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SIMULTANEOUS ESTIMATION OF LOSARTAN POTASSIUM AND HYDROCHLOROTHIAZIDE IN COMBINED DOSAGE FORM BY UV SPECTROPHOTOMETRY

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ABSTRACT

A simple and rapid UV spectrophotometric method was developed for simultaneous estimation of losartan potassium (LOS) and hydrochlorothiazide (HCTZ) employing absorbance correction method. In this method methanol was used as solvent. From the UV overlain spectra of both the drugs 271 nm and 316 nm were selected as wavelengths for analysis. The method was validated as per ICH guidelines. The linearity was established from 10-100 µg/ml for losartan and 5-50 µg/ml for hydrochlorothiazide with correlation coefficient (r^2) greater than 0.990. The developed method was considered to be accurate as % recovery at 80%, 100% and 120% levels were found to be within the acceptance criteria. The results of precision were within the limits of % RSD. The % assay of losartan and hydrochlorothiazide were found to be 104.036% and 100.868% by

absorption correction method which were within the acceptable limit. Hence the developed method can be employed as a simple, analytical method for simultaneous estimation of two drugs.

Key words: Losartan, Hydrochlorthiazide

INTRODUCTION

Losartan **(Figure 1)**, is [2-butyl-5-chloro-3-[[4-[2-(2*H*-tetrazol-5-yl) phenyl] phenyl]methyl]imidazol-4-yl]methanol. It is a non-peptide angiotensin II antagonist with antihypertensive activity. Losartan and its active metabolite selectively and competitively blocks the binding of angiotensin II to the angiotensin I receptor. This blocks the vasoconstricting and aldosterone-secreting actions of angiotensin II, leading to a decrease in blood pressure.

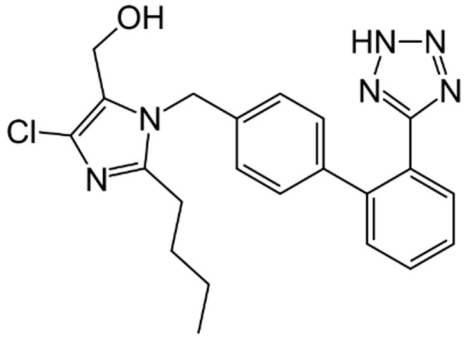

Figure 1: Structure of Losartan

Hydrochlorothiazide **(Figure 2)** is 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide. It is a diuretic medication used to treat high blood pressure and swelling due to fluid build up. Drug is also used to treat renal tubular acidosis and to decrease risk of kidney stones in those with a high calcium level in the urine. It reduces blood volume by acting on kidneys to reduce sodium reabsorption in the distal convoluted tubule.

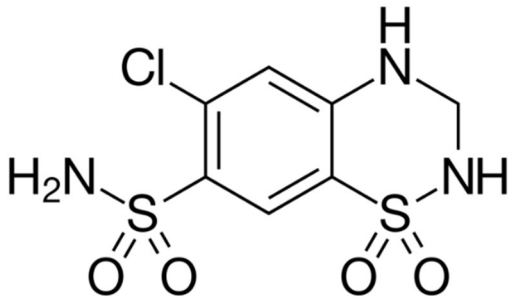

Figure 2: Structure of Hydrochlorothiazide

Many methods using sophisticated instruments have been reported for the estimation of these two drugs in single or in combined pharmaceutical dosage forms. This work was directed towards the development of a simple, sensitive and an accurate UV method for the estimation of these two drugs in combination **[1-11]**.

MATERIALS AND METHODS

Instrumentation and equipment:

A Double beam UV-Visible spectrophotometer (Shimadzu 1800) with 10 mm matched quartz cells was used. All weighing was done on a single pan balance.

Table 1: Instruments and equipments

Sr. No.	Instrumentation and Equipment	Model/Make
1.	UV Spectrophotometer	Shimadzu UV-1800
2.	Cuvettes	Paired Quartz Cuvettes
3.	Electronic Weighing Balance	CITIZON CY 204
4.	Sonicator	ULTRASONICS
5.	Glassware (volumetric flasks, pipettes, measuring cylinders, funnels, etc.)	Borosil
6.	Filter Paper	Whatman Filter paper no.1

Reagents and chemicals

Active pharmaceutical ingredients, losartan and hydrochlorothiazide were obtained as a gift samples from Unichem and Microlabs Pvt. Ltd. respectively. Distilled water and methanol of spectroscopic grade were used in the experiment. All the reagents used were of analytical grade. Distilled water was used throughout the experiment.

Tablets were purchased from local market sold under the brand name "COSART H" containing 50 mg of LOS and 12.5 mg of HCTZ.

Method Development

Selection of Diluent [12]

The choice of solvent was arrived at by dissolving drugs in different solvents like NaOH, HCl, methanol, ethanol etc. Depending upon the solubility and a stable absorbance reading methanol was selected as a solvent and distilled water was selected as a diluent.

Selection of wavelength [13, 14]

The working standard solutions of the drugs were scanned in the UV region 200nm to 400nm using diluent as blank. Using the peak point function λmax for drugs were obtained. The two spectra were then overlaid. From the overlain spectra, the two wavelengths for the absorption correction method were selected as follows: λ_1 absorbance due to HCTZ only (LOS shows zero absorbance) and λ_2 absorbance due to both drugs.

Preparation of stock solution of LOS and HCTZ (1000 µg/ml): About 25 mg of LOS and HCTZ were accurately weighed and transferred two 25 ml volumetric flasks and volume was made by dissolving with sufficient amount of methanol to get the concentration of 1000 µg/ml.

Preparation of working stock solutions:

From the above two stock solutions 10 ml of each of the solution was pipetted out and made upto 100 ml with the diluent i.e. distilled water to get the concentration of (100 µg/ml). From the above appropriate volumes were taken into a series of 10 ml volumetric flasks and volume was made up with diluent to obtain the required concentrations of both the drugs.

Preparation of sample solutions:

For estimation of LOS and HCTZ in tablet formulation, 10 tablets were weighed and average weight was calculated. These

tablets were finely crushed to obtain fine powder. Tablet powder equivalent to 50 mg losartan and 12.5 mg of hydrochlorothiazide was weighed and transferred in 25 ml volumetric flask with addition of about 10 ml of methanol and was sonicated for 8 minutes and volume was made with methanol. The resulting solution was filtered and from this 0.25 ml was pipetted out in 10ml volumetric flask and volume was made up to the mark using distilled water. The absorbance of resulting solution was recorded at predetermined wavelengths and percentage purity was calculated.

RESULTS AND DISCUSSION

Method Validation [15]

Linearity

Aliquots of working stock solutions of LOS and HCTZ were taken into a series of 10 ml volumetric flasks and volume was made up with diluent to obtain concentrations 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml for LOS and 5, 10, 15, 20, 25, 30, 35 µg/ml HCTZ.

Absorbance of these solutions was recorded at predetermined wavelengths λ1 and λ2 and calibration curves were plotted of absorbance v/s concentration. The Beer Lambert's range for LOS and HCTZ was established from 10-100 µg/ml and 5-50 µg/ml **(Table 2)**.

Precision

The precision of the method was performed by analyzing the formulation for repeatability and intraday and interday variation studies **(Table 3, 4, 5)**.

Accuracy

Accuracy was performed at three different concentration levels of 80, 100 and 120 % with three replicates at each level in which the amount of sample was kept constant i.e. 50µg/ml of LOS and 12.4 µg/ml of HCTZ. The percent recovery, mean SD and %RSD were calculated and found to be within the limits as per the ICH guidelines **(Table 6)**.

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ for losartan and hydrochlorothiazide were determined from the calibration curves by calculating the signal to noise ratio(S/N) **(Table 7)**.

Robustness

Robustness of the method was conducted by performing the assay of the formulation by deliberately introducing minor changes in experimental conditions like, change of UV spectrometer instrument, change of wavelengths and change of analyst **(Table 8)**.

Assay

Assay was carried out on the marketed formulation name containing 50 mg of LOS and 12.5 mg of HCTZ **(Table 9)**.

Table 2: Linearity data of LOS and HCTZ

Sr No.	Parameters	Losartan	Hydrochlorothiazide	
		271 nm	271 nm	316 nm
1.	Linearity (µg/ml)	10-100	5-50	
2.	Slope	10.444	58.1610	10.0040
3.	Regression coefficient (r^2)	0.999	0.9990	0.9980

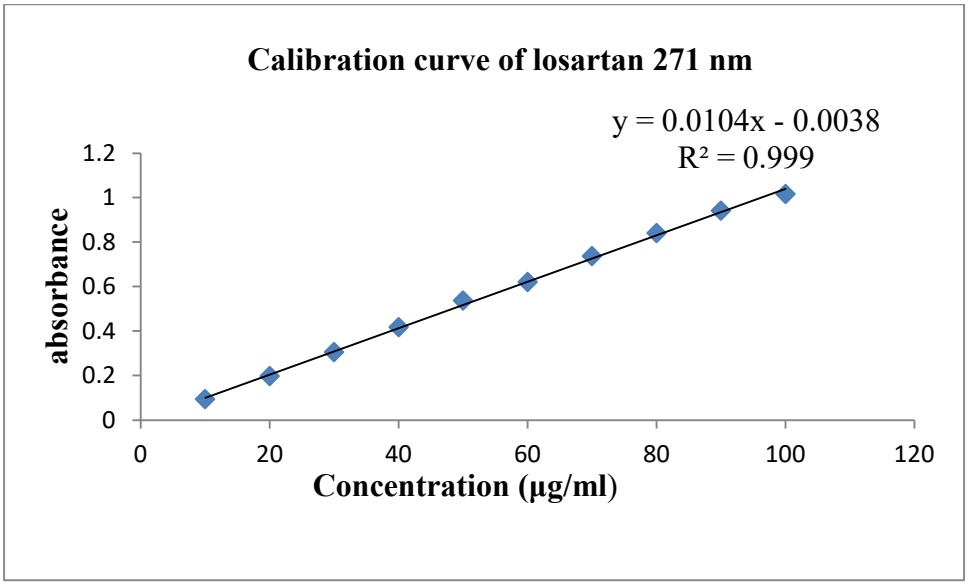

Figure 2: Calibration curve of LOS

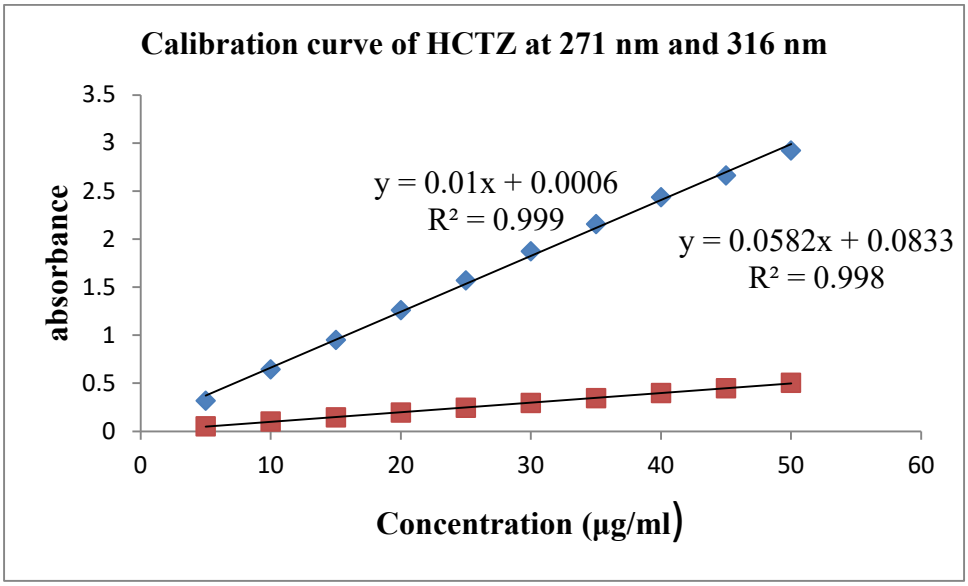

Figure 3: Calibration curve of HCTZ

Table 3: Repeatability data

	Repeatability (n=6)	
Sr no.	**Abs at 271 nm (X)**	**Abs at 316 nm (Y)**
1	**0.1230**	**1.3270**
2	**0.1320**	**1.2293**
3	**0.1220**	**1.3210**
4	**0.1210**	**1.2400**
5	**0.1239**	**1.2280**
6	**0.1225**	**1.2251**
Average	**0.1224**	**1.2310**
SD	**0.0014**	**0.0079**
% RSD	**1.1842**	**0.6417**

Table 4: Intra-day data LOS and HCTZ

Morning		
	Absorbance at 271 nm	**Absorbance at 316 nm**
Average	**0.1220**	**1.2566**
SD	**0.0005**	**0.0037**
% RSD	**0.4719**	**0.3012**
Afternoon		
	Absorbance at 271 nm	**Absorbance at 316 nm**
Average	**0.1204**	**1.26660**
SD	**0.0010**	**0.00450**
% RSD	**0.80640**	**0.35590**
Evening		
	Absorbance at 271 nm	**Absorbance at 316 nm**
Average	**0.1253**	**1.2746**
SD	**0.0005**	**0.0020**
% RSD	**0.4606**	**0.1633**

Table 5: Inter day data of LOS and HCTZ

Day 1		
Sr No.	**Absorbance at 271 nm**	**Absorbance at 316 nm**
Average	**0.1316**	**1.3340**
SD	**0.0005**	**0.0010**
% RSD	**0.4384**	**0.0749**
Day 2		
Sr No.	**Absorbance at 271 nm**	**Absorbance at 316 nm**
Average	**0.1253**	**1.2786**
SD	**0.0005**	**0.0030**
% RSD	**0.4606**	**0.2385**
Day 3		
Sr No.	**Absorbance at 271 nm**	**Absorbance at 316 nm**
Average	**0.1223**	**1.2553**
SD	**0.0005**	**0.0049**
% RSD	**0.4719**	**0.3929**

Table 6: Accuracy data of LOS and HCTZ

	Concentration level	Amount of pure Drug added (µg/ml)	Amt. of std recovered (µg/ml)	Mean %recovery
LOS	80%	40	41.60	104.00
		40	41.60	104.00
		40	40.80	102.02
	100%	50	51.40	102.80
		50	50.31	100.63
		50	49.92	99.84
	120%	60	62.53	104.22
		60	61.94	103.24
		60	62.51	104.19
HCTZ	80%	10	9.56	95.60
		10	9.512	95.12
		10	9.666	96.66
	100%	12.5	12.47	99.81
		12.5	12.30	98.42
		12.5	12.50	100.01
	120%	15	14.65	97.73
		15	14.65	97.73
		15	14.75	98.39

Table 7: LOD and LOQ data for LOS and HCTZ

Parameters	LOS	HCTZ	
	271nm	271 nm	316 nm
LOD (µg/ml)	3.333	1.688	2.737
LOQ (µg/ml)	9.981	4.995	1.380

Table 8: Robustness data for LOS and HCTZ

Parameters	Mean % ± SD		% RSD	
	LOS	HCTZ	LOS	HCTZ
Change of UV Instrument				
Instrument 1	100.104 ± 0.457	99.21 ± 0.510	0.457	0.514
Instrument 2	100.100 ± 0.444	99.54 ± 0.411	0.441	0.413
Change of Analyst				
Analyst 1	99.57 ± 0.457	98.45 ± 0.533	0.459	0.541
Analyst 2	99.47 ± 0.458	99.10 ± 0.234	0.458	0.236
Change of Wavelength				
Wavelength +2 nm	98.78 ± 0.457	96.40 ± 0.644	0.463	0.668
Wavelength -2 nm	100.89 ± 0.457	99.44 ± 0.612	0.453	0.615

Table 9: Results of assay of marketed formulation

Sr. No.	Amount present (µg)		Amount found (µg)		% purity	
	LOS	HCTZ	LOS	HCTZ	LOS	HCTZ
1.	50	12.5	50.700	12.676	103.799	101.411
2.	50	12.5	51.900	12.550	103.800	100.405
3.	50	12.5	52.225	12.598	104.510	100.790

CONCLUSION

A simple and novel absorption correction method has been developed for the simultaneous estimation of losartan and hydrochlorothiazide without observing any interference by the excipients.

The developed method employed methanol as solvent and distilled water as diluent. The method was evaluated as per ICH guidelines. The linearity was established over a concentration range of 10-100 µg/ml for losartan and 5-50 µg/ml for HCTZ with correlation coefficient greater than 0.990 for both the drugs. % RSD calculated for precision was found to be < 2. For accuracy % recovery study and robustness results were obtained within the limit. By performing LOD and LOQ study method was observed to be sensitive. The technique was found to be accurate, precise, sensitive and robust.

The established method was applied for assay of losartan and hydrochlorothiazide in synthetic mixture. The % assay in synthetic mixture was found to be 104.065% for losartan and 100.8686% for HCTZ which was within the acceptance criteria.

Hence the developed method can be used as a cost-effective alternative to HPLC method for the simultaneous estimation of the drugs.

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