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Spectrophotometric and HPTLC-densitometric determination of lisinopril and hydrochlorothiazide in binary mixtures

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Abstract

Different spectrophotometric and HPTLC-densitometric methods are presented for the simultaneous determination of lisinopril and hydrochlorothiazide in pharmaceutical tablets. The spectrophotometric methods include third derivative (^{3}D) ultraviolet spectrophotometry with zero crossing measurement at 217.4 and 233.4 nm, second derivative of the ratio spectra with measurement at 214.3 and 228.0 nm; both classical least squares and principal component regression were applied to the UV absorption and first derivative spectra of the mixture. The HPTLC method was based on separation of both drugs followed by densitometric measurements of their spots at 210 and 275 nm for lisinopril and hydrochlorothiazide, respectively. The separation was carried out on Merck HPTLC aluminum plates of silica gel 60 F_{254}, using chloroform–ethylacetate–acetic acid (10:3:2 by vol.) as mobile phase. The linear and second order polynomial were used for the regression equation of lisinopril and hydrochlorothiazide, respectively.
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1. Introduction

Lisinopril, (*S*)-1-[N^2-(1-carboxy-3-phenylpropyl)-L-lysyl]-L-proline dihydrate, (I) is an angiotensin converting enzyme inhibitor. Hydrochlorothiazide, 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide1,1dioxide, (II) is one of the oldest and widely used thiazide diuretics. The binary mixture of the two drugs is used in the treatment of hypertension. Various spectrophotometric methods have been reported for the simultaneous determination of both drugs in pharmaceutical tablets by measuring the UV

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absorbance at 205 and 225 nm [1]. The Vierordt method uses the absorbance at 259.8 and 272.7 nm, first derivative spectrophotometry uses zero crossing points at 269.6 and 279.8 nm and ratio-spectra first derivative spectrophotometry measures the signals at 253.7 and 243.6 nm for (I) and 280.1 and 270.8 nm for (II) [2]. The mixture was also analyzed through derivatization of (I) with acetylacetone and formaldehyde to form colored condensation products measured at 356 nm while (II) is determined by first derivative spectrophotometry measured at 326 nm [3]. A HPLC method has been reported for the simultaneous determination of the two drugs using an ODS Hypersil column [4]. No HPTLC and chemometric methods have been reported for the determination of this mixture.

By comparison with HPLC, high performance thin layer chromatography (HPTLC) still has the advantage of a rapid, reliable and economical analysis method. In recent years, HPTLC has been improved to incorporate the following features: HPTLC grade stationary phase, automated sample application devices, controlled development environment, automated developing chamber, computer-controlled densitometry and quantitation, and fully validated procedures. These features result in methods that are not only convenient, fast, robust and cost-efficient, but also reproducible, accurate and reliable.

The greatest difficulties with UV-Vis multidetermination methods arise when the analytes to be determined give partial or fully overlapped spectra, as is the case with the ingredients of most pharmaceutical preparations. The application of multivariate calibration such as classical least squares and principal component regression to the absorbance signals produced by drugs during their simultaneous determination in pharmaceutical preparations is an effective means for quality control of their manufacture. Control analyses on pharmaceutical preparations using multivariate methods have been proved to be a valid alternative to HPLC, even with highly complex samples. The performance of several graphical methods such as zero-crossing and derivative ratio spectra with standardized devisor and numerical methods such as classical least squares and principal component regression for the resolution of binary mixtures was compared. Numerical methods were found to be specially suited to multicomponent analysis, particularly for mixtures with highly overlapped spectra.

The present work presents HPTLC; third derivative spectrophotometry (^{3}D) ; second derivative of the ratio spectra (^{2}DD) ; classical least squares (CLS) and principal component regression (PCR) applied to the UV absorption (A) and first derivative (^{1}D) spectra for simultaneous determination of (I) and (II) in tablets. The proposed methods are more simple and sensitive than the other published methods, reduced the duration of the analysis and are suitable for routine determination of the two drugs.

2. Experimental

2.1. Instrumentation

A double-beam Shimadzu (Japan) UV-Visible spectrophotometer, model UV-1601 PC connected to an IBM compatible computer and a HP600 inkjet printer was used. The bundled software was UVPC personal spectroscopy software version 3.7 (Shimadzu). The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min^{-1}. The absorption spectra of test and reference solutions were recorded in 1-cm quartz cells over the range 200–350 nm. The first, second and third derivatives of the measured spectra were obtained using the accompanying software with $\Delta\lambda = 4$ nm and scaling factor of 10 and 100 for ^{1}D and ^{3}D, respectively.

HPTLC plates (20 × 10 cm, aluminum plates precoated with silica gel 60 F_{254}) were purchased from E. Merck (Darmstadt, Germany). The samples were applied to the plates using a 10-μl Hamilton microsyringe. A Shimadzue dual wavelength flying spot densitometer model CS-9000 was used. The experimental conditions were: wavelength = 210 and 275 nm for (I) and (II), respectively, photomode = reflection, scan mode = zigzag, swing width = 12.

2.2. Materials and reagents

Pharmaceutical grades of (I) and (II) were kindly supplied by Sedico (6 October, City, Egypt) and certified to contain 100.00 and 99.99 %, respectively. Methanol, chloroform, ethylacetate, acetic and hydrochloric acids were analytical grade.

The commercial Zestoretic tablets used (Batch No. 1197102) was manufactured by Sedico (6 October, City, Egypt) under license from Zenica Ltd, England. Each tablet contains lisinopril dihydrate equivalent to 20 mg anhydrous lisinopril and 12.5 mg hydrochlorothiazide, in addition to tablet excipients consisting of maize starch, calcium hydrogen phosphate, magnesium stearate and mannitol.

2.3. HPTLC conditions

The HPTLC plates were developed with chloroform–ethylacetate–acetic acid (10:3:2 by vol.) as mobile phase. For detection and quantification 10 μl of test and 10 μl of different concentrations of the standard solution within the quantitation range were applied as separate compact spots 15 mm apart and 10 mm from the bottom of the plate using a 10-μl Hamilton microsyringe. The plate was developed up to the top (over a distance of 8 cm) in the usual ascending way. The chromatographic tank was saturated with mobile phase in the usual manner. After elution, the plate was air dried and scanned at 210 and 275 nm for (I) and (II), respectively, as under the described instrumental parameters.

2.4. Standard solutions and calibration

Stock standard solutions were prepared separately by dissolving 100 mg of (I) or 125 mg of (II) in 50 ml methanol.

The standard solutions were prepared by dilution of the stock standard solutions with 0.1 M hydrochloric acid (for spectrophotometric methods) or methanol (for HPTLC method) to reach the linearity range specified for each method stated in Tables 1 and 2.

2.4.1. For ^{3}D method

The values of the ^{3}D amplitudes were measured at 217.4 nm (zero-crossing of II) and 233.4 nm (zero-crossing of I) and found to be proportional to the concentration of (I) and (II), respectively.

Table 1
Characteristic parameters for the regression equations of third derivative (^{3}D) and second derivative of the ratio spectra (^{2}DD) methods for determination of lisinopril (I) and hydrochlorothiazide (II)

Parameters	^{3}D		^{2}DD	
	I	II	I	II
Linearity (μg ml^{-1})	8–56	2.5–17.5	8–40	5–20
Regression equation (Y)[a]: slope (b)	1.45×10^{-2}	4.26×10^{-2}	4.85×10^{-3}	3.97×10^{-2}
Standard deviation of the slope (S_b)	1.31×10^{-4}	1.50×10^{-4}	4.00×10^{-5}	4.30×10^{-4}
Relative standard deviation of the slope (%)	0.90	0.35	0.82	1.08
Confidence limit of the slope[b]	1.42×10^{-2}	4.22×10^{-2}	4.75×10^{-3}	3.87×10^{-2}
	-1.48×10^{-2}	-4.29×10^{-2}	-4.94×10^{-3}	-4.07×10^{-2}
Intercept (a)	5.87×10^{-3}	2.88×10^{-2}	8.00×10^{-4}	1.7×10^{-2}
Standard deviation of the intercept (S_a)	3.41×10^{-4}	1.46×10^{-3}	3.4×10^{-4}	5.40×10^{-3}
Confidence limit of the intercept[b]	5.08×10^{-3}	2.54×10^{-2}	1.46×10^{-5}	4.52×10^{-3}
	-6.66×10^{-3}	-3.22×10^{-2}	-1.59×10^{-3}	-2.94×10^{-2}
Correlation coefficient (r)	0.9998	0.9999	0.9998	0.9998
Standard error of estimation	7.4×10^{-3}	2.2×10^{-3}	1.2×10^{-3}	6.3×10^{-3}

[a] $Y=a+bC$,where C is the concentration of drug in μg ml^{-1} and Y is the amplitude at the specified wavelength.
[b] 95% confidence limit.

Table 2
Characteristic parameters for the linear regression equation of lisinopril (I) and the second order polynomial regression equation of hydrochlorothiazide (II) of the HPTLC method

Standard deviation of the first regression coefficient (S_b)	4.85×10^3–4.95×10^3	-2.84×10^2–(-3.76×10^2)
Parameters	I	II
Linearity range (μg/spot)	4–20	2.5–25
Regression equation (Y)[a]:	4.90×10^3	20.82×10^3
First regression coefficient (b)		
Standard deviation of the first regression coefficient (S_b)	0.2×10^2	5.6×10^2
Confidence limit of the first regression coefficient[b]	4.85×10^3–4.95×10^3	19.53×10^3–22.11×10^3
Second regression coefficient (c)	–	-3.3×10^2
Standard deviation of the second regression coefficient (S_c)	–	0.2×10^2
Confidence limit of the second regression coefficient[b]	–	-2.84×10^2–(-3.76×10^2)
Intercept (a)	1.24×10^3	71.41×10^3
Standard deviation of the intercept (S_a)	2.30×10^2	3.39×10^3
Confidence limit of the intercept[b]	7.08×10^2–1.77×10^3	63.58×10^3–79.24×10^3
Correlation coefficient	0.9999	0.9998
Standard error of estimation	2.8×10^{-3}	3.8×10^{-5}

[a] $Y=a+bX$ for (I) and $Y=a+bX+cX^2$ for (II) where X is the concentration of drug in μg/spot and Y is the peak area of the spot.
[b] 95% confidence limit.

2.4.2. For ^{2}DD method

For (I): the UV absorption spectra of standard solutions of (I) were divided by a normalized spectrum of (II) [a spectrum of unit concentration]. The second derivative was calculated for the obtained spectra with $\Delta\lambda=4$ nm. The second derivative of the ratio spectra obtained was smoothed with 16 experimental points. The amplitudes at 214.3 nm were measured and found to be proportional to the concentrations of (I).

For (II): the UV absorption spectra of standard solutions of (II) were divided by a normalized spectrum of (I). The second derivative was calculated for the obtained spectra with $\Delta\lambda=4$ nm. The second derivative of the ratio spectra obtained was smoothed with 16 experimental points. The amplitudes at 228 nm was measured and found to be proportional to the concentrations of (II).

2.4.3. For CLS and PCR methods

A training set of 16 synthetic mixtures with different concentrations in the range of 15–30 g ml^{-1} for (I) and 8–20 g ml^{-1} for (II) within the concentration ratio ranging from 1:0.4 to 1:1 for (I):(II), were prepared in 0.1 M hydrochloric acid.

The UV absorption and first derivative were recorded over the range 200–350 nm. The data points of the spectra were collected every 2 nm. The data were then exported into EXCEL®. The computations were made in MICROSOFT® EXCEL 97-Arabic edition using macros written in VISUAL BASIC FOR APPLICATION. The multivariate calibration models were applied to these mixtures to calculate the concentrations of each component.

2.4.4. For HPTLC method

Ten microliters of each standard solution were applied to the HPTLC plate. The plate was developed using the previously described mobile phase. The peak areas were plotted against the corresponding concentrations to obtain the calibration graph.

2.5. Sample preparation

Twenty tablets were weighed and finely powdered. A portion of the powder equivalent to 40 mg of (I) and 25 mg of (II) was weighed accurately, dissolved and diluted to 50 ml with methanol. The sample solution was filtered.

2.5.1. For spectrophotometric methods

Further dilutions of the sample solution were carried out with 0.1 M hydrochloric acid to reach the linearity range specified for each method. The general procedures for ^{3}D, ^{2}DD, CLS and PCR methods described under calibration were followed and the concentration of (I) and (II) were calculated.

2.5.2. For HPTLC method

Ten microliters of the sample solution were applied to the HPTLC plate. The plate was developed in the previously described chromatographic conditions. The peak area of the spots were measured at 210 nm for (I) and 275 nm for (II) and their concentrations in the sample were determined using multilevel calibration developed on the same plate under the same conditions, using a linear regression equation for (I) and a second order polynomial regression equation for (II).

2.6. Percent recovery study

This study was performed by addition of known amounts of (I) and (II) to a known concentration of the commercial tablets (standard addition method). The resulting mixtures were assayed and results obtained were compared with expected results (Table 3).

3. Results and discussion

3.1. ^{3}D method

The main instrumental parameters that affect the shape of the derivative spectra are the wavelength scanning speed, the wavelength increment over which the derivative is obtained ($\Delta\lambda$) and the smoothing. These parameters need to be opti-

Table 3
Determination of lisinopril(I) and hydrochlorothiazide (II) in synthetic mixtures and commercial tablets using HPTLC, third derivative (^{3}D), second derivative of the ratio spectra (^{2}DD) methods; classical least squares (CLS) and principal component regression (PCR) methods applied to UV absorption (A) and first derivative (^{1}D) spectra; and reference HPLC methods

	Mean found ± S.D.[a]							
				CLS		PCR		HPLC
	HPTLC	^{3}D	^{2}DD	A	^{1}D	A	^{1}D	
Synthetic mixtures								
For (I)	100.1 ± 0.83	100.2 ± 1.22	100.0 ± 1.24	99.9 ± 0.91	99.8 ± 1.10	99.9 ± 0.78	100.0 ± 0.72	
For (II)	100.8 ± 0.91	100.2 ± 1.06	100.1 ± 1.19	100.0 ± 0.72	100.1 ± 0.92	100.0 ± 0.71	100.1 ± 0.69	
Commercial tablets								
For (I)	99.8 ± 1.10	99.9 ± 1.37	100.0 ± 1.60	100.0 ± 0.70	99.9 ± 1.22	100.3 ± 0.78	100.0 ± 0.86	99.7 ± 0.81
	$t = 0.16$	0.28	0.37	0.63	0.31	1.19	0.57	$t = (2.31)$[b]
	$F = 1.84$	2.86	3.93	1.34	2.27	1.08	1.13	$F = (6.39)$[b]
For (II)	100.5 ± 0.97	100.2 ± 1.33	100.3 ± 1.39	99.8 ± 0.72	100.2 ± 1.11	99.9 ± 0.69	100.2 ± 0.77	100.0 ± 0.95
	$t = 0.82$	0.27	0.40	0.38	0.31	0.19	0.37	$t = (2.31)$[b]
	$F = 1.04$	1.96	2.14	1.74	1.36	1.90	1.52	$F = (6.39)$[b]
Recovery[c]								
For (I)	99.8 ± 0.91	99.9 ± 1.32	100.4 ± 1.31	99.6 ± 0.69	99.9 ± 0.89	100.1 ± 0.78	99.7 ± 0.75	
For (II)	100.6 ± 0.77	100.2 ± 0.92	99.7 ± 1.47	99.7 ± 0.56	100.2 ± 0.99	99.9 ± 0.71	100.2 ± 0.76	

[a] Mean and S.D. for percentage recovery from the label claim amount.
[b] Theoretical values for t and F.
[c] For standard addition of 50% of the nominal content ($n = 5$).

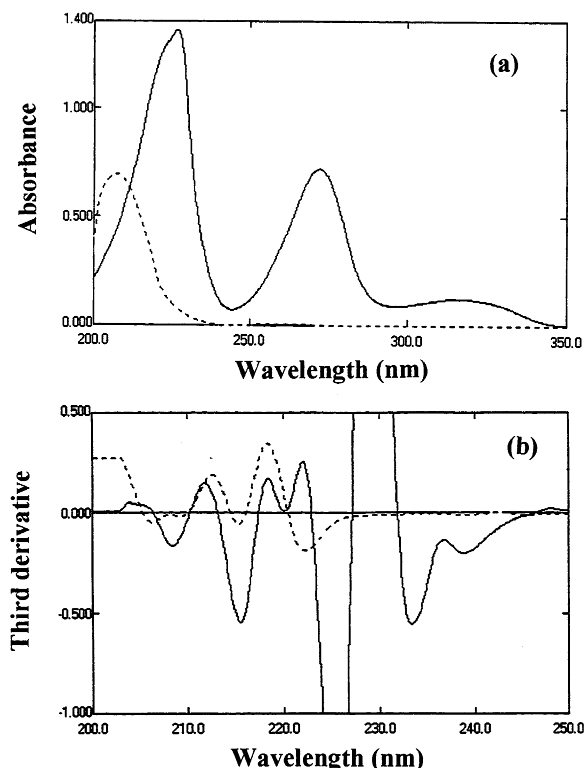

Fig. 1. UV absorption spectra (a) and third derivative spectra (b) of 20 μg ml^{-1} of lisinopril (-----) and 12.5 μg ml^{-1} of hydrochlorothiazide (—) in 0.1 M hydrochloric acid.

mized to give a well-resolved large peak and to give good selectivity and larger sensitivity in the determination. Generally, the noise level decreases with an increase in $\Delta\lambda$ thus decreasing the fluctuation in the derivative spectrum. However, if the value of $\Delta\lambda$ is too large, the spectral resolution is very poor. Therefore, the optimum value of $\Delta\lambda$ should be determined by taking into account the noise level and the resolution of the spectrum. Some values of $\Delta\lambda$ were tested; $\Delta\lambda = 4$ nm and wavelength scanning speed = 2800 nm min^{-1} were selected for the ^{3}D method as the optimal conditions to give a satisfactory signal to noise ratio.

(I) Possesses a very low absorption in the UV region while (II) exhibits a large absorption in the same region (Fig. 1a). The conventional UV method for the assay of (I) is susceptible to interference from (II). Third derivative spectrophotometry can be used to overcome this problem. The third derivative spectra of (I) and (II) in 0.1 M hydrochloric acid (Fig. 1b) showed significant differences in some areas that permits the determination of both drugs. The zero-crossing method is the most common procedure for the preparation of the analytical calibration graph [5]. The ^{3}D value at 217.4 nm (zero-crossing of II) has been used for quantitation of (I) for maximum sensitivity and decreasing the error resulted from the high slope of the third derivative spectrum of (II). Also, the ^{3}D value at 233.4 nm (zero-crossing of I) has been used for quantitation of (II). The plots of the absolute values of third derivative at 217.4 and 233.4 nm against concentrations of (I) and (II), respectively, showed a linear relationship.

3.2. ^{2}DD method

To optimize the simultaneous determination of the lisinopril and hydrochlorothiazide by using the ^{2}DD method, it is necessary to test the influence of the divisor standard concentration and the $\Delta\lambda$ and smoothing function. All these variables were studied. The influence of the $\Delta\lambda$ for obtaining the second derivative of the ratio spectra was tested and $\Delta\lambda = 4$ nm was selected as the optimum value. A correct choice of the divisor standard concentration is fundamental for several reasons. Among these, in the wavelength range were the absorbance of the standard spectrum used as divisor is zero or below the base line, the noise of ratio spectra is greatly increased. Hence, a certain overlap of spectra in the working wavelength region is actually desirable to avoid an increase of the error. If the concentration of divisor is increased or decreased, the resulting derivative ratio values are proportionality decreased or increased with the consequent variation of both sensitivity and linearity range. From several tests, the best results in terms of signal to noise ratio, sensitivity and repeatability followed using normalized spectra as divisor. Due to the extent of the noise levels on the ratio spectra, a smoothing function was used and 16 experimental points were considered as suitable.

The second derivative of the ratio spectra was preferred than the first derivative for a better

resolution of the ratio spectra and more accurate and precise results. In this method, the UV absorption spectra of (I) were divided by a normalized spectrum [6] of (II) (obtained by dividing the spectra for several standards of different concentrations by their corresponding concentrations and subsequently averaging them, in order to obtain a spectrum of unit concentration). The second derivative was calculated for the ratio spectra obtained with $\Delta\lambda = 4$ nm. These spectra were smoothed with 16 experimental points due to the high noise of the signals obtained [6] (Fig. 2). The concentration of (I) was proportional to the amplitude at 214.3 nm, in the concentration range 8–40 μg ml^{-1}. Similarly, for determination of (II), the UV absorption spectra of (II) were divided by a normalized spectrum of (I); from the ratio spectra obtained, a second derivative was calculated with $\Delta\lambda = 4$ nm. These spectra were also smoothed with 16 experimental points (Fig. 3). The concentration of (II) was proportional to the amplitude at 228 nm, in the concentration range of 5–20 μg ml^{-1}.

For the ^{3}D and ^{2}DD methods, the characteristic parameters of regression equations and correlation coefficients are given in Table 1.

The accuracy of ^{3}D and ^{2}DD methods were checked by analyzing six synthetic mixtures of (I) and (II) at various concentrations within the linearity range, at concentration ratios ranging from 1:0.4 to 1:0.8 for (I):(II). Satisfactory recoveries with small standard deviations were obtained (Table 3), which indicated the high repeatability and accuracy of the two methods.

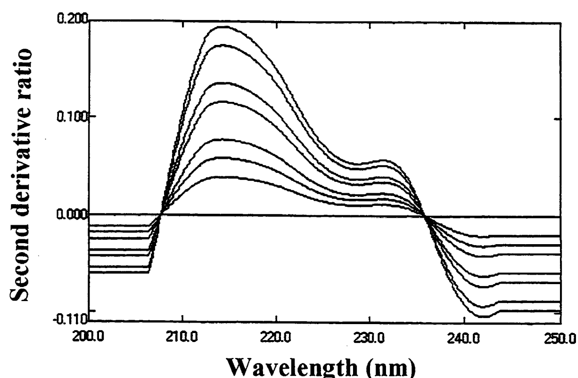

Fig. 2. Second derivative of the ratio spectra for different concentrations (8, 12, 16, 24, 28, 36, 40 μg ml^{-1}) of lisinopril, using normalized spectrum of hydrochlorothiazide as a devisor.

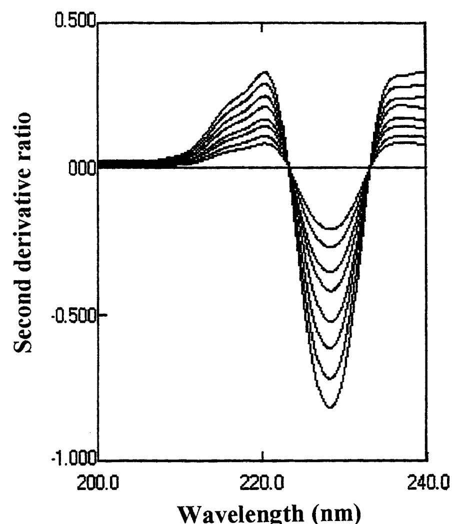

Fig. 3. Second derivative of the ratio spectra for different concentrations (5, 6.5, 8.5, 10, 12.8, 15, 17.5, 20 μg ml^{-1}) of hydrochlorothiazide, using normalized spectrum of lisinopril as a devisor.

3.3. CLS and PCR methods

To select the number of factors in the PCR method , the cross-validation method, leaving out one sample at a time was used. The predicted residual error sum of squares (PRESS) was calculated each time a new factor was added giving rise to different PCR models. The model that gives the best results was chosen.

CLS and PCR methods were applied to the UV absorption and first derivative spectra. For application of these methods, a training (calibration) set of 16 synthetic mixtures with different concentrations in the range of 15–30 μg ml^{-1} for (I) and 8–20 μg ml^{-1} for (II) within a concentration ratio ranging from 1:0.4 to 1:1 for (I):(II) were prepared. The CLS and PCR models developed were validated using a cross-validation procedure (leave one out) [7]. Given the set of 16 training spectra corresponding to synthetic mixtures, the CLS and PCR calibrations on 15 training spectra

were performed, and using these calibrations, the concentration of the left out mixture during calibration was determined. This process was repeated 15 times until each training synthetic mixture had been left once. Satisfactory recoveries with small standard deviations were obtained (Table 3), indicating an adequate model for CLS and PCR methods applied on UV absorption and first derivative spectra of (I) and (II).

3.4. HPTLC method

Experimental conditions, such as mobile phase, scan mode and wavelength of detection were optimized to provide accurate, precise and reproducible results for simultaneous determination of (I) and (II). The chosen scan mode was zigzag mode and the wavelengths of scanning were chosen to be 210 nm for (I) and 275 nm for (II). The greatest differences between the R_f values of the two drugs [0.31 for (I) and 0.88 for (II)], with minimum tailing were obtained by using the mobile phase consisting of chloroform–ethyl acetate–acetic acid in a ratio of 10:3:2 by vol., respectively. The separated spots of the two drugs were scanned separately on the same plate at the specified wavelengths.

The relationship between the concentration of each drug and peak area of the spot was investigated. The linear relationship was tested and found to be accepted for (I) and unaccepted for (II). The second order polynomial fit was found to be more suitable for (II). The characteristic parameters of the linear regression equation of (I) and the second order polynomial regression equation of (II) are shown in Table 2.

To assess the specificity, accuracy and selectivity of the HPTLC method for assay of both drugs without interference from one another, six synthetic mixtures of (I) and (II) at various concentrations within the linearity range at concentration ratio ranging from 1:0.4 to 1:1.5 for (I):(II) were prepared and analyzed. Satisfactory recoveries with small standard deviations were obtained (Table 3), which indicate the high repeatability and accuracy of the HPTLC method.

3.5. Method validation

Spiked placebos were prepared according to the manufacturing formula. The spiked placebos were tested at five levels: 50, 75, 100, 125 and 150% of label claim for each individual drug. Assays were performed in duplicate on two samples at the five levels. This was repeated with a second instrument, standard and sample preparation and analyst on different days. The complete set of validation assays was performed for each drug, determined by the proposed methods. Spiked placebo assays were used to determine accuracy and precision of the proposed methods for determination of each drug. The recoveries ranging from 99.8 to 100.8% of the amount of active ingredient spiked into the placebo. The bias showed only minor variation in recovery at each level with 0.6% the maximum variation observed. The proposed methods were tested for repeatability, reproducibility, selectivity, specificity, robustness and ruggedness. Satisfactory results were obtained. The proposed methods complied with USP [8] validation guidelines.

The non-instrumental methods for determination of the detection limit and the quantitation limit were applied [8], the limit of detection is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. While the limit of quantitation is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be determined with acceptable accuracy and precision. The detection limits of the proposed methods were found to be 1.5, 0.7, 0.8, 0.6 and 0.5 μg ml^{-1} for (I) and 0.3, 0.2, 0.4, 0.3 and 0.1 μg ml^{-1} for (II), detected by ^3D, ^2DD, CLS, PCR and HPTLC methods, respectively. While the quantitation limits of the proposed methods were found to be 4, 3, 2, 1.5 and 0.9 μg ml^{-1} for (I) and 1.5, 1.0, 0.9, 0.6 and 0.5 μg ml^{-1} for (II), determined by ^3D, ^2DD, CLS, PCR and HPTLC methods, respectively.

The stability of lisinopril dihydrate and hydrochlorothiazide during the analytical procedures were studied and found to be stable. The

two analytes were stable for at least 24 h in solution.

3.6. Tablet analysis

The proposed methods were applied to the determination of (I) and (II) in commercial tablets. Five replicate determinations were made. Satisfactory results were obtained for both drugs and were in good agreement with the label claims (Table 3). Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding (I) and (II) to the previously analyzed tablets. The recovery of each drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. The results of analysis of the commercial tablets and the recovery study (standard addition method) of both drugs (Table 3) suggested that there is no interference from any excipients, which are present in tablets. The results of determination of (I) and (II) in tablets obtained from the ^3D, ^2DD, CLS, PCR and HPTLC methods were compared with those of the reference HPLC method using a Hypersil column [4]. A statistical comparison of the results was performed with regard to accuracy and precision using Student's t-test and the F-ratio at a 95% confidence level (Table 3). There is no significant difference between the proposed methods with regard to accuracy and precision.

4. Conclusion

The proposed HPTLC, ^3D, ^2DD, CLS and PCR methods provide simple, accurate, and reproducible quantitative analysis for simultaneous determination of lisinopril and hydrochlorothiazide in tablets. The ^3D method is more rapid and simple than ^2DD method. While the ^2DD method has greater sensitivity and accuracy. The easier treatment of the data by using CLS and PCR methods makes them preferable to the ^2DD method. The proposed methods are suitable for routine determination of both drugs in their formulations, but they can not be considered as stability indicating assays. The proposed methods are more simple and sensitive than the published method and do not need any chemical derivatization or measurement of the UV spectra of the drug at short critical wavelengths. The HPTLC method has some advantages over HPLC such as a short run time, large sample capacity and minimal volume use of solvent. The use of CLS and PCR methods for the multicomponent analysis of complex mixtures with highly overlapped spectra offers considerable advantages over conventional multicomponent analysis which requires a separation technique such as HPLC. With these two methods, one can gain the advantages of speed, lower cost, and environmental protection without sacrificing accuracy.

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