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DEVELOPMENT AND VALIDATION OF RP-HPLC AND UV METHODS FOR SIMULTANEOUS ESTIMATION OF ENALAPRIL MALEATE AND HYDROCHLOROTHIAZIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

To develop simple, accurate, precise and rapid reverse phase high performance liquid chromatography method and Two UV-Spectrophotometric methods have been developed for the simultaneous estimation of Enalapril maleate and Hydrochlorothiazide in bulk and pharmaceutical dosage form. In RP-HPLC analysis is carried out using Methanol: Acetonitrile (9:1, v/v) as the mobile phase at a flow rate of 0.5mL/min. this method includes Shimadzu LC-2010 instrument, Zodiac C_{18} (250 4.6mm, 5µm) column as stationary phase with detection wavelength of 238nm. Retention time of Enalapril maleate and Hydrochlorothiazide was found to be 2.340 & 2.992 respectively. The linearity of proposed method in the range of 1-5µg/mL (HPLC) & 2-10µg/mL (UV) for both Enalapril maleate and Hydrochlorothiazide respectively. The LOD of Enalapril and Hydrochlorothiazide was 0.091&0.072µg/mL respectively. The LOQ of Enalapril and Hydrochlorothiazide was 0.314&0.219µg/mL. The first UV method was determination using the Q-absorbance ratio method at 238nm & 262nm over the concentration range 2-10µg/mL respectively. The second UV method using determination of the Area under the curve method at 222-232 & 262-272nm over concentration range of 2-10µg/mL for Enalapril maleate and Hydrochlorothiazide respectively. The Regression equation of both drugs were 0.999.

KEYWORDS: Enalapril maleate, Hydrochlorothiazide, RP-HPLC, UV-Methods, Stability studies.

INTRODUCTION

Enalapril maleate is an angiotensin converting enzyme inhibitor. Chemically as (s)-1-[N-[1-(Ethoxycarbonyl)-3-phenyl propyl]-L-proline, (Z)-2-butenedioate salt.[1,2]

Normally, angiotensin I is converted to angiotensin II by angiotensin converting enzyme (ACE). Angiotensin II constricts blood vessels, increasing blood pressure. Enaloprilat the active metabolite of enalapril, inhibits ACE. Inhibition of ACE decreases levels of angiotensin II, leading to less vasoconstriction and decreased BP.[3]

Hydrochlorothiazide, is a first line diuretic drug of the thiazide class. Chemically as 6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfanamide 1,1-dioxide.

It reduces blood volume by acting on the kidneys to reduce sodium (Na^+) reabsorption in the distal convoluted tubule. The major site of action in the nephron appears on an electroneutral NaCl co-transporter by competing for the chloride site on the transporter. By impairing Na^+ transport in the distal convoluted tubule, hydrochlorothiazide induces a natriuresis and concomitant water loss. Thiazides increase the reabsorption of calcium in this segment in a manner unrelated to sodium transport. Additionally, by other mechanisms, HCTZ is believed to lower peripheral vascular resistance.

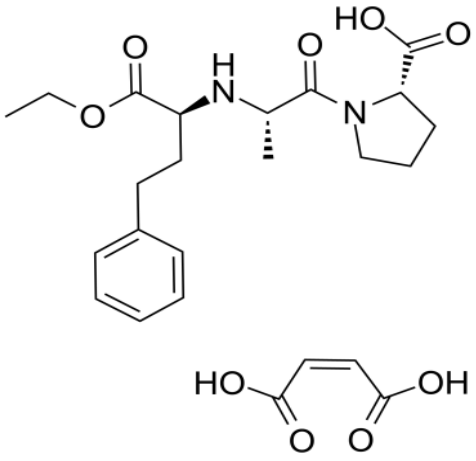

Fig. 1: Enalapril maleate

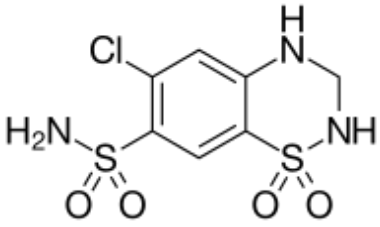

Fig. 2: Hydrochlorothiazide

MATERIALS AND METHODS

Chemicals& Reagents

Enalapril maleate and Hydrochlorothiazide procured from the KP Laboratories. Commercial pharmaceutical preparation Enalapril-HT, manufactured by Intas pharmaceuticals, containing 10mg of Enalapril maleate and 25 mg of Hydrochlorothiazide was collected from local market, Acetonitrile, methanol were used of analytical grade.

Instrumentation

The proposed was carried on a shimadzu UV-Visible Spectrophotometer (UV-1800 series). All weighing was done on Digital balance (Shimadzu). A fast clean Ultra sonicator was used for degassing the mobile phase.[4-7]

Selection of Solvents

On the basis of the solubility study Acetonitrile & Methanol was selected solvent for degassing the ENA& HCZ.

HPLC

Preparation of Standard solutions

Accurately weighed 10mg Enalapril maleate bulk and Hydrochlorothiazide bulk transferred into a 10mL clean and dry volumetric flask, add mobile phase and sonicate to dissolve and degas completely and make volume up to mark with the mobile phase.

Chromatographic conditions

The mobile phase consists of Methanol and Acetonitrile (HPLC grade). The chromatograph was operated in isocratic mode starting at mobile phase of methanol: acetonitrile (90:10 v/v). Eluent was delivered at a flow rate 0.5mL/min. Absorbance was monitored at λ_{max}=238nm.[8-10]

UV-SPECTROSCOPY

Preparation of Standard Solutions

Weigh accurately 10mg of ENA and HCZ separately into a 100ml volumetric flask. Add 10ml of solvent (or)mobile phase and shake well to dissolve the drug completely. Make up the volume to 100ml with solvent to get 100µg/ml of both ENA and HCZ.

Preparation of Sample Solution

15 Tablets were taken, crushed to fine powder. An accurately weigh powder sample equivalent to 10mg of Enalapril maleate powder as weighed and transferred to 100ml volumetric flask, dissolved in sufficient solvent and filtered through whatmann filter paper. The filtrate was made up to volume of 100ml with solvent get 100µg/mL of both ENA and HCZ.

Determination of λ_{max}

Standard solutions of ENA and HCZ were prepared and scanned in UV- spectrophotometer in the range of 200-400nm to determine the λ_{max} of each drug λ_{max} of ENA and HCZ were found to be 222nm and 262nm respectively.[11-13]

METHOD DEVELOPMENT

1. Q-Absorbance ratio method

According to Q-absorption ratio method, use the ratio of absorption at two selected wavelengths.

$C_x = \{(Q_m-Q_y)/ (Q_x-Q_y)\}* (A_1/ax_1)$

$C_y = \{(Q_m-Q_x)/ (Q_y-Q_x)\}* (A_1/ay_1)$

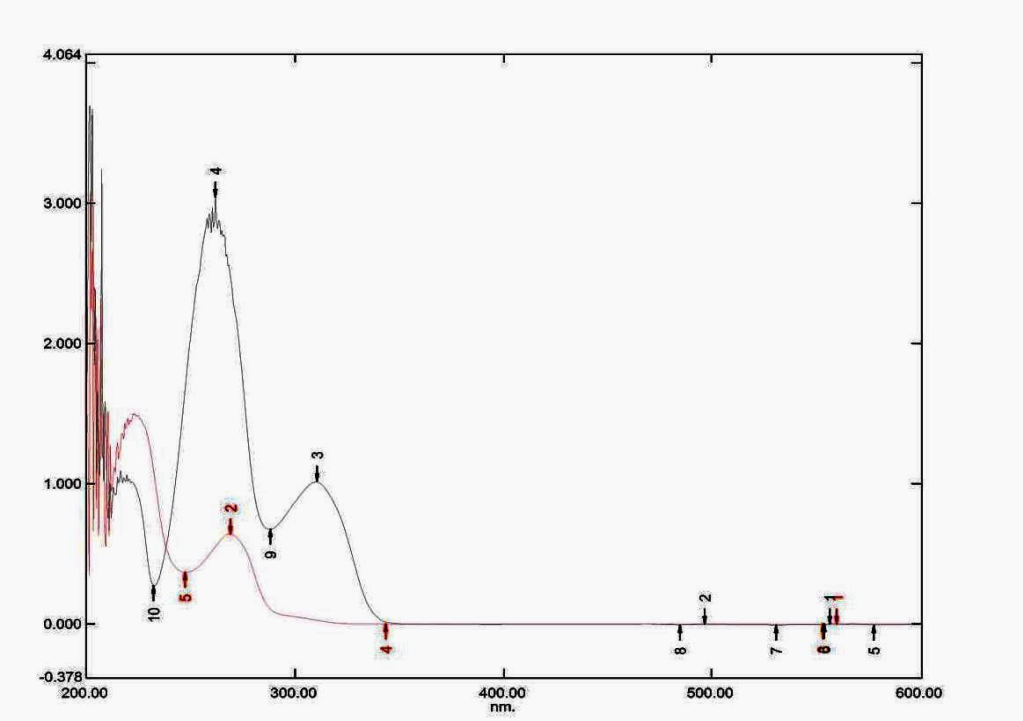

Fig. 3: Overlay UV spectrum of Enalapril maleate and Hydrochlorothiazide.

2. Area Under The Curve Method

The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained.

$$C^M = X^N_{\lambda1\text{-}\lambda2}\, AUC_{\lambda3\text{-}\lambda4} - X^N_{\lambda3\text{-}\lambda4}\, AUC_{\lambda1\text{-}\lambda2} / X^N_{\lambda1\text{-}\lambda2} = X^M_{\lambda3\text{-}\lambda4} - X^N_{\lambda3\text{-}\lambda4}\, X^M_{\lambda1\text{-}\lambda2}$$

$$C^N = X^M_{\lambda1\text{-}\lambda2}\, AUC_{\lambda3\text{-}\lambda4} - X^M_{\lambda3\text{-}\lambda4}\, AUC_{\lambda1\text{-}\lambda2} / X^N_{\lambda1\text{-}\lambda2} = X^M_{\lambda3\text{-}\lambda4} - X^N_{\lambda3\text{-}\lambda4}\, X^M_{\lambda1\text{-}\lambda2}$$

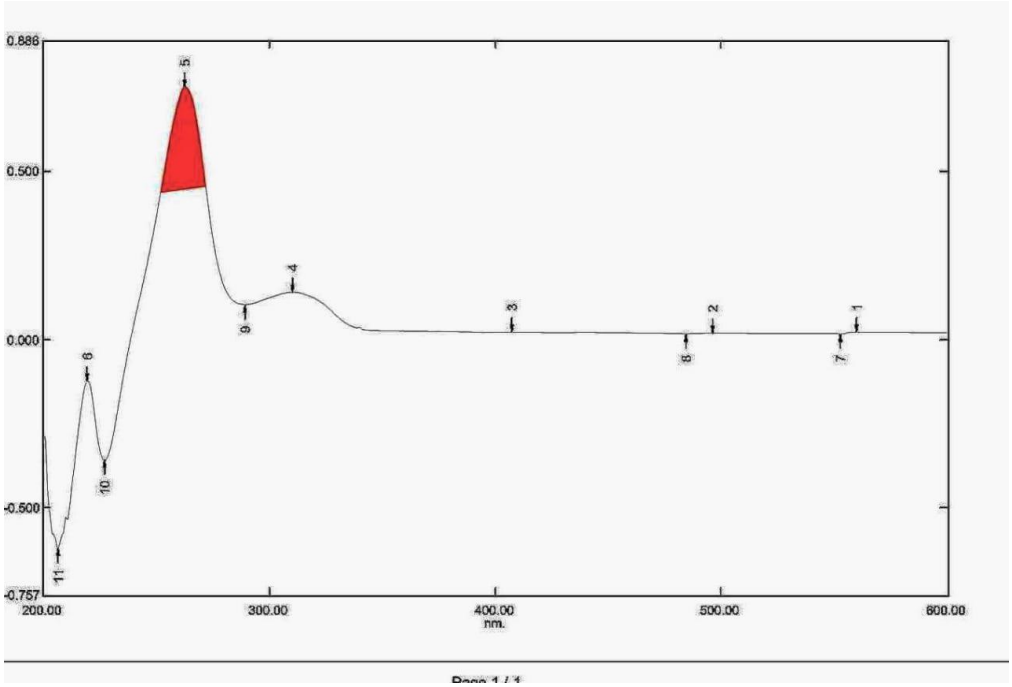

Fig. 4: Area under the curve of Hydrochlorothiazide.

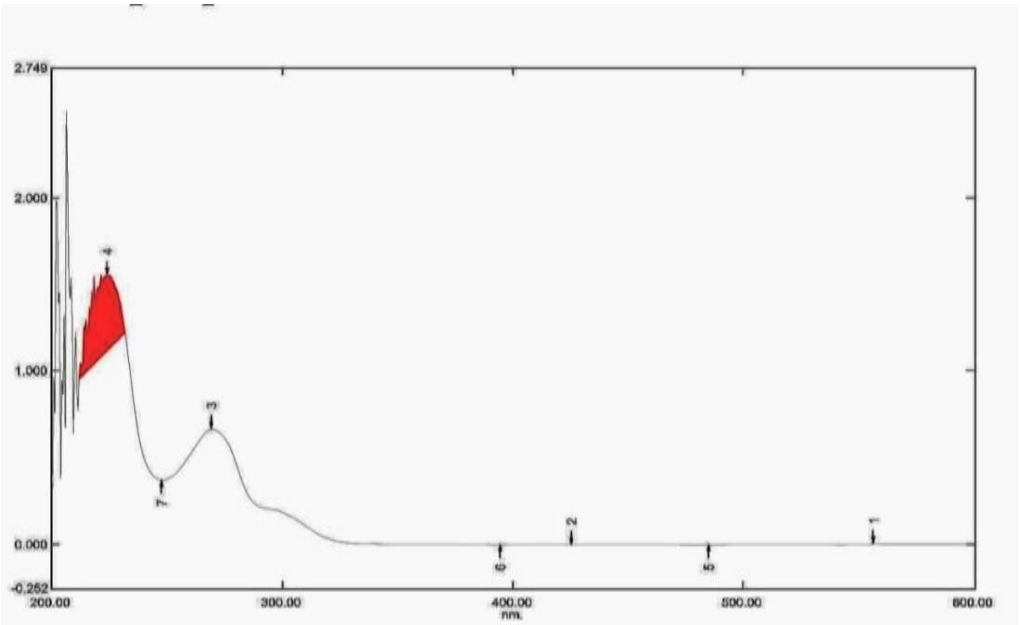

Fig. 5: Area under the curve of Enalapril maleate.

Validation of the Method

RP-HPLC and UV Spectroscopic method was validated according to International Conference on Harmonization (ICH) guidelines. The following characteristics were considered for validation: linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ).

Linearity

The methods were validated according to International conference on Harmonization guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte. Calibration curves were generated with appropriate volumes of working standard solutions for both UV and HPLC with the range of 1-5 and 2-10µg/mL respectively.

Accuracy and Precision

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as %RSD for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assay on 3 different days and the results documented as standard deviation and %RSD.

Accuracy is the percent of analyte recovered by assay from a known added amount.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as composition mobile phase ratio and wavelength and flow rate.

LOD and LOQ

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentration of ENA and HCZ. The LOD and LOQ values were calculated by using the following formula:

LOD = 3.3 X σ/S

LOQ = 10X σ/S

Where σ= the standard deviation of the response

S = Slope of calibration curve.

RESULTS AND DISCUSSION

Table 1: Q-Absorbance values of Enalapril maleate and Hydrochlorothiazide.

Concentration (µg/mL)	ENA 262nm	ENA 238nm	HCZ 262nm	HCZ 238nm
2	0.105	0.102	0.17	0.12
4	0.198	0.185	0.32	0.254
6	0.301	0.285	0.494	0.397
8	0.398	0.379	0.641	0.483
10	0.498	0.456	0.798	0.619

The two wavelength were used for the analysis of the drugs were 238nm (isoabsorptive point) and 262 nm(λmax of HCZ) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of ENA (222nm) and HCZ (262nm) in methanol is shown in Fig.3.

Table 2: Area under the curve of Enalapril maleate and Hydrochlorothiazide.

Concentration(µg/mL)	AUC of ENA	AUC of HCZ
2	0.05455	0.07654
4	0.11983	0.15041
6	0.19231	0.25621
8	0.24327	0.30411
10	0.29940	0.39872
12	0.34672	0.47521
Linearity	0.029X+0.01	0.039X+0.02
R^2	0.996	0.996

Area under curve values for both ENA and HCZ of 2-10µg/mL concentrations were noted. The AUC values for ENA and HCZ of concentration 2µg/mL was found to be 0.054551 and 0.07654 respectively.

Method Development

A series of trails were carried out using different ratios of mobile phases such as Methanol: Water (5:5 v/v), Acetonitrile: Methanol (7:3 v/v), Methanol: Acetonitrile (5:5 v/v) and change in flow rate method for simultaneous estimation of Enalapril maleate and Hydrochlorothiazide in marketed tablet dosage form. Finally, a typical chromatogram was obtained using Methanol: Acetonitrile as mobile phase in a ratio of 9:1 v/v on Zodiac C_{18} (250 mmX4.6 mm, 5µ) column and injection volume of 20µL. The flow rate was 0.5 mL/min and the run time was 7 min. The column temperature was 30 and detection was carried out at 238nm. The retention time was 2.340 and 2.992 min for Enalapril maleate and

Hydrochlorothiazide respectively. Typical chromatograms of standard and sample solutions of Enalapril and Hydrochlorothiazide are shown in Fig.4&5.[14-16]

The optimized chromatographic conditions are tabulated

Instrument: HPLC SHIMADZU, Column: C 18 (250 X 4.6mm, 5µm), Mobile phase: Methanol : Acetonitrile (9:1 v/v), Flow rate: 0.5 mL/min, Detector : UV-detector, Injector: Rheodyne injector, Type of elution : Isocratic, Run time: 7min.

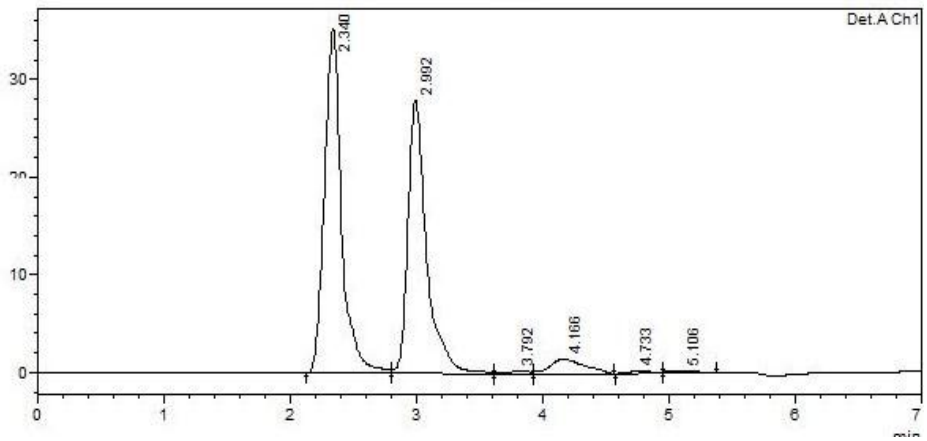

Fig. 6: Chromatogram of Enalapril maleate and Hydrochlorothiazide.

Linearity

Table 3: Linearity results of Enalapril maleate and Hydrochlorothiazide.

HPLC		UV-Spectroscopy				HPLC		UV-Spectroscopy			
Enalapril maleate		Enalapril maleate				Hydrochlorothia-zide		Hydrochlorothiazide			
Concen-tration (µg/mL)	Peak area of ENA	Concentration (µg/mL)	ENA 262 nm	ENA 238 Nm	ENA 222 nm (AUC)	Concentration (µg/mL)	Peak Area of HCZ	Concen-tration (µg/mL)	HCZ 262 nm	HCZ 238 nm	HCZ 262nm (AUC)
1	10724	2	0.10	0.10	0.0545	1	97198	2	0.17	0.12	0.0765
2	21517	4	0.19	0.18	0.1198	2	18412	4	0.32	0.25	0.1504
3	31243	6	0.30	0.28	0.1923	3	27171	6	0.49	0.39	0.2562
4	41718	8	0.39	0.37	0.2432	4	35171	8	0.64	0.48	0.3041
5	50913	10	0.49	0.45	0.2984	5	44168	10	0.79	0.61	0.3987

The linearity of Enalapril & Hydrochlorothiazide was in the range of 1-5µg/mL (HPLC) & 2-10µg/mL (UV). After this concentration deviation in Beer's law has been occurred. The data was represented in Table 3.

Accuracy

Table 4: Accuracy values of Enalapril maleate and Hydrochlorothiazide.

HPLC					UV-Spectroscopy						
Level of Recovery	Recovery Of ENA	Recovery Of HCZ	MeanRecovery Of ENA	MeanRecovery Of HCZ	Methods	HCZ			ENA		
						Amount taken	Amount found	% Recovery	Amount taken	Amount found	% Recovery
15%	110%	150%	95%	125%	Method-A	5	4.54	100.02	5	4.52	99.4%
20%	82%	122%				10	8.99	99.9	10	8.90	98.0%
						15	15.02	99.0	15	14.8	101.0%
25%	75%	117%			Method-B	5	5.092	101.4	5	4.92	99.4%
						10	9.89	99.0	10	10.2	102.5%
						15	15.20	101.4	15	14.8	98.8%

The % Recovery of Enalapril maleate is 95% (HPLC) & 99.6% (UV) and Hydrochlorothiazide is 125% (HPLC) & 99.4% (UV). The data was represented in Table 4.

Precision

Table 5: Precision values of Enalapril maleate &Hydrochlorothiazide.

HPLC		UV-Spectroscopy				HPLC		UV-Spectroscopy			
Enalapril maleate		Enalapril maleate				Hydrochlorothia-zide		Hydrochlorothiazide			
Concen-tration (µg/mL)	Peak area of ENA	Concentration (µg/mL)	ENA 262 nm	ENA 238 Nm	ENA 222 nm (AUC)	Concentration (µg/mL)	Peak Area of HCZ	Concen-ration (µg/mL)	HCZ 262 Nm	HCZ 238 nm	HCZ 262nm (AUC)
5	509139	10	0.498	0.456	0.2984	5	44168	10	0.798	0.599	0.398
5	508724	10	0.499	0.458	0.2989	5	44279	10	0.795	0.592	0.398
5	506352	10	0.494	0.452	0.2979	5	44532	10	0.792	0.596	0.399
5	509242	10	0.496	0.459	0.2980	5	44574	10	0.799	0.594	0.398
Average	509139	Average	0.413	0.380	0.248	Average	4438.8	Average	0.663	0.496	0.332
S.D	1360.0	S.D	0.002	0.001	0.0012	S.D	1962.6	S.D	0.003	0.002	0.0016
%RSD	0.267	%RSD	0.483	0.489	0.482	%RSD	0.441	%RSD	0.488	0.472	0.487

The %RSD of Enalapril maleate and Hydrochlorothiazide was found to be < 2, which indicates that the developed are highly precise and reproducible. The data was represented in Table 5.

Robustness

Table 6: UV values of Enalapril maleate &Hydrochlorothiazide (change in wavelength).

ENA							HCZ						
Concentration (µg/mL)	262nm		238nm		222nm		Concentration (µg/mL)	262nm		238nm		262nm (AUC)	
	260 Nm	265 Nm	230 nm	235 nm	220 nm	225 nm		260 nm	265 Nm	230 nm	235 Nm	264 Nm	266 nm
10	0.462	0.504	0.40	0.432	0.298	0.342	10	0.79	0.810	0.629	0.657	0.3682	0.399
10	0.469	0.527	0.40	0.437	0.299	0.346	10	0.78	0.820	0.635	0.656	0.3690	0.421
10	0.475	0.535	0.41	0.440	0.301	0.354	10	0.78	0.824	0.639	0.674	0.3741	0.425
10	0.480	0.539	0.42	0.449	0.302	0.364	10	0.77	0.804	0.644	0.678	0.3782	0.435
10	0.487	0.551	0.42	0.451	0.313	0.367	10	0.78	0.807	0.649	0.683	0.3834	0.438
Mean	0.474	0.531	0.41	0.441	0.303	0.354	Mean	0.78	0.809	0.639	0.669	0.3746	0.426
%RSD	0.20	0.32	0.29	0.18	0.192	0.324	%RSD	0.59	0.92	0.12	0.18	0.92	1.42

Table 7: HPLC Robustness values of Enalapril maleate and Hydrochlorothiazide.

Change in Flow rate (mL/min)					Change in Mobile phase ratios				
S.NO	Mobile phase ratio(v/v)	Flow rate (mL/min)	%RSD of ENA	%RSD of HCZ	S.NO	Mobile phase ratio(v/v)	Flow rate (mL/min)	%RSD of ENA	%RSD of HCZ
1	9:1	0.7	0.291	0.576	1.	8.5:1.5	0.7	0.445	0.674
2	9:1	1	0.452	0.765	2.	8:2	1	0.787	0.998

The results of robustness study of the developed methods are established in Tables 6 &7. Since %RSD is< 2, the developed methods are robust.

LOD and LOQ

Table 8: LOD & LOQ values of Enalapril maleate & Hydrochlorothiazide.

HPLC			UV-Spectroscopy					
LOD (µg/mL)	0.091µg/mL of ENA	0.072µg/mL of HCZ	Method-A		Method-B	Method-A		Method-B
			262nm	238nm	222nm	262nm	238nm	262nm
LOQ (µg/mL)	0.314µg/mLof ENA	0.219µg/mL HCZ	0.136 of ENA	0.136 of ENA	0.101 of ENA	0.135 of HCZ	0.133 of HCZ	0.182 of HCZ
			0.412 of ENA	0.448 of ENA	0.307 of ENA	0.410 of HCZ	0.405 of HCZ	0.555 of HCZ

The LOD and LOQ values of ENA and HCZ are reported in the Table 8. These data shows that the methods developed are highly sensitive and specific.

Assay

Table 9: Assay values of Enalapril maleate & Hydrochlorothiazide.

HPLC			UV-Spectroscopy		
Drug	**Retention time(min)**	**% Purity**	**Dosage Form**	**Method-A**	**Method-B**
Enalapril maleate	3.005	99.9%	Enalapril maleate	97.70%	97.8%
Hydrochlorothiazide	4.194	99.8%	Hydrochlorothiazide	98.8%	96.5%

The % purity of Enalapril maleate and Hydrochlorothiazide were found to be 99.9% (HPLC) & 97.7-97.8% (UV) and 99.8% (HPLC) & 98.8-96.5% (UV). The data was represented in Table 9.

Table 10: Forced degradation studies.

Stress Degradation condition	Area under the curve		%Assay purity	Active drug present after Degradation (%)
	ENA	HCZ		
Standard drug	3.876	4.108	0	100
Acid degradation	1.843	2.051	64.31	57.3
Base degradation	0.932	1.123	79.98	34.4
Peroxide oxidation	1.234	2.032	61.67	44.8
Photolytic degradation	2.721	3.095	12.2	77.8

Forced degradation of Enalapril-HT was performed as per ICH guidelines in various conditions like alkaline, acidic, oxidation and photolytic degradation. The results in Table 10. Drug degradation was confirmed by observing the changes in the area of sample spectrum and degraded spectrums. It was found that the degradation in Base condition is greater than the other degradation conditions.

CONCLUSION

A simple, sensitive, fast, economical method has been developed using method as mobile phase. It can be concluded that the developed RP-HPLC method represents a good technique for the simultaneous determination of Enalapril maleate and Hydrochlorothiazide in bulk and pharmaceutical dosage form with good sensitivity, precision and reproducibility. The proposed study describes two different spectrophotometric methods for the simultaneous estimation of Enalapril maleate and Hydrochlorothiazide in bulk and pharmaceutical dosage form; Q-absorbance ratio method, Area under the curve methods. The proposed UV spectrophotometric methods are simple, fast, sensitive, accurate, precise, less time-consuming and economic. They are validated in terms of Linearity, Precision, Accuracy, Robustness, LOD and LOQ. All the parameters were found to be within limits according ICH guidelines.

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