Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(16):33-41
(http://www.derpharmachemica.com/archive.html)

Validated Spectrophotometric Methods for Simultaneous Determination of Lercanidipine HCl and Enalapril MALEATE in their Binary Mixture

Hanan A Merey*, Nesrin K Ramadan, Sherine S Diab, Azza A Moustafa

Analytical Chemistry Department, Cairo University, 11562, Cairo, Egypt

ABSTRACT

Four, simple, accurate, precise, sensitive and rapid spectrophotometric methods were employed for the simultaneous determination of Lercanidipine HCl (LER) and Enalapril Maleate (ENA) in their binary mixture. Method (A), dual wavelength, depended on measuring absorbance difference (ΔA) between 225.8 and 241.2 nm for determination of ENA (ΔA of LER is zero at the same two wavelengths). Method (B), second derivative spectrophotometry, adopted for the determination of ENA by measuring the peak amplitude of second derivative at 221.0 nm (zero crossing of LER). Method (C), absorptivity factor, based on determining the total concentration at 220.8 nm, where the absorptivity of LER is double the absorptivity of ENA. In methods (A), (B) and (C), the determination of LER was achieved directly by measuring its absorbance at λ_{max} 358.6 nm. Method (D), mean centering, based on measuring the mean centered ratio spectra of LER and ENA at 292.0 and 210.0 nm, respectively. The specificity of the proposed methods was tested by analyzing laboratory prepared mixtures of both drugs in different ratios and their combined dosage form (Zanipress® tablets). The validity of the developed methods was further assessed by applying the standard addition technique. Statistical comparison revealed that there was no significant difference between the results obtained from the proposed methods and those obtained by official or reported ones in terms of accuracy and precision.

Keywords: Lercanidipine HCl, Enalapril maleate, Dual wavelength, Absorptivity factor, Second derivative, Mean centering

INTRODUCTION

Lercanidipine HCl (LER) (Figure 1a) is chemically designated as 2-{(3,3-diphenyl-propyl) methylamine}-1,1-dimethylethyl,methyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate monohydrochloride, molecular formula: $C_{36}H_{41}N_3O_6$.HCl, molecular weight: 648.20 [1]. LER HCl, a calcium channel blocker, is used for treatment of angina pectoris and hypertension. It inhibits cellular influx of calcium leading to the maintenance of the plateau phase of the action potential. Following oral administration, LER is completely absorbed from the gastrointestinal tract. It is extensively metabolized in liver mainly to inactive metabolites and excreted in the urine [2].

Enanlpril Maleate (ENA) (Figure 1b), is a chemically named as (N-{N-[(S)-1-ethoxycrbonyl-3-phenylpropyl]L-alanyl}-proline hydrogen maleate) Molecular formula: $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$, molecular weight: 492.50. ENA acts as a prodrug of the diacid enalaprilat, its active form, which is poorly absorbed by mouth. Following oral administration about 60% of a dose of enalapril is absorbed from the gastrointestinal tract. ENA is extensively hydrolysed in the liver to enalaprilat [2].

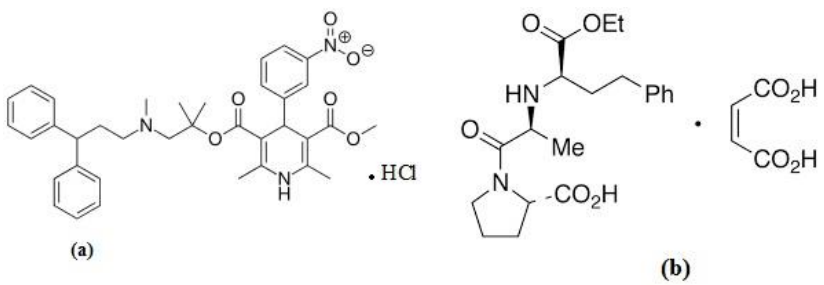

Figure 1: Chemical structures of (a) Lercanidipine HCl, (b) Enalapril maleate

ENA maleate is classified as Angiotensin Converting Enzyme (ACE) inhibitor. Its antihypertensive effect is achieved by reduction of peripheral vascular resistance without causing relative increase in cardiac output, rate or contractility, thus lowering blood pressure. ENA proved to be beneficial in the management of essential hypertension especially in patients with diabetes and chronic renal diseases. It has been also shown to be effective in the treatment of congestive heart failure [3].

LER HCl and ENA are recently introduced in the market as a new combined tablet dosage form (Zanipress®), taken once-daily for the treatment of hypertension. ENA is an official drug, while LER HCl is unofficial one. Determination of ENA is described in British Pharmacopoeia (BP) [4] by potentiometric titration method while in the United State Pharmacopeia (USP) [5] by RP-HPLC.

Literature survey represented that LER and ENA were determined by several methods either alone or in mixture with other drugs in pharmaceutical preparations or in biological fluids. These methods include; spectrophotometry [6-12], voltammetry [13,14], TLC [15,16], HPLC [6,17-27] and UPLC [20,28]. Few methods were reported for the determination of LER and ENA in combination including: HPLC [29-32] and three spectrophotometric methods involving solving simultaneous equation, Q analysis [33] and first derivative spectrophotometry [29].

The primary purpose of this work is to develop reliable, simple, sensitive, accurate, precise and relatively inexpensive analytical methods for the determination of the two drugs with no need for prior separation steps.

EXPERIMENTAL SECTION

Apparatus

Spectrophotometer: Shimadzu dual beam UV-Visible spectrophotometer (Kyoto/Japan), Model UV-1650 PC connected to IBM compatible and a HP1020 laser jet printer. The bundled software, UV-Probe personal spectroscopy software version 2.21 (Shimadzu) is used. The spectral band is 2 nm and scanning speed is 2800 nm/min with 0.1 nm interval, Software**:** Matlab® version 7, release 14.

Pure samples

Lercanidipine HCl (LER) was kindly supplied by Pharma Care Company, Cairo, Egypt**.** Its purity was found to be 99.98 ± 0.933 in accordance to the reported method [34]. Enalapril Maleate (ENA) was kindly supplied by National Organization for Drug Control and Research (NODCAR), Cairo, Egypt, its purity was found to be 100.07 ± 0.815 according to the official method [5].

Pharmaceutical formulations

Zanipress® tablets (batch number CC0CC43), manufactured by Recordati, Inc. from local pharmacy (Union) in Beirut, Lebanon. Each tablet was labeled to contain 10 mg Lercanidipine HCl (LER) and 10 mg ENA.

Reagents

Methanol of spectroscopy grade was purchased from Sigma-Aldrich, (St. Louis, MO USA).

Standard stock and working solutions: LER and ENA stock solutions (1 mg/ml of each in methanol). LER and ENA working solutions (100 µg/ml of each in methanol).

Laboratory prepared mixtures containing LER and ENA in different ratios: Aliquots of LER and ENA were transferred from their corresponding standard working solutions (100 µg/l of each into a series of 10 ml volumetric flasks. The volume was then completed with methanol to prepare mixtures containing both drugs in different ratios.

Procedures

Spectral characteristics of LER and ENA

The zero-order (^{0}D) absorption spectra of LER and ENA (10 µg/ml, of each) were scanned against methanol as a blank and stored.

Linearity

Aliquots of LER and ENA were separately transferred from their working standard solutions (0.1 mg/ml) into two separate sets of 10 ml volumetric flasks. The volume was then completed with methanol to give samples having concentration in the range of 6-22 µg/ml for LER and 4-22 µg/ml for ENA. The zero order absorption spectra of these solutions were recorded in the range of 200-400 nm and were stored.

(^{0}D) Direct spectrophotometric method for LER

For the determination of LER for methods A, B and C, the zero order absorption of the stored spectra of LER were measured at its λ_{max} 358.6 nm (no interference from ENA). A linear calibration curve was constructed relating the absorbance at 358.6 nm to the corresponding concentration of LER and the regression equation was computed.

Method A: Calibration curve relating the difference between the absorbance (ΔA) at 225.8 and 241.2 nm for ENA versus its corresponding concentrations was constructed and the regression equation was computed. All the stored spectra of LER were measured at 225.8 and 241.2 nm to ensure that the difference between these two wavelengths is zero.

Method B: For the determination of ENA, the second derivative spectra at Δλ=8 and scaling factor=100 were recorded and calibration curve was constructed relating the peak amplitude of the obtained second derivative spectra at 221.0 nm (zero crossing point for LER) versus its corresponding concentrations and the regression equation was computed.

Method C: For the determination of ENA, the recorded spectra of LER were measured at 220.8 nm (absorptivity factor point). Calibration graph was constructed by plotting the zero order absorbance of LER at 220.8 nm against its corresponding concentrations and the regression equation was computed.

Method D: For the determination of LER, the stored zero order absorption spectra of LER in the range of 200-400 nm, were divided by the spectrum of 10 µg/ml ENA, and the obtained ratio spectra were then mean centered.

For the determination of ENA, the stored scanned spectra of ENA in the range of 200-400 nm, were divided by the spectrum of 10 µg/ml LER to obtain the ratio spectra, and the obtained ratio spectra were then mean centered. The calibration graphs of LER and ENA were constructed by plotting the peak amplitude of the mean centered values at 292.0 and 210.0 nm for LER and ENA, respectively, against their corresponding concentrations and the regression equations were then computed.

Analysis of laboratory-prepared mixtures

For methods A-C: The absorption spectra of laboratory prepared mixtures (2.7.), were recorded at 200-400 nm and stored in the computer. For the determination of LER, the absorbance of zero order spectra of the mixtures were recorded at 358.6 nm and their concentrations were calculated by substituting in the corresponding regression equation as mentioned under linearity.

For the determination of ENA (Methods A and B), the stored spectra of the laboratory prepared mixtures (2.7.) were recorded. Then procedures were performed as described under linearity. While for method C, the zero order absorbance of the stored spectra was measured at 220.8 nm (absorptivity factor point). The total concentration (½ ENA+LER) was calculated by substituting in the corresponding regression equation. The concentration of ENA could be obtained after subtracting the concentration of LER and multiplying by 2 (F=½), using the following equation:

$$C_{ENA} = [\text{Total concentration } (½\ C_{ENA}+C_{LER}) – C_{LER}] \times 2$$

For method D: For the determination of LER and ENA, The absorption spectra of laboratory prepared mixtures [27] were scanned and stored. Then procedures were performed as described under linearity.

Application of the proposed methods for the determination of LER and ENA in Zanipress® tablets

Ten tablets of Zanipress® were accurately weighed and finely powdered. A portion equivalent to 10 mg of each LER and ENA was accurately weighed, accurately transferred into a beaker, dissolved in suitable amount of methanol by shaking in ultrasonic bath for about 30 min. The solution was filtered and transferred quantitatively into a 100 ml volumetric flask. The volume was then completed to the mark with the same solvent and finally filtered. Further dilutions with methanol were done to obtain final concentrations of 10 µg/ml for both LER and ENA. Then the procedure was completed as described under analysis of laboratory prepared mixture (2.9). The concentrations of the cited drugs were obtained from the corresponding regression equations and the validity of the methods was further assessed by applying the standard addition technique

RESULTS AND DISCUSSION

Zanipress® is a pharmaceutical dosage form containing LER and ENA and used for treatment of hypertension [3]. By reviewing the literature review, it was found that only three spectrophotometric methods were published for simultaneous determination of LER and ENA. This attracted our attention to develop accurate, precise, reliable, simple, fast and less mathematically manipulated spectrophotometric methods for the simultaneous determination of LER and ENA in their pure and combined dosage form.

The zero order absorption spectra of LER and ENA showed spectral overlap in the range of 200-300 nm (Figure 2). Only LER can be determined by measuring its absorbance at 358.6 nm. Linearity relationship obtained between the absorbance of LER at this wavelength and its corresponding concentrations in the range of 6-22 µg/ml (Table 1). Since the absorption spectra of ENA showed complete overlapping by LER, which prevents its direct determination. Therefore, dual wavelength was tried to solve this problem (method A). For the determination of ENA using dual wavelength, two wavelengths (225.8 and 241.2 nm) were selected where the difference in absorbance (ΔA) between these two wavelengths was directly proportional to the concentration of ENA in the range of 8-22 µg/ml (Table 2), while (ΔA) of LER at these wavelengths was equal to zero (Figures 3).

Table 1: Regression and validation parameters for the determination of pure LER samples by applying the direct and the mean centering spectrophotometric methods

Parameters	Zero order method (at 358.6 nm)	Mean centering method (at 292.0 nm)
Range (µg/ml)	June 22	February 20
Slope[a]	0.0183	0.0872
Intercept[a]	0.0147	0.128
SE of the slope	0.0001	0.0006
SE of the intercept	0.0016	0.0073
Correlation coefficient (r)	0.9998	0.9997
LOD[b] (µg/ml)	0.282	0.385
LOQ[b] (µg/ml)	0.856	1.168
Accuracy Mean ± SD, RSD%	99.85 ± 0.625 -0.626	99.82 ± 1.078 -1.08
Precision (RSD%) Repeatability[c] Intermediate precision[d]	 0.697 -0.905	 0.87 -1.047

[a]Average of three determinations; [b]Average of three determinations; [c]The intraday (n=3) standard deviation of concentrations (8, 15, 20 µg/ml) of LER repeated three times within the same day; [d]The interday (n=3) relative standard deviation of concentrations (8, 15, 20 µg/ml) of LER repeated three times in three successive days

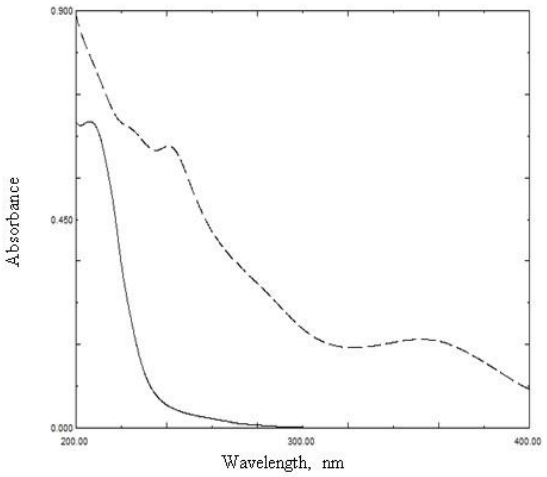

Figure 2: Zero order absorption spectra of 10 µg/ml of each LER (----) and ENA (——), using methanol as a blank

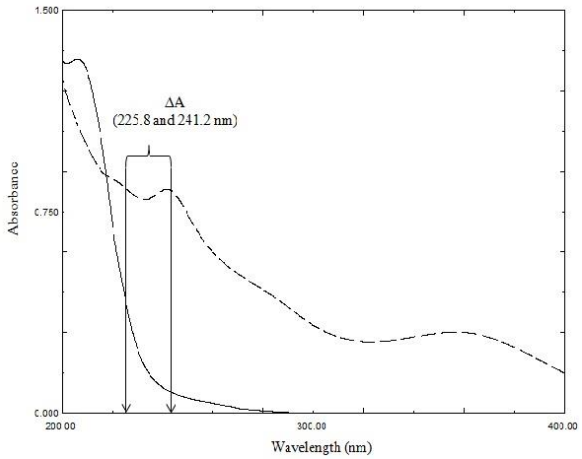

Figure 3: The absorption spectra of 20 µg/ml (——) of ENA and 16 µg/ml (----) LER, using methanol as a blank

Derivative spectrophotometry was also investigated for the determination of ENA in the presence of LER. The first derivative spectrophotometry (1D) failed to resolve the binary mixture (Figure 4), while the second derivative spectrophotometry (2D) was successfully applied for the determination of ENA. This was achieved by measuring the peak amplitude of the second derivative spectra of ENA at 221.0 nm (Method B), where LER had no contribution and showed zero crossing (Figure 5). The influence of Δλ for the second derivative spectra was tested and it was found that it is very appropriate to use the values of Δλ=8, in the determination of ENA. Furthermore the scaling factor of 10 and 100 was investigated and it was revealed that the scaling factor of 100 was optimum for the determination of ENA. A linear relationship was obtained in the concentration range of 4-22 µg/ml using the peak amplitudes measured for ENA at 221.0 nm versus its concentrations (Table 2).

Parameters	**Method A (ΔA 225.8-241.2)**	**Method B (at 221.0 nm)**	**Method C (at 220.8 nm)**	**Method D (at 210.0 nm)**
Range (µg/ml)	8 – 22	4 – 22	Apr-22	4-22
Slope[a]	0.017	0.0288	0.0515	0.0734
Intercept[a]	-0.031	0.0116	0.056	-0.0604
SE of the slope	0.0002	0.0002	0.0003	0.0004
SE of the intercept	0.0026	0.003	0.0045	0.006
Correlation coefficient (r)	0.9996	0.9997	0.9997	0.9998
LOD[b] (µg/ml)	0.386	0.415	0.35	0.328
LOQ[b] (µg/ml)	1.17	1.256	1.058	0.994
Accuracy Mean ± SD RSD%	100.08 ± 0.937 0.936	100.02 ± 1.003 1.003	100.02 ± 0.901 0.9	100.06 ± 0.926 0.925
Precision (RSD%) Repeatability[c] Intermediate precision[d]	1.017 0.806	993 1.057	0.96 1.059	0.611 1.033

Table 2: Regression and validation parameters for the determination of pure ENA samples by applying the proposed spectrophotometric methods

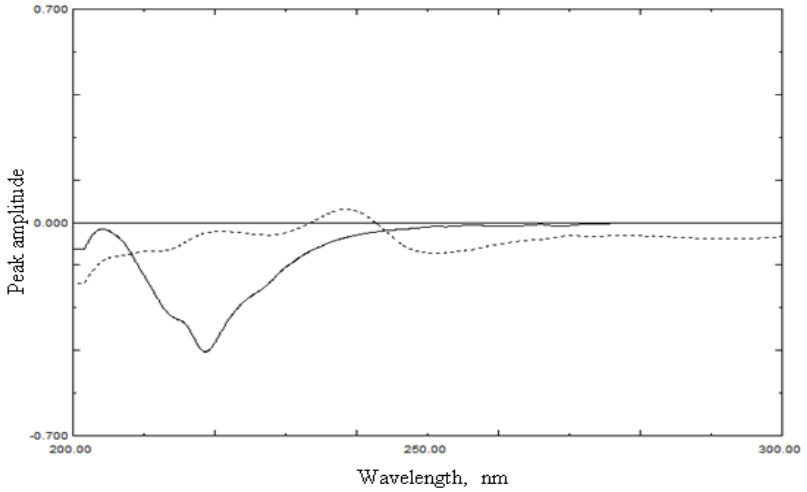

Figure 4: First derivative absorption spectra of 12 µg/ml of each LER (----) and ENA (—)

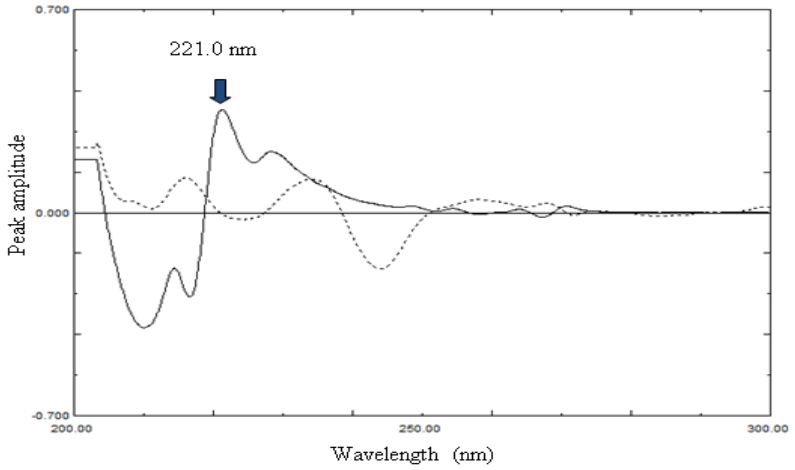

Figure 5: Second derivative absorption spectra of 12 µg/ml of each LER (----) and ENA (—)

Absorptivity factor (Method C) is a newly established method [35,36] that can be adopted for the determination of the concentrations of two components of different absorptivity's in their drug mixture. Compared to the classical isoabsorptive point method, this method has advantage of wide application for resolving different mixtures, since the latter is not restricted for the components of similar absorptivity's at certain wavelength [37-39].

For the two drugs X and Y, in the mixture, where concentration of Y can be obtained using any well-established spectrophotometric method. For the determination of the concentration of X, the absorptivity factor method is applied, which depends on the calculation of the absorptivity factor. The absorptivity factor point (λ_F) is the ratio between the two absorptivity's (a_x, a_y) at intersection point with the same absorbance value. This can be summarized as follows:

$$A_x = A_y$$
$$a_x b_x C_x = a_y b_y C_y, \text{ where, } b_x = b_y = 1 \text{ cm}$$
$$a_x C_x = a_y C_y$$
$$a_x/a_y = C_y/C_x$$
$$a_x/a_y = Fa_x = Fa_y$$

Where, F is the absorptivity factor, a_x, a_y are the absorptivity's of X and Y, respectively. For mixture of X and Y, the total absorbance of X and Y at absorptivity factor point λ_F can be expressed as follows:

$$A_m = A_x + A_y$$
$$A_m = a_x b_x C_x + a_y b_y C_y, \text{ where, } b_x = b_y = 1 \text{ cm}$$
$$A_m = a_x C_x + a_y C_y$$

Where, A_x, A_y and A_m are the absorbance of X, Y and their mixture at λ_F, respectively, Cx and C_y are the concentrations of X and Y, respectively, and ax and ay are the absorptivity's of X and Y at λ_F, respectively. If ax is substituted by Fa_y:

$$Am = a_y F C_x + a_y C_y$$
$$Am = a_y (F C_x + C_y)$$

So, the total content of the mixture ($FC_x + C_y$) can be calculated by using a regression equation representing the linear relationship between the absorbance of Y versus its corresponding concentration at the absorptivity factor point.

For the determination of ENA, a crossing point (isoabsorptive point) was obtained where the concentration of ENA (20 µg/ml) was double that of LER (10 µg/ml) and the absorptivity of LER was double that of ENA (F=½) (Figure 6). Thus by measuring the absorbance value at the chosen absorptivity factor point at 220.8 nm, the concentration of LER and half the concentration of ENA could be calculated, while the concentration of LER alone could be obtained at its λ_{max} at 358.6 nm without any interference from the other drug. The concentration of ENA could be calculated by subtraction and multiplying by (1/F=2).

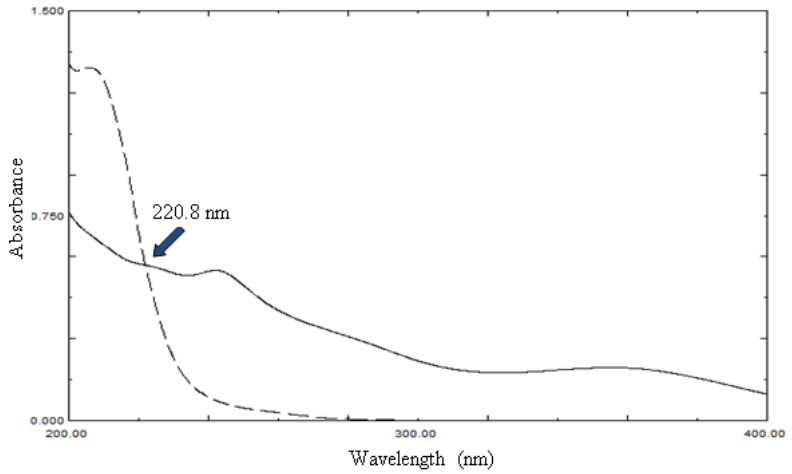

Figure 6: Zero-order absorption spectra of Lercanidipine HCl (—) (10 µg/ml) and Enalapril maleate (----) (20 µg/ml) in methanol, showing isoabsorptive point at 220.8 nm

Calibration graphs were plotted, relating the absorbance at the selected wavelength (220.8 nm) versus the corresponding concentrations of LER and the concentrations were obtained by substituting in the computed regression equation (Table 2).

For further enhancement of the selectivity to resolve the highly overlapped spectra of LER and ENA, a simple, sensitive method was applied based on manipulating the ratio spectra, which is Mean centering method. It has the advantage of omitting the derivative step and therefore the signal-to-noise ratio is enhanced [40]. The overlapped spectra of LER and ENA in the wavelength region of 200-300 nm (Figure 2), suggested that Mean centering method is suitable for their simultaneous analysis. For the determination of LER, the absorption spectra of the standard solutions in the range of (2-20 µg/ml) were recorded in the range of 200-400 nm, divided by the spectrum of 10 µg/ml ENA and the obtained ratio spectra were then mean centered. The concentration of LER was determined by measuring the peak amplitude at 292.0 nm (Figure 7).

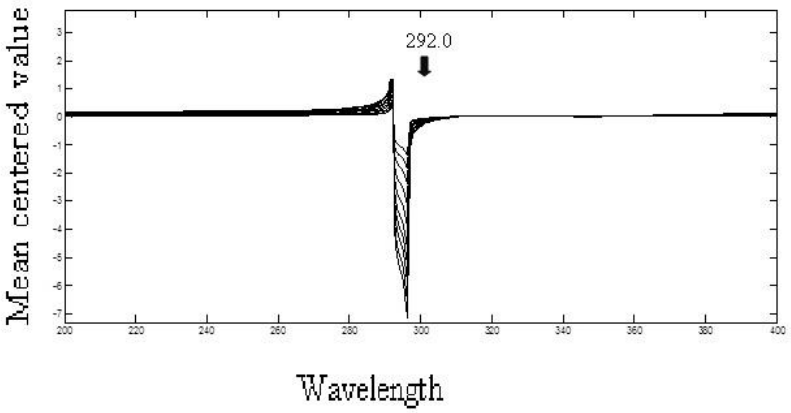

Figure 7: The mean centered ratio absorption spectra of ratio spectra of LER in the range of 2-20 µg/ml using methanol as a blank

For the determination of ENA, similarly, the absorption spectra of the standard solutions of ENA in the range of (4-22 µg/ml) were recorded in the range of 200-300 nm, divided by the spectrum of 10 µg/ml LER and the obtained ratio spectra were then mean centered. The concentration of LER was determined by measuring the amplitude at 210.0 nm (Figure 8). The effect of divisor concentration on the analytical parameters such as slope, intercept and correlation coefficient of the calibration graphs was also investigated. Three different divisors' concentrations were tested (5, 10 and 20 µg/ml). The concentration 10 µg/ml of each of the LER and ENA was found suitable and therefore it was the divisor of choice in the proposed method. The calibration curve relating the peak amplitude of the mean centered values at the selected wavelengths to the corresponding concentrations of each component were constructed and the concentrations were calculated from corresponding regression equations (Tables 1 and 2).

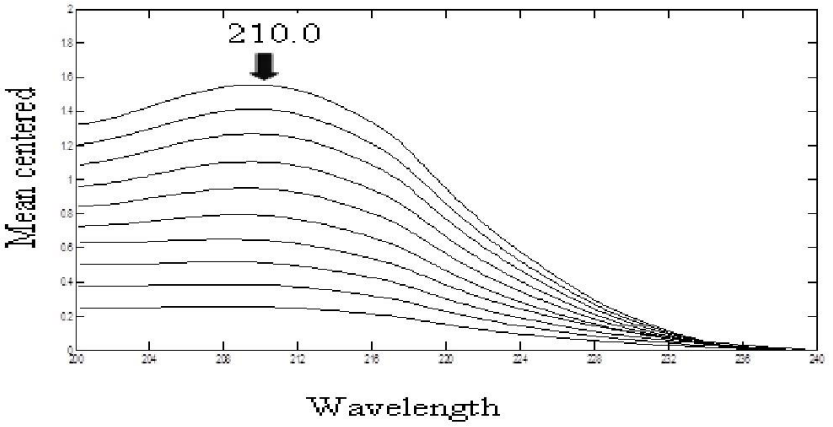

Figure 8: The mean centered ratio absorption spectra of ratio spectra of ENA in the range of 4-22 µg/ml using methanol as a blank

The analysis of laboratory prepared mixtures of LER and ENA in different ratios, proved the specificity of the developed methods (Tables 3 and 4). All the proposed methods were successfully applied for the determination of LER and ENA in Zanipress® tablets**,** and the validity of the methods was assessed by standard addition technique (Tables 5 and 6).

Table 3: Determination of LER in laboratory prepared mixtures by the direct and the mean centering spectrophotometric methods

Lab. Mix. No.	Concentration (µg/ml)		Recovery* (%)	
	LER	ENA	Direct method	Mean centering
1	10	10	98.16	98.53
2	20	10	100.08	99.35
3	10	20	99.07	100.58
4	10	15	99.95	99.25
5	15	10	100.8	99.34
Mean ± SD			99.61 ± 1.018	99.41 ± 0.738
RSD%			1.022	0.742

*Average of three determinations

Table 4: Determination of ENA in laboratory prepared mixtures by applying the proposed spectrophotometric methods

Lab. Mix. No.	Concentration (µg/ml)		Recovery* (%)			
	LER	ENA	Method A	Method B	Method C	Method D
1	10	10	98.17	101.17	101.17	99.87
2	20	10	99.87	98.91	98.67	98.97
3	10	20	99.44	101	99.79	101.02
4	10	15	100.14	100.21	101.32	100.88
5	15	10	100.04	100.1	99.03	99.89
Mean ± SD			99.64 ± 0.585	100.26 ± 0.874	99.99 ± 1.198	100.13 ± 0.840
RSD%			0.587	0.872	1.198	0.839

Method validation

Validation was done according to International Conference on Harmonisation (ICH) recommendations [41]. Linearity, accuracy, selectivity ranges, Limit of Detection (LOD), Limit of Quantification (LOQ) and precision (repeatability and intermediate precision) were determined. Satisfactory results were obtained within global validation reference values (Tables 3 and 6).

Table 5: Results obtained for the determination of LER in Zanipress® tablets by the proposed methods and results of application of standard addition technique

Pharmaceutical formulation	Method	Found (%)* ± SD	Standard addition technique		
			Pure added (µg/ml)	Found (µg/ml)	Recovery**
Zanipress® Tablets (Claimed to contain 10 mg LER and 10 mg ENA) B. N. CC0C43	Direct method	100.30 ± 1.023	6	6.02	100.33
			8	8.16	102
			10	9.98	99.8
	Mean ± SD				100.71 ± 1.148
	Mean centering	99.82 ± 1.078	6	6.01	100.17
			8	8.07	100.88
			10	10.1	101
	Mean ± SD				100.68 ± 0.449

*Average of three determinations; **Average of three determinations; Claimed concentration 6 µg/ml of each LER

Table 6: Results obtained for the determination of ENA in Zanipress® tablets by the proposed methods and results of application of standard addition technique

Pharmaceutical formulation	Methods	Found (%)* ± SD	Standard addition technique		
			Pure added (µg/ml)	Found (µg/ml)	Recovery**
Zanipress® Tablets (Claimed to contain 10 mg LER and 10 mg ENA). B. N. CC0C43	Method A	101.15 ± 0.970	4	3.96	99
			8	7.98	99.75
			16	16.03	100.19
	Mean ± SD				99.65 ± 0.602
	Method B	100.53 ± 1.063	4	3.97	99.25
			8	8.12	101.5
			16	16.09	100.56
	Mean ± SD				100.44 ± 1.130
	Method C	99.79 ± 1.161	4	3.96	99
			8	8.07	100.87
			16	15.9	99.37
	Mean ± SD				99.75 ± 0.990
	Method D	99.56 ± 1.050	4	3.94	98.5
			8	7.98	99.75
			16	15.91	99.44
	Mean ± SD				99.23 ± 0.651

*Average of three determinations; **Average of three determinations; Claimed concentration 6 µg/ml of each ENA

Stability

The stability of LER and ENA in methanol has been tested by keeping a sample of each drug in the refrigerator and another one in a tightly capped volumetric flask, covered with aluminum foil and placed at ambient temperature. The samples were checked for assay in fourteen successive days of storage and compared with freshly prepared sample. The RSD values of assay were found to be below 2.0% in both cases. This indicates that LER and ENA are stable in the solutions for 2 weeks.

Statistical analysis

Statistical comparison was carried out between the results obtained for the analysis of LER and ENA by the developed spectrophotometric methods in their pure form and those obtained by applying the reported method [34] (for LER) or official method [5] (for ENA). Calculated t and F values are less than the tabulated ones, which reveals that there is no significant difference between the two methods with respect to accuracy and precision (Table 7).

Table 7: Statistical comparison of the results obtained by the proposed method and the reported [34] or official [5] methods for the determination of LER and ENA in their pure form

Item	LER		ENA				Reported Method [34]	Official Method [5]
	Direct method	Mean centering	Method A	Method B	Method C	Method D	LER[(a)]	ENA[(b)]
Mean	99.85	99.82	100.08	100.02	100.02	100.06	99.98	100.55
SD	0.626	1.078	0.937	1.003	0.901	0.926	0.933	0.71
Variance	0.391	1.162	0.878	1.006	0.812	0.857	0.87	0.504
n	9	10	8	10	10	10	10	5
Student's t test*	0.352	0.355	0.957	1.048	1.143	1.034		
	-2.11	-2.101	-2.201	-2.16	-2.16	-2.16		
F-value*	2.23	1.34	1.74	1.99	1.61	1.7		
	-3.39	-3.18	-6.09	-6	-6	-6		

[a]First derivative UV spectroscopy using Δλ= 4 and scaling factor=10 at λ=332.0 nm; [b]HPLC method (USP 29-NF24); C_8 column (5 µm) (25 × 4.6 mm i.d), temperature maintained at 50°, mobile phase: 75 ml phosphate buffer solution and 25 ml acetonitrile, flow rate: 1.5 ml/min, UV detection: 215 nm

CONCLUSION

The proposed direct, dual wavelength, absorptivity factor and derivative spectrophotometric methods were found to be simple, sensitive and selective. No sophisticated techniques or special software was required, furthermore no hazardous solvents or long sample preparation is needed and therefore it can be employed for the routine analysis of LER and ENA with high degree of accuracy and precision in their pure forms as well as in their marketed dosage form without prior separation in quality control laboratories.

The most prominent disadvantage of dual wavelength method is that the choice of the selected wavelengths is highly restricted to those with constant absorbance of the interfering component. Therefore the measurement of the absorbance of the component of interest is highly critical, since any minor change in the chosen wavelength will affect the results leading to poor reproducibility and robustness. Another disadvantage is the poor values obtained from absorbance differences of the two components which lead to poor sensitivity. So, lowest concentrations should be excluded from the concentration range.

Absorptivity factor method has the advantage of applying simple mathematical equation to calculate the concentration of cited drugs in their binary mixture, using their zero order spectra with no need for advanced or expensive software or device. Furthermore the proposed method can solve the problem of large difference between the absorptivity's of the mixture components and shows high sensitivity as it can use the zero order spectra.

The proposed Mean centering method was successfully adopted for the determination of the two components in their drug mixture, with high sensitivity due to the enhancement of the signal-to-noise ratio. The only limitation to this method is the need of special software (Matlab®). However, the advantage of the accuracy, precision and reproducibility of the results obtained by MCN overweighs this drawback.

REFERENCES

[1] S. Budavari, J. Maryadele, A. Smith, Merk & Co., Inc. Ruchway USA, **1989**, (Monograph No 5465), 973.
[2] S.C. Sweetman, The Pharmaceutical Press; Electronic Version, London, **2007**.
[3] J.A. Oates, N.J. Brown, In: J.G. Hardman, L.E. Limbird, McGraw Hill, NY, USA, **2008**, 893.
[4] British Pharmacopoeia, Her Majesty's Stationery Office, London, UK, **2010**.
[5] M.D. Roukville: The United States Pharmacopeia and National Formulary (USP 35-NF 30), The United States Pharmacopoeial Convention, **2012**.
[6] P.S. Bansode, M.S. Ranawat, R. Kamble, C.S. Chauhan, *Indian J. Pharm. Pharmacol*., **2014**, 1, 37.
[7] V.P. Choudhari, V.M. Suryawanshi, R.H. Mahabal, G. S. Deshchougule, P.K. Bhalerao, S.B. Kuchekar, *Int. J. Pharm.Rev.Res.*, **2010**, 3, 73.
[8] N. Jain, R. Jain, N. Thakur, S. Jain, D.K. Jain, *J. Anal. Chem.*, **2011**, 6, 84.
[9] G. Sowjanya, P. Gangaghar, P.R. Rao, P. Subrahmanyam, P. Suresh, *J. Chem. Pharm. Res.*, **2012**, 4, 3483.
[10] F. Wedian, A. Lataifeh, *Int. J. Chem.*, **2013**, 5, 29.
[11] K. S. Raveendra, K. R. Vinayakrao, *World J. Pharm. Pharm.Sci*., **2014**, 4, 852.
[12] G. Garg, S. Saraf, S. Saraf, *Trends Appl. Sci. Res.*, **2008**, 3, 278.
[13] C.F. Valezi, E.H. Duarte, G.R. Mansano, L.H. Dall'Antonia, C.R. Tarley, E.R. Sartori, *Sens. Actuators B: Chem*., **2014**, 205, 234.
[14] C.A. Salamanca-Neto, P.H. Hatumura, C.R. Tarley, E.R. Sartori, *Ionics.*, **2014**, 21, 1615.
[15] K. Manish, G. Rajdeep, A. Vishesh, R. Amit, *Int. J. PharmTech. Res*., **2011**, 3, 1454.
[16] A.M. Tamboli, N.I. Khan, R.S. Bathe, A.M. Ansari, *Int. J. Biomed. Adv. Res*., **2014**, 5, 237.
[17] G.S. Devika, M. Sudhakar, J.V. Rao, *Res. J. Pharm. Tech*., **2011**, 4, 592.
[18] D.K. Jain, P. Patel, A.S. Khan, N. Jain, *Int. J. ChemTech. Res.*, **2011**, 3, 766.
[19] S. Vidyadhara, B.V. Rao, K. Tejaswi, A.L. Rani, *Der Pharma Chemica*., **2014**, 6, 217.
[20] B. Uslu, T. Özden, *Chromatographia.*, **2013**, 76,1487.
[21] V.N. Maslarska, J.D. Tencheva, *Int. J. Adv. Pharm. Res.*, **2013**, 4.
[22] M.E. Hammouda, M.A. Abu El-Enin, D.T. El-Sherbiny, D.R. El-Wasseef, S.M. El-Ashry, *J. Chromatogr. Sci*., **2015**, 53, 90.
[23] C. Vishnuvardhan, P. Radhakrishnanand, S.G. Navalgund, K.R. Atcha, N. Satheeshkumar, *Chromatographia*., **2014**, 77, 265.
[24] N. Sultana, M.S. Arayne, S. Naveed, *J. Chil. Chem. Soc*., **2011**, 56, 734-737.
[25] M.S. Arayne, N. Sultana, A. Tabassum, S.N. Ali, S. Naveed, *Med. Chem. Res.*, **2012**, 21, 4542.
[26] N. Shafi, F.A. Siddiqui, N. Sultana, M.S. Arayne, *J. Liq. Chromatogr. Related Technol*., **2015**, 38, 1466.
[27] O. Gonzalez, G. Iriarte, E. Rico, N. Ferreiros, M.I. Maguregui, R.M. Alonso, R.M. Jimenez, *J. Chromatogr. B.*, **2010**, 878, 2685.
[28] H.O. Kaila, M.A. Ambasana, A.K. Shah, *J. AOAC*., **2013**, 96, 295.
[29] M. Gumustas, S. Sanli, N. Sanli, S.A. Ozkan, *Talanta.*, **2010**, 82, 1528.
[30] S. Kurbanoglu, M. Gumustas, B. Uslu, S.A. Ozkan, *Chromatographia.*, **2013**, 76, 1477.
[31] P.N. Shah, B.N. Patel, C.N. Patel, J.B. Dave, *Int. J. Res. Pharm. Chem*., **2011**, 1, 308.
[32] D.R. El-Wasseef, D.T. El-Sherbiny, M.A. Abu El-Anin, S.M. El-Ashry, *J. Liq. Chromatogr. Related Technol.*, **2010**, 34, 48.
[33] P.N. Shah, B.N. Patel, C.N. Patel, *Res. J. Pharm. Technol.*, **2011**, 4, 1118.
[34] A. Agrawal, K. Mittal, A.R. Thakkar, *J. Pharma. Res. Health*., **2010**, 2, 263.
[35] A. Samir, H. Salem, M. Abdelkawy, *Bull. Fac. Pharm. Cairo Univ*., **2012**, 50, 121.
[36] S.S. Saleh, H.M. Lotfy, N.Y. Hassan, S.M. Elgizawy, *Saud. Pharm. J*., **2013**, 21, 411.
[37] V.S. Erram, H.P. Tipnis, *Indian Drugs*., **1993**, 30, 319.
[38] V.S. Erram, H.P. Tipnis, *Indian Drugs,* **1993**, 30, 462.
[39] N. Erk, *J. Pharm. Biomed. Anal.*, **2002**, 37, 901.
[40] A. Afkhami, M. Bahram, *Talanta.*, **2005**, 66, 712.
[41] ICH, Q2 (R1) Validation of analytical procedures, Proceeding the International Conference of Harmonization, Geneva. **2003**.