Simultaneous estimation and validation of losartan potassium and hydrochlorothiazide in bulk and tablet dosage form by using different spectrophotometric method

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ABSTRACT

Three simple, accurate and precise spectrophotometric methods have been developed for simultaneous determination of Losartan potassium and hydrochlorothiazide in a binary mixture. In method I absorbance were measured at 235nm and 271nm corresponding to the absorbance maxima of losartan potassium and hydrochlorothiazide. Concentration of each drug was obtained by using the absorptive values calculated for both drugs at wavelengths 235nm and 271nm. Method II by dual wavelength method, losartan potassium and hydrochlorothiazide were quantified using principle that absorbance difference between two points on mixture spectra was directly proportional to concentration of component of interest and independent of interfering component. Method III describes Area Under Curve and involves measurement of area under curve in the range of 265-282nm(For LOS) and 229-242nm(For HCTZ) for the analysis in methanol. Linearity range was observed in the concentration range of 5-30µg/ml for losartan potassium and hydrochlorothiazide. Developed methods were applied to marketed formulation. The methods were validated statistically and recovery study was performed to confirm the accuracy of both methods.

Keywords: Losartan potassium, Hydrochlorothiazide, Ultraviolet spectroscopy, Simultaneous equation Method, Dual Wavelength Method, Area under Curve Method.
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INTRODUCTION

Losartan potassium(LOS) is chemically described as 2-butyl-4-chloro-1{[2'-(1H-tetrazol-5-yl)][1,1'-biphenyl]-4-yl]methyl}-1H-imidazol and is mainly used to treat high blood pressure(hypertension) as it is a competitive antagonist and inverse agonist of Angiotensin-II receptor. Hydrochlorothiazide (HCTZ) is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide used as diuretic(1). In the chemical analysis of multicomponent dosage form one drug may interfere with estimation of other drug. Hence analytical methods are developed to estimate all the drugs simultaneously in multicomponent formulations.

Many analytical methods like HPLC, HPTLC, electrochemical, radioimmunoassay were reported for determination of LOS and HCTZ alone and combination with other antihypertensive drugs. The RP-HPLC method has been reported for simultaneous estimation of LOS and HCTZ.

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However no Dual wavelength and Area under Curve methods reported till date for simultaneous determination of these drugs. In this communication we report a new UV spectrophotometric methods.

MATERIALS AND METHODS

Materials
UV-visible double beam spectrophotometer, Jasco model 680 with spectral bandwidth of1nm, wavelength accuracy of ±0.3nm and a pair of 10mm matched quartz cells was used. The commercially available tablet, Arrow (Label claim: Losartan potassium I.P.-50mg, Hydrochlorothiazide I.P-12.5mg) was procured from commercial market.

Selection of common solvent
After assessing the solubility of drugs in different solvents Methanol was used as common solvent for developing spectral characteristics.

Preparation of standard stock solution
The standard stock solutions (100µg/ml) of each of Losartan Potassium and hydrochlorothiazide were prepared separately by dissolving accurately about 10mg of drug in 20ml of methanol and volume was made up to 100ml with methanol. Working standard solutions of 10µg/ml were scanned in the entire UV range of 200-400 nm to obtain the absorbance.

Preparation of calibration curves
Solutions of 10 µg/ml of LOS and HCTZ each were prepared separately. Both the solutions were scanned in the spectrum mode from 200-400 nm. The maximum absorbance of LOS and HCTZ were at 235 and 271 nm, respectively. LOS and HCTZ obeys Beers-Lamberts law in the concentration range of 5-30 µg/ml at their respective maxima. Accurately measured standard stock solution of LOS and HCTZ (0.5,1.0, 1.5, 2.0, 2.5, 3.0 ml) were transferred to a separate series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbance of each solution was measured at wavelength 235 nm and 271 nm. The coefficient of correlation was found to be 0.996 and 0.998 for LOS and HCTZ respectively.

Method I: Simultaneous Equation Method
Sample stock solution was appropriately diluted with methanol to obtain final concentration of 10µg/ml for LOS and HCTZ. These solutions were scanned in the wavelength range of 200-400 nm. From the overlain spectrum, two wavelengths namely 235 nm and 271 nm, λ-max of LOS and HCTZ respectively were selected. The calibration curves were constructed in the concentration range of 5-30 µg/ml for LOS and HCTZ. The concentration of drugs was determined by using the Equations 1 and 2. Mention in paragraph where would be; Figure 1.

$A_1 = 0.0861C_x + 0.0341C_y$……. (1)
$A_2 = 0.00094C_x + 0.0271C_y$…… (2)

Where, A_1 and A_2 are absorbance of sample at 235 nm and 271 nm respectively. 0.0861 and 0.0341 are absorptivities of LOS at 235 nm and 271 nm respectively. 0.00094 and 0.0271 are absorptivities of HCTZ at 235 nm and 271 nm respectively. C_x and C_y are concentrations of LOS and HCTZ respectively.

Method II: Dual Wavelength Method
In this method, LOS wasdetermined by plotting the difference in absorbance at 229 nm)and 242 nm (difference was zero for HCTZ) against the concentration of LOS (Figure 2). Similarly for the determination of HCTZ, the difference in absorbance at 265 nm and 282 nm (difference was zero for LOS)was plotted against the concentration of HCTZ (Figure 3). Standard solutions were prepared having concentration 5-30 µg/ml for both drugs. The difference in absorbance at 229 nm and 242 nm were plotted against the concentration of LOS and that at 265 nm and 282 nm were plotted against the concentration of HCTZ to constructtwo separate calibration curves for LOS and HCTZ.

Method III: Area under Curve Method
For the simultaneous determination using the Area under Curve method, suitable dilutions of the standard stock solutions (100µg/ml) of LOS and HCTZ were prepared separately in methanol. The solutions of drugs were scanned in the range of 200 nm-400 nm**.** For Area under Curve method, calibration curve was plotted and the sampling

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wavelength ranges selected for estimation of LOS and HCTZ are 229 nm-242 nm (λ1-λ2) and265 nm-282 nm (λ3-λ4) respectively (Figure 4) and area were integrated between these selected wavelength ranges for both drugs, which showedlinear response with increasing concentration hence the same wavelength range were used for estimation of tablet formulations. By using integrated areas two simultaneous equations were constructed and solved to determine concentrations of analytes. Concentration of two drugs in mixed standard and the sample solution were calculated using equation (3) and (4).

C_{LOS} = A2ay1-A1ay2/ax2ay1-ax1ay2…. (3)
C_{HCTZ} = A1ax2-A2ax1/ax2ay1-ax1ay2… (4)

Where, ax1 (861.80) and ax2 (341.83) are absorptivities of LOS at (λ1-λ2) and (λ3-λ4) respectively, ay1 (94.26) and ay2 (271.13) are absorptivities of HCTZ at (λ1-λ2) and (λ3-λ4) respectively. A1 and A2 are absorbance of mixed standard at (λ1-λ2) and (λ3-λ4) respectively. C_{LOS} and C_{HCTZ} are concentrations in g/100ml.

Analysis of Tablet Formulation
Twenty tablets were weighed and crushed to a fine powder. An accurately weighed powder sample equivalent to 100 mg of Losartan potassium was transferred to a 100 ml volumetric flask, dissolved in 100 ml methanol, shake for 10 minutes and the volume wasmade up to the mark with methanol. The solution was then filtered through Whatman filter paper no.41. The solution was further diluted to get different concentrations in the range of 5-30 µg/ml of both the drugs. The spectra of LOS and HCTZ when overlaid indicated that the spectra of LOS and HCTZ satisfied this condition. The result of analysis of the formulation is shown in Table 1 and 2.

Validation of Method
Validationof the proposed methods was carried out for its accuracy, precision, specificity and linearity according to ICH guidelines.

Accuracy
Recovery studies were carried out at three different levels by adding the pure drug to previously analyzed tablet powder sample. Accurately weighed quantities of tablet powder equivalent to 80%, 100% and 120% of label claim of LOS were taken in a series of 100 ml volumetric flasks and dilutions were made as under sample solution. The graphs of % label claim vs. absorbance were plotted for Method I,II and III. From the amount of drug total drug found, percentage recovery was calculated by proposed four methods and results are shown in Table 3.

Precision
Inter-day precision
It was done by analyzing the solutions bysame analyst on alternate day till 5th day. Results indicate that the solution is stable up to 1 day, thereafter degradation may have taken place leading lower percent label claim.

Intra-day precision
It was done by analyzing the solutions by same analyst within a day. Results indicate that the solution is stable up to 4 hours and thereafter, degradation may have taken place in the solution.

Linearity
Linearity was checked by diluting standard stock solution at six different concentrations. LOS was with the concentration range of 5-30 µg/ml at 235 nm. HCTZ was linear in the concentration range of 5-30 µg/ml at 271 nm. Calibration curves (n=6) were plotted between concentration and absorbance of drugs. Optical parameters were calculated.

Limit of Detection
The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula and shown in Table 1.

LOD = 3.3 (σ/S)

Where, S = slope of calibration curve, σ = standard deviation of the response.

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Limit of Quantification
The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in Table 1.
LOQ – 10 (σ/S)

Where, S = slope of calibration curve, σ = standard deviation of the response.

RESULTS AND DISCUSSION

LOS and HCTZ hasestimated at 235 nm and 271 nm in methanol, both drugs obey Beer-Lamberts law in concentration range of 5-30 µg/ml. The method was validated as per ICH and USP guidelines. The result of recovery study was found to be within the prescribed limit of 98-101%. The assay results obtained by proposed methods as shown in Table 1 and 2. The % R.S.D. Linearity was observed by linear regression equation method for LOS and HCTZ in different concentration range. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity, hence it can be used for routine analysis of two drugs in combined dosage forms. There was no interference from tablet excipients was observed in these methods. These methods were accurate, simple, rapid, precise, reliable, sensitive, reproductive and economic.

Table 1: Spectral and linearity characteristics data

Parameters	Method I		Method II		Method III	
	LOS	**HCTZ**	**LOS**	**HCTZ**	**LOS**	**HCTZ**
Beer's Law limit (µg/ml)	5-30	5-30	5-30	5-30	5-30	5-30
Coefficient of correlation	0.9956	0.996	0.994	0.998	0.9949	0.9995
Intercept	0.0491	0.0078	0.005	0.008	0.3425	0.0029
Slope	0.0789	0.0283	0.007	0.013	0.5437	0.0866
LOD	0.3054	0.3153	0.3102	0.3363	0.3178	0.5084
LOQ	0.9255	1.9564	1.9400	1.7010	1.9631	1.1540
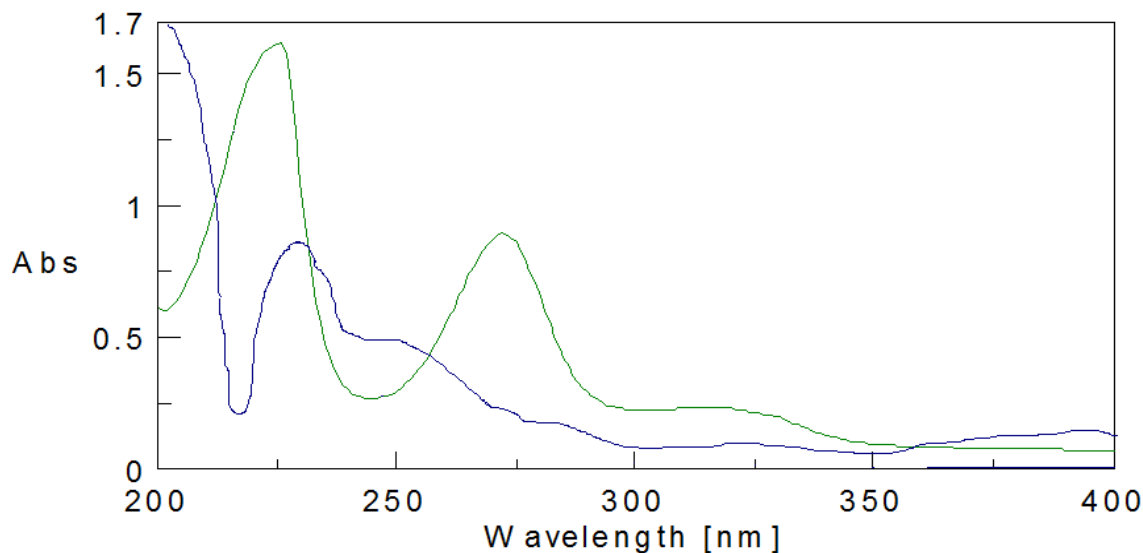
Figure 1: Overlay Spectra for Losartan potassium and Hydrochlorothiazide

Table 2: Result of analysis of tablet formulation

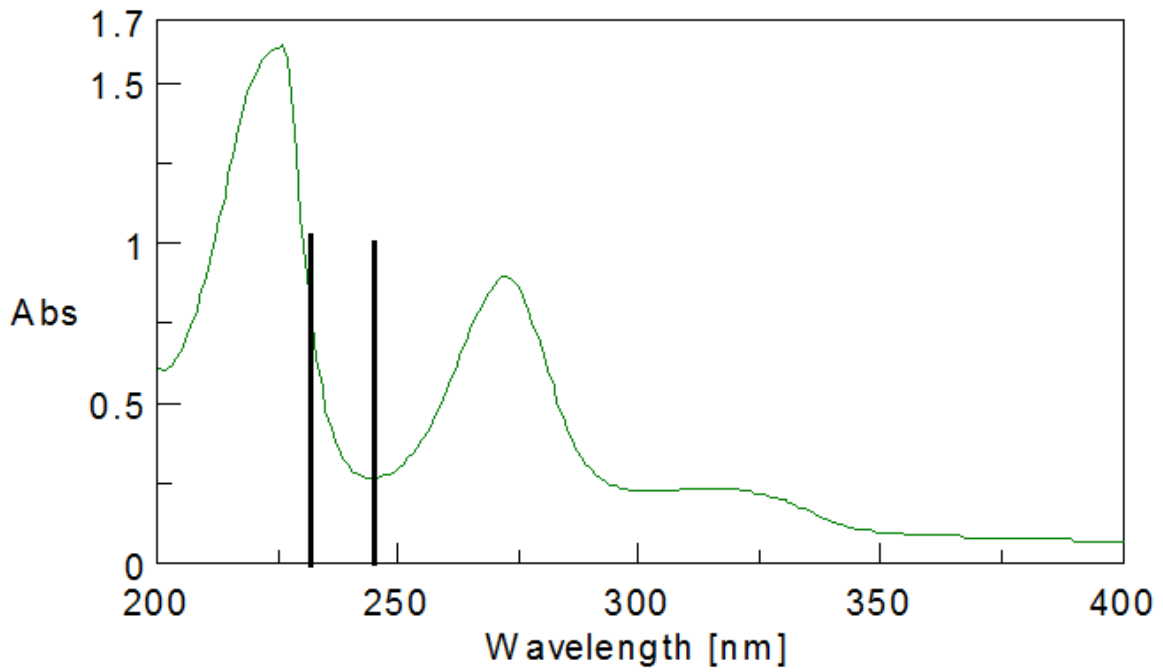| Methods	Standard Deviation		% Relative Standard Deviation		Standard Error Mean	
	LOS	**HCTZ**	**LOS**	**HCTZ**	**LOS**	**HCTZ**
Method I	0.7219	0.2676	0.5019	0.5514	0.2947	0.1092
Method II	0.0653	0.1325	0.4853	0.4853	2.1353	0.3305
Method III	5.2304	0.8095	0.5737	0.5328	0.0267	0.0541

Figure 2: Spectra for hydrochlorothiazide in Dual wavelength method
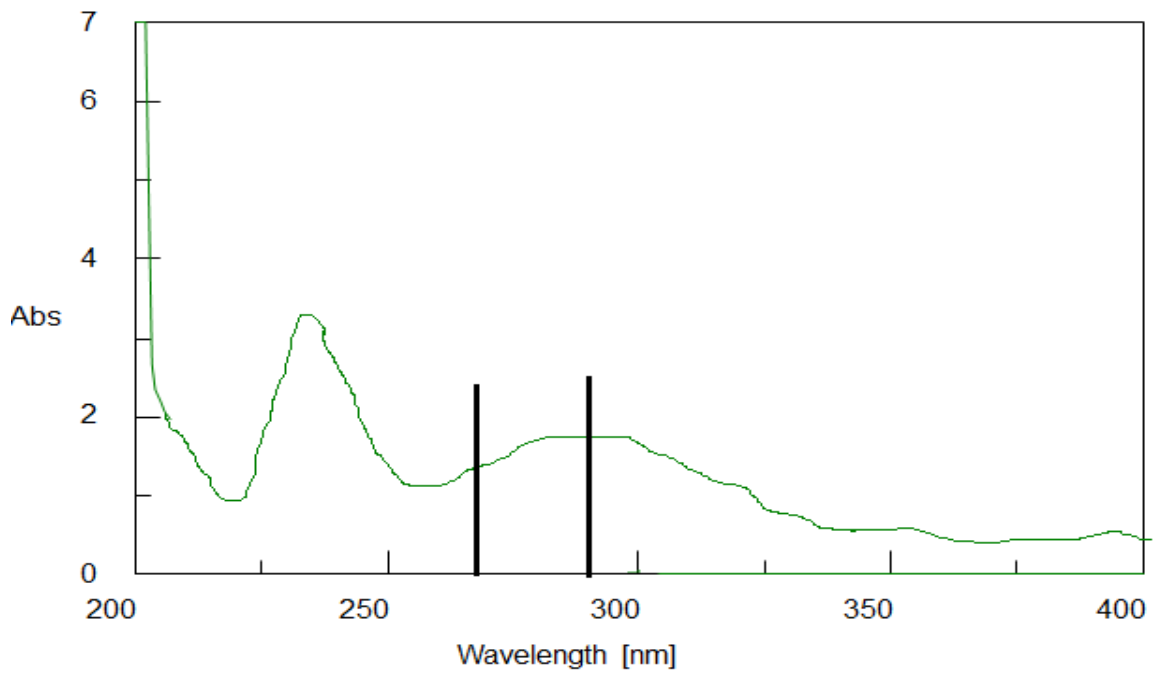

Figure 3: Spectra for Losartan potassium in dual wavelength method
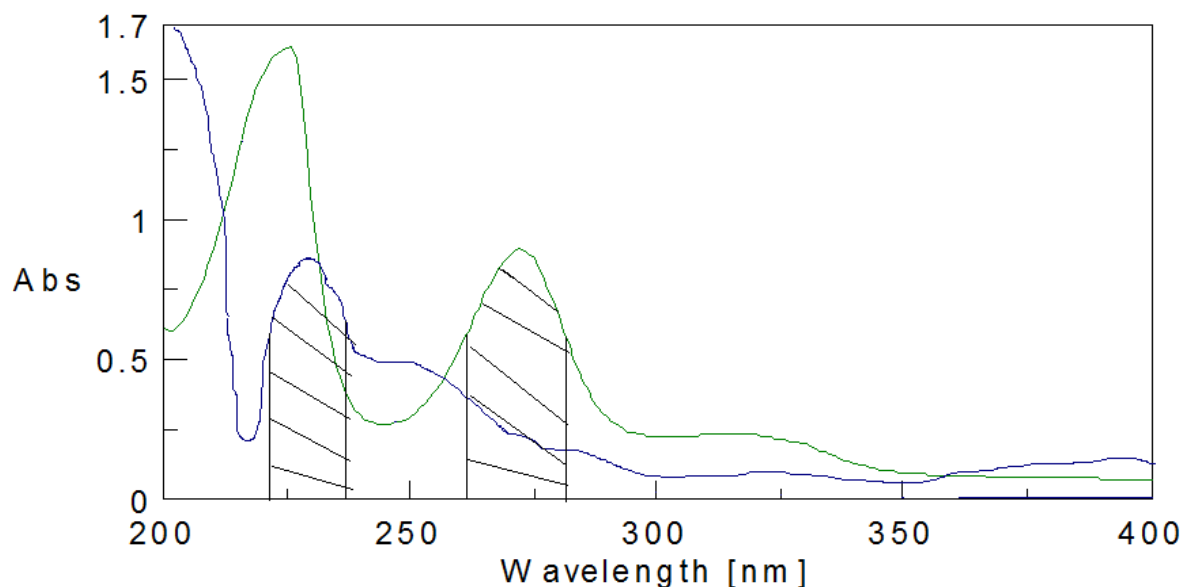
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Figure 4: Overlay spectra for Losartan potassium and hydrochlorothiazide for AUC

Table 3: Result of recovery study

Method	Label Claim(mg/tab)		Amount Claim (mg/tab)		% Label Claim found		% Recovery	
	LOS	HCTZ	LOS	HCTZ	LOS	HCTZ	LOS	HCTZ
I	50	12.5	49.1	12.1	97.8	98.2	99.53	99.90
II	50	12.5	49.4	12.2	97.6	98.8	98.73	100.31
III	50	12.5	49.7	12.3	98.4	99.2	98.50	100.12

CONCLUSION

Simple UV spectrophotometric methods were developed for the simultaneous determination of Losartan potassium and Hydrochlorothiazide in bulk and tablet formulation, both the results of our study indicate that drugs analyzed at wavelength 235 nm and 271 nm respectively, The main recovery was 98.7 % for LOS and 100.3 % for HCTZ respectively. statistical analysis proves that, these methods are repeatable and selective for the analysis of LOS and HCTZ.

Abbreviations:
LOS-Losartan potassium
HCTZ-Hydrochlorothiazide

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