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Derivative Spectrophotometric Method for Determination of Losartan in Pharmaceutical Formulations

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ABSTRACT

Losartan, a highly effective blood pressure lowering agent, has been widely used for the treatment of hypertension. A fast and reliable method for the determination of losartan was highly desirable to support formulation screening and quality control. A first derivative UV spectroscopic method was developed for determination of losartan in the tablet dosage form. The first derivative spectrum recorded between 220 and 320 nm, and a zero-crossing technique for first derivative measurement at 232.5 nm was selected. It is found that the selectivity and sensitivity of method to be in desirable range. In comparison with the direct UV method, the first derivative UV spectroscopy has a definite through without any interference from UV absorbing excipients. This method is also fast and economical in comparison to the more time-consuming HPLC method regularly used for formulation screening and quality control and can be used routinely by any laboratory possessing a spectrophotometer with a derivative accessory. The linear concentration ranges were 2-50 µg/mL, (DI = -0.0159C - 0.0056, r =0.9994, n=6). Between days CV% ≤ 2.9, within day CV% ≤ 2.1, analytical recovery close to 98.1 % shows the suitability of the method for determination in quality control.

Keywords: *Losartan, First-Derivative Spectrophotometry, HPLC, Tablet*

Technological and scientific progress has led to the development of numerous synthetic drugs. It is therefore imperative to dispose of analytical methods to determine these drugs both in the quality control manufacturing phase of the pharmaceutical formulations and their determination in the human body [1]. Derivative UV spectroscopy has been widely used as a tool for quantitative analysis, characterization, and quality control in agricultural, pharmaceutical, and biomedical fields [2, 3]. This technique offers various advantages over the conventional absorbency methods such as the discrimination of the sharp spectral features over the large bands and the enhancement of the resolution of overlapping spectra. As a result, derivative spectroscopy usually provides much better fingerprints than the traditional absorbency spectra [4-8]. This outstanding feature coupled with zero crossing, least square deconvolution, or Fourier transform data processing technique has received increasing attention in single and multi-component quantitative analysis of pharmaceutical drug substances, especially in UV absorbing matrices [9]. For example, derivative UV spectroscopy has been used for the quantification of acyclovir, celecoxib, amiloride and furosemide in the presence of degradation products and other ingredients [10-12]. Losartan is a synthetic orally active compound which binds selectively to the AT1 receptor (same as angiotensin II) (Fig 1). This drug was developed as tablet dosage form for the treatment of hypertension.

At initial formulation screening stage, formulation composition was constantly varied during a highly compressed time frame. A fast and reliable method for the dissolution and release testing of losartan was highly desirable. Losartan has no any maximum in its normal spectrum and therefore we can not use a wavelength for quantitative analysis at zero order spectrums. Losartan has been studied and determined by several procedures such as high-performance liquid chromatography (HPLC), capillary electrophoresis, and super critical fluid chromatography in biological materials and tablets [13-17], spectrofluorimetric in human urine [18].

Derivative spectrophotometric method was recommended for losartan in tablet [19], but the recommended linear range of the method is very narrow (4-6 µg/mL)

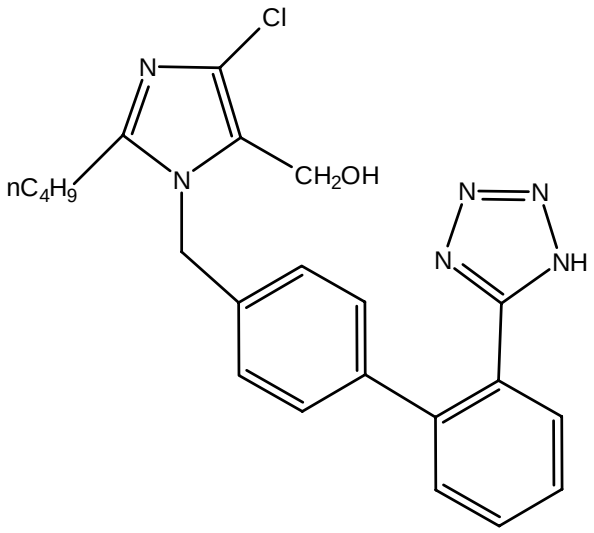


Fig 1. Structure of losartan.

and the results were not compared to a standard method such as HPLC. The aim of this study was to develop an alternative analytical method, to the more time consuming HPLC method, which can be used regularly and for formulation screening. A first derivative UV spectroscopy was developed to support formulation development of losartan in an immediate release solid dosage form.

EXPERIMENTAL

Materials

All reagents used were of analytical reagent grade. Pharmaceutical grade losartan was obtained from Hetero (India). Losartan tablets and the placebo product were manufactured by Pharmaceutical Research and Development of Daru Pakhsh laboratories (Iran). Cozaar tablets labeled to contain 25mg losartan potassium manufactured by MSD (Lot No. 210464, UK) were prepared from the Shafayab Co (Iran). Acetonitrile, potassium dihydrogen phosphate from Merck (Darmstadt, Germany) and used as received. Doubly distilled water was used in all stages.

Apparatus

Spectrophotometric analyses were performed on a Shimadzu, 2100, UV-Vis spectrophotometer, with a 1.00 cm quartz cells. The optimized operating conditions for recording the first derivative spectra were: scan speed, fast; spectral slit width, 2 nm; $\Delta\lambda$, 10 nm; and an ordinate maximum-minimum of 0 to -0.5. Measurements were carried out using the first derivative of the absorbance spectra, measuring the amplitude of the through at 232.5nm.

The HPLC analysis was performed on a Waters 515 liquid chromatograph with a 717 plus autosampler, 2996-photodiode-array detector and the Millennium 32 automation system software was used for the chromatographic analysis of losartan [20]. Measurements were made with a 50-µL-injection volume at ambient temperature; the detector wavelength was set at 254 nm. Routine analyses were carried out isocratically on a Nova pack 5 micron ODS (15 cm, 4 mm), with a mobile phase mixture containing of phosphate buffer pH 3: acetonitrile (60:40 v/v) at a flow rate of 1 mL/min.

Methods

Preparation of losartan standard solutions and calibration. A stock solution containing 200 µg/mL of losartan was prepared by dissolving 0.020 g of losartan in doubly distilled water, then transferring into a 100 ml calibrated flask and diluting to the mark with water. Losartan solution containing of 20 µg/mL were tested for stability in solution and during the actual analysis. The behavior of the analytes remained unchanged up to about 3 months from their preparation. Further tests of stability (i.e., over 3 months) were found unnecessary and were not made. All measurements were made at room temperature. The standard solutions were prepared by the proper dilutions of the stock standard solution with doubly distilled water to reach concentration range of 2-50 µg/mL.

Sample solution preparation to content uniformity testing. One tablet was placed into each of ten 100-mL volumetric flasks. 50 mL water was added. After ultrasonic vibration for 10 min until the tablets were dispersed in the solution, the mixture was diluted to volume with water and filtered (Filter membrane of 0.45 µm, Millipore, USA). After discarding few first milliliters of the filtrate, 2 mL of filtrate transferred to a calibrated 25 mL volumetric flask to obtain concentration of 20 µg/mL. These samples were analyzed by UV and HPLC methods.

Possible interfering effect of tablet excipients. For the studying possible interfering effect of excipients, excipients that reported to be used in tablets were analyzed by the spectrophotometric method in concentration range that can be used in tablets separately and in combination.

Precision assays. Losartan standard solutions were prepared and analyzed six times within the same day to

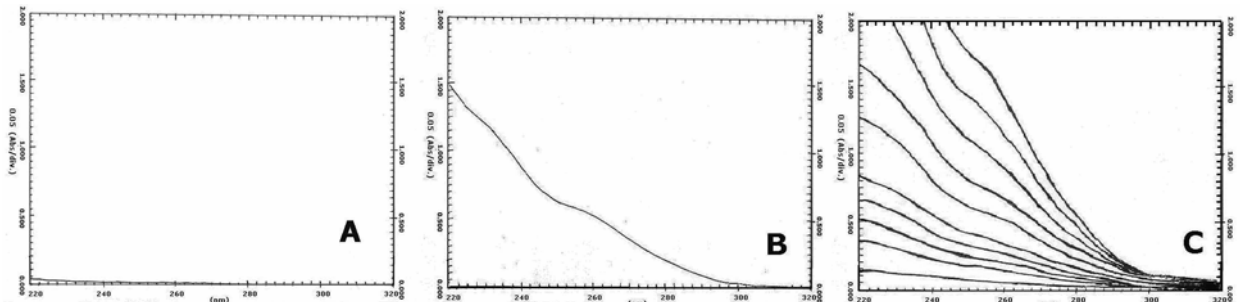


Fig 2. Normal spectra of: A) pure losartan solution in water (2, 4, 6, 8, 10, 15, 20, 30, 40, and 50 µg/mL); B) losartan solution (20 µg/mL) prepared from losartan tablet; and C) solution prepared from placebo tablet.

obtain the repeatability and six times over different days to obtain the reproducibility. Each assay was carried out on a different sample of losartan. The percentage relative standard deviation (RSD %) of the data obtained was calculated.

Accuracy. Known concentrations of losartan were analyzed by spectrophotometric and HPLC methods and the results were compared. The samples were analyzed and the mean recovery as well as the repeatability was calculated on six assays for each concentration added.

Linearity of the method. Linearity of first derivative spectra of losartan concentration was established by preparing one series of losartan solution ranging from 2 to 50μg/mL. The first derivative spectra were recorded using the diluents as a blank. All solutions were measured for absorbency from 220 to 320 nm.

Limit of detection (LOD) and limit of quantitation (LOQ). LOD determined by measuring the D1 absorbance's at 232.5 nm of at least 25 separate base placebo tablet samples. Average and SD of blank responses were calculated, LOD and LOQ were estimated by calculating of 3 SD and 10 SD of blanks, respectively.

RESULTS AND DISCUSSION

The zero order, first, second, third, and fourth derivative spectra for all investigated ingredients of the losartan placebo tablets were recorded in the wavelength range from 220-320 nm. The zero and D1 spectra of losartan in the wavelength range of 220-320 nm are shown in Fig 2 and Fig 3. It can be seen that in Fig 2A, there is no peak in zero order spectrum of losartan, and its breakthrough wavelength at 250 nm changes with different concentrations. The D1 spectra (Fig 3A) have a through at 232.5 nm with a good sensitivity and linearity. Spectra with higher order of derivation had lower sensitivity and linearity, and because of this, only first order derivative spectra were selected for quantitative analysis.

However, under most circumstances, pronounced interference from other excipients was observed. A typical UV spectrum of a placebo tablet is also shown in Fig 2C, which indicates no significant UV response at 250 nm. Based upon the direct UV spectroscopic data, there is no wavelength where losartan can be accurately quantified. This problem does not exist in first derivative spectra of above mentioned materials. Losartan potassium is soluble in mobile phase and their solution was found to be stable for 2 weeks at least. As shown in Fig 4, at a flow rate of 1 mL/min, the retention time of losartan was 2.8 min.

The reversed–phase HPLC method was used to provide a proper procedure for the rapid quality control analysis of losartan dosage forms. For quantitative analysis, the analytical data for the calibration graphs were obtained with correlation coefficients of 0.999997. The good precision of the HPLC procedure was indicated by the relative standard derivation (< 2%).

The excipients (lactose, microcrystalline cellulose, HPMC, CMC, PVP, corn starch, magnesium starch, lactose and talc) were added to the drug for recovery studies according to manufacturer's batch formula for per tablets. The data shown in Table 1 indicate good accuracy and precision of the proposed procedure. The detection limit (LOD) and quantification limit (LOQ) were 0.4 μg/mL and 1.5 μg/mL, respectively.

Furthermore, the proposed method does not require the elaboration of treatment and procedures, which are usually associated with chromatographic methods.

Table 1. First-derivative spectrophotometric determination of losartan tablet.

Parameters	*Losartan (λ=232.5 nm)*
Concentration range (μg/mL)	2-50
Y=aX+b	Dl = -0.0159C - 0.0056
Correlation coefficient	0.9994
Standard error of the slope	0.002
Standard error of the intercept	0.002
P value for correlation coefficient	0.0001
Within day CV%	2.1
Between day CV%	2.9
Limit of detection(μg/mL)	0.4
Limit of quantitation (μg/mL)	1.5
Losartan tablet (taken) (mg)	25
Losartan tablet (found) (%)	99.2-110.5
Recovery (%)	98.1

Method Validation

Using regression analysis the following equation was obtained for standard calibration curve of losartan:

$$Dl = -0.0159C - 0.0056 \quad (r=0.9994)$$

Where D1 is the value of the first derivative of losartan absorbency at 232.5 nm and C is the concentration of losartan (mg/L). The method was linear in the range of 2 to 50 μg/mL (r=0.9994). The calibration curve was in agreement with beer's law. The regression equations for the losartan were calculated including the standard

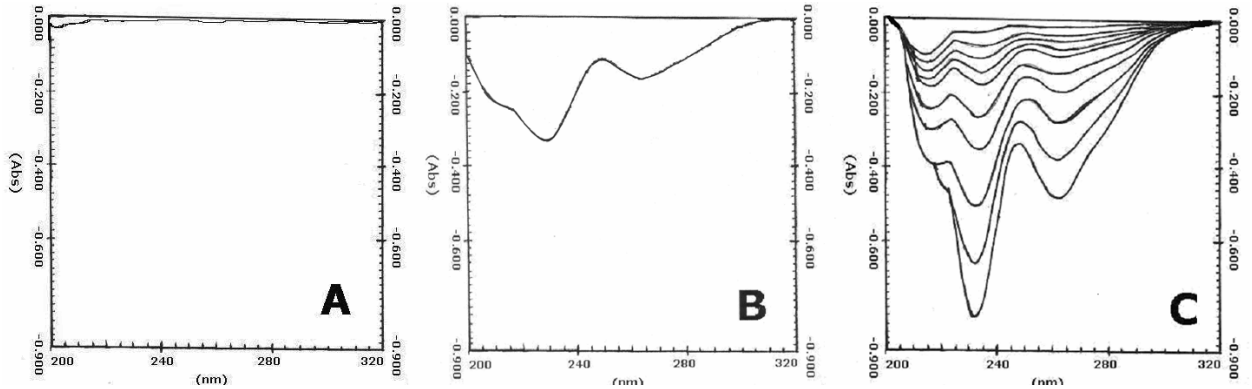
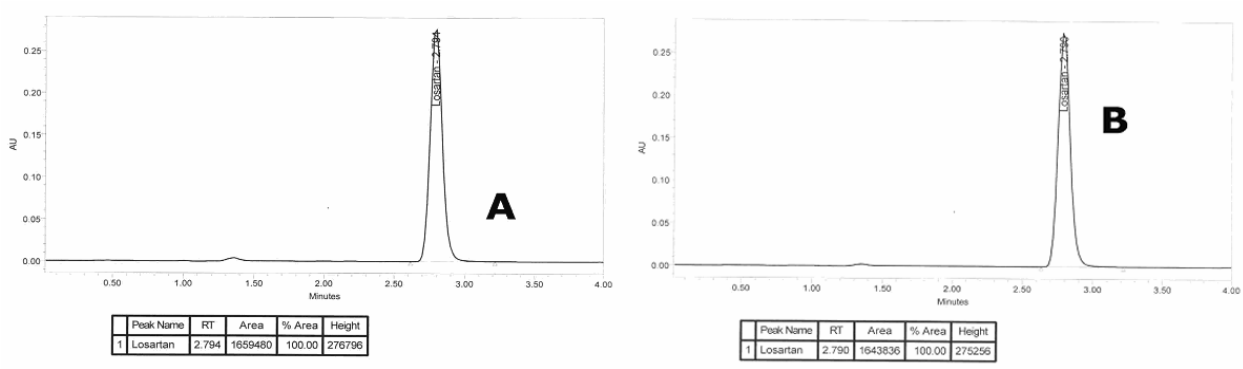


Fig 3. First derivative spectra of A) pure losartan solution in water (2, 4, 6, 8, 10, 15, 20, 30, 40, and 50 μg/mL); B) losartan solution (20 μg/mL) prepared from losartan tablet; and C) solution prepared from placebo tablet with the same concentration of excipients as in losartan tablet.



Peak Name	RT	Area	% Area	Height
1 Losartan	2.794	1659480	100.00	276796

Peak Name	RT	Area	% Area	Height
1 Losartan	2.790	1643836	100.00	275256

Fig 4. Chromatograms of: A) losartan standard solution (20µg/mL); and B) losartan solution prepared from Cozaar tablet (20µg/mL).

error of the slope, standard error of the intercept, correlation coefficient(r), p of the correlation coefficient were taken in Table 1.

The validation parameters (linearity, selectivity, recovery, precision, limit of detection and limit of quantitation) were also determined (Table 1). The derivative spectrophotometric method is selective because the excipients did not interfere during the determination of losartan. Relatively small amount of CV% (2.1 and 2.9%) confirms a precision of the method, and recovery (greater than 98%) show good accuracy. As demonstrated, interferences do not exist between tablet excipients and losartan. Therefore first derivative spectra can be used for quantitation of the drug. Meanwhile, the D1 spectrum displayed a trough at 232.5 nm without any interference. Stability of samples prepared in water was studied. Results showed that samples are stable at least for one month, and changes during sample preparation and time of reading are found to be negligible. For quantitative analysis of the losartan tablets, ten solutions were prepared by dissolving ten individual tablets in water or mobile phase and analyzed by derivative UV and HPLC procedure. The amount of the losartan was calculated by the method of standard. Results (Table 2) show that all of the formulations that analyzed by these methods are in the acceptable ranges (85-115% of label claimed).

Table 2. Determination of losartan in its tablet dosage form by derivative UV spectrophotometric, and HPLC methods

	Percent of label claimed	
Tablet No	UV Derivative	HPLC
1	99.2	106.9
2	102.0	103.8
3	103.9	106.5
4	107.4	108.7
5	106.4	104.8
6	101.7	106.0
7	108.6	106.0
8	108.3	105.5
9	108.0	107.8
10	108.6	103.4
Mean	105.4	105.9
SD	3.4	1.6
RSD	3.3	1.6

CONCLUSION

In conclusion, the proposed D1- method provides simple and sensitive method suitable for the quality control analysis of losartan in dosage forms. This method have some advantages in comparison to similar studies, such as wide range of linearity that makes it suitable for all in vitro studies of the tablet formulations such as dissolution studies, study of the excipients interferences, stability studies and comparison of the method with a validated HPLC one as a method of choice for determination of losartan in tablet. The proposed method does not require the elaboration of treatment and procedures, which are usually associated with chromatographic methods. It is very efficient and offers high sample throughput by comparison with HPLC method. Therefore, it undoubtfully renders in-time data turnaround during formulation development. The first-derivative order of the spectra of the losartan was found to be suitable for determination of it in tablets. The obtained results are accurate and precise and confirmed by statistical parameters. There was no interference of the excipients in the tablet. The described first derivative spectrophotometric method is a simple, rapid, selective, accurate, precise and an excellent alternative to HPLC method for determination of the losartan in tablet or a corresponding mixture.

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