Analytical Method development and validation for the estimation of Losartan by first order derivative spectroscopy.

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ABSTRACT:

Losartan a prodrug is a angiotensin II receptor antagonist , IUPAC Name (2-butyl-4-chloro-1-{[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl}-1H-imidazol-5-yl)methano. It is soluble in water, slightly soluble in isopropyl alcohol, methanol, its melting point is 183.5. Objective of the present study is to develop a simple, sensitive, accurate, precise and rapid First order derivative spectrophotometric method for the estimation of Losartan in pure form. For the estimation of Losartan, solvent system employed was absolute methanol and wavelength of detection (λ_{det}) was 267 nm. The linearity was obtained in the range $10 - 20 \mu g/ml$. The limit of detection is $0.5 \mu g/ml$ and limit of quantification was fund to be $1.54 \mu g/ml$. Obtained results showed that there is minimum intra day and inter day variation. The developed method was validated and recovery studies were also carried out. Sample recovery using the above method was in good agreement with their respective labeled claims, thus suggesting the validity of the method and non-interference of formulation excipients in the estimation. First order derivative spectroscopy method is simple, rapid and reproducible and further it can be used for the analysis.

INTRODUCTION:

Losartan is an angiotensin II receptor antagonist drug used mainly to treat high blood pressure (hypertension). Losartan was the first angiotensin II receptor antagonist to be marketed. Losartan potassium is marketed by Merck & Co. Inc. under the trade name Cozaar. As of 2009, losartan is available in generic form. As with all angiotensin II type 1 receptor (AT1) antagonists, losartan is indicated for the treatment of hypertension. It may also delay progression of diabetic nephropathy, and is also indicated for the reduction of renal disease progression in patients with type 2 diabetes, hypertension and microalbuminuria (>30 mg/24 hours) or proteinuria (>900 mg/24 hours)

Although clinical evidence shows calcium channel blockers and thiazide-type diuretics are preferred first-line treatments for most patients (from both efficacy and cost points of view), an angiotensin II receptor antagonist such as losartan is recommended as first-line treatment in patients under the age of 55 who cannot tolerate an ACE inhibitor. The Life study demonstrated losartan was significantly superior to atenolol in the primary prevention of adverse cardiovascular events (myocardial infarction or stroke), with a significant reduction in cardiovascular morbidity and mortality for a comparable reduction in blood pressure. A study hints that losartan has a beneficial effect on mitochondria by reversing age related dysfunction in maintaining normal blood pressure and cellular energy usage. The maximal effects on blood pressure usually occur within 3-6 weeks upon starting losartan.
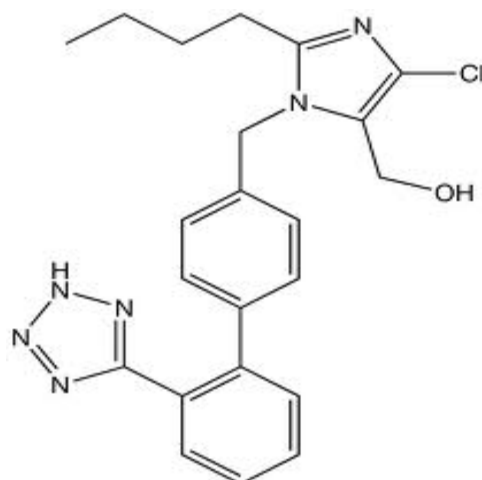
FIG. 1: STRUCTURE OF LOSARTAN

Mechanism of action[3]: Losartan is a prodrug that has little pharmacological activity until it Losartan is a selective, competitive angiotensin II receptor type 1 (AT$_1$) receptor antagonist, reducing the end organ responses to angiotensin II. Losartan administration results in a decrease in total peripheral resistance (afterload) and cardiac venous return (preload) All of the physiological effects of angiotensin II, including stimulation of release of aldosterone, are antagonized in the presence of losartan. Reduction in blood pressure occurs independently of the status of the renin-angiotensin system. As a result of losartan dosing, plasma renin activity increases due to removal of the angiotensin II feedback.

Literature review suggested several analytical methods that have been reported for the estimation of Losartan in bulk or pharmaceutical formulations include High Performance Liquid Chromatography, and UV-Visible Spectrophotometry. Literature review also suggested that there is no First Order Derivative Spectroscopic method. The objective of the work was to develop simple, accurate, precise and economic First order derivative Spectroscopic method to estimate the Losartan in bulk. The method should be simple, accurate, precise, reproducible and statistically valid.

UV spectrophotometry is generally preferr especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (190-380nm). The fundamental principle of operation of spectrophotometer covering UV region consists in that light of definite interval of wavelength passes through a cell with solvent and falls on to the photoelectric cell that transforms the radiant energy into electrical energy.

OBJECTIVES
Thus, the objectives of project:
I. To develop a simple, precise, accurate method, less time consuming & economical derivative spectroscopic method.

II.   Under derivative spectroscopy, the development of First Order derivative Method.
III.   Validation of developed method using common parameters:
       a) Linearity
       b) Precision
       c) Accuracy
       d) Sensitivity
       e) Limit of Detection (LOD)
       f) Limit of Quantification (LOQ)

MATERIALS AND METHODS:

Year of experimentation: 2011
Site of experimentation: Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg, Chhattisgarh.

Drug
The standard sample of LOSARTAN was obtained as gift sample from Dr. Reddy's Laboratory Pvt. Ltd., Hyderabad, A.P., India. The losartan tablets were procured from local market, Merck & Co. Inc. under the brade name Cozaar(25 mg) manufactured by RANBAXY Laboratories, India.

Instrument specifications
UV Spectrophotometer, Shimadzu, model 1800.

Chemicals and reagents used
Methanol obtained from local market, manufactured by Merck Pharmaceuticals.

Preparation of stock solution
The stock solution of Losartan is prepared by dissolving 100 mg of drug in 100 ml methanol in volumetric flask with continuous shaking. 1 ml of sample was withdrawn and diluted to 100 ml methanol to get 10 μg/ml of solution. The solution was than scanned in UV range between 200-400nm UV-VIS Spectrophotometer, Shimadzu, Japan to determine the absorption maxima of the drug against blank as methanol.

Wavelength scanning and determination of absorption maximum
From the stock solution of Losartan, known concentration of 10μg/ml is prepared by suitable dilution with methanol. Wavelength scanned for the maximum absorption of drug solution using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against blank methanol. Convert the normal mode obtained spectra to first order derivative. The wavelength that shows the peak with a highest absorbance is considered as absorbance maximum of the drug. The result is presented in fig. 3.

Linearity studies for Losartan analytical method
Stock solution was subsequently diluted with methanol to get 2μg/ml, 4μg/ml, 6μg/ml, 8μg/ml, 10μg/ml, 12μg/ml 14μg/ml, 16μg/ml, 18μg/ml 20μg/ml, 22μg/ml 24μg/ml, 26μg/ml 28μg/ml, 30μg/ml. Convert the normal mode obtained spectra to first order derivative. The results are

tabulated and the linearity curve was constructed by plotting concentration Vs. D^1 value. The result is presented in table 1 and fig. 2.
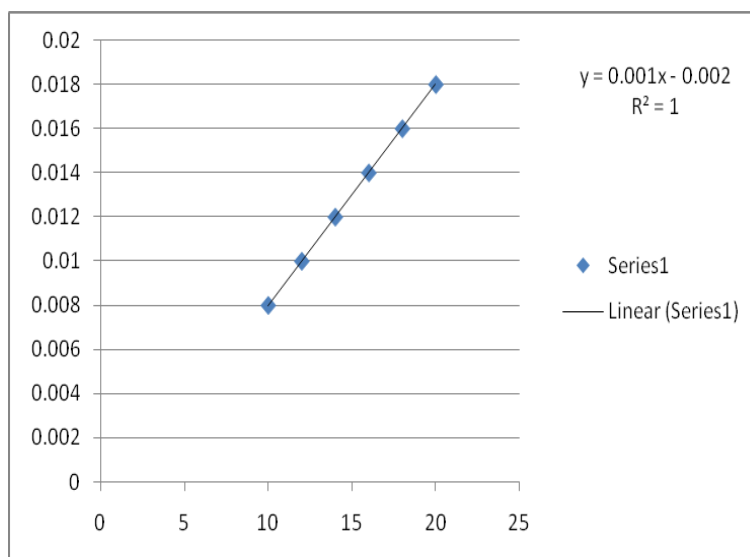

FIG. 2: STANDARD GRAPH OF LOSARTAN
X – Axis: Concentration
Y- Axis: D^1 value

Precision
The precision of method was ascertained; the percent relative standard deviation were calculated and presented.

Inter day and intraday studies for Losartan analytical method
The prepared stock solution was subsequently diluted to get 2μg/ml, 4μg/ml, 6μg/ml 8μg/ml, and 10μg/ml. The resulting solutions absorbance was measured at detection wavelength of 267 nm using double beam UV spectrophotometer against blank of methanol. The findings was made at different time intervals in a day times in a day and performed continuously for six days. Convert the normal mode obtained spectra to first order derivative. The results obtained were tabulated and studied for inter day and intraday variation.

Accuracy studies
The accuracy/recovery studies were carried out by adding a known amount of drug from the pre-analyzed tablet powder and percentage recoveries were calculated. Convert the normal mode obtained spectra to first order derivative. The reproducibility of estimation was determined by performing the tablet drug content of different samples. The results of accuracy studies were expressed in %. The result is presented in table 2.

RESULTS AND DISCUSSION

Losaran is a novel, potent, highly selective non peptide angiotensin II type 1 (AT1) receptor blocker which is administered orally as Losartan which is rapidly and completely hydrolyzed to Losartan, the active moiety, during absorption from the gastrointestinal tract. The physico-chemical characteristic study of Losartan like melting point is 183.5°C nearer to the literature In.

In our laboratory we developed first order derivative spectroscopic method for the analysis of Losartan. The known concentration of Losartan is prepared and scanned for absorption maximum. The detection wavelength according to spectra is 267 nm. Different measured D^1 values at detection wavelength 267 nm is plotted as the curve as D^1 value versus concentration. Losartan obeys the beers law in the concentration range 10 to 20 µg/ml. Linearity study indicates the curve is linear in the range of 2 to 10 µg/ml. The linear regression equation is $Y = 0.001*X – 0.002$ with correlation coefficient (r^2) = 1. The developed method is validated for repeatability, reproducible and the accuracy and precision. In the inter day and intraday study of standard graph, the %SD is less than 2% indicating the developed method is reproducible. The different levels of standard concentration solutions are measured for D^1 value and actual concentration is calculated. The results showed that the amount recovered is 100% indicating the first order derivative spectroscopic method is accurate and precise.
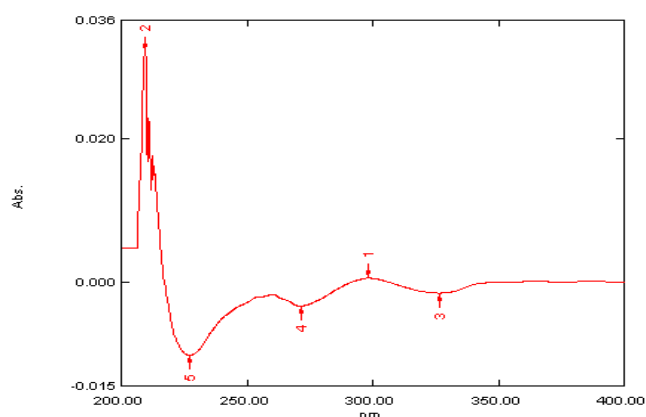

FIG. 3: Wavelength scanning and determination of absorption maximum

S.NO.	CONCENTRATION (µg/ml)	D^1 VALUE AT DETECTION WAVELENGTH (267 nm)
1	2	0.003
2	4	0.004
3	6	0.006
4	8	0.007
5	10	0.008
6	12	0.010
7	14	0.012
8	16	0.014
9	18	0.016
10	20	0.018

TABLE 1: Linearity of Losartan

S.NO	CONCENTRATION (μg/ml)	D^1 VALUE AT DETECTION WAVELENGTH (267 nm)				
		TIME (MINS)	I	II	III	MEAN
1	18	10:30 PM	0.020	0.019	0.019	0.0196
2	18	10:45 PM	0.021	0.022	0.022	0.0213
3	18	11:00 PM	0.022	0.022	0.022	0.022
4	18	11:30 PM	0.021	0.021	0.021	0.021
5	18	1:30 PM	0.021	0.021	0.021	0.021
6	18	2:30 PM	0.021	0.021	0.021	0.021
					MEAN =	0.021
					SD =	0.000191
					% RSD =	0.90

TABLE 2 (a) intraday precision

S.NO	CONCENTRATION (μg/ml)	DAYS & DATE	D^1 at 267nm
1	18	13.02.2012	0.021
2	18	14.02.2012	0.021
3	18	15.02.2012	0.019
4	18	16.02.2012	0.021
5	18	17.02.2012	0.021
6	18	18.02.2012	0.021
		MEAN =	0.021
		SD =	0.00028
		% RSD =	1.33

TABLE 2 (b) Inter day precision

S.NO.	TEST (μg/ml)	STANDARD (μg/ml)	D^1 VALUE AT 267nm	CONC. (μg/ml)	AMOUNT OF TEST RECOVERED (μg/ml)	% RECOVERY
1	5	10	0.012	15	5	100
2	10	10	0.017	20	10	100
3	15	10	0.024	25	15	100

TABLE 3 Accuracy studies

CONCLUSION

The developed analytical method for Losartan by using first order derivative spectroscopy is found to simple, rapid and selective and the amount of drug recovered will be same as the label claimed and precise. It can be conveniently employed for the routine analysis and quantification of Losartan.

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