
		Peak Area	RT	Peak Area	RT
ID Precision - 1	1	368833	2.089	2722206	5.132
	2	370351	2.083	2736569	5.221
	3	370941	2.083	2736932	5.203
ID Precision - 2	1	369580	2.080	2670969	5.097
	2	369641	2.082	2709378	5.112

	3	370013	2.078	2712355	5.151
MEAN		369893.2	2.0825	2714734.83	5.152
STDEV		722.155	0.003	24392.912	0.049
% RSD		0.195	0.179	0.898	0.965

2.10. Analysis of a commercial formulation:

Experimentally the results for the amount of HTZ and ENL in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interaction from the excipients which are commonly present in formulation of tablets.

2.11. Degradation study:

Acid degradation study: In acidic degradation study, sample was treated with 3ml of 1N hydrochloric acid and kept for 10hrs at 60ºC. After 10hrs the solution was neutralized with 3ml of 1N sodium hydroxide, made the volume upto the mark with biorelevant media and analyzed using HPLC. The drug content was found to be degrading up to 4.618% in acidic condition (Figure 7 & 8, Table 10 & 11).

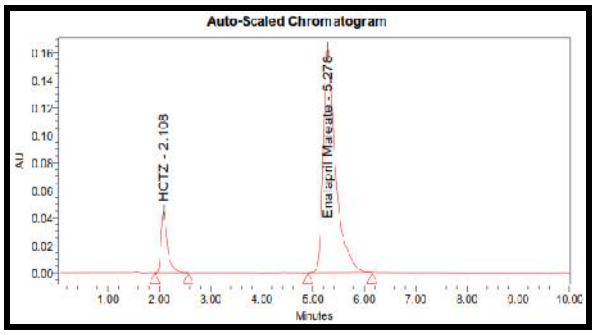


Figure 7: Chromatogram of acidic forced degradation of HTZ and ENL

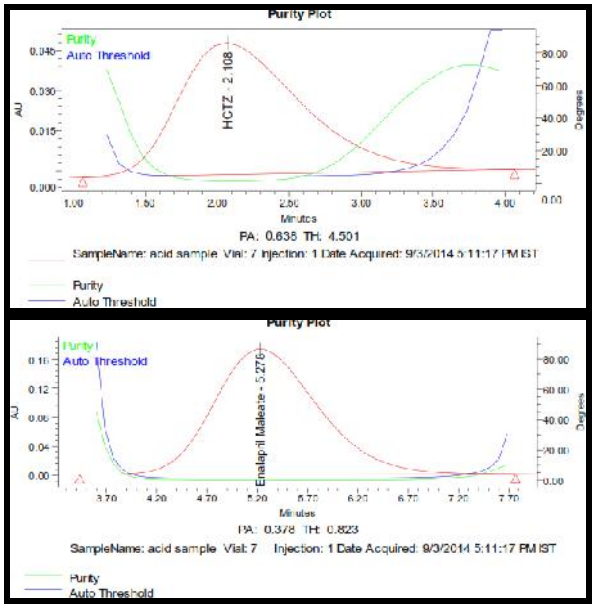


Figure 8: Purity Plots for HTZ and ENL in acidic forced degradation

Alkaline degradation study: Alkaline degradation study was performed by the sample was treated with 3ml of 1N sodium hydroxide and kept for 10hr. After 10hr the solution was neutralized with 3ml of 1N hydrochloric acid, made the volume upto the mark with biorelevant media and analyzed using HPLC. In alkali degradation, it was found that around 6.591% of the drug degraded (Figure 9 & 10, Table 10 & 11).

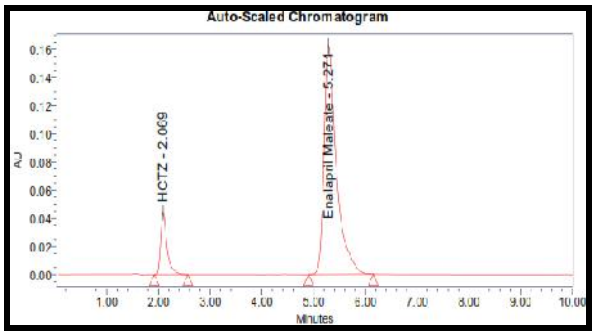


Figure 9: Chromatogram of alkali forced degradation of HTZ and ENL

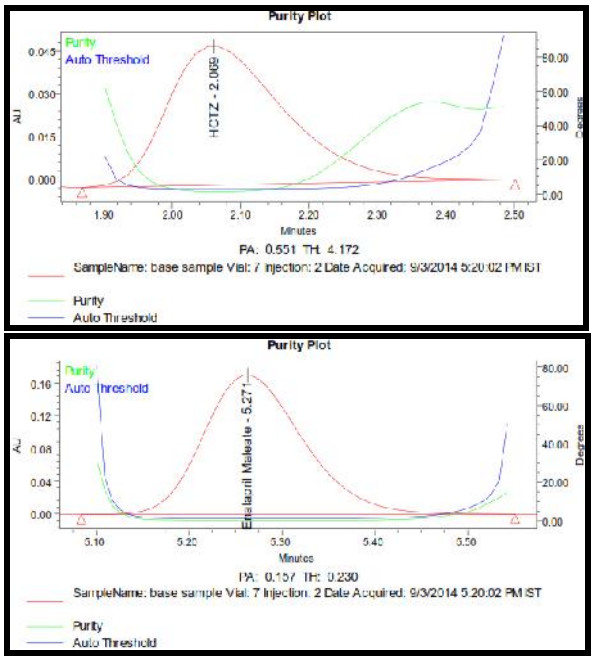


Figure 10: Purity Plots for HTZ and ENL in alkali forced degradation

Oxidative degradation study: Oxidation degradation study was performed by the sample solutions were mixed with 3mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10hrs. After 10hrs made the volume upto the mark with biorelevant media and analyzed using HPLC. In oxidative degradation, it was found that around 4.049% of the drug degraded (Figure 11 & 12, Table 10 & 11).

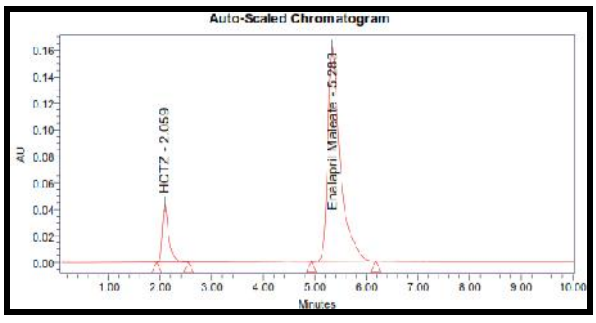


Figure 11: Chromatogram of oxidative forced degradation of HTZ and ENL

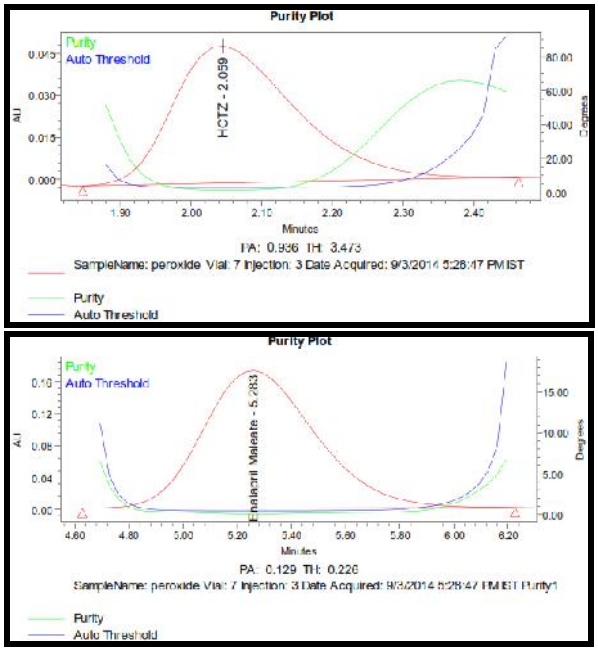


Figure 12: Purity Plots for HTZ and ENL in oxidative forced degradation

Photolytic degradation study: Photolytic degradation study was performed by exposing the drug content in UV light for 15mins to 7days. There is 2.822% degradation observed in above specific photolytic condition (Figure 13 & 14, Table 10 & 11).

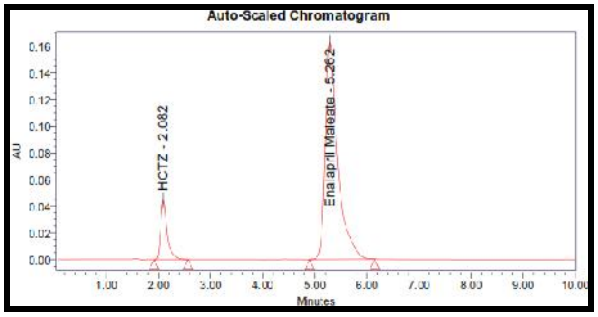
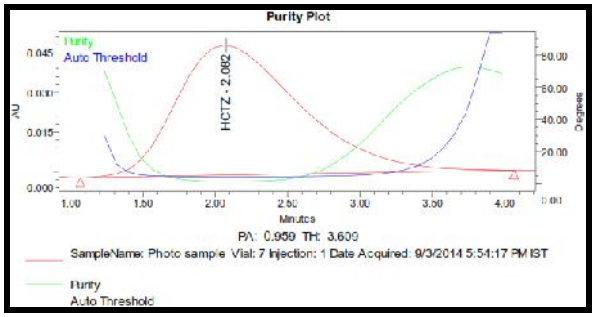


Figure 13: Chromatogram of UV-light degradation of HTZ and ENL



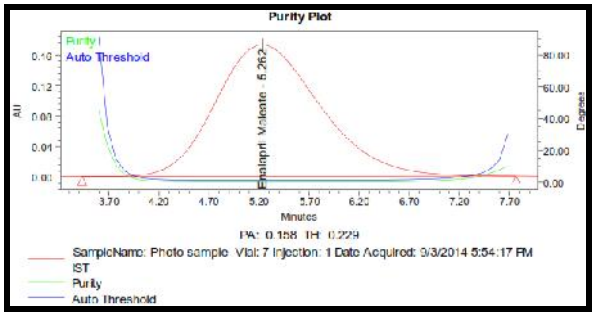


Figure 14: Purity Plots for HTZ and ENL in UV-light degradation

Thermal degradation study: Thermal degradation was performed by exposing solid drug at 80°C for 15mins to 60mins and at 220°C for 2-5mins. Resultant chromatogram of thermal degradation study (Figure 15 & 16, Table 10 & 11) indicate that drug is found to be slightly stable under thermal degradation condition. Only 7.273% drug content were degraded.

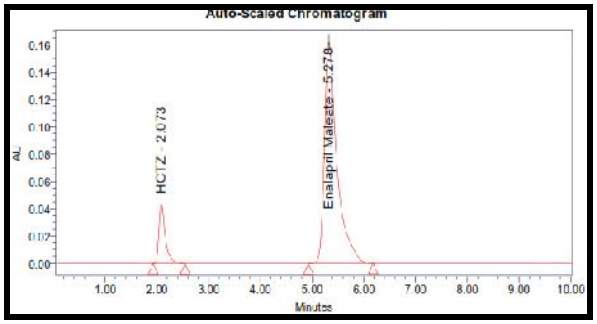


Figure 15: Chromatogram of thermal degradation of HTZ and ENL

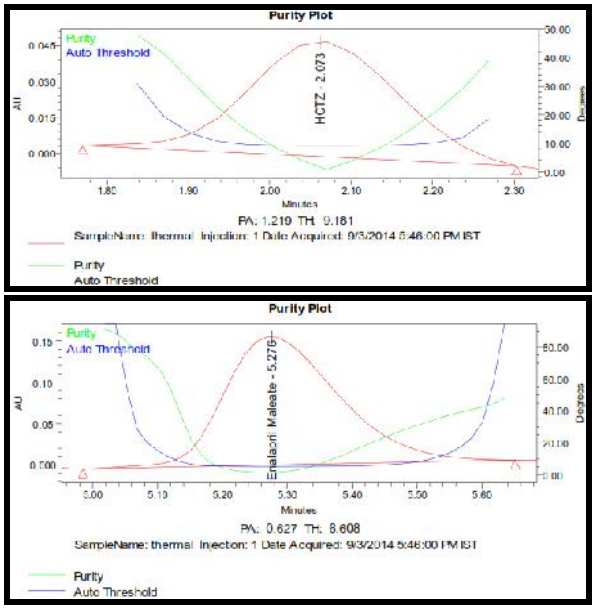


Figure 16: Purity Plots for HTZ and ENL in thermal degradation

Table 10: Peak purity results of HTZ and ENL

Stress	Purity Angle		Purity Threshold	
Condition	HTZ	ENL	HTZ	ENL
Acid Degradation	0.638	0.378	4.501	0.823
Alkali Degradation	0.551	0.157	4.172	0.230
Oxidative Degradation	0.936	0.129	3.473	0.226
Photolytic Degradation	0.959	0.158	3.609	0.229
Thermal Degradation	1.219	0.627	9.181	6.608

Table 11: Percentage of degradation of HTZ and ENL

Drug Name		Acid	Alkali	Oxidative	Photolytic	Thermal
HTZ	Std Area			379701		
	Sample Area	359116	359116	359116	359116	359116
	% of Degradation	5.421	5.421	5.421	5.421	5.421
ENL	Std Area			2723309		
	Sample Area	2619391	2619391	2619391	2619391	2619391
	% of Degradation	3.815	3.815	3.815	3.815	3.815
Average of % Degradation		4.160	4.618	4.618	4.618	4.618

CONCLUSION

A new RP-HPLC method described in this manuscript provides a simple, convenient and reproducible approach for the simultaneous estimation and quantification of Cilnidipine and Metoprolol Succinate in routine quality control analysis.

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