The National Ribat University

Faculty of Graduate Studies & Scientific Research

Development of Absorption Factor Spectrophotometric method for the Estimation of Losartan potassium and Hydrochlorothiazide in Tablets

A Thesis Submitted in Partial Fulfilment of the Requirement for Master Degree in Drug Quality Control

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2018-1439

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Acknowledgement

This research project would not have been done without ALLAH almighty God and the support of many people.

I would like to express my appreciation and grateful to my supervisor Dr. Imad Osman Abu Reid, the faithful advisor and the proud father for his patience, and for given me knowledge and strength of determination and spared no effort or precious-time.

Furthermore I would like to thank all the staff of the National Al Ribat University who gave me the permission to use all required equipment and the necessary materials to complete my research.

Abstract

A simple, specific, accurate and precise spectrophotometric method was developed for the simultaneous determination of losartan potassium and hydrochlorothiazide in tablets dosage form. Beer-lambart law was obeyed in the range of 1.5-7.5µg/ml and 4.5-22.5µg/ml for losartan potassium and hydrochlorothiazide respectively at the selected wavelengths 266.9nm and for hydrochlorothiazide at 325nm.The proposed methods was based on calculating absorption factor by measuring the absorbance of losartan potassium and hydrochlorothiazide at two different wavelengths, in the first wavelength both losartan potassium and hydrochlorothiazide have absorbance while in the other only hydrochlorothiazide has absorbance. The concentration of the two drugs in the mixture was calculated using factor and their corresponding calibration curves at 266.9 nm. The accuracy of this method was confirmed by analyzing laboratory synthetic mixtures mean recovery was 99.78±0.65 for losartan potassium and 101.31±1.47for hydrochlorothiazide. The precision of the method was evaluated by analyzing commercial tablets on two different days, the repeatability RSD% was found to be 1.64% and 1.67% for losartan potassium and hydrochlorothiazide respectively, the intermediate precision RSD% was 0.78% for losartan potassium and 0.91 %for hydrochlorothiazide.

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1.1 Introduction

Combination drug products occupy a time-honored and important role in therapeutics. When rationally formulated, fixed-combination drugs may produce greater convenience, lower cost, and sometimes greater efficacy and safety [1].

Analysis of samples with numerous components presents a major challenge in modern analysis [2]. Multicomponent analysis has become one of the most appealing topics for analytical chemists in the last few years [3]. Thus, analytical procedures are needed to rapidly and reliably determine ingredients of such products. Spectrophotometric methods are most frequently used for such purposes.

Ultraviolet-visible spectrophotometry is a method of analysis that depends on therelation between the analyte concentration and the amount of light absorbed. The amount ofthis light depends on the electron excitation from low energy level to a higher one within themolecule after passing a beam of light in the range of 200nm-700nm. The leaner relationbetween the concentration and the absorption is expressed mathematically by Beer-lambertlaw:

$$A=abc$$

Where: **A** is absorbance and its unit is dimensionless, **c** concentration has units of moles perliter (M) or gram per 100ml, **b** is path length in centimeters (cm), **a** is the absorptivity expressed in the units of concentration used [4].

For the simultaneous spectrophotometric determination of twosample components, the choice of an analytical procedure is strictly related to the observed resolution between the individual absorption peaks of these components and the following methods are commonly used [5].

- Simultaneous equation method
- Derivative spectrophotometric method
- Absorbance ratio method (Q-Absorbance method)
- Difference spectrophotometry

The assay of samples containing more than one analyte presents a challenge for theanalyst specially when the analytes absorbs light in the same spectral region which will result inan overlapping of the spectrum making it difficult to use one of the previous procedures in thedetermination of their concentrations. Traditionally methods of extraction were used toseparate the analytes from each other but this was difficult and troublesome as these methodsconsume large volume of solvent along with the risk of sample loss, contamination orincomplete separation, time consuming and expensive methods [5].

There exist several powerful, well established multivariate calibration techniqueswhich can be used in the modem spectrophotometry: classical least squares (CLS),inverse least squares (ILS), principal component regression (PCR) or partial least squares (PLS) [6,7]. These techniques have been widely used for the simultaneous determination of the two or more components of the sample and excellent analytical results have been reported. On the other hand, derivative spectrophotometry has also been satisfactorily applied for such kind of analyses. However, most of the techniques mentioned above require full-spectrum information and the spectral data have to be processed using highly specialized software[8-15]. Accordingly there is everlasting demand for simpler spectrophotometric techniques to overcome this technical demand.

Losartan (LSP) Figure 1, chemicallyis (2-butyl-4-chloro-1-{[2'- (1*H*-tetrazol-5-yl)biphenyl-4-yl] methyl}-1*H*- Imidazol-5-yl) methanol. Itis an angiotensin II receptor antagonist withantihypertensive activity due mainly to selectiveblockade of AT_1 receptors and the consequent reducedpressure effect of angiotensin II. It is used in the management of

hypertension, particularly in patients who develop cough with ACE inhibitors and toreduce the risk of stroke in patients with left ventricularhypertrophy, and in the treatment of diabetic nephropathy. It has also beentried in heart failure and in myocardial infarction [16].

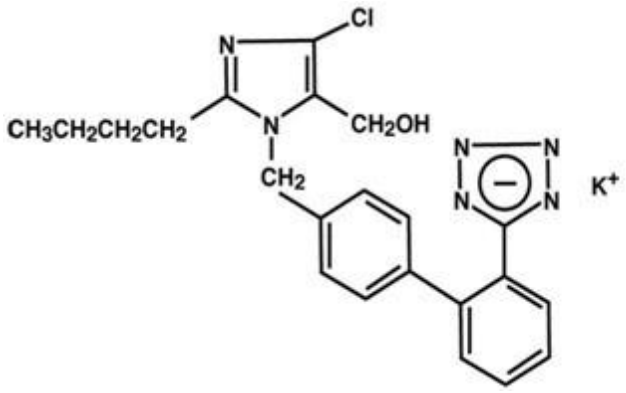

Figure1: Chemical structure of losartan potassium

Hydrochlorothiazide (HCT) Figure 2, chemically is 6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide.It is used in the treatment of hypertension, either alone or with other antihypertensive such asACE inhibitors and beta blockers. They are also used to treat oedema associated with heartfailure and with oedema associated with heart failure and with renal and hepatic disorders. Otherindications have included the treatment of oedema accompanying thepre menstrual syndrome,the prevention of water retention associated with corticosteroids and oestrogens, the treatment ofdiabetes insipidus , andthe prevention of renal calculus formation in patientswith hypercalciuria [16].

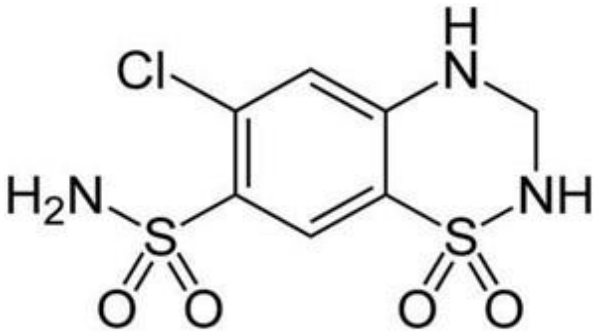

Figure 2: Chemical structure of hydrochlorothiazide

1.2 Objectives

The individual spectrum of LSP and HCT show considerable overlapping in the range of 200nm to 280 nm; accordingly application of the classical spectrophotometric techniques for their determination incombined dosage form is not possible.

The objectives of this research were:

- To develop a new spectrophotometric method based on absorption factor to overcome the problem of spectral overlap.
- To validate the developed method.
- To apply the developed method for the estimation of LSP and HCT combined in tablet dosage form.

1.3 Literature review

The United States Pharmacopeia [17] official method for the determination of LSP and HCT in combination, is based on linear gradient elution using solution A: made of 7:93 v/v (acetonitrile :phosphate buffer 7.5) and solution B: acetonitrile as mobile pumped at flow rate of 1ml/minute over 35 minutes utilizing reversed phase C_8 column and 280 nm as detection wavelength.

Literature review also indicated that several methods employing different analytical techniques were also used for the determination of the two drugs combination:

Different UV spectrophotometric methodstogether with a reversed phase high performance liquid chromatographic method were developed for the determination of the two drugs in combination, according to the first derivative method LSP was determined at 261 nm while the determination of HCT was carried out at 259.7 nm and 279 nm,the determination by the ratio-first derivativemethod was carried at(225.6nm , 255.9nm)and at (265nm, 283nm)for LSP and HCT respectively, while the ratio-second derivative determination was carried at (274.8nm, 287nm) for LSPand (247.8 nm, 287nm) for HCT. The chromatographic determination was carried out on a C_{18} column using 225 nm as detection wavelength [18].

Three spectrophotometric methods have been developed for simultaneous determination of LSP and HCT in a combination. In the first method the concentration of each drug was determined its absorbance values at its maximum absorbance wavelength using235nm and 271nm for LSP and HCT respectively.In method two dual wavelength methods using the direct proportionality of the absorbance difference between two points on mixture spectra with concentration of component of interest and its independence of the other component.Area under the curve in the range of 265-282nm for LSP and 229-242nm for HCTwas also used [19].

Reverse phase liquid chromatographic method using a mobile phase consisting buffer and acetonitrile (65:35%v/v)adjusted to pH3.0 with orthophosphric acid with flow rate of 1.0 ml min and detection at 254 nm was also reported [20].

Simultaneous equation using 218 nm and 272 nm as analytical wavelengths, graphical absorbance ratio method (Q-analysismethod) at 266.5 nm (isosbestic wavelength) and 272 nm (Wavelength of maximum absorption of HCT) and first order derivative methodat zero crossing wavelengths, 222 nm and 332 nmfor HCT and LSP, respectively [21].

A high performance liquid chromatographic method using water (containing 0.25 ml/L triethylamine), methanol and acetonitrile (60:38:30, pH adjusted to 2.7±0.1) as mobile phase flown through C_{18} columnat a flow rate of 1.0ml/min, the analytes were detected at 271nm was also developed [22].

Another reversed phase liquid chromatographic method on Inertsil ODS -3V Column (150 × 4.6 mm i.d., 5µm particle size) using mobile phase of acetonitrile and phosphate buffer adjusted to pH2.0 with orthophosphoric acid pumped at a flow rate of 1 ml/min and 226 nm as detection wavelength was also described [23].

GRACE C_{18}(4.6 x 250 mm) column as a stationary phase and acetonitrile: phosphate buffer (50:50adjusted pH 3.1 with orthophosphricacid) pumped at 1 ml/min and detection wavelength 226 nm; was used for the determination of the two drugs in combination [24].

LSP and HCT in combinedpreparations were quantitated by using the first-derivative responses at 271.6 nm for LSP and335.0 nm for HCT in spectra of their solutions inwater. HPTLC method, a mobile phase of chloroform–methanol–acetone–formic acid (7.5 + 1.5 +0.5 + 0.03, v/v) and a prewashed Silica Gel G60 F_{254} TLC plate as the stationary phase were used to resolve LSP and

HCT in a mixture. Two wellseparated and sharp peaks for LSP and HCT were obtained at Rf values of 0.61 and 0.41,respectively. LST and HCTwere quantitated at254.0 nm [25].

A chemo metric calibration technique based on the artificialneural network (ANN) was proposed for losartan potassium(LSP) and hydrochlorothiazide (HCT) in their mixturewithout using chemical separation and mathematicalgraphical treatment [26].

Anew method for the simultaneous determination of losartan potassium and hydrochlorothiazide, with minimum sample pretreatment, is described. The procedure based on the multivariate analysis of spectral data in the 220−274 nm region by the partial least squares Algorithm[27].

1.4 Theoretical background

Absorbance subtraction[28]depend on the fact that if you have a mixture of two drugs X and Y having overlapped spectra intersect at isoabsorptive point and Y is extended more than X, while X doesn't show any absorbance(A_2) at another wavelength (λ_2).The isoabsorptive point (λ_{iso}) used for separate quantitative estimation of each X & Y in their mixture (X+Y). The determination can be done using mathematically calculated factor of one of these components.

The absorbance values corresponding to X and Y at (λ iso) were calculated by using absorbance factor (A_{iso} / A_2) which is a constant for pure Y representing the average of the ratio between the absorbance values of different concentrations of pure Y at λ_{iso} (A_{iso}) to those at λ_2 (A_2).

Absorbance of Y in the mixture at λ_{iso}=$\left(\frac{\text{abs}_{\text{iso}}}{\text{abs2}}\right) \times \text{abs}_{\lambda_2}(x + y)$

Absorbance of X in the mixture at λ_{iso}= $\text{abs}_{\text{iso}}(X + Y) - \left(\frac{\text{abs}_{\text{iso}}}{\text{abs2}}\right) \times \text{abs}_{\lambda_2}(x + y)$

Where: absorption value $\left(\frac{\text{abs}_{\text{iso}}}{\text{abs2}}\right)$: is the absorption ratio and it is constant for pure Y at $\lambda_{iso}$and λ_2 is the absorbance factor and abs λ_{iso} (X + Y) and abs λ_2(X + Y) are the absorbance of the mixture at these wavelengths(λ_{iso}, λ_2).

The concentration of each X or Y, separately, is calculated using the isoabsorptive point.

2. 1Material

2.1.1 Chemicals

- Losartan potassium and hydrochlorothiazide working standards were supplied byAmipharma Laboratories – Sudan.
- Methanol was purchased from the market.
- Distilled water was provided by the laboratory of the university.
- Loscar-H ® tablets(Batch no: G701397) manufactured by Cadila HealthcareLimited, India, it is labeled to contain 50mg Losartan potassium and 12.5mg Hydrochlorothiazide, and were purchased from the local market.

2.1.2 Reagents:

50% v/vmixture of distilled water: methanol (solvent mixture).

2.2 Instruments

- Asingle beam UV-Visible spectrophotometer UV 1800 (Shimadzu-Japan) with the use of 1.0 cm quartz cell.
- Ultrasonic (Berlin- Germany).
- Analytical balance (Sartorius –Germany).

2.3 Preparation of standard solutions & sample

2.3.1 Losartan potassium standard stock solution

About 7.5 mg oflosartan potassium working standard were accurately weighed and transferred into a 100 ml volumetric flask; the volume was completed to the mark by methanol to obtain a final concentration of 75µg/ml.

2.3.2 Hydrochlorothiazide standard stock solution

About 22.5 mg of hydrochlorothiazide working standard were accurately weighed and transferred into a 100 ml volumetric flask; the volume was completed to the mark using methanol to obtain a final concentration of 225µg/ml.

2.4 Procedure

2.4.1 Selection of analytical wavelengths

Twoseparate solutions were prepared from the standard stock solution oflosartan potassiumand hydrochlorothiazide by diluting 5ml from each toa separate 50 ml volumetric flask using the solvent mixture.The individual spectrum were obtained over the wavelength range of 200-400nm in the overlay mode.

2.4.2 Linearity and calibration

Serial dilutions were prepared from standard stock solutionsof the two analytes by transferring aliquot volumes (1- 5 ml) of each to a separate set of 50 ml volumetric flasks and dilution with the solvent mixture to obtain losartan potassiumconcentration in the range of 1.5-7.5µg/ml and hydrochlorothiazide concentration in the range of 4.5-22.5µg/ml. The absorbance for these solutions was measured at the selected wavelengths and the calibration curves were obtained by plotting the absorbance values obtained against their corresponding concentrations.

2.4.3 Preparation of the laboratory synthetic mixtures

Nine laboratory synthetic mixtures with different concentration ratios of losartan potassiumand hydrochlorothiazide were prepared according to [29] by mixing different volumes from the two stock solutions in nine separate 50 ml volumetric flask and the volume completedto the mark with the mixture.The scheme describing the method of preparation of these mixtures and the correspondingtheoretical concentrations of the two drugs in the mixtures are shown in Tables 1and 2 below.

Table1: Synthetic mixtures preparation scheme

Mixture	LSP	HCT
1	0	0
2	-1	1
3	1	1
4	1	0
5	0	1
6	1	-1
7	-1	-1
8	-1	0
9	0	-1

Table 2: Theoretical concentrations of the analytes in the synthetic mixtures

Mixture	LSP (µg/ml)	HCT (µg/ml)
1	45	13.5
2	30	18.0
3	60	18.0
4	60	13.5
5	45	18.0
6	60	9.0
7	30	9.0
8	30	13.5
9	45	9.0

2.4.4 Sample preparation

Ten tablets were accurately weighed and crushed to a fine powder,weight equivalent to one tablet was taken and transferred to 100 ml volumetric flask ,1ml of distilled water was added to disperse the powder thenthe disperse was dissolved inmethanol with sonication for 15 minutes; cooled and made to volume with methanol.The resulting solution was filtered using 0.45 micron syringe filter; 5 ml of the filtrate were diluted to 50ml using the solvent mixture.

3.1 Selection of analytical wavelength

From overlay spectraof LSP and HCT the isoabsorptive wavelength was found at to be 266.9 nm and the wavelength ofmaximum absorption of HCT where LSP is not having any interference was identified at 325 nm(Figure 3).

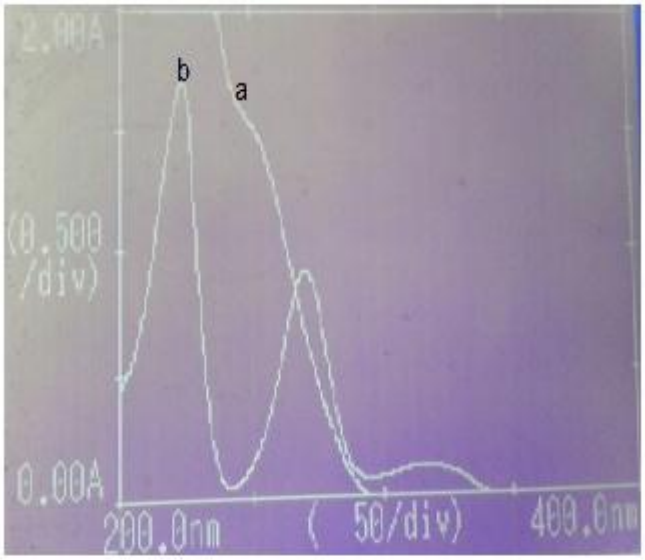

Figure 3:Absorption spectraof (a) LSP (75µg/ml) and (b) HCT (22.5µg/ml) in diluents

3.2 Linearity at the selected wavelengths

The absorbance valuesof the linearity solution were measured at 266.9nm and 325 nm for hydrochlorothiazide and at 266.9nm for losartanpotassium. The absorbance valueswere plotted against the correspondingconcentrations. The regression data for the two drugs are shown in Tables3,4and5,Figures4, 5and 6.

Table 3:Losartan standard solution linearity data at 266.9nm

LSP (µg/ml)	A at 266.9nm
15	0.270
30	0.535
45	0.792
60	1.073
75	1.304
Slop (B)	0.0174
Intercept (A)	0.013
Correlation coefficient (R)	0.9996

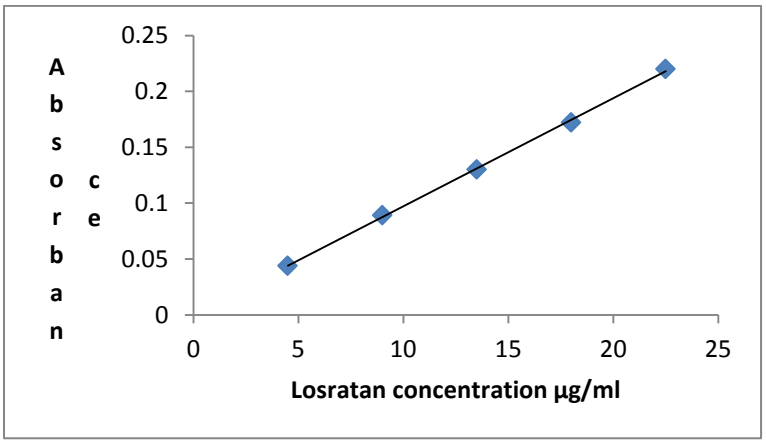

Figure 4: Losratan potassium standard calibration curve at 266.9nm

Table 4:Hydrochlorothiazide standard solution linearity data at 266.9nm

HCT (µg/ml)	A at 266.9nm
4.5	0.277
9.0	0.538
13.5	0.795
18.0	1.070
22.5	1.304
Slop (B)	0.05747
Intercept (A)	0.021
Correlation coefficient (R)	0.9997

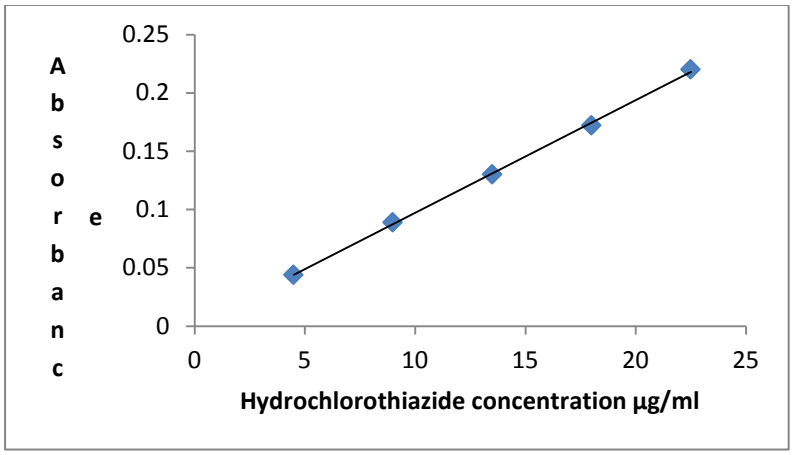

Figure 5: Hydrochlorothiazide standard calibration curve at 266.9nm

Table 5: Hydrochlorothiazide standard solution linearity data at 325 nm

HCT (µg/ml)	A at 325nm
4.5	0.044
9.0	0.089
13.5	0.130
18.0	0.172
22.5	0.220
Slop (B)	0.0097
Intercept (A)	0.0005
Correlation coefficient (R)	0.9996

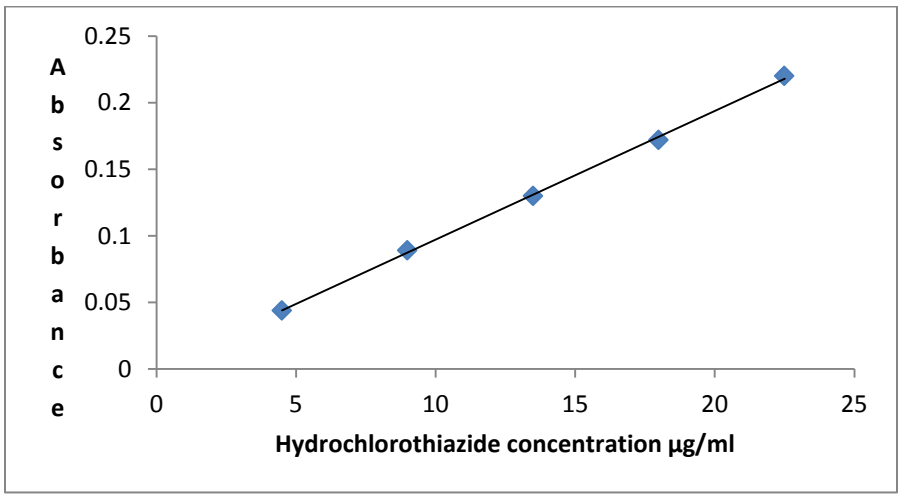

Figure 6: Hydrochlorothiazide standard calibration curve at 325 nm

Absorption ratio = 6.252 was calculated as an average from the absorbance data of hydrochlorothiazide standard solution at the two wavelengths (266.9 and 325 nm)respectively.

3.3Accuracy:

The accuracy of the method wastested by analyzing nine laboratory prepared synthetic mixtures containing different concentrations of losartan potassium and hydrochlorothiazide. Using the corrected absorbance at 266.9 nm obtained; using the absorption factor of 6.252, the results of the determination of the two analytesshowed good agreement between the theoretical and actual concentrations of the two analytes. Losartanpotassiumaverage recovery from the synthetic mixtures was (99.78±0.65), while the hydrochlorothiazide average recovery was (101.31±1.47). The small relative standard deviations (< 2%) support the accuracy of the method [30].

Table 6:Absorbance valuesof the synthetic mixture at the selected wavelengths

Mixture	Abs $_{266.9\ nm}$	Abs $_{325\ nm}$
1	1.581	0.125
2	1.572	0.163
3	2.100	0.163
4	1.850	0.125
5	1.807	0.163
6	1.570	0.084
7	1.048	0.083
8	1.315	0.125
9	1.316	0.083

Table 7: The calculated absorbance of LSP and HCT in the mixtures at 266.9nm

Mixture	**Abs $_{LSP*}$**	**Abs $_{HCT*}$**
1	0.7995	0.7814
2	0.5529	1.0190
3	1.0809	1.0190
4	1.0685	0.7814
5	0.7879	1.0190
6	1.0448	0.5251
7	0.5290	0.5189
8	0.5335	0.7814
9	0.7970	0.5189

*calculated using the absorption factor

Table 8: The accuracy results of the synthetic mixtures

Mixture	**LSP(µg/ml)**		**%content**	**HCT (µg/ml)**		**%content**
	Theoretical	**Actual**		**Theoretical**	**Actual**	
1	45	45.27	99.40	13.5	13.23	102.04
2	30	31.07	96.55	18.0	17.36	103.68
3	60	61.47	97.60	18.0	17.36	103.68
4	60	60.75	98.76	13.5	13.23	102.04
5	45	44.60	100.89	18.0	17.36	103.68
6	60	59.39	101.02	9.00	8.773	102.58
7	30	29.70	101.01	9.00	8.664	103.87
8	30	29.96	100.13	13.5	13.23	102.04
9	45	45.13	99.71	9.00	8.664	103.87
Average			99.45			103.05
SD			1.573			0.8526
RSD%			1.58			0.82

3.4 Precision:

Repeatability of the method was evaluated by calculating the % RSD for six concentrations of the test sample and was found to be 1.64 % and 1.67% for losartanpotassiumand hydrochlorothiaziderespectively;results are shown in Tables 9,10 and11.

Table 9: samples weight taken and corresponding amount of analytes (repeatability)

Sample	Weight taken (gm)	Active (mg)	
		LSP	HCT
1	0.1862	50	12.5
2	0.1862	50	12.5
3	0.1862	50	12.5
4	0.1862	50	12.5
5	0.1862	50	12.5
6	0.1862	50	12.5

Table 10: the absorbance data at the selected wavelengths of the mixture and of theindividual LSPand HCT

Sample	Sample		Individual analytes	
	Abs $_{266.9\ nm}$	Abs $_{325\ nm}$	Abs LSP $_{266.9\ nm*}$	Abs HCT $_{266.9\ nm*}$
1	1.648	0.117	0.9165	0.7314
2	1.654	0.119	0.9100	0.7439
3	1.639	0.120	0.8887	0.7502
4	1.658	0.118	0.9202	0.7377
5	1.635	0.120	0.8847	0.7502
6	1.625	0.115	0.9060	0.7189

*calculated using the absorption factor

Table 11: The assay results of the samples(repeatability)

Mixture	LSP(µg/ml)		%content	HCT (µg/ml)		%content
	Theoretical	Actual		Theoretical	Actual	
1	50	52.00	104.00	12.5	12.36	98.88
2	50	51.63	103.26	12.5	12.58	100.64
3	50	50.40	100.80	12.5	12.68	101.44
4	50	52.22	104.44	12.5	12.47	99.76
5	50	50.17	100.34	12.5	12.68	101.44
6	50	51.40	102.80	12.5	12.14	97.12
Average			102.60			99.88
SD			1.683			1.677
RSD%			1.64			1.67

The intermediate precision was studied by assaying another set of sample in different day following the same method. The %RSD was 0.78% for losartan potassiumand0.91%for hydrochlorothiazide as shown in Tables 12, 13 and 14.

Table 12: samples weight taken and corresponding amount of analyte (intermediate precision)

Sample	Weight taken (gm)	Active (mg)	
		LSP	HCT
1	0.1835	50	12.5
2	0.1835	50	12.5
3	0.1835	50	12.5
4	0.1835	50	12.5
5	0.1835	50	12.5
6	0.1835	50	12.5

Table 13: the absorbance data at the selected wavelengths of the mixture and of theindividual LSP and HCT

Sample	Sample		Individual analytes	
	Abs $_{266.9\ nm}$	Abs $_{325\ nm}$	Abs LSP $_{266.9\ nm*}$	Abs HCT $_{266.9\ nm*}$
1	1.655	0.119	0.7439	0.9110
2	1.655	0.118	0.7377	0.9172
3	1.653	0.118	0.7377	0.9152
4	1.651	0.117	0.7314	0.9195
5	1.643	0.118	0.7377	0.9052
6	1.651	0.116	0.7252	0.9257

*calculated using the absorption factor

Table 14:The assay results of the samples(intermediate precision)

Mixture	LSP(µg/ml)		%content	HCT (µg/ml)		%content
	Theoretical	Actual		Theoretical	Actual	
1	50	51.68	103.36	12.5	12.58	100.64
2	50	52.04	104.08	12.5	12.47	99.76
3	50	51.93	103.86	12.5	12.47	99.76
4	50	52.17	104.34	12.5	12.36	98.88
5	50	51.35	102.70	12.5	12.47	99.76
6	50	52.53	105.06	12.5	12.25	98.00
Average			103.9			99.46
SD			0.812			0.908
RSD%			0.78			0.91

The average %RSD for the twelve determinations at the tow different days was 1.21% and1.29% for losartan potassiumand hydrochlorothiazide respectively, which are less than 3% as specified by ICH [30].

3.5 Conclusion:

The obtained results from linear regression for simultaneous estimation of losartan potassium and hydrochlorothiazide in their pharmaceutical dosage forms by absorption factor method indicate that the method is accurate, precise,simple and sensitive,it show acceptable linearity,so it can be used for the routine analysis.

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