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Journal of Pharmaceutical and Biomedical Analysis
26 (2001) 43–52

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Comparison of spectrophotometric and an LC method for the determination perindopril and indapamide in pharmaceutical formulations

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Received 27 September 2000; received in revised form 29 December 2000; accepted 3 January 2001

Abstract

A new sensitive, simple, rapid and precise reversed-phase high performance liquid chromatographic (HPLC) and two spectrophotometric methods have been developed for resolving binary mixture of perindopril and indapamide in the pharmaceutical dosage forms. The first method is based on HPLC on a reversed-phase column using a mobile phase of phosphate buffer pH 2.4 and acetonitrile (7:3 v/v) was used. Linearity range for perindopril and indapamide was 5.0–70.0 and 8.0–35.0 μg ml^{-1}. In the second method, the first derivative spectrophotometry with a zero-crossing technique of measurement is used for the simultaneous quantitative determination of perindopril and indapamide in binary mixtures without previous separation step. Linear calibration graphs of first derivative values at 225.7 and 255.4 nm for peridopril and indapamide, respectively. The third method is based on ratio derivative spectrophotometry, the amplitudes in the first derivative of the ratio spectra at 226.5 and at 255.3 nm were selected to determine perindopril and indapamide in the binary mixture. All the proposed methods showed good linearity, precision and reproducibility. The proposed methods were successfully applied to the pharmaceutical dosage forms containing the above-mentioned drug combination without any interference by the excipients.

Keywords: Perindopril; Indapamide; Simultaneous determination; High performance liquid chromatography; Zero-crossing derivative spectrophotometry; Ratio derivative spectrophotometry

1. Introduction

Perindopril (2*S*,3a*S*,7a*S*)-1-[(*S*)-*N*-[(*S*)-1-carboxybutyl]alanyl] hexahydro-2-indolinecarboxylic acid 1-ethyl ester, is the first member of a new chemical class of a non-peptide angiotensin II receptor antagonist. The first approved indication for perindopril is for hypertension.

Indapamide, or 3-(aminosulfonyl)-4-chloro-*N*-(2,3-dihidro-2-methyl-1*H*-indol-1-yl) benzamide, is a diuretic of the class of benzothiadiazines.

Perindopril (I) and indapamide (II) have the following structural formulae.

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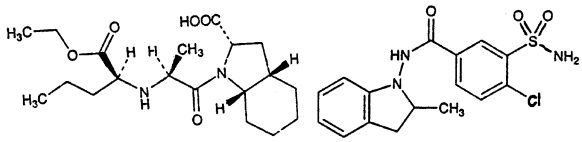
0731-7085/01/$ - see front matter
PII: S0731-7085(01)00388-0



(I) (II)

Recently, perindopril has been marketed in combination with indapamide in tablets. The tablet manufacturer claims that the combined oral administration of perindopril with indapamide has been found to be more effective than either drug alone in the treatment of hypertension. The perindopril–indapamide mixture is not yet official in any pharmacopoeia. To knowledge, neither high performance liquid chromatographic (HPLC) method nor spectrophotometric methods have been described for the simultaneous determination of both drugs in tablets. Therefore, it has created a need for new analytical methods for their simultaneous determination.

There are a number of reported methods for the determination of the two pharmaceuticals. Thus, perindopril has been determined spectrophotometrically [1,2], and chromatographic methods [3,4], and indapamide by use of spectrophotometrically [5,6], and chromatographic methods [7,8], and titrimetry [9].

In pharmaceutical analysis, Fell [10] has demonstrated the possibilities offered by derivative spectrophotometry. This technique has proven to be useful in the assay of single components in the presence of excipients [10,11] or degradation products [12,13] and in the analysis of two-component mixtures [14–16]. A spectrophotometric method based on the use of the first derivative of the ratio spectra was developed by Salinas et al. [17], for resolving binary mixtures.

In this paper, three new methods, based on selective zero-crossing derivative and ratio first derivative method and HPLC were reported and the optimum experimental parameters for each method were described. The proposed methods were applied to the determination of both analytes in synthetic mixtures and pharmaceutical preparations, with satisfactory results in both cases.

2. Experimental

2.1. Apparatus

A chromatographic system consisted of a JASCO model PU-980 pump with a 7725 Rheodyne value injector 20-μl fixed loop, equipped with a JASCO UV-975 UV/VIS detector. The detector was set at 215.0 nm (0.02 a.u.f.s.) and peak areas were integrated automatically by computer using Borwin software programme.

Spectrophotometric analysis was carried out on a Shimadzu 1601 double beam spectrophotometer with a fixed slit width (2 nm) connected to an IBM-PC computer loaded with a Lexmark printer was used for all the absorbance signals and treatment of data.

Other apparatus used included a Radiometer NEL pH 890 pH meter digital equipped with a combined glass–calamol electrode and ultrasound generator.

2.2. Chemicals used

Perindopril and indapamide were kindly supplied by Servier Pharm. Ind. Methanol and acetonitrile were of HPLC grade (Merck Chem. Ind.). All other chemicals were of analytical-reagent grade.

2.3. Pharmaceutical preparation

A commercial pharmaceutical preparation (PRETERAX® tablet Servier Pharm. Ind., Turkey) was assayed. Its declared content was as follows.

Perindopril	2.000 mg
Indapamide	0.625 mg per tablet

3. Procedures

3.1. Procedure for high performance liquid chromatography

3.1.1. Chromatographic Conditions

Chromatographic separation was carried out at

ambient temperature on a RP-YMC pack ODS A-132 C_{18} (5 μm, 15 cm × 6.0 mm i.d.) column. The compounds were separated isocritically with a mobile phase of phosphate buffer pH 2.4 and acetonitrile (7:3 v/v). The flow rate was 1.0 ml min^{-1}. The mobile phase was degassed for 15 min in an ultrasonic bath before use. The analysis was usually started after the passage of mobile phase to reach equilibrium. The injection volume was 20 μl.

All solvents were filtered through 0.45 μm milipore filter to use and degassed in an ultrasonic bath.

3.1.2. Calibration

An external standard method was used for quantitative determinations. Calibration graphs were prepared from authentic samples of perindopril and indapamide in the mobile phase. Triplicate 20-μl injections were made for each solution. The final concentrations of perindopril and indapamide in the samples were calculated by comparison of sample and standard peak area obtained with the average of three injections of standard solutions.

3.1.3. Analysis of tablets for HPLC

Ten commercial tablets and the contents of ten-tablet ingredients were separately weighed and powdered in a different mortars. A portion of the powder equivalent to about one tablet and the content of one tablet was weighed accurately, transferred to a 100-ml calibrated flask and suspended in mobile phase for HPLC method. The flasks were completed to volume with the same solvent. The samples were filtered through a 0.45-μm membrane filter, then further diluted to suit the calibration graphs.

3.2. Procedure for spectrophotometric methods

3.2.1. Calibration

Stock solutions were prepared by dissolving perindopril and indapamide in methanol to obtain a concentration of 1.0 mg ml^{-1} for each compound. The standard solutions were prepared by dilution of stock solutions in methanol to reach concentration ranges of 10.0–50.0 and 10.0–30.0 μg ml^{-1} for perindopril and indapamide, respectively.

3.2.2. Assay procedure for tablet

An accurately weighed amount of powdered tablets equivalent to about one tablet was transferred into a 100-ml conical flask in methanol. After 30 min of mechanical shaking, the solution was filtered in a 100-ml calibrated flask through Whatman No. 42 filter paper. The residue was washed three times with 10 ml of solvent and then the volume was completed to 100 ml with the same solvent. Appropriate solutions were prepared by taking suitable aliquots of the clear filtrates and diluting them with methanol.

4. Results and discussion

4.1. HPLC method

The reversed-phase HPLC method was developed to provide a specific procedure for the rapid quality control analysis of binary mixtures containing perindopril–indapamide. As shown in Fig. 1, at a flow rate of 1.0 ml min^{-1}, the retention times were 3.20 min for perindopril and 5.80 min for indapamide in combined pharmaceutical dosage. The retention times for the investigated drugs were found to be 3.20 (perindopril) and 5.80 min (indapamide). To find the appropriate HPLC conditions for separation of the examined drugs, various reversed phase columns, isocratic and gradient mobile phase systems were tried. Successful attempts were performed using a reversed phase RP-YMC pack ODS A-132 C_{18} (5 μm, 15 cm × 6.0 mm i.d.) column. The mobile phases used were phosphate buffer pH 2.4 and acetonitrile (7:3 v/v). The optimum wavelength for detection was 215.0 nm at which much better detector responses for two drugs were obtained. Under the described HPLC parameters, the respective compounds were clearly separated and their corresponding peaks were sharply developed at reasonable retention times. For quantitative analysis, the analytical data for the calibration graphs are listed in Table 1. The linearity of the detector response for both drugs was determined

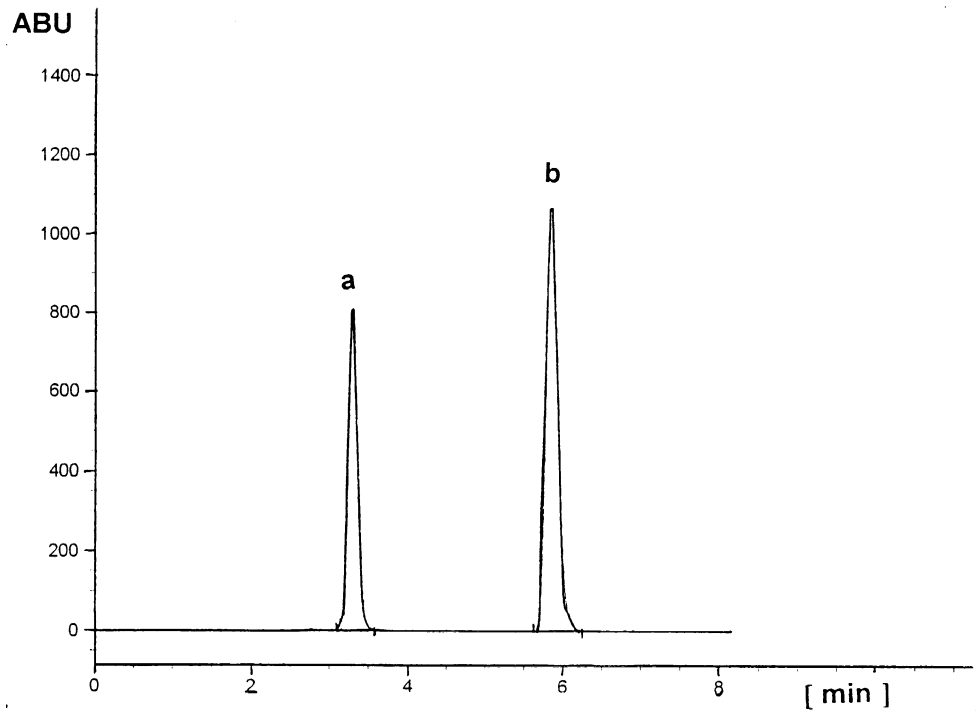


Fig. 1. Chromatogram of a diluted solution of tablet formulation containing (a) perindopril and (b) indapamide.

by plotting peak area ratios versus concentration. The detection limit was calculated as the intercept of the calibration graphs are listed in Table 1. The relative standard deviations (R.S.D.) were found to be less than 0.39%. To prove the validity and applicability of the proposed method, ten synthetic mixtures in the concentration range stated in Table 2 were assayed using procedures. The excipients (corn starch, magnesium stearete, lactose and talc) were added to the drug for recovery studies according to manufacturer's batch formula for per tablets. The data shown in Table 2 indicate good accuracy and precision of the proposed procedure.

LOD [18] were 0.028 μg ml^{-1} for perindopril, and 0.051 μg ml^{-1} for indapamide while LOQ [19] were 0.78 μg ml^{-1} for perindopril, 0.67 μg ml^{-1} for indapamide.

4.2. First derivative spectrophotometry

The stability of working solutions of perindopril and indapamide was studied by recording their absorption spectra. At first these spectra were measured. No changes in the spectra were observed for at least 2 days when the solutions were stored at room temperature in the dark.

Fig. 2a shows the absorption (zero-order) spectra of (a) perindopril (30.0 μg ml^{-1}) with a maximum at 216.0 nm and (b) indapamide (20.0 μg ml^{-1}) with a maximum at 248.0 nm. Perindopril and indapamide solutions showed overlapping UV spectra in methanol studied, making it difficult to resolve mixtures by classical spectrophotometry. However, derivative spectrophotometry can be used for resolving this problem satisfactorily. Fig. 2b shows the first derivative spectra (^1D) of perindopril and indapamide; the

Table 1
Statistical analysis of calibration graph in the determination of indapamide and perindopril by HPLC, first derivative spectrophotometry and ratio first derivative spectrophotometry

Parameters	Perindopril			Indapamide		
	HPLC	First derivative spectrophotometry	Ratio derivative spectrophotometry	HPLC	First derivative spectrophotometry	Ratio derivative spectrophotometry
Range (μg ml^{-1})	5.0–70.0	10.0–50.0	10.0–50.0	8.0–35.0	10.0–30.0	10.0–30.0
Detection limits (μg ml^{-1})	0.028	0.073	0.098	0.051	0.10	0.68
Regression equation (Y)[a]						
Slope (b)	0.090	1.42×10^{-2}	1.91×10^{-4}	0.093	8.43×10^{-2}	4.25×10^{-2}
S.D. on slope(S_b)	3.87×10^{-5}	7.72×10^{-5}	3.12×10^{-4}	4.89×10^{-5}	1.35×10^{-4}	1.24×10^{-4}
Intercept (a)	2.85	1.24×10^{-4}	8.45×10^{-2}	1.56	9.72×10^{-5}	4.87×10^{-2}
S.D. on intercept (S_a)	1.19×10^{-5}	8.24×10^{-6}	2.19×10^{-4}	7.41×10^{-5}	6.27×10^{-6}	1.11×10^{-4}
Standard error of estimation (S_e)	6.01×10^{-5}	1.98×10^{-3}	9.12×10^{-3}	4.47×10^{-5}	8.25×10^{-3}	3.15×10^{-2}
Correlation coefficient (r)	0.9998	0.9989	0.9990	0.9985	0.9985	0.9999
R.S.D. (%)[b]	0.21	0.56	1.58	0.39	0.54	0.67
%Range of error[b] (95% confidence limit)	0.38	0.48	0.65	0.45	0.29	0.93

[a] $Y=a+bC$ where C is concentration in μg ml^{-1} and Y in absorbance units.
[b] Five replicate samples.

Table 2
Assay results of perindopril and indapamide in laboratory-made mixtures and in commercial tablets

Sample	Recovery (mean ± S.D.)%[a]					
	Perindopril			Indapamide		
	HPLC	First derivative spectrophotometry	Ratio derivative spectrophotometry	HPLC	First derivative spectrophotometry	Ratio derivative spectrophotometry
Synthetic mixtures	98.4 ± 0.74 $t = 0.59$ (2.26)[b] $F = 2.30$	99.1 ± 1.5 0.91 1.50	99.9 ± 0.8	99.1 ± 1.28 $t = 0.48$ $F = 1.87$	98.5 ± 1.9 1.02 1.04	98.9 ± 0.4
Commercial tablets[c]	99.7 ± 1.43 $t = 0.97$ $F = 1.84$	98.8 ± 1.9 0.84 2.41	99.1 ± 0.9	99.7 ± 0.57 $t = 0.87$ $F = 2.30$	99.7 ± 1.4 0.48 0.77	99.8 ± 1.3

[a] Mean and R.S.D. for ten determinations; percentage recovery from the label claim amount.
[b] Values in parentheses are the theoretical values at $P = 0.95$. Theoretical values at 95% confidcence limits. $F = 3.18$; $t = 2.26$.
[c] Preterax® tablets were labeled to contain 2.0 mg perindopril, 0.625 mg indapamide per tablets, respectively.

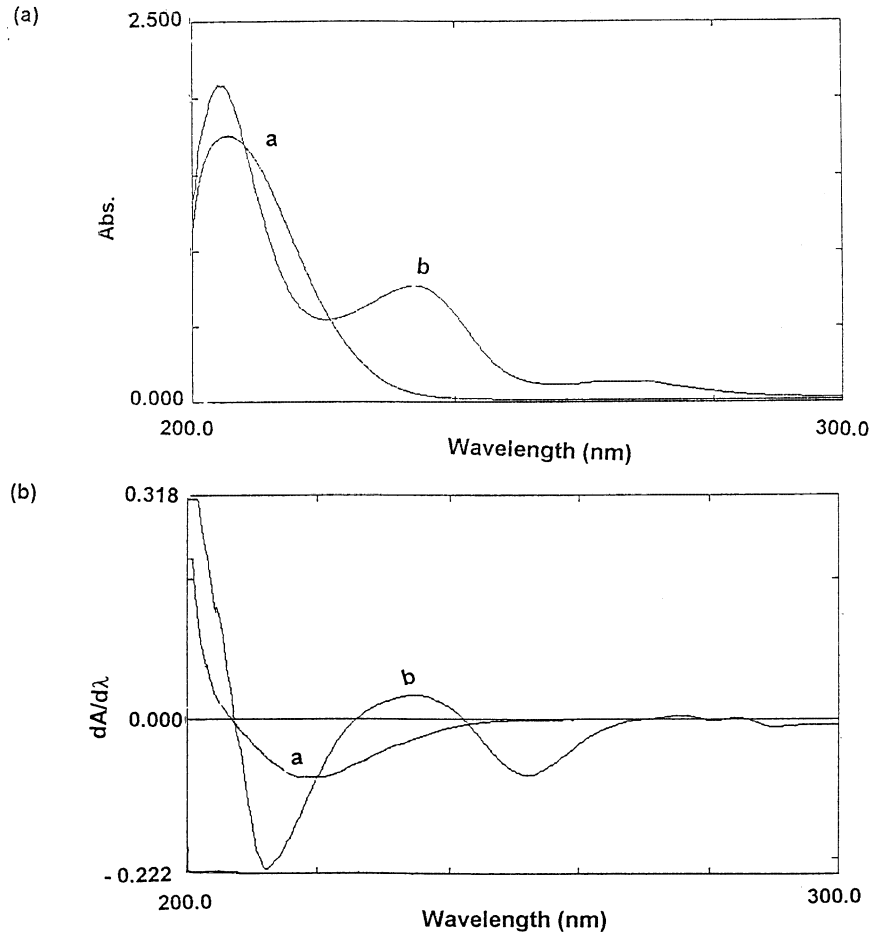


Fig. 2. a. Zero-order, (a) 30.0 μg ml^{-1} perindopril; (b) 20.0 μg ml^{-1} indapamide in methanol. b. First derivative spectra, (a) 30.0 μg ml^{-1} perindopril; (b) 20.0 μg ml^{-1} indapamide in methanol.

^1D spectra of both drugs display features, which may permit the determination of both drugs in the presence of each other. The ^1D amplitudes at 225.7 nm (zero-crossing of indapamide) and at 255.4 nm (peak-to-baseline, nil contribution from perindopril) were chosen for the simultaneous determination of perindopril and indapamide, respectively, in a mixture. Linear relationships between the selected amplitudes from the ^1D spectra and drug concentration were observed. Under the described experimental conditions, the graphs obtained by plotting the derivative values of each drug in this mixture versus concentration, in the range stated in Table 1, show linear relationships. A critical evaluation of the proposed method was performed by the statistical analysis of the data, where slopes intercepts and correlation coefficients were shown in Table 1. R.S.D. values of the slope and intercepts of the calibration graphs indicated the high reproducibility of the proposed method. LOD were 0.081 μg ml^{-1} for perindopril and μg ml^{-1} for indapamide; while LOQ were 0.79 μg ml^{-1} for perindopril and 0.899 μg ml^{-1} for indapamide. The selected methods were successfully applied to the determination of these drugs in laboratory-prepared mixtures and commercial tablets. The excipients (corn starch, magnesium stearete, lactose and talc) were added to the drug for recovery studies

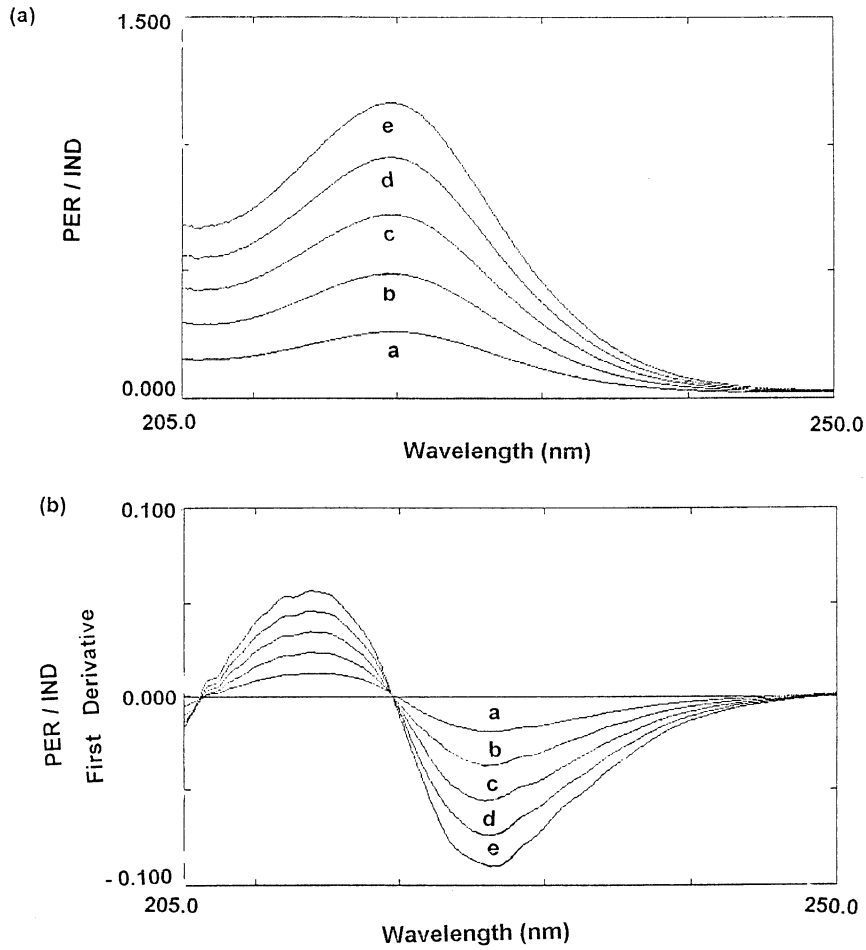


Fig. 3. a. Ratio spectra of the perindopril, (a) 10.0; (b) 20.0; (c) 30.0; (d) 40.0; (e) 50.0 μg ml^{-1}, where μg ml^{-1} indapamide used as divisor in methanol. b. First ratio derivative spectra of the perindopril, (a) 10.0; (b) 20.0; (c) 30.0; (d) 40.0; (e) 50.0 μg ml^{-1}, where 20.0 μg ml^{-1} indapamide used as divisor in methanol.

according to manufacturer's batch formula for per tablets. The results are summarized in Table 2.

4.3. Ratio spectra derivative spectrophotometry

The method of derivative ratio spectra with a standardized divisor, described in detail elsewhere [17], involves dividing the spectrum for a mixture into the standardized spectra for each of the analytes and deriving the ratio to obtain a spectrum that is independent of the analyte concentration used as the divisor.

Fig. 2a shows the absorption spectra of perindopril and indapamide. The absorption spectra of the two components are strongly overlapped. This spectral overlapping was sufficiently enough to demonstrate the resolving power of the proposed method. Fig. 3a and b shows the ratio spectra of different perindopril standards (spectra divided by the spectrum of a 20.0 μg ml^{-1} indapamide solution) and their first derivatives. The ratio first derivative amplitudes at 226.5 ($^1DD_{226.5}$)[1] nm cor-

[1] Derivative Divided$_{\text{wavelength measure}}$.

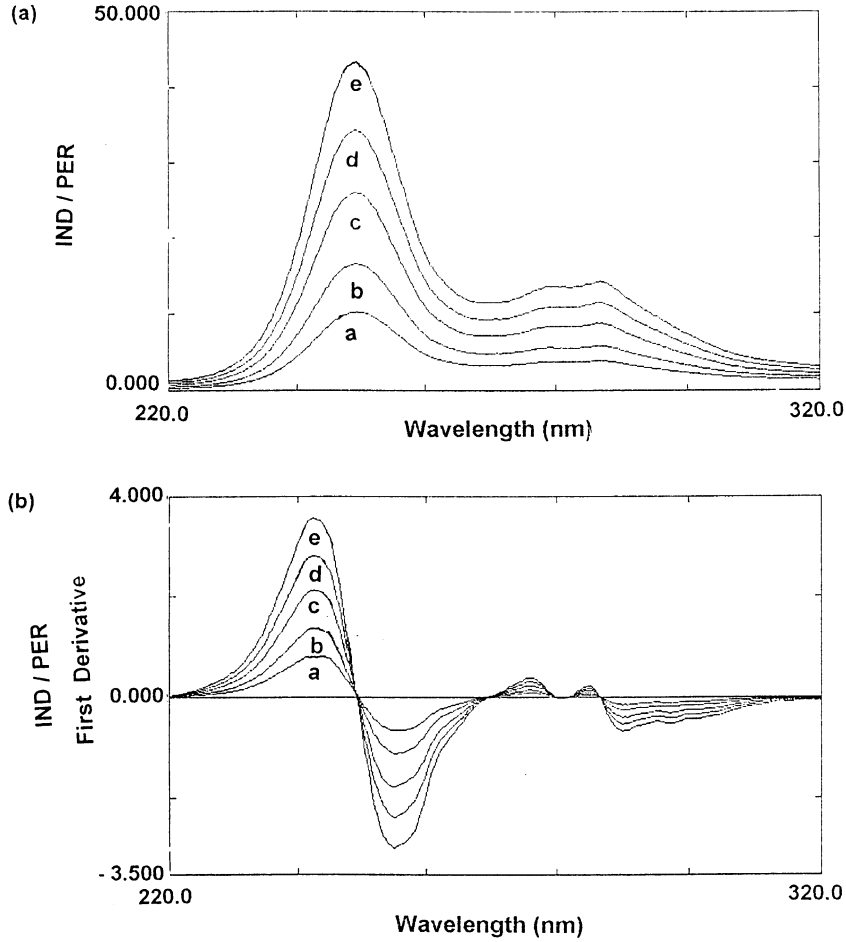


Fig. 4. a. Ratio spectra of the indapamide, (a) 10.0;(b) 15.0; (c) 20.0; (d) 25.0; (e) 30.0 μg ml^{-1}, where 30.0 μg ml^{-1} perindopril used as divisor in methanol. b. First ratio derivative spectra of the indapamide, (a)10.0; (b) 15.0; (c) 20.0; (d) 25.0; (e) 30.0 μg ml^{-1}, where 30.0 μg ml^{-1} perindopril used as divisor in methanol.

responding to a minimum wavelengths are proportional to the perindopril concentration. For determining indapamide, the stored spectra of the mixtures were divided by a standard spectrum of perindopril of 30.0 μg ml^{-1}. In the same way as describe above, the content of indapamide was determined by selecting the first derivative of the ratio spectrum in the range 220.0–320.0 nm and measuring the signals at 255.3 nm ($^1DD_{255.3}$) Fig. 4. The influence of $\Delta\lambda$ for obtaining the first derivative of the ratio spectra was tested to obtain the optimum wavelength interval; $\Delta\lambda = 6$ nm was considered to be suitable. Under the experimental conditions described, standard calibration curves for and indapamide were constructed by plotting absorbance versus concentration. Conformity with Beer's law was evident in the concentration range of the final dilution cited in Table 1. The correlation coefficients were 0.9990 and 0.9999 indicating good linearity. Five replicate determinations at different concentration levels were carried out to test the precision of the methods.

R.S.D. were found to be less than 1.58%, indicating reasonable repeatability of the proposed method. LOD were 0.036 μg ml^{-1} for perindopril and 0.056 μg ml^{-1} for indapamide; while LOQ

were estimated 0.58 μg ml^{-1} for perindopril and 0.512 μg ml^{-1} for indapamide. Calibration graphs were made from the minimum at 226.5 ($^1DD_{226.5}$) nm and a minimum at 255.3 ($^1DD_{255.3}$) nm wavelengths perindopril and indapamide.

Under the described experimental conditions, the graphs obtained by plotting the ratio derivative values of each drug in this mixture versus concentration, in the range stated in Table 1, show linear relationships. A critical evaluation of the proposed method was performed by the statistical analysis of the data, where slopes intercepts and correlation coefficients were shown in Table 1. R.S.D. values of the slope and intercepts of the calibration graphs indicated the high reproducibility of the proposed method. The selected method were successfully applied to the determination of these drugs in laboratory-prepared mixtures and commercial tablets.

The HPLC method was chosen as the analytical reference method. First derivative spectrophotometry and ratio derivative spectrophotometry were compared with HPLC method. The results obtained were summarized in Table 2. No significant differences were found between the results obtained by the HPLC method, the first derivative spectrophotometry and ratio derivative spectrophotometry, for same batch at the 95% confidence level (student's t-test and F-variance ratio test).

5. Conclusion

HPLC and the spectrophotometric methods are suitable techniques for the simultaneous determination of perindopril and indipamide in multi-component formulations without interference of each other. The ratio spectra derivative method, and derivative spectrophotometric method are rapid, simple and sensitive. In the ratio spectra derivative spectrophotometry separate peaks and higher values of measurements can be obtained owing to the advantages of the selectivity of divisor concentration. This higher values of measurements on the separate peak, no need to work only at zero-crossing points is an advantage for the ratio derivative spectrophotometry in comparison to the derivative spectrophotometry. HPLC method gives a good resolution between perindopril and indapamide within a short analysis time (< 5.8 min). The HPLC method may be considered to be more specific than other methods, but also more expensive, requiring sophisticated chromatographic instrumentation for its performance. All the developed methods may be recommended for routine and quality control analysis of the investigated drugs in two-component pharmaceutical preparations.

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