Journal of Advanced Biomedical and Pharmaceutical Sciences

Journal Homepage: http://jabps.journals.ekb.eg

Two smart spectrophotometric methods for simultaneous determination of Lisinopril and Hydrochlorothiazide in binary mixtures

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Received: December 19, 2018; revised: January 12, 2019; accepted: January 15, 2019

Abstract

Two new, simple, fast and sensitive spectrophotometric methods were developed for analysis of binary mixtures of Lisinopril (LIS) and hydrochlorothiazide (HCT). These methods were absorptivity factor method and ratio subtraction method. In the first method, calculations were made using the absorbance of the mixtures at 211 nm where LIS absorptivity is half HCT absorptivity. On the other hand, the second method was based on obtaining the original spectrum of LIS from the zero order spectrum of the binary mixture then determining the concentration of LIS from its linear regression equation. HCT concentration in both methods was determined from the absorbance at 270 nm where a lack of interference of LIS occurs. The methods were validated according to ICH and applied successfully on different ratios of laboratory mixtures and on pharmaceutical tablets with excellent accuracy and precision.

Key words
Spectrophotometric, Lisinopril, Hydrochlorothiazide, Absorptivity factor, Ratio subtraction

1. Introduction

LIS (**Figure 1**), the third ACE inhibitor approved for use in the United States, is the lysine analogue of enalaprilat; unlike ENA, LIS itself is active. In vitro, LIS is a slightly more potent ACE inhibitor than is enalaprilat. They are both used for treatment of heart failure and hypertension [1]. Diuretic drugs e.g. HCT (**Figure 1**) and angiotensin converting enzyme (ACE) inhibitors e.g. LIS are amongst the major therapeutic advances of modern medicine due to their dramatic influence in the treatment of congestive cardiac failure and arterial hypertension. Diuretics achieve hypotension by eliminating salt and water, thus reducing blood volume. The renin-angiotensin system is immediately stimulated as a reflex response in order to conserve blood volume. When ACE inhibitors are used at the same time, activation of the renin-angiotensin system is prevented, enhancing the effect of both therapies and decreasing their side effects as lower doses are needed [2, 3].

LIS was previously analyzed by titrimetric [4], spectrophotometric [5-14], Spectrofluorimetric [15-19], chromatographic [20-38], electrophoretic [39, 40] and electrochemical methods [4, 41, 42].

Mixtures of LIS and HCT were analyzed simultaneously by spectrophotometric [43-45], chromatographic [21, 22, 29, 46-48], electrophoretic [39] and electrochemical methods [49].

However, chromatographic, electrophoretic and electrochemical methods require expensive instruments and large volumes of solvents, and limited number of spectrophotometric methods was available in literature to for simultaneous determination of both drugs including multivariate [43], ratio spectra derivative, derivative and Vierordt's spectrophotometric methods [44, 45]. So this work aimed to develop new spectrophotometric methods that have the advantages of being rapid, more sensitive, cheap and simple to perform.

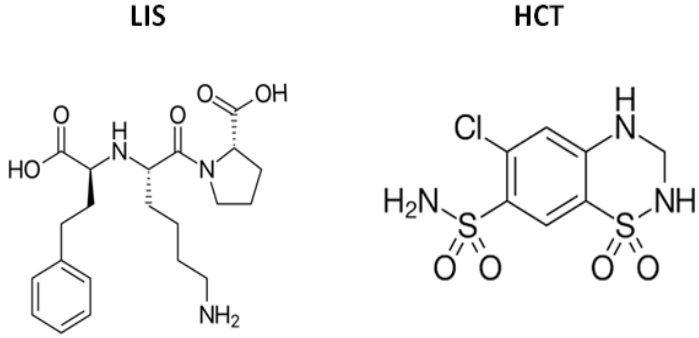

Figure 1: Chemical structures of LIS and HCT.

2. Theories of the proposed methods

2.1. The absorptivity factor method

The absorptivity factor is the ratio between the two absorptivities (a_x, a_y) of two mixed substances X & Y at intersection point with the same absorbance value. This point is called the absorptivity factor point (λ_F). If Y can be determined using well-known spectrophotometric methods, the absorptivity factor method is applied for the determination the concentration of X. The final equation that is applied in this method is:

$A_m = a_y (FC_x + C_y)$ (1)

Where, A_m are the absorbance of X, Y and their mixture at the absorptivity factor point (λ_F), F is the absorptivity factor, a_x and

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a_y are the absorptivities of X and Y respectively. C_x and C_y are the concentrations of X and Y respectively.
From equation (1) we can calculate the total concentration ($FC_x + C_y$) via the regression equation representing the linear relationship between the absorbance of Y and its corresponding concentration at the absorptivity factor point. The concentration of X can be determined after subtraction of concentration of Y and multiplication by the inverse of F. [50, 51]

2.2. Ratio subtraction method

When two drugs X and Y were combined and have overlapping spectra but one drug (Y) has an extended part in its zero order spectrum where no interference from drug (X) exists. Then drug X can be determined using ratio subtraction technique by dividing the zero order spectrum of the mixture by the zero order spectrum of a certain concentration of Y named as the divisor (Y'). The obtained constant is subtracted then the resulting spectra is multiplied by the divisor (Y') to isolate the original spectrum of X that can be determined by applying the linear regression equation of its calibration at its λ_{max}. On the other hand drug (Y) can be determined by applying the linear regression equation of its calibration at λ_{max} of the extended curve [50, 52].

3. Experimental

3.1. Apparatus

The instrument used throughout the work was Spectronic Genesis 2PC UV/visible (Milton Roy Co, USA) connected to IBM computer, that is uploaded with winspec software (version 1.22), and using 1cm quartz cuvette.

3.2. Materials

3.2.1. Pure authentic samples

LIS dihydrate powder (purity = 98.9 %) was generously provided by Sedico Co for pharmaceuticals (Cairo, Egypt) while HCT powder (purity = 98.2 %) was generously supplied by Amoun Co for pharmaceuticals (Cairo, Egypt). No further purification was done on the obtained powders.

3.2.2. Solvent

Methanol (analytical grade) was purchased from El Nasr chemical Co (Cairo, Egypt) and it was used as the main solvent for dissolution and dilution for all analyzed drugs.

3.2.3. Commercial tablets

Sinopril Co® tablets (Global Napi pharmaceuticals, Egypt) that were labeled to contain 20 mg LIS + 12.5 mg HCT were analyzed in this work (batch no. E23305).

3.3. Standard solutions

LIS and HCT powders were accurately weighed and dissolved separately in methanol to prepare a stock standard solution of concentration 100.0 µg mL^{-1}. Working solutions were prepared by further dilution with methanol in a series of 10-mL volumetric flasks to give final concentration ranges of 1.0 - 30.0 and 2.5 - 30.0 µg mL^{-1} in order to construct calibration curve for LIS and HCT, respectively.

3.4. General methods of analysis

3.4.1. The absorptivity factor method

Zero order spectrum of LIS (10.0 µg mL^{-1}) and HCT (5.0 µg mL^{-1}) were recorded. There were two points of intersections at 204 and 211 nm (Two absorptivity points). Wavelength of 211 nm was chosen for analysis because at this wavelength accurate results and better % recoveries were obtained. Two calibration curves were constructed for HCT by measuring the absorbance at both 270 nm and 211 nm to obtain two linear regression equations.
In order to analyze mixture containing LIS and HCT, the mixture is scanned and the absorbance values at both 211 nm (absorptivity factor point) and 270 nm are recorded. The concentration of HCT is determined through the linear regression equation at 270 nm where no interference from LIS occurs. Then LIS concentration is determined by subtracting HCT concentration from the value obtained using the linear regression equation at 211 nm and finally multiplying the result by 2.

3.4.2. Ratio subtraction method

Five different concentrations of LIS in the range of 5.0 – 30.0 µg mL^{-1} were scanned and calibration curve was constructed by measuring the absorbance at 210 nm (its λ_{max}) relative to the concentration. The divisor (Y') was the zero order spectrum of 5.0 µg mL^{-1} solution of HCT. To perform the analysis on a binary mixture, the spectrum of the mixture is divided by the divisor. A constant value appears in the region where extension of HCT spectrum occurred (260-280 nm). Subtraction of this constant from the spectrum and then multiplication of the obtained spectrum by the divisor (Y') isolates the original spectrum of LIS. Determination of LIS concentration can be finally made by applying the linear regression equation at 210 nm while HCT concentration is determined from the linear regression equation of its calibration at 270 nm by measuring the absorbance of the binary mixture at 270 nm.

3.5. Analysis of laboratory prepared mixtures

Accurate aliquots of either LIS or HCT were transferred into 10-mL volumetric flasks to prepare mixtures containing different ratios including 1:1, 1:2, 2:1, 1:3, 3:1, 2:3 and 3:2 then the solutions were completed to the mark with methanol. The final solutions were analyzed according to the general methods.

3.6. Analysis of pharmaceutical tablets

Ten tablets of Sinopril Co® tablets were accurately weighed, finely minced and mixed carefully. An accurately weighed portions of the powdered tablets equivalent to 10 mg LIS + 6.25 mg HCT are mixed in adequate portion of methanol, sonicated

for 10 minutes then filtration is done into 100-mL volumetric flask and the volume was completed to the mark with methanol to form stock solutions of 100.0 µg $mL^{-1}$of LIS + 62.5 µg mL^{-1} HCT. Different aliquots were taken and diluted to prepare working solutions and the general procedure was followed.

4. Results and discussion

The purpose of the present work is to develop new, sensitive, simple and fast analytical methods for simultaneous determination of LIS and HCT in their bulk powders and pharmaceutical formulations with high degree of accuracy and precision and without the need for preliminary separation of each drug individually such as extraction or filtration procedures. As well, to construct a statistical comparison between the ability of the proposed methods to determine both drugs in their pure form, laboratory prepared mixtures and in their pharmaceutical formulations. A serious spectral overlap was observed when the mixture of LIS and HCT in methanol was scanned in the wavelength region of 200 - 300 nm which prevent the direct determination of LIS (**Figure 2**). Therefore different methods were applied for attaining high separation and quantitative estimation of both drugs with no significant interference.

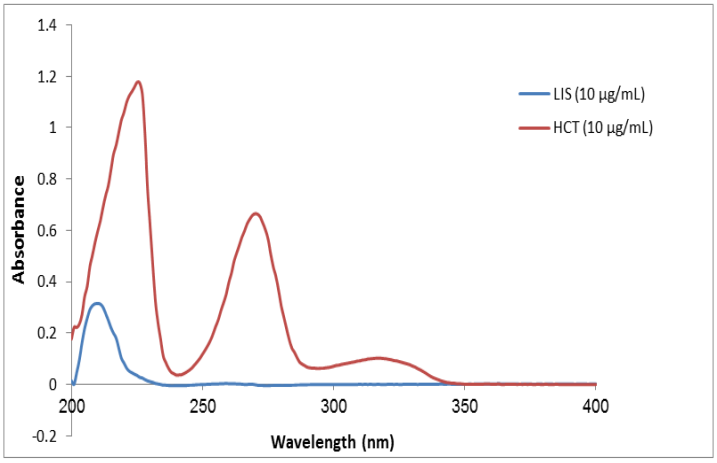

Figure 2: Zero order spectrum of LIS (10 µg mL^{-1}) and HCT (10 µg mL^{-1}) showing overlapped spectra.

4.1. Absorptivity factor point

The absorption spectra of LIS and HCT in the ratio (1:1); recorded in methanol show a severe spectral overlap in the wavelength region of 200 – 300 nm (**Figure 2**) without any point of intersection. But if the concentration of LIS is doubled (20 µg mL^{-1}), two intersection points will appear at 204 and 211 nm (**Figure 3**). At the absorptivity factor point $a_x/a_y = C_y/C_x = F$, then the absorptivity factor point (F) = 1/2.

HCT concentration was determined from the linear regression equation at 270 nm without interference (**Figure 4**). The wavelength 211 nm was chosen in the present work because it had given more accurate results. The linear regression equation obtained from the calibration of HCT at 211 nm (**Figure 4**) gave a value equals ($FC_x + C_y$). The concentration of LIS was obtained by subtracting HCT concentration (C_y) and multiplying the result by 2 (the inverse of F).

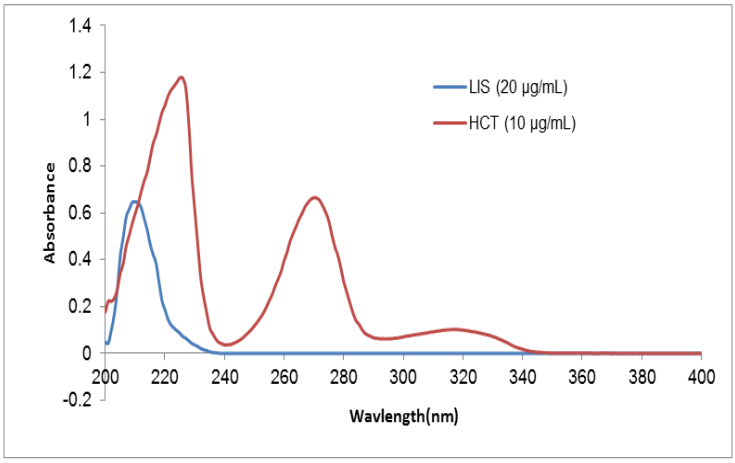

Figure 3: Zero order spectrum of LIS (20 µg mL^{-1}) and HCT (10 µg mL^{-1}) showing two intersection points at 204 and 211 nm.

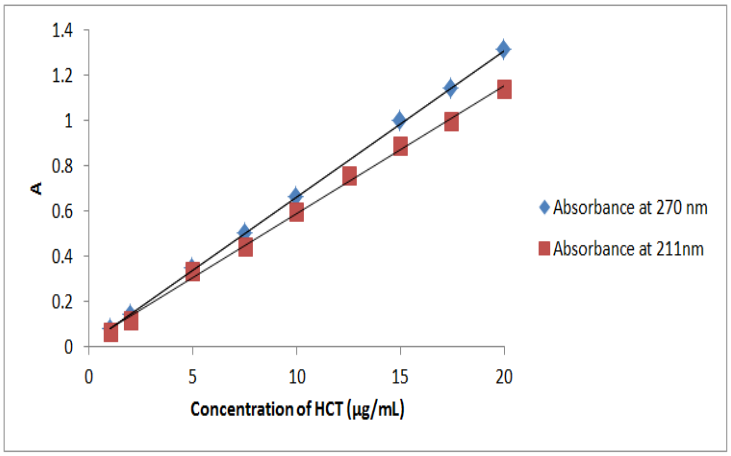

Figure 4: Calibration curve of HCT at 270 nm and at 211 nm (Absorptivity factor point).

4.2. Ratio subtraction method

The mixture of LIS and HCT which have overlapping spectra could be resolved by using spectra ratio subtraction method. The spectrum of the mixture was recorded in the region of 200 - 300 nm and divided by the spectrum of a known concentration of HCT (5 µg mL^{-1}) as a divisor. A new curve was obtained representing $\frac{LIS}{HCT} + constant$ (**Figure 5**). The constant can be obtained from the plateau in the region of 260 –300 nm. If the constant is subtracted from the curve in **Figure 5**, the ratio spectrum of LIS/HCT will be given. The sole spectrum of LIS in the mixture could be obtained through multiplying the ratio spectrum by the divisor spectrum (5 µg/mL HCT)). This gave the original zero order spectra of LIS in the mixture, which can be used for direct determination of LIS at 210 nm (**Figure 6**). The concentration of LIS could be calculated from the corresponding regression equation (obtained by plotting the absorbance values of the zero order curves of LIS at 210 nm against the corresponding drug concentrations) (**Figure 7**).

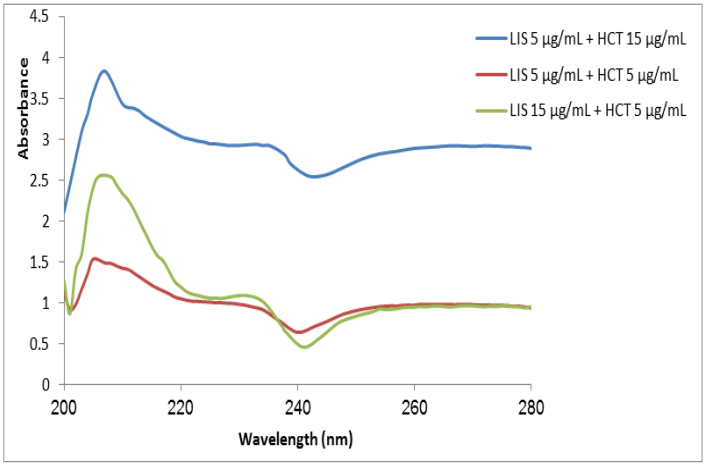

Figure 5: The division spectra of three analysed binary mixtures of LIS: HCT after division by the divisor (Y') showing plateau in the extended region (260-280).

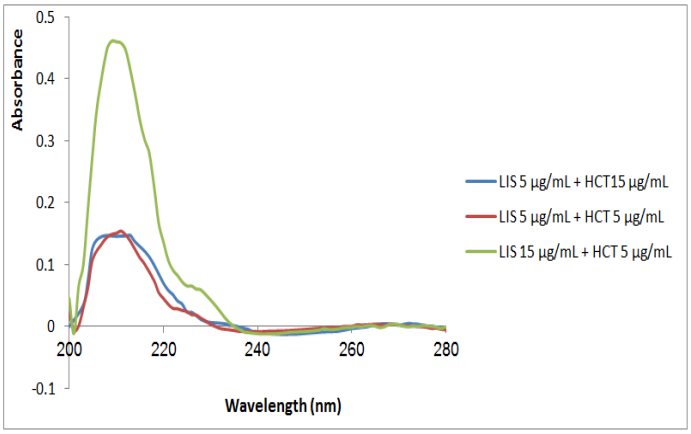

Figure 6: The Final spectra of three analysed binary mixtures of LIS: HCT after constant subtraction and multiplication by the divisor (Y') showing the original spectrum of LIS.

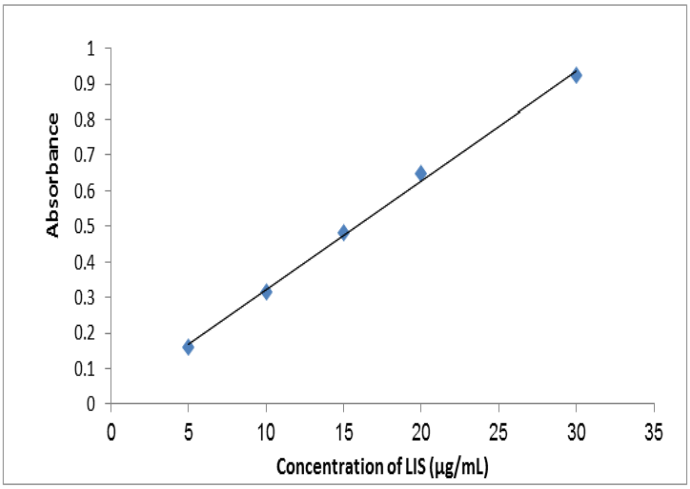

Figure 7: Calibration curve of LIS at 210 nm (λmax).

4.3. Validation of the proposed methods

ICH recommendations (53) were adopted to assess validation of the proposed method. Linearity, accuracy, precision, specificity, LOD and LOQ were evaluated.

4.3.1. Linearity

Method I: Two calibration curves were constructed for HCT at both 211 (absorptivity point) and 270 nm and statistical parameters were calculated (**Table 1**). LOD and LOQ values were found to be 0.297 and 0.90 µg mL^{-1} at 270 nm while at 211 nm they were found to be 0.790 and 2.40 µg mL^{-1}, respectively.
Method II: LIS working solutions were analysed in the concentration ranges of 5.0-30.0 µg mL^{-1} and calibration curve was constructed from the absorbance values at 210 nm (Table 1). LOD and LOQ values were found to be 1.517 and 4.596 µg mL^{-1}, respectively.

4.3.2. Accuracy

Accuracy of the proposed methods was evaluated by applying the proposed methods for determination of different laboratory prepared mixtures of LIS and HCT. The concentration of each drug was calculated from the corresponding regression equations. The obtained % recoveries indicate suitable accuracy of the proposed methods (**Table 2**).
Standard addition method was also performed to check the accuracy of the methods. It was applied for analysis of commercial tablets by addition of different amounts of standard LIS or HCT to certain concentration of the extracted tablet solution. The results showed excellent % recoveries with low % RSD (**Table 3**).

4.3.3. Precision

Repetitive analysis of three different laboratory prepared mixtures was performed three successive days in order to evaluate intra- and inter-day precision of the proposed methods. % RSD lower than 2 % were obtained that proved that the proposed methods were precise (**Table 4**).

4.3.4. Specificity

The proposed methods determined successfully LIS and HCT simultaneously without interference from each other (**Table 2**). Also they were applied for their determination in tablets where no interferences from tablet excipients occurred (**Table 5**). This proves the proposed methods specificity for the studied drugs.

4.4. Application on analysis of commercial tablets

The proposed spectrophotometric methods were utilized for the determination of LIS and HCT in their combined pharmaceutical formulation Sinopril Co® tablets and the results were present in table 5, the same dosage form was analyzed by the reported method [45]. The spectra of the extracted tablet solutions showed no interferences from tablet excipients that proves the proposed methods' selectivity and specificity (**Figure 8**). The good % recoveries prove the suitable accuracy of the proposed methods for the routine determination of these drugs in their combined dosage form. Also comparison to a reported method [45] was done by repetitive analysis of certain tablet solution of LIS five times with both the proposed and the

Table 1: Statistical parameters for the calibration of HCT at 211 and 270 nm and LIS at 210 nm.

Parameter	HCT at 270 nm	HCT at 211 nm (Absorptivity point)	LIS at 210 nm
Linear range (μg mL^{-1})	1.0 – 20.0	2.5 – 20.0	5.0-30.0
Slope	0.0642	0.0543	0.0307
Standard deviation of slope (S_b)	0.0004	0.0009	0.0008
Intercept	0.0194	0.0634	0.0145
Standard deviation of the intercept (S_a)	0.0058	0.0131	0.0141
Correlation Coefficient*	0.9999	0.9995	0.9990
Standard deviation of residuals ($S_{y,x}$)	0.0104	0.0108	0.0149
LOD (μg mL^{-1})	0.297	0.790	1.517
LOQ (μg mL^{-1})	0.90	2.40	4.59

Table 2: Determination of LIS and HCT concentration in laboratory mixtures by the two proposed methods

Mix	Ratio	% Recovery [a]		
		HCT	LIS	
		(at 270 nm in both methods)	Method I (at absorptivity point 211 nm)	Method II (at λ_{max} 210 nm)
1	1:1	101.56	101.32	99.07
2	1:2	102.00	101.67	97.67
3	2:1	101.56	98.098	100.18
4	1:3	100.83	99.82	102.34
5	3:1	99.69	97.72	102.06
6	2:3	101.46	100.72	101.35
7	3:2	100.94	97.66	99.96
Mean		**101.15**	**99.57**	**100.38**
SD		**0.76**	**1.74**	**1.68**

a the value is the mean of three determinations.

Table 3: Standard addition method for the determination of LIS and HCT in Sinopril Co® tablets (20 mg LIS + 12.5 mg HCT) by addition of standard LIS or HCT

Amount taken (μg mL^{-1})		Amount added (μg mL^{-1})	Found [a] (μg mL^{-1})			% Recovery [a]		
LIS	HCT	LIS	HCT	LIS		HCT	LIS	
			$M_{1,2}$	M_1	M_2	$M_{1,2}$	M_1	M_2
10	6.25	5	6.250	14.860	15.11	100.00	99.07	100.75
10	6.25	10	6.359	20.479	19.84	101.75	102.39	99.21
10	6.25	15	6.265	25.481	24.79	100.25	101.93	99.16
Mean						**100.667**	**101.13**	**99.70**
SD						**0.946**	**1.79**	**0.90**
LIS	**HCT**	**HCT**						
5	3.125	2.5	5.62	5.12	4.84	99.91	102.19	96.86
5	3.125	5	8.07	5.01	4.91	99.32	100.14	98.18
5	3.125	7.5	10.51	5.06	4.95	98.89	101.29	98.90
Mean						**99.37**	**101.21**	**97.98**
SD						**0.51**	**1.03**	**1.03**

a the value is the mean of three determinations, M_1 and M_2 are Method I (absorptivity factor method) and Method II (Ratio subtraction method) respectively.

Table 4: Intra- and inter- day precision for the analysis of LIS and HCT in three laboratory mixtures by the proposed methods.

Concentration level (μg mL^{-1})		% Recovery ± RSD			
		Intra-day precision		Inter-day precision	
LIS	**HCT**	**LIS**	**HCT**	**LIS**	**HCT**
Absorptivity factor point method					
10	12.5	100.24±1.86	101.63±1.44	99.14±1.57	101.32±1.04
5	10	99.69±1.19	100.59±1.82	100.39±1.19	101.31±0.78
10	5	102.12±0.65	100.72±0.97	99.87±1.25	100.68±1.95
Ratio subtraction method					
10	12.5	100.93±0.65	102.42±0.99	100.36±1.26	99.32±1.95
5	10	97.67±1.17	100.83±1.85	97.50±1.17	99.64±0.78
10	5	98.54±1.83	100.26±1.42	101.97±1.59	102.53±1.07

[a] The value is the mean of three determinations.

Table 5: Determination of LIS and HCT in Sinopril Co® tablets and comparison with reported method.

Conc. taken (μg mL^{-1})		% Recovery [a] ± SD				
		Proposed methods			Reported method [45]	
		M_1	M_2	$M_{1,2}$		
LIS	**HCT**	**LIS**	**LIS**	**HCT**	**LIS**	**HCT**
10	6.25	101.03±1.78	101.23±1.21	99.75±1.88	102.71±0.88	99.33±1.24
t- value[b]		1.901	2.216	0.415		
F- value		4.103	1.921	2.277		

a the value is the average of five measurements for both the proposed and reported methods.
b Tabulated values at 95% confidence limit are t = 2.306, F = 6.338.

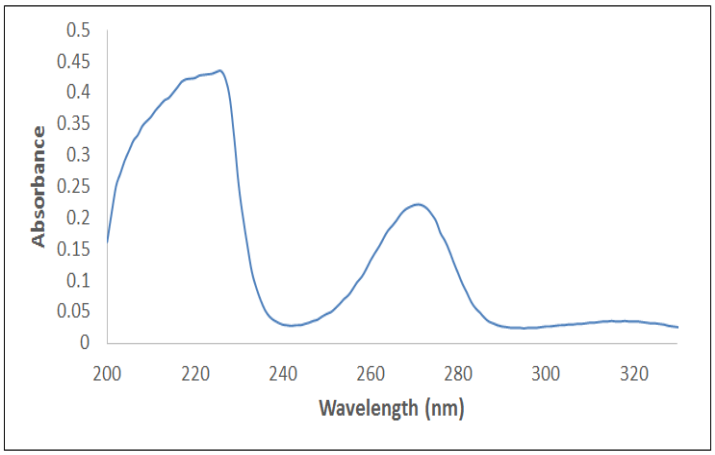

Figure 8: The spectrum of extracted tablet solution containing 5 µg mL^{-1} LIS + 3.125 µg mL^{-1} HCT.

reported methods (Table 5). t- and F- values were calculated and was found to be lower than the tabulated indicating that there is no significant differences between both the proposed methods and the reported method.

5. Conclusion

It could be concluded that the proposed methods of analysis represent new, rapid, simple, sensitive methods for simultaneulsy determine the studied drugs in their binary mixtures. The proposed methods have several advantages over the previously reported methods as they are faster and cheaper than chromatographic, electrophoretic and electrochemical methods that require expensive and sophisticated instruments. In addition, They are more simple and cover wider concentration ranges than the reported multivariate spectrophotometric method [43] that needs special mathematical program. Furthermore, the proposed methods are more sensitive than the reported ratio spectra derivative, derivative and Vierordt's spectrophotometric methods [45]. The methods were successfully validated showing high degree of accuracy and precision.

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