Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(7):3483-3488

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Simultaneous UV spectrphotometric estimation of enalapril maleate and hydrochlorothiazide in tablets

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ABSTRACT

A simple, rapid and sensitive UV spectrophotometric method was developed for the simultaneous estimation of Enalapril maleate (ENM) and Hydrochlorothiazide (HCTZ) in pharmaceutical dosage forms. This method employs solving of simultaneous equations based on the measurement of absorbance at the λ_{max} values of ENM (207nm) and HCTZ (272nm).Calibration curves were linear in the range of 5-50µg/mL and 5-30µg/mL for ENM and HCTZ respectively. Recovery studies for ENM and HCTZ were performed and the percentage recovery for both the drugs was obtained in the range of 98.4-99.1 % and 98.8-99.1 % respectively. The method showed good reproducibility and recovery with % RSD less than 2. The proposed method can be successfully applied for the determination of ENM and HCTZ in pharmaceutical formulations and is validated according to ICH guidelines.

Keywords: Enalapril maleate, Hydrochlorothiazide, UV spectrophotometry, Simultaneous equations, Validation, ICH.

INTRODUCTION

Enalapril maleate [1] is the maleate salt of enalapril, the ethyl ester of a long-acting angiotensin converting enzyme inhibitor, enalaprilat. ENM is chemically described as (S)-1-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline, (Z)-2-butenedioate salt (1:1). Enalapril is a pro-drug which following oral administration is bioactivated by hydrolysis of the ethyl ester to enalaprilat, the active angiotensin converting enzyme inhibitor [2]. Its structural formula is:

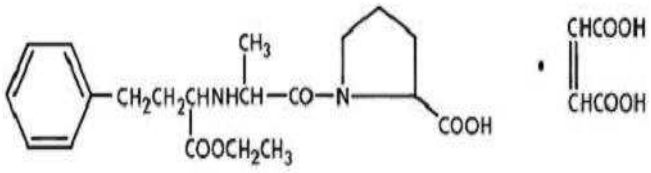


Fig 1: Chemical Structure of Enalapril maleate (ENM)

Hydrochlorothiazide [1] is 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide. HCTZ is a diuretic and antihypertensive. It affects the distal renal tubular mechanism of electrolyte reabsorption. HCTZ increases excretion of sodium and chloride in approximately equivalent amounts. Its structural formula is:

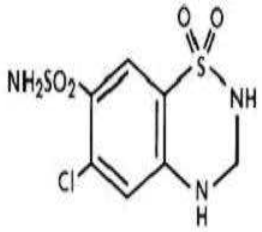


Fig 2: Chemical Structure of Hydrochlorothiazide (HCTZ)

ENM and HCTZ are often used in combination to achieve better curative effects in case of hypertension and hence there is a need for developing a simple and rapid spectroscopic method for the simultaneous estimation of these two drugs in marketed formulations. Literature survey revealed that several analytical methods were developed for the estimation of ENM and HCTZ separately but very few methods were reported for the simultaneous estimation of these two drugs in combined dosage forms which includes Derivative spectroscopy [3], Chromatography [4-6] and Bio analytical methods [7].Hence an attempt has been made to develop a simple and rapid spectroscopic method for the simultaneous determination of ENM and HCTZ without prior separation in commercial tablets and validated as per the ICH guidelines [8].

EXPERIMENTAL SECTION

Instrumentation

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to a computer loaded with spectra manager software UV Probe was employed with a spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Shimadzu AUX 220, Japan).

Chemicals and reagents

All chemicals were of analytical reagent grade and solutions were prepared using double distilled water. Potassium dihydrogen ortho phosphate, Sodium hydroxide and Methanol were purchased from Merck. ENM and HCTZ were obtained as gift samples from Dr.Reddy's Laboratories Ltd, India. Combined tablets of ENM and HCTZ were procured from the local pharmacy.

Preparation of Phosphate buffer (pH 6.8):

Accurately weighed about 0.896 gm of Sodium hydroxide and 6.804 gm of Potassium dihydrogen phosphate were dissolved in sufficient water and the volume made up in a 1000 mL volumetric flask.

Preparation of stock solution :(1000µg/mL)

Accurately weighed quantity of pure ENM (10mg) and pure HCTZ (10mg) were transferred into two separate 10mL volumetric flasks, dissolved in methanol and made up the volume to 10mL with the same solvent. The stock solution was sonicated for 2min.

Preparation of working standard solution :(100µg/mL)

From the above stock solution 1mL each of ENM and HCTZ was taken, transferred to separate 10mL volumetric flasks and the volume was made up to 10 mL with phosphate buffer (pH 6.8)

Simultaneous equation's method:

10µg/mL each of ENM and HCTZ was prepared separately in phosphate buffer p^H 6.8 and the solutions scanned in the UV region from 200-380 nm. UV spectrum of ENM and HCTZ was recorded and it was found that drugs showed maximum absorbance at 207 nm and 272 nm respectively. Hence these wavelengths were chosen as the λ_{max} values for each drug respectively (Fig 3).

Standard solutions of ENM and HCTZ in the concentration range of 5-50 µg/mL and 5-30 µg/mL respectively were prepared in phosphate buffer pH 6.8 and the absorbance of these solutions was measured at 207 nm and 272 nm against blank. Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. Two simultaneous equations [9] as below were formed using these absorptivity values, A (1%, 1cm).

$$A_1 = 612bC_X + 762bC_Y$$

$$A_2 = 4.5bC_X + 781bC_Y$$

Where, C_X and C_Y are the concentrations of ENM and HCTZ measured in gm/100mL in sample solutions. A_1 and A_2 are the absorbances of mixture at selected wavelengths 207 nm and 272 nm respectively.

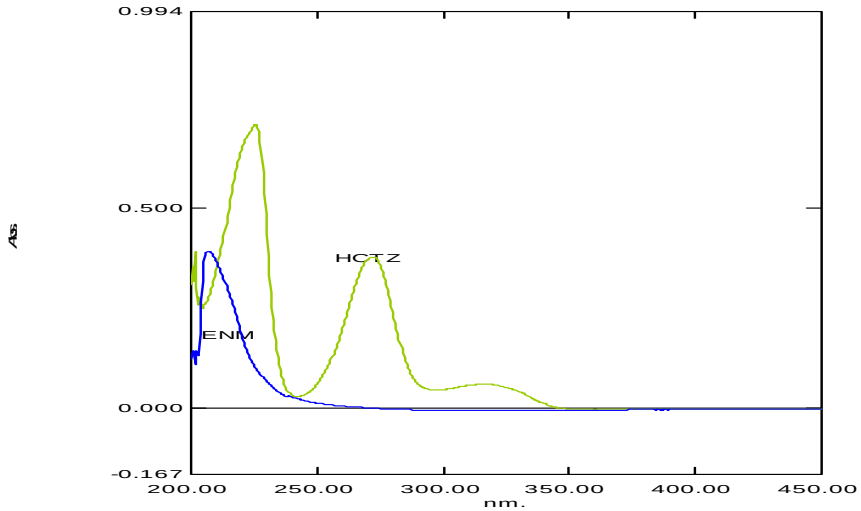


Fig 3: **Overlay Spectrum of Enalapril maleate (5µg/mL) and Hydrochlorthiazide (5µg/mL)**

Table 1: Absorptivity values (A 1%, 1 cm) for ENM and HCTZ

Conc (µg/ml)	Absorptivity (A 1%, 1 cm)			
	ENM		HCTZ	
	207nm	272nm	207nm	272nm
5	606	4	762	830
10	616	4	767	809
15	606	4.67	782	766
20	586	4.50	765.5	765
25	630	4.40	755.6	754
30	603	4.67	742	760
35	615	4.57	-	-
40	610	4.50	-	-
45	629	4.67	-	-
50	619	4.60	-	-
Mean	612	4.50	762	781

Analysis of Tablets:

Twenty tablets (VASERETIC®, ENVAS-H®) were weighed and finely powdered. Accurately weighed portion of this powder equivalent to 10 mg of HCTZ was transferred to a 10mL volumetric flask containing 5 mL of methanol. The contents of flask were sonicated for 10 mins for complete solubility of drug and the volume was made up with methanol. Then the mixture was filtered thoroughly using What man filter paper. 1mL of this filtrate was further diluted to 10mL with phosphate buffer. From the above tablet solution 1mL was taken in a 10mL volumetric flask and volume was made up with phosphate buffer pH 6.8 to obtain ENM and HCTZ in the ratio of formulation.

Method validation

a)Linearity:

Linearity was performed by preparing standard solutions of ENM and HCTZ at different concentration levels. Aliquots of the working standard solution for ENM and HCTZ were taken in the range of 0.5mL-5.0mL and 0.5mL-3.0mL to obtain final concentrations in the range of 5-50 µg/mL and 5-30 µg/mL respectively. Absorbance was measured individually for ENM and HCTZ solutions at the fixed wavelengths of 207 nm and 272 nm against phosphate buffer p^H 6.8 as blank. In each case the absorbance was plotted against the concentration to obtain a linearity plot individually for ENM and HCTZ at 207 nm and 272 nm. The regression of the plot was computed by least square regression method.

b)Precision:

Precision study was carried out for the method at three different concentration levels (6, 8, 10µg/mL), each concentration prepared three times for ENM and HCTZ in the ratio of the formulation. The absorbance of each

solution was measured at 207 and 272nm. Assay calculations were carried out at each level from the absorbance values obtained. The results of statistical analysis of precision are reported in terms of relative standard deviation.

b)Accuracy:

The accuracy of the method was determined by standard addition method at 80%, 100% and 120%. Known concentrations of standard drug solutions were added to fixed concentration of pre analyzed tablet solution. The solutions were prepared in triplicate at each level and absorbance of all the solutions was measured at 207 nm and 272 nm. The percent recovery and relative standard deviation at each level was calculated.

RESULTS AND DISCUSSION

The overlain spectra of ENM and HCTZ reveal that they exhibit maximum absorbance at 207 nm and 272 nm respectively while contributing some absorbance at the other λ max. Hence these two wavelengths were selected for simultaneous estimation of ENM and HCTZ. Calibration curves for ENM and HCTZ at both the wavelengths were obtained by plotting concentration Vs absorbance, regression analysis was performed and the optical characteristics summarized in Table 2.

Table-2: Regression analysis of calibration curves, summary of validation parameters

S.No	Parameter	ENM		HCTZ	
		207nm	272nm	207nm	272nm
1	Beer's law limit (µg ml^{-1})	5-50	5-50	5-30	5-30
2	Molar absorptivity (l mol^{-1} cm^{-1})	30145	219	22698	23242
3	Sandell's sensitivity (µg/cm^2/0.001)	0.0165	2.5	0.01303	0.01314
4	Intercept(c)	0.062	-	0.017	0.075
5	Slope (m)	-0.017	-	0.074	0.027
6	Correlation coefficient (r^2)	0.999	0.998	0.999	0.999

ENM obeyed Beer's law in the concentration range of 5-50 µg/mL (0.999, 0.998) while HCTZ obeyed the Beer's law in the concentration range of 5-30 µg/mL (0.999, 00.999) at both the wavelengths. The calibration curves are shown in Figures 4-7.

Figure 4: Linearity plot of ENM at 207nm

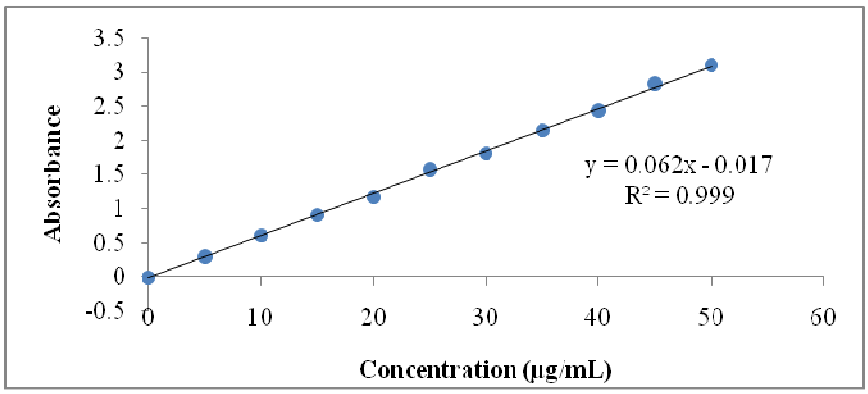


Figure 5: Linearity plot of ENM at 272nm

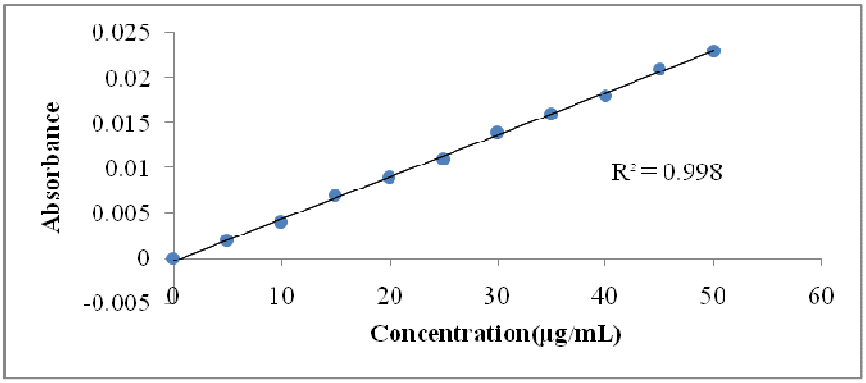


Figure 6: Linearity plot of HCTZ at 207nm

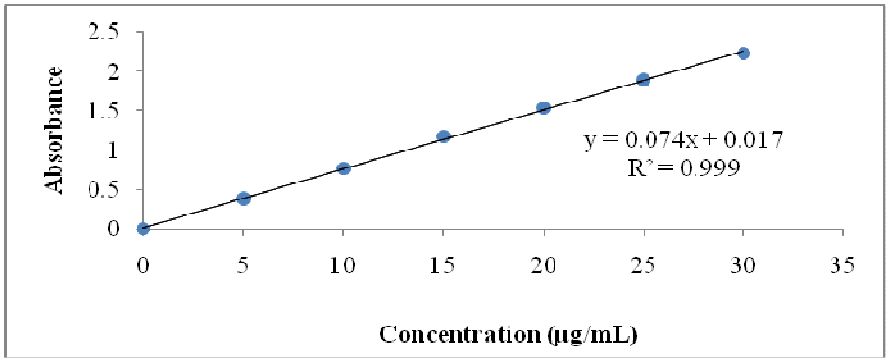
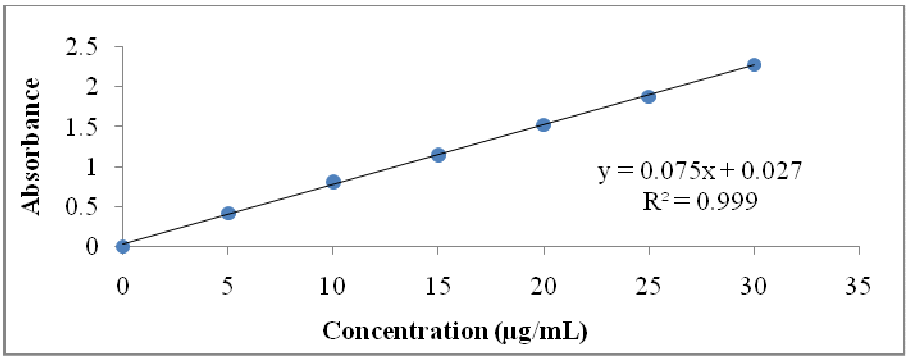


Figure 7: Linearity plot of HCTZ at 272nm



The RSD values in precision studies were found to be 0.38-0.59 and 0.34-0.62 for ENM and HCTZ respectively which are less than 2.0 % indicating that the method is more precise. The results are shown in Table 3.

Table 3: Results for Precision studies

S.No	Conc (µg/mL)		*Assay(%) ± SD		*RSD (%)	
	ENM	HCTZ	ENM	HCTZ	ENM	HCTZ
1	6	15	98.7±0.58	98.9±0.34	0.59	0.34
2	8	20	98.7±0.43	99.3±0.61	0.44	0.62
3	10	25	99.1±0.38	98.6±0.48	0.38	0.49

**Mean of three estimations; ENM = Enalapril maleate; HCTZ = Hydrochlorothiazide*

The recovery studies done by standard addition method had given satisfactory results with an average percentage recovery of 98.4-99.1% and 98.8-99.1% for ENM and HCTZ respectively. The results are shown in Table 4.

Table 4: Results for Recovery studies

Level of Recovery (%)	Drug in tablet (µg)		Drug added (µg)		*Drug recovered (µg)		Recovery(%)	
	ENM	HCTZ	ENM	HCTZ	ENM	HCTZ	HCTZ	ENM
80	4	10	3.2	8	7.11	17.8	98.9±0.13	98.6±0.17
100	4	10	4	10	7.93	19.82	99.1±0.26	99.1±0.18
120	4	10	4.8	12	8.66	21.74	98.8±0.45	98.4±0.42

**Mean of three estimations; ENM = Enalapril maleate; HCTZ = Hydrochlorothiazide*

Table 6: Assay of commercial formulations

S.No	**Brand**	**Label claim (mg)**		**Amount obtained(mg)**		***Recovery(%)±SD**	
		ENM	HCTZ	ENM	HCTZ	ENM	HCTZ
1.	Brand I	10	25	9.87	24.71	98.7±0.58	98.8±0.76
2.	Brand II	5	12.5	4.96	12.39	99.2±0.65	99.1 ±0.57

**Mean of three estimations; ENM = Enalapril maleate; HCTZ = Hydrochlorothiazide*

The method was applied for the assay of ENM and HCTZ in a combined tablet. The results obtained for the assay of ENM and HCTZ in commercial tablets by the proposed method were comparable with the corresponding label claim. The results of assay given in Table 6.

CONCLUSION

The present proposed method can be successfully employed for the determination of Enalapril maleate and Hydrochlorothiazide in pharmaceutical formulations and the method is validated as per the ICH guide lines.

Acknowledgement
The authors are grateful to M/S GITAM University for providing necessary research facilities to carry out the research work and to Dr.Reddy's Laboratories Ltd, India for providing the gift samples of the drugs.

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