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SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF ENALAPRIL MALEATE AND LOSARTAN POTASSIUM IN TABLET DOSAGE FORM AND IT'S APPLICATION TO DISSOLUTION STUDY

Thomas AB*, Chaudhari AA, Nanda RK, Kothapalli LP, Chavan UB and Deshpande AD.
Department of Pharmaceutical chemistry, Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune - 411 018, Maharashtra, India. Phone No. - 9881236220

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ABSTRACT
Two spectrophotometric methods have been developed for the simultaneous estimation of Enalapril Maleate (Ena) and Losarton Potassium (Los) in combined tablet dosage forms. The first method involves determination using the absorbance correction method, the sampling wavelengths selected are, 222 nm and 250 nm over the concentration ranges of 2-32 mcg/mL and 1-60 mcg/mL for Ena and Los respectively. The second method is the second order derivative method, the sampling wavelength selected for estimation of Ena and Los are 219.5 nm and 264 nm which show linearity in the concentration ranges of 2-32 mcg/mL and 1-60 mcg/mL respectively. The results of the analysis were validated statistically and recovery studies were carried out as per ICH guidelines. Also the developed methods were successfully employed for dissolution studies in tablet dosage form.

Keywords: *Enalapril; Losartan; Absorbance correction method; Second order derivative method; dissolution studies.*

INTRODUCTION

Enalapril Maleate (Ena) is an angiotensin converting enzyme inhibitor which is official in B. P.[1] and U.S.P.[2] Losartan Potassium (Los) belongs to the angiotensin II inhibitor class of drugs and is official in I.P.[3] Literature survey reveals several methods such as HPLC [4-8] and U.V. spectrosctropy [9-12] which have been reported for the estimation of individual drugs as well as in combination with other drugs. Not a single UV or HPLC method is reported so far for the simultaneous analysis of Ena and Los in their combined dosage form. Ena and Los are available in combined tablet dosage form as antihypertensive agents. So a need was felt to develop new methods to analyze the drugs simultaneously. An attempt has been made to estimate the two drugs simultaneously by spectrophotometric analysis. This paper describes two methods for the simultaneous determination of Ena and Los in tablet formulations using absorbance correction method and second order derivative method.

MATERIAL AND METHODS

A Shimadzu UV/Visible spectrophotometer, model 1700 (Japan) was employed with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction employing a pair of quartz cells. Enalapril Maleate (Intas Pharmaceutical Pvt. Ltd.) and Losartan Potassium (Cipla Ltd) were used in the study. The tablets were procured from the market (ENVAS – RB 25, Cadila Pharma).

EXPERIMENTAL

Preparation of standard stock solution

Standard stock solutions (100µg/mL) of Ena and Los were prepared by dissolving separately 10 mg of drug each in distilled water.

Preparation of sample stock solutions

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed powder sample equivalent to 10 mg of Losartan was transferred to a 100ml volumetric flask and dissolved in 50 ml of distilled water. After the immediate dissolution, the volume was made up to the mark with same solvent. The solution was sonicated for about 5 mins and was then filtered through Whatmann filter paper No.41. The solution was suitably diluted with distilled water to obtain sample solutions containing Ena and Los in the concentrations ratio of 5:25 mcg/mL respectively.

Method A: Absorption correction method [13]

Standard solutions (10 µg/mL) of Ena and Los were scanned in spectrum mode of the instrument from 400 to 200 nm. The overlain spectrum of the two drugs (Fig. 1) indicated that both the drugs exhibited strong absorbance at about 222 nm. However Los exhibited strong absorbance at 250 nm, at which Ena showed zero absorbance. Hence 250 nm was selected for the determination of Los without interference of Ena. The linearity range for Ena is 2-32 µg/mL at 222 nm with a co-efficient of correlation of 0.998. Los exhibits linearity

***Correspondence :** dypharmachem@yahoo.co.in

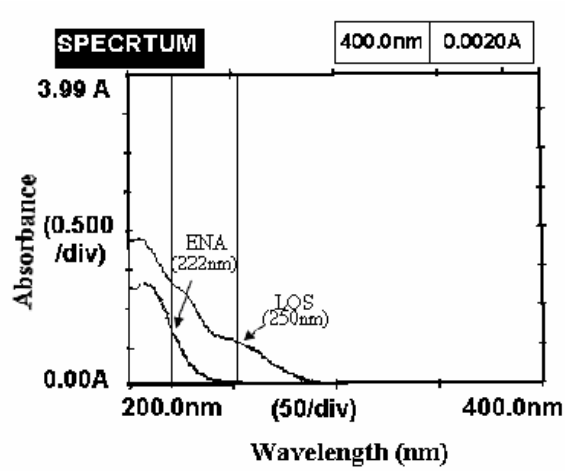

Fig. 1: *Overlain spectra of ena and los in absorbance correction method*

over a concentration range of 1 -60 µg/mL both at 222 nm and 250 nm. The co-efficient of correlation for Los were found to be 0.9998 and 0.9990 at 222 nm and 250 nm respectively.

Absorbances of both the drugs recorded were found to be practically additive at 222 nm. An accurate estimation of Ena at 222 nm has been achieved after correction for absorption of Los. The molar absorptivity values for each drug at selected wavelengths were calculated. Since Ena does not absorb at 250 nm, the concentration of Los at 250 nm is given by the formula

$A_{Los250} = \varepsilon_{Los250} \times b \times C_{Los}$

$C_{Los} = A_{Los250} / 1 \times (1.436 \times 10^4)$ ..... (1)

The absorbance of Los at 222 nm was calculated as,

$A_{Los222} = \varepsilon_{Los222} \times b \times C_{Los}$

$A_{Los222} = (3.0834 \times 10^4) \times 1 \times C_{Los}$ ..... (2)

The corrected absorbance of Ena at 222 nm was found to be –

Corrected absorbance of

Ena at 222 nm = $A_{222} - A_{Los222}$ ..... (3)

The corrected absorbance of Ena was then substituted in the formula given below to determine the concentration of Ena.

$A_{Ena222} = (2.804 \times 10^3) \times 1 \times C_{Ena}$ ..... (4)

Where, A_{Los250} and A_{Los222} – Absorbances of Los at 250 and 222 nm respectively,

ε_{Los250} and ε_{Los222} – Molar absorptivity of Los at 250 and 222 nm respectively,

C_{Los250} and C_{Los222} – Concentrations of Los at 250 and 222 nm respectively,

A_{250} and A_{222} – Absorbance of standard mixture at 250 and 222 nm respectively,

A_{Ena} – Absorbance of Ena at 222 nm,

ε_{Ena222} – Absorptivity of Ena at 222 nm,

C_{Ena} – Concentration of Ena at 222 nm.

Estimation from marketed preparation

Suitable dilutions of tablet sample solution were scanned in the range of 400–200 nm and their absorbances were recorded at selected wavelengths. The concentrations of each drug in sample solutions were calculated using equations 1–4. The results of the analysis and statistical validation data of the tablet formulation are given in Table 1.

TABLE 1. *Statistical validation data of tablet formulation*

Component	Amount present (mg)	Method	% Amount found	*Standard deviation	*Relative standard Deviation	Standard error
Ena	50	A	99.98	0.044	0.044	0.018
	50	B	100.23	0.354	0.353	0.146
Los	400	A	99.95	0.083	0.083	0.034
	400	B	100.12	0.197	0.196	0.081

* *Denotes average of six estimations*

Tablet Formulation: ENVAS – RB 25, manufactured by Cadila Pharma

Method B - Second order derivative method

The standard stock solutions were prepared as discussed in Method A. suitable dilution of both drug solutions (10 µg/mL of Los and 10 µg/mL of Ena) were scanned between 400 to 200 nm using the spectrum mode of the instrument. The absorption spectras thus obtained were derivatised from first to fourth order. The second order derivative spectras were selected for the analysis of both the drugs. From the overlain derivative spectras obtained, the wavelengths were selected in a manner such that at the zero crossing wavelength of one drug, the other should show substantial absorbance. The second order overlain spectrum of both drugs (Fig.2) reveals that Ena and Los show zero crossing points at 214 and 254 nm, respectively. Mixed standards of Ena and Los were prepared and their absorbances were measured at the

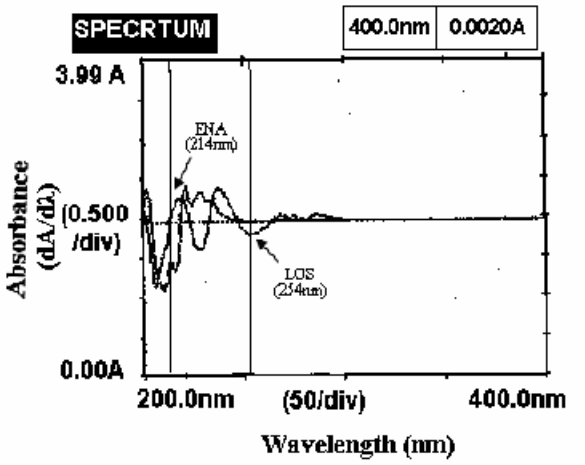

Fig. 2: *Overlain spectra of Ena and Los in first order derivative method*

selected wavelengths in the second order derivative mode. The absorbances were plotted against concentration to obtain standard calibration curves. Los and Ena exhibited linearity with absorbances in the concentration range of 1–60 µg/mL and 2–32 µg/mL at their respective wavelengths. Coefficients of correlations were found to be 0.9996 and 0.9995 for Ena and Los respectively. The optical characteristics and validation data for Ena and Los are presented in Table 2.

TABLE 2. *Optical characteristics and validation data of enalapril maleate and losartan potassium*

Parameters	Enalapril Maleate		Losartan Potassium	
	Method-A	Method-B	Method-A	Method-B
Working wavelengths	222 nm	219.5 nm	250 nm	264 nm
Beer-Lamberts Law range	2-32 mcg/mL	2-32 mcg/mL	1-60 mcg/mL	1-60 mcg/mL
Precision*				
Inter day	0.1498	0.6724	0.1256	0.1415
Intra day	0.1079	0.3805	0.1021	0.0494
LOD (mcg/mL)*	0.1118	0.2968	0.1626	0.2559
LOQ (mcg/mL)*	0.3389	0.9055	0.4930	0.7756
Regression Values:				
I. Slope*	0.0311	-0.0042	0.031	-0.0011
II. Intercept*	-0.004	0.0059	0.0012	0.0001
III. Regression coefficient (r)*	0.9981	0.9956	0.9992	0.9993

** Denotes average of six estimations*
Where, Method-A – Absorbance correction method
Method-B – Second order derivative method

Estimation from marketed preparation

The tablet sample solution was scanned in the spectrum mode in range of 400 – 200nm. The absorbances of the sample solutions were recorded at 214 nm and 254 nm in the second order derivative mode. By using the standard calibration curves, the unknown concentration of the drugs in sample solutions were obtained. The analysis procedure was repeated six times with the same batch of tablets. The results of the tablet analysis and its statistical validation data are given in Table 1.

DISSOLUTION STUDIES[3]

The release kinetics of Enalapril Maleate and Losartan Potassium from tablet dosage forms were studied by performing dissolution studies. Dissolution tests were performed using USP type II dissolution test apparatus and 900 mL of distilled water as the dissolution medium set at 37 □ 0.5 ^{0}C at 100 rpm. 10 mL of sample solutions were withdrawn at intervals of 5 min for 60 min, each time replacing the withdrawn volume with fresh 10 mL distilled water (sink condition) maintained at the same temperature (37 □ 0.5^0 C). The withdrawn samples were filtered through Whatmann filter paper No.41. and suitably diluted to obtain solutions within the Beer's concentration range for both drugs. The resulting solutions were then analyzed by both absorbance correction method and second order derivative method. By applying both methods for the dissolution studies, % cumulative drug release was calculated for Ena and Los. The dissolution study was carried out in triplicate. The graph of dissolution time Vs % cumulative drug release was plotted, which are shown in Figs. 3 and 4.

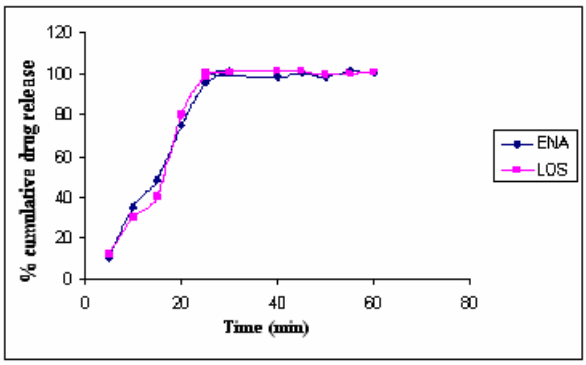

Fig. 3: *Dissolution profile of Ena and Los by absorbance correction method*

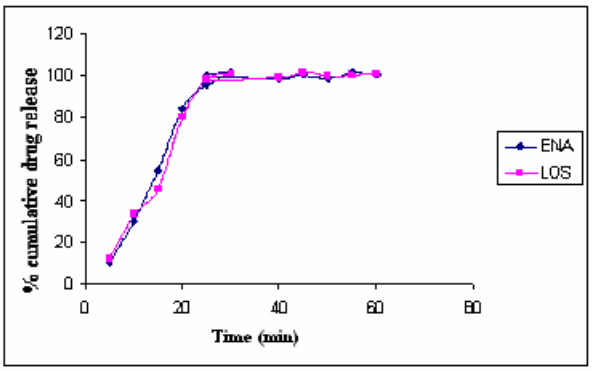

Fig. 4: *Dissolution profile of Ena and Los by second order derivative method*

RESULTS

The optical characteristics and regression values of the calibration curves for the developed methods are presented in Table 2. The mean % content of Ena and Los by both methods was 100.10% and 100.03% respectively. Also the mean % recoveries of Ena and Los by both methods were 100.23% and 99.79% respectively. The results of the tablet analysis, its statistical validation data and recovery studies by both the methods are given in Table-2 and 3 respectively. Also the results of the proposed methods were evaluated using t test and F test to determine if there exist any significant difference between these methods for the analysis of Ena and Los, the results of which are given in Table 4. The dissolution study also indicated that Ena and Los show an average release of 99.59% and 98.56% in 25 min by both methods.

TABLE 3: *Statistical validation of recovery studies*

Level of % recovery	Methods	% Recovery		*Relative standard Deviation		Standard error	
		Ena	Los	Ena	Los	Ena	Los
80	A	100.49	99.97	0.525	0.768	0.303	0.444
	B	100.1	99.69	0.401	0.395	0.233	0.226
100	A	100.49	99.06	0.843	0.901	0.487	0.521
	B	99.76	99.81	0.470	0.319	0.272	0.184
120	A	100.78	99.88	0.503	0.162	0.289	0.093
	B	99.79	100.3	0.581	0.189	0.344	0.167

** Denotes average of three estimations at each level of recovery.*

TABLE 4: *Statistical significance of difference between two methods*

	Ena	Los
t value	1.762	2.041
F value	0.015	0.177

t =1.762, t= 2.041 for Ena and Los respectively, at 10 degrees of freedom are < 2.22
F= 0.015, F=0.177 for Ena and Los respectively, at 5 degrees of freedom are < 5.05

CONCLUSIONS

Enalapril Maleate and Losartan Potassium are available in combined tablet dosage form for the treatment of Hypertension. No single UV spectrophotometric method has been reported for the estimation of the two drugs in combination. Here two simple UV spectrophotometric methods (Absorbance correction method and Second order derivative method) were developed for their simultaneous estimations. The standard deviation, RSD and standard error calculated for both the methods are low, indicating high degree of precision of the methods. The RSD is also less than 2% as required by ICH guidelines. The % recovery was between 98-102% indicating high degree of accuracy of the proposed methods. The results of the t test and F test also indicated that there is no significant difference between the two methods for the analysis of Enalapril Maleate and Losartan Potassium in bulk and formulation. The application of both methods for dissolution study of Ena and Los showed reproducible results.

Hence the developed methods are simple, rapid, precise, accurate and can be employed for the routine estimation of Enalapril Maleate and Losartan Potassium in both bulk and tablet dosage form and can also be employed for the dissolution studies of it's tablet dosage form.

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