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Stability Indicating UV-Spectrophotometric Methods for Simultaneous Determination of Losartan Potassium and Hydrochlorothiazide in Pharmaceuticals

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Abstract

Two new stability indicating UV-Spectrophotometric methods have been described for the simultaneous assay of Losartan Potassium and Hydrochlorothiazide in bulk drug and in tablet dosage forms using 0.01 N HCl as the solvent. Method A is based on simultaneous equation or Vierodt's method and Method B is Q-analysis or Q-absorbance ratio method. The λ_{max} values for Losartan Potassium and Hydrochlorothiazide in the solvent medium were found to be 227.4 nm, 270.4 nm and 256.4 nm, 270.4 nm for Method A and Method B respectively. The systems obey Beer's law in the range of 2.02-22.22 μg mL^{-1}, 3.03-27.27 μg mL^{-1} and 5.05-50.50 μg mL^{-1}, 3.03-27.27 μg mL^{-1} for Losartan Potassium and Hydrochlorothiazide for Method A and Method B respectively. Repeatability, Intra-day and interday precision were found to be 0.202 and 0.670, 0.566-1.31, 0.608- 1.35 for Method A and 0.989 and 0.586, 0.561-1.30, 0.602- 1.33 for Method B. No interference was observed from common tablet adjuvants. t –test and F-test have been applied for the recovery studies of the two methods. The changes in the λ_{max} values (Physical parameter) for Losartan Potassium and Hydrochlorothiazide was evaluated as for stability study. The methods were successfully applied to the assay of Losartan Potassium and Hydrochlorothiazide in tablet formulations.

Keywords:

Losartan Potassium; hydrochlorothiazide; UV Sspectrophotometry; stability indicating.

1. Introduction

Losartan Potassium1 (LOP), [2-Butyl-4-chloro-1-[{2`-(1H-tetrazol-5-yl)-1, 1`-biphenyl}-4-yl] methyl]-1H-imidazole-5-methanol, is an angiotensin II receptor antagonist drug used mainly to treat high blood pressure (hypertension). Hydrochlorothiazide [2] (HCZ), 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamode -1, 1-diaxide, is a diuretic agent used mainly for treatment of hypertension, heart failure, renal or hepatic failure.

The literature survey reveals that the methods available for estimation of Losartan Potassium and Hydrochlorothiazide includes HPLC [3-9], RP-HPLC [10-12], HPTLC [13], ratio derivative spectrophotometry [14,15] and Spectrophotometric [16], LC–MS and LC–MS/MS [17], capillary electrophoretic [18] and capillary electrochromatographic [19].

In the present method 0.01 N HCl is used as solvent. HCl (0.01 N) has an advantage of being inexpensive, non-volatile and relatively less hazardous. Moreover, in the dissolution tests for Hydrochlorothiazide tablets, 0.1 mol L^{-1} HCl is prescribed in various pharmacopoeias

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as dissolution media. Moreover the maximum wavelength (λ_{max}) for both the drugs remains stable by changing the concentration of the drugs.

Stability Indicating Assay Methods [20] may be defined as validated, quantitated analytical method that can detect the change with timing chemical, physical and microbiological properties of drug substances and drug products, and that are specific so that the content of active ingredients, degradation products and other component of interest can be accurately measured without interference.

1.1. Introduction to the Simultaneous Equation Method [21]:

The simplest possible case would be where at a certain wavelength analyte X does not absorb at all and analyte Y strongly absorbs, and at another wavelength the converse is true. Then one could simply measure the absorbances at those two wavelengths to determine the concentration of the individual analytes directly. However, in most cases this does not hold strictly true, and both analytes absorb, if only, weakly, across the spectrum. Fortunately, by choosing wavelengths where the absorption of X is strong and Y is weak, and vice versa, it is still possible to determine their concentrations because the absorbances are additive. This will give the concentrations of X and Y in an unknown mixture. First, the absorption of the analytes is documented individually (Beer's Law curve over some range of concentration is developed for each analyte) and then wavelengths are chosen that will best differentiate their responses.

According to Beer's Law,

$A = \varepsilon bC$

Where, A is the absorbance, ε is the molar absorptivity (a physical constant of the substance), b is the path length through the cell containing the analyte (usually 1 cm), and C is the concentration. By using the same or matched cuvette to hold the sample, b is held constant, so that εb is a constant,

$A = kC$

k is referred to as the absorptivity constant. It is the proportionality factor that relates A and C for some particular substance at some particular wavelength. For a plot of A vs. C (Beer's Law plot), k is the slope of line.

1.2. Simultaneous Equations Method/ Vierodt's Method:

If a sample contains two absorbing drugs (Losartan Potassium and Hydrochlorothiazide) each of which absorbs at the λ_{max} (Fig.1) of the other, it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method) [22].

The information required is:

- The absorptivities of Losartan Potassium at λ_1 and λ_2 , a_{x1} and a_{x2}, respectively
- The absorptivities of Hydrochlorothiazide at λ_1 and λ_2 , a_{y1} and a_{y2}, respectively
- The absorbance of the diluted sample at λ_1 and λ_2 , A_1 and A_2 respectively

Let c_x and c_y be the concentrations of Losartan Potassium and Hydrochlorothiazide respectively in the diluted sample

Two equations are constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of the individual absorbances of Losartan Potassium and Hydrochlorothiazide.

At λ_1

$$A_1 = a_{x1}\, b\, c_x + a_{y1}\, b\, c_y \qquad (1)$$

At λ_2

$$A_2 = a_{x2}\, b\, c_x + a_{y2}\, b\, c_y \qquad (2)$$

For the measurements in 1 cm cells, b =1

On rearranging equation (2)

$$C_y = \frac{A_2 - a_{x2} c_x}{a_{y2}} \qquad (3)$$

Substituting for c_y in equation (1) and rearranging gives

$$c_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \qquad (4)$$

$$c_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \qquad (5)$$

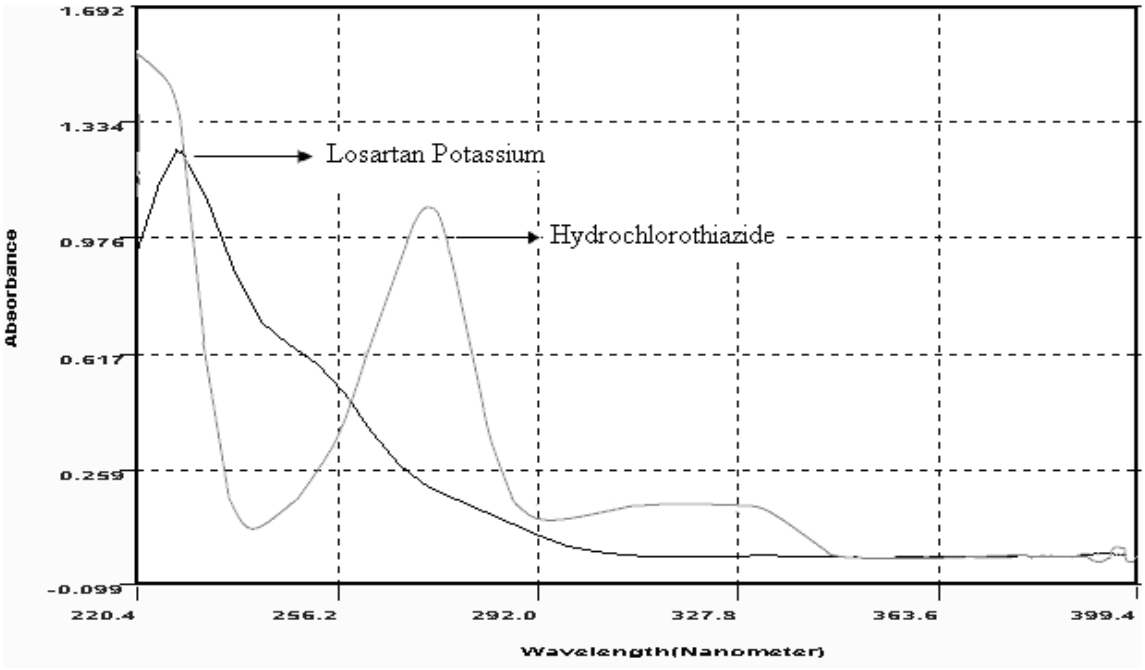

Fig.1: Overlain spectra of Losartan Potassium and Hydrochlorothiazide at 227.4 nm and 270.4 nm for Method A (Simultaneous equations Method)

1.3. Introduction to the Absorbance Ratio Method (Q Ratio Method):

The absorption ratio method is a modification of the simultaneous equation method. It depends on the property that, for substance which obeys beer's law at all wavelengths, the ratio of absorbances at any two wavelengths is constant value independent of concentration or path length. In the USP, this ratio is referred to as a Q value.

In the quantitative assay of two components in admixture by the absorption ratio method, absorbance's are measured at two wavelengths one being the λ max of one of the components (λ_2) and the other being a wavelength of equal absorptivity of the two components (λ_1), i.e., an Iso-absorptive point (Fig.2). Two equations are constructed which are as follows:

$$C_1 = (Q_0\text{-}Q_2) / (Q_1\text{-}Q_2) \times (A/a_1) \qquad (6)$$

$$C_2 = (Q_0\text{-}Q_1) / (Q_2\text{-}Q_1) \times (A/a_2) \qquad (7)$$

Q_0 = Absorbance of sample at λ_1/ Absorbance of sample at λ_2.

Q_1 = Absorptivity of drug 1 at λ_1/ Absorptivity of drug 1 at λ_2.

Q_2 = Absorptivity of drug 2 at λ_1/ Absorptivity of drug 2 at λ_2.

Where C_1 and C_2 are concentrations of Losartan Potassium and Hydrochlorothiazide respectively in gm/liter in the sample solutions, A is the absorbance of the mixture at 256.4 nm (Iso-absorptive point). a_1 and a_2 are absorptivities of drug 1 and drug 2 at Iso-absorptive point respectively.

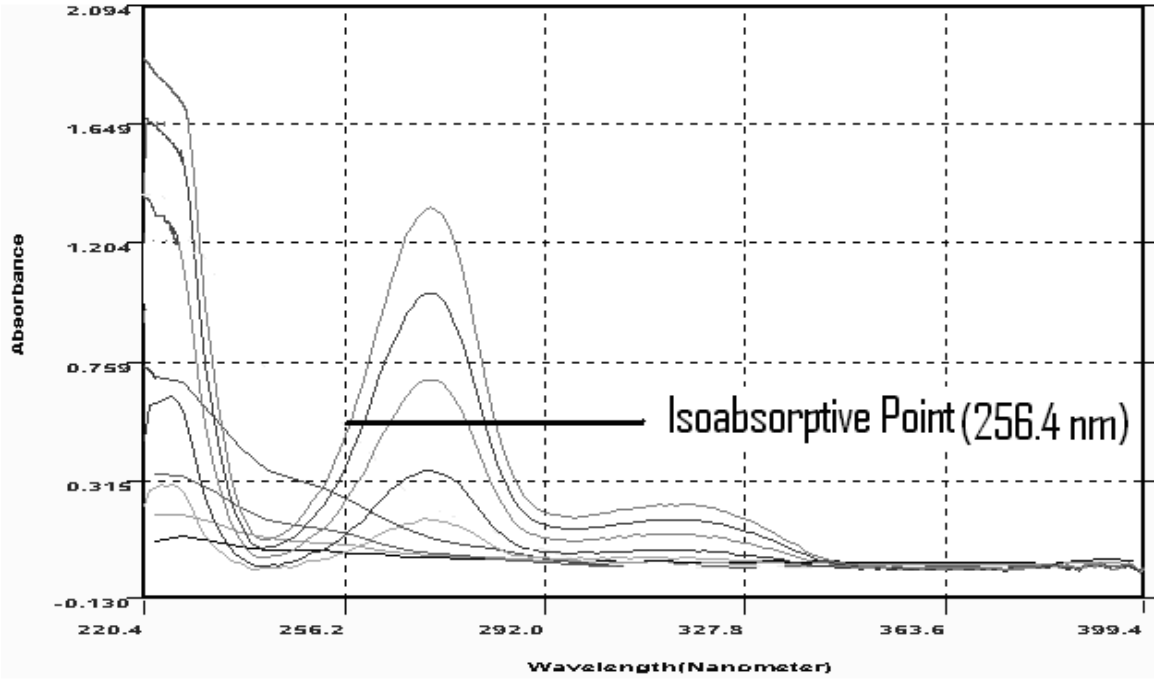

Fig.2: Overlain spectra of LOS and HCZ showing 256.4nm as Isoabsorptive point for Method B (Absorbance ratio method or Q ratio method)

2. Experimental

2.1. Instrument

ELICO SL 160 Double beam UV-VIS Spectrophotometer with spectral band width of 1.8 nm, wavelength accuracy of ±2 nm and matched quartz cells of 10 mm optical path length was used for all spectral and absorbance measurements.

2.2. Reagents and materials

All chemicals used were of analytical reagent grade and double distilled water was used to prepare the solvent medium. Concentrated HCl (Lobachem Ltd.) was diluted with double distilled water so as to obtain 0.01 N HCl. Pharmaceutical grade LOP and HCZ procured from Intas pharmaceutical Ltd., Ahmedabad and Unichem labs. Ltd., Mumbai, India were used as received. A 101.0 μg mL^{-1} solution of LOP and HCZ were prepared by dissolving 10.1 mg of pure drugs in 0.01 N HCl and diluting to 100 mL with 0.01 N HCl. These stock solutions (100.1 μg mL^{-1}) were diluted with 0.01 N HCl to get working concentrations of 2.02-22.22 μg mL^{-1} for LOP and 3.03-27.27 μg mL^{-1} for HCZ for Method A and 5.05- 50.50 μg mL^{-1} for LOP and 3.03-27.27 μg mL^{-1} for HCZ for Method B.

2.3. Method

Method A indicates simultaneous equation or Vierodt's method and Method B indicates Q-analysis or Q-absorbance ratio method.

Aliquots of pure LOP and HCZ solutions (0.2 to 2.2 mL and 0.5 to 5.5 mL; 100.1 μg mL^{-1}) and (0.3 to 2.7 mL; 100.1 μg mL^{-1}) were transferred into a series of 10 mL calibrated flasks and the total volume was adjusted upto the mark with 0.01 N HCl. The absorbances of the resulting solutions were then measured at 227.4 nm, 270.4 nm and 256.4nm, 270.4 nm for Method A and Method B respectively in triplicate against 0.01 N HCl blank and calibration curves were plotted between absorbance v/s concentrations.

2.4. Assay of formulations

Twenty tablets each of two brands were weighed and ground into a fine powder. Powder equivalent to 50 mg of LOP and 12.5 mg of HCZ was weighed accurately and transferred into a 500 mL calibrated volumetric flask; 250 mL of 0.01 N HCl was added and sonicated for 15 minutes and the solution was filtered through Whatmann No. 40 filter paper. The residues were washed and the washings were added to the filtrate and the volume was made up to the mark with 0.01 N HCl. From these solutions, suitable aliquots (0.8 mL; 100 μg mL^{-1} for Method A and 2.5 mL; 100 μg mL^{-1} for Method B) were transferred to three different 10 mL volumetric flasks and volumes were made upto mark with the same solvent and were used for analysis. The absorbances of these solutions were measured in triplicate at 227.4 nm, 270.4 nm and 256.4 nm, 270.4 nm for Method A and Method B respectively using 0.01 N HCl as blank.

2.5. Mathematical Calculations

Concentrations of Losartan Potassium and Hydrochlorothiazide were determined by solving the simultaneous equations**.** Two simultaneous equations (i and ii) were formed using absorptivity coefficient values for Method A.

$$C_1 = \frac{A_1 x 34.5 - A_2 x 55.2}{894.24} \qquad (i)$$

$$C_2 = -\frac{A_2 x 9.80 - A_2 x 41.6}{894.24} \qquad (ii)$$

Where C_1 and C_2 are concentrations of Losartan Potassium and Hydrochlorothiazide respectively in gm/liter in the sample solution, A_1 and A_2 are the absorbances of the mixture at 227.4 nm and 270.4 nm respectively.

Whereas for Method B equations (iii and iv) were used for determining the concentrations of Losartan Potassium and Hydrochlorothiazide

$$C_1 = \frac{Q_0 - Q_2}{Q_1 - Q_2} x \frac{A}{a_1} \qquad (iii)$$

$$C_2 = \frac{Q_0 - Q_1}{Q_2 - Q_1} x \frac{A}{a_2} \qquad (iv)$$

Q_0 = Absorbance of sample at λ_1/ Absorbance of sample at λ_2.

Q_1 = Absorptivity of drug 1 at λ_1/ Absorptivity of drug 1 at λ_2.

Q_2 = Absorptivity of drug 2 at λ_1/ Absorptivity of drug 2 at λ_2.

Where C_1 and C_2 are concentration of Losartan Potassium and Hydrochlorothiazide respectively in gm/liter in the sample solution, A is the absorbance of the mixture at 256.4 nm (Iso-absorptive point). a_1 and a_2 are absorptivities of drug 1 and drug 2 at Iso-absorptive point respectively. λ_1 = isoabsorptive point (256.4 nm) and λ_2 = 270.4 nm.

3. Results and Discussion:

3.1. Analytical data

A linear correlation was found between absorbances at λ_{max} and concentrations of LOP and HCZ. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values are given in Table 1 and Table 2 for Method A and Method B respectively. Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values are presented in Table 1 and Table 2. The graph shows negligible intercept as described by the regression equation Y = a + bX where Y is the absorbance and x concentration in μg mL^{-1}. The limit of detection and quantification calculated according to ICH guidelines [23] are also given in Table 1 and Table 2 and reveals a very high sensitivity of the methods.

Table 1. Analytical parameters of the spectrophotometric Method A

S. No.	Parameters	LOS	HCZ
1.	λ_{max}, nm	227.4	270.4
2.	Beer's Law limits, μg mL^{-1}	2.02 -22.22	3.03-27.27
3.	Molar absorptivity, 1/mol/cm	19.2×10^3	10.3×10^3
4.	Sandell sensitivity, μg/cm^2	0.0240	0.0289
5.	Regression equation (Y)*		
	Intercept (a)	-0.0052	-0.0093
	Slope (b)	0.0424	0.0355
6.	S_a	0.016	0.011
7.	S_b	0.002	0.002
8.	Correlation co-efficient (r)	0.9993	0.9992

S_a = Standard deviation of intercept
S_b = Standard deviation of slope
(Y)* = a + bX where Y is the absorbance and x concentration in μg mL^{-1}.

Table 2. Analytical parameters of the spectrophotometric Method B

S. No.	Parameters	LOS	HCZ
1.	λ_{max}, nm	256.4	270.4
2.	Beer's Law limits, μg mL^{-1}	5.05 -50.50	3.03-27.27
3.	Molar absorptivity, 1/mol/cm	9.92×10^3	10.3×10^3
4.	Sandell sensitivity, μg/cm^2	0.0465	0.0289
5.	Regression equation (Y)*		
	Intercept (a)	-0.0036	-0.0093
	Slope (b)	0.0217	0.0355
6.	S_a	0.0002	0.011
7.	S_b	0.0001	0.002
8.	Correlation co-efficient (r)	0.9999	0.9992

S_a = Standard deviation of intercept
S_b = Standard deviation of slope
(Y)* = a + bX where Y is the absorbance and x concentration in μg mL^{-1}.

3.2. Method Validation

3.2.1. Accuracy and precision

To evaluate the accuracy and precision of the methods, pure drug solutions at three different levels (within the working limits) were analyzed, each determination being repeated three times. The relative standard deviations (%) were less than 1 and indicate the high accuracy and precision for the methods (Table 3 and Table 5). For intra-day and interday precision the relative standard deviation values were in the range of 0.566 -1.35% and represent the best appraisal of the methods in routine use.

Table 3. Summary of validation parameters for Method A

S. No.	Parameters	LOS	HCZ
1.	Specificity:		
	-% interference	≤ 0.5%	≤ 0.5%
	-% agreement	100.37-100.45	100.33- 100.49
2.	Range (μg/ml):		
	-Working range	0.708-22.22	0.563-27.27
	-Linearity range	4.04-22.22	6.06-27.27
	-Target range	10.4- 15.6	13.2- 19.8
	-Test conc. (100%)	13.0	16.5
3.	Precision: (RSD)		
	-Repeatability (n =7)	0.202	0.670
	-Intraday (n=3)	0.566	1.31
	-Interday (3 days)	0.608	1.35
4.	Accuracy %	98.59-99.69	98.13-99.50
5.	Limit of detection, μg mL^{-1}	0.233	0.186
6.	Limit of quantification, μg mL^{-1}	0.708	0.563

Table 4. Summary of estimation of LOS and HCZ in different brands for Method A

S. No.	Brand	Labeled amount (mg)	Amount found [a] (mg)	% of Labeled amount [a]	RSD
1.	Losacar-H	50	49.78 ± 0.256	99.56 ± 0.513	0.515
		12.5	12.67 ± 0.14	101.33 ± 1.18	1.166
2.	Losar –H	50	49.97 ± 0.194	99.93 ± 0.388	0.388
		12.5	12.58 ± 0.233	100.61 ± 1.87	1.857

a: data represents mean ± SD ; n = 3.

3.2.2. Interference study

To investigate the effect of tablet fillers on the measurements involved in the methods, a standard solution each of LOP and HCZ (100 μg mL^{-1}) were diluted further to obtain concentrations of 12, 16 and 20 μg mL^{-1} for LOP and 3, 4 and 5 μg mL^{-1} for HCZ and the absorbances of the resulting solutions were measured in triplicate at 227.4 and 270.4 nm for Method A and 256.4 nm and 270.4 nm for Method B respectively and then a mixture containing lactose, starch, talc, magnesium stearate and bulk drug solutions in the ratio 80: 7: 2.5: 0.5:10 was prepared in 0.01 N HCl and filtered using Whatmann no. 40 filter paper. The absorbances of the resulting solutions were then measured in triplicate at 227.4 and 270.4 nm for Method A and 256.4 nm and 270.4 nm for Method B respectively and results obtained

were treated statistically. The % interference ranged from 0.345 to 0.501 for Method A and from 0.352 to 0.503 for Method B. From this study, it is apparent that the usual co-formulated substances would seldom interfere in the method (Table 3 and Table 5).

Table 5. Summary of validation parameters for Method B

S. No.	Parameters	LOS	HCZ
1.	Specificity:		
	-% interference	≤ 0.5%	≤ 0.5%
	-% agreement	100.35-100.50	100.33- 100.49
2.	Range (μg/ml):		
	-Working range	0.772-50.50	0.563-27.27
	-Linearity range	5.05-50.50	6.06-27.27
	-Target range	22.2- 33.3	13.2- 19.8
	-Test conc. (100%)	27.8	16.5
3.	Precision: (RSD)		
	-Repeatability (n =7)	0.989	0.586
	-Intraday (n=3)	0.561	1.30
	-Interday (3 days)	0.602	1.33
4.	Accuracy %	98.13-99.20	98.11-99.84
5.	Limit of detection, μg mL^{-1}	0.159	0.186
6.	Limit of quantification, μg mL^{-1}	0.483	0.563

3.3. Application to analysis of commercial samples

In order to check the validity of the proposed methods, LOP and HCZ were determined in some commercial formulations. Table 4 and Table 6 present the results of the determination from which it is clear that there is close agreement between the results obtained by the proposed methods and the labeled claim.

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powders were spiked with pure LOP and HCZ standard solutions at three different levels and the concentration of the sum total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug solution added were quantitative (99.56–100.61 % for Method A and 99.15-99.77 % for Method B) and revealed that co-formulated substances did not interfere in the determination.

Table 6. Summary of estimation of LOS and HCZ in different brands for **Method B**

S. No.	**Brand**	**Labeled amount (mg)**	**Amount found [a] (mg)**	**% of Labeled amount [a]**	**RSD**
1.	**Losacar-H**	50	49.57 ± 0.083	99.15 ± 0.167	0.168
		12.5	12.47 ± 0.012	99.77 ± 0.098	0.102
2.	**Losar –H**	50	49.69 ± 0.122	99.39 ± 0.244	0.246
		12.5	12.47 ± 0.014	99.74 ± 0.115	0.116

a: data represents mean ± SD ; n = 3.

2.4. Forced degradation studies

The standard solution of drugs was prepared by dissolving 10.0 mg of Losartan and 10.0 mg of Hydrochlorothiazide separately in 50 mL of 0.01N HCl solution and the final

volume was made upto 100 mL with 0.01N HCl solution. The reactions were carried out using a concentration of 20.0 μg/mL for Losartan Potassium and 5.0 μg/mL for Hydrochlorothiazide. Hence the synthetic mixture contains 4:1 ratio of both the drugs. The stress conditions employed were acidic and basic hydrolysis, oxidative conditions using 3% H_2O_2, thermal stress and stress under UV light. The experiments/ scans /assays of the synthetic mixtures of Losartan Potassium and Hydrochlorothiazide indicates that the conditions under which the drugs clearly degraded was basic hydrolysis, thermal stress and stress under UV light. Since the acidic solvent was used for assay, hence microbiological studies were not performed. The results are shown in Tables 7 and Table 8 for Method A & Method B, respectively.

Table 7. Summary of degradation studies of spectrophotometric Method A

S. No.	Conditions applied	Conc. taken (μg/ml)	Conc. Found (μg/ml)	Observation
1.	Acidic hydrolysis (0.1 -1.0 N)	20.0 μg/ml	19.6 μg/ml	No change
		5.0 μg/ml	4.86 μg/ml	No change
2.	Basic hydrolysis (0.02-1.0 N)	20.0 μg/ml	39.03 μg/ml	Degraded
		5.0 μg/ml	12.01 μg/ml	Degraded
3.	Thermal stress (60 ^{0}C, 24 hrs)	20.0 μg/ml	Change in λ max	Degraded
		5.0 μg/ml	Change in λ max	Degraded
4.	3% H_2O_2	20.0 μg/ml	16.07 μg/ml	Degraded
		5.0 μg/ml	3.56 μg/ml	Degraded
5.	UV-treatment (7 days, 4 hrs daily)	20.0 μg/ml	15.64 μg/ml	Degraded
		5.0 μg/ml	3.82 μg/ml	Degraded

Table 8. Summary of degradation studies of spectrophotometric Method B

S. No.	Conditions applied	Conc. taken (μg/ml)	Conc. Found (μg/ml)	Observation
1.	Acidic hydrolysis (0.1 -1.0 N)	20.0 μg/ml	19.6 μg/ml	No change
		5.0 μg/ml	4.86 μg/ml	No change
2.	Basic hydrolysis (0.02-1.0 N)	20.0 μg/ml	41.03 μg/ml	Degraded
		5.0 μg/ml	12.01 μg/ml	Degraded
3.	Thermal stress (60 ^{0}C, 24 hrs)	20.0 μg/ml	Change in λ max	Degraded
		5.0 μg/ml	Change in λ max	Degraded
4.	3% H_2O_2	20.0 μg/ml	15.97 μg/ml	Degraded
		5.0 μg/ml	3.56 μg/ml	Degraded
5.	UV-treatment (7 days, 4 hrs daily)	20.0 μg/ml	16.5 μg/ml	Degraded
		5.0 μg/ml	3.82 μg/ml	Degraded

3. Conclusion

The methods for the determination of Losartan Potassium and Hydrochlorothiazide have been developed and validated. These are applicable over a range of 2.02- 22.22 μg mL^{-1} for LOP and 3.03-27.27 μg mL^{-1} for HCZ in Method A and 5.05-50.50 μg mL^{-1} for LOP and 3.03-27.27 μg mL^{-1} for HCZ in Method B and molar absorptivity of 1.90 x 10^3 L $mole^{-1}$ cm^{-1} for LOP and 10.3 x 10^3 L $mole^{-1}$ cm^{-1} for HCZ in Method A and 9.92 x 10^3 L $mole^{-1}$ cm^{-1} for LOP and 10.3 x 10^3 L $mole^{-1}$ cm^{-1} for HCZ in Method B. The recoveries of LOS and HCZ from two brands have been compared by using t- test and F-test for both the methods and results are shown in Table 9 and 10. The methods rely on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus these can be used as alternatives for rapid and routine determination of bulk sample and tablets.

Table 9. Results of t-test and F-test applied for Losartan Potassium and Hydrochlorothiazide for Losacar- H brand

S. No.	Parameters	Losartan Potassium		Hydrochlorothiazide	
		Method A	Method B	Method A	Method B
1	Mean	99.44	99.00	99.35	98.92
2	Variance	0.051	0.242	0.028	0.183
3	t Stat	1.41		1.63	
4	P (T<=t) two-tail	0.23		0.18	
5	t Critical two-tail	2.78		2.78	
6	$F_{(2,2)}$ Statistical	4.75		6.54	
7	$F_{(2,2)}$ critical	19.0		19.0	

Here, t_{crit} and F_{crit} is greater than t_{stat} and F_{stat}; hence significant difference between the recoveries of Losartan Potassium and Hydrochlorothiazide (Losacar-H) using the two methods does not exist.

Table 10. Results of t-test and F-test applied for Losartan Potassium and Hydrochlorothiazide for Losar- H brand

S. No.	Parameters	Losartan Potassium		Hydrochlorothiazide	
		Method A	Method B	Method A	Method B
1	Mean	99.49	98.89	99.35	98.92
2	Variance	0.340	0.226	0.028	0.157
3	t Stat	1.38		1.73	
4	P (T<=t) two-tail	0.24		0.16	
5	t Critical two-tail	2.78		2.78	
6	$F_{(2,2)}$ Statistical	1.51		5.61	
7	$F_{(2,2)}$ critical	19.0		19.0	

Here, t_{crit} and F_{crit} is greater than t_{stat} and F_{stat}; hence significant difference between the recoveries of Losartan Potassium and Hydrochlorothiazide (Losar-H) using the two methods does not exist.

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