

A Comparative Study of First-Derivative Spectrophotometry and High-Performance Liquid Chromatography Applied to the Determination of Losartan Potassium in Tablets

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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 and HPLC were developed for the 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 were selected. The selectivity and sensitivity of the method was in desirable range. In comparison with the direct UV method, first-derivative UV spectroscopy has a definite trough without any interference from UV absorbing-excipients. This method is also fast and economical in comparison with 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 $\mu\text{g ml}^{-1}$, ($D_1 = -0.0159C - 0.0056$, $r = 0.9994$, $n = 6$). Between-days CV of $\leq 2.9\%$, within-day CV of $\leq 2.1\%$, and analytical recovery close to 98.1% show the suitability of the method for determination in quality control.

Key words losartan; first-derivative spectrophotometry; HPLC; tablet

Technological and scientific progress has led to the development of numerous synthetic drugs. It is therefore imperative to develop 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 the 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 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 techniques has received increasing attention in single and multicomponent 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 that binds selectively to the AT1 receptor (the same as angiotensin II) (Fig. 1). This drug was developed in tablet form for the treatment of hypertension.

At the 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 maximum in its normal spectrum and therefore we can't use a wavelength for quantitative analysis at zero-order spectra. Losartan has been studied and determined by several procedures such as HPLC, capillary electrophoresis, and supercritical fluid chromatography in biological materials and tablets,^{13–17)} and spectrofluorimetrically in human urine.¹⁸⁾

No simple derivative spectrophotometric method for the analysis of losartan in pharmaceutical preparations has been reported in the literature. 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 (a generic product claimed to contain losartan potassium 25 mg) and the placebo product were manufactured by Pharmaceutical Research and Development of Daru Pakhsh laboratories (Iran). Cozaar tablets labeled to contain losartan potassium 25 mg manufactured by MSD (lot no. 210464, U.K.) were prepared by Shafayab Co. (Iran). Acetonitrile and potassium dihydrogen phosphate were from Merck (Darmstadt, Germany) and used as received. Double-distilled water was used in all stages.

Apparatus Spectrophotometric analyses were performed on a Shimadzu 2100 UV-Vis spectrophotometer, with 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 trough at 232.5 nm.

HPLC analysis was performed on a Waters 515 liquid chromatograph with a 717 plus autosampler and 2996 photodiode-array detector, and the Millennium 32 automation system software was used for the chromatographic analysis of losartan. Measurements were made with a 50- μl injection.

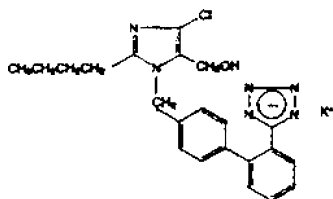


Fig. 1. Chemical Structure of Losartan

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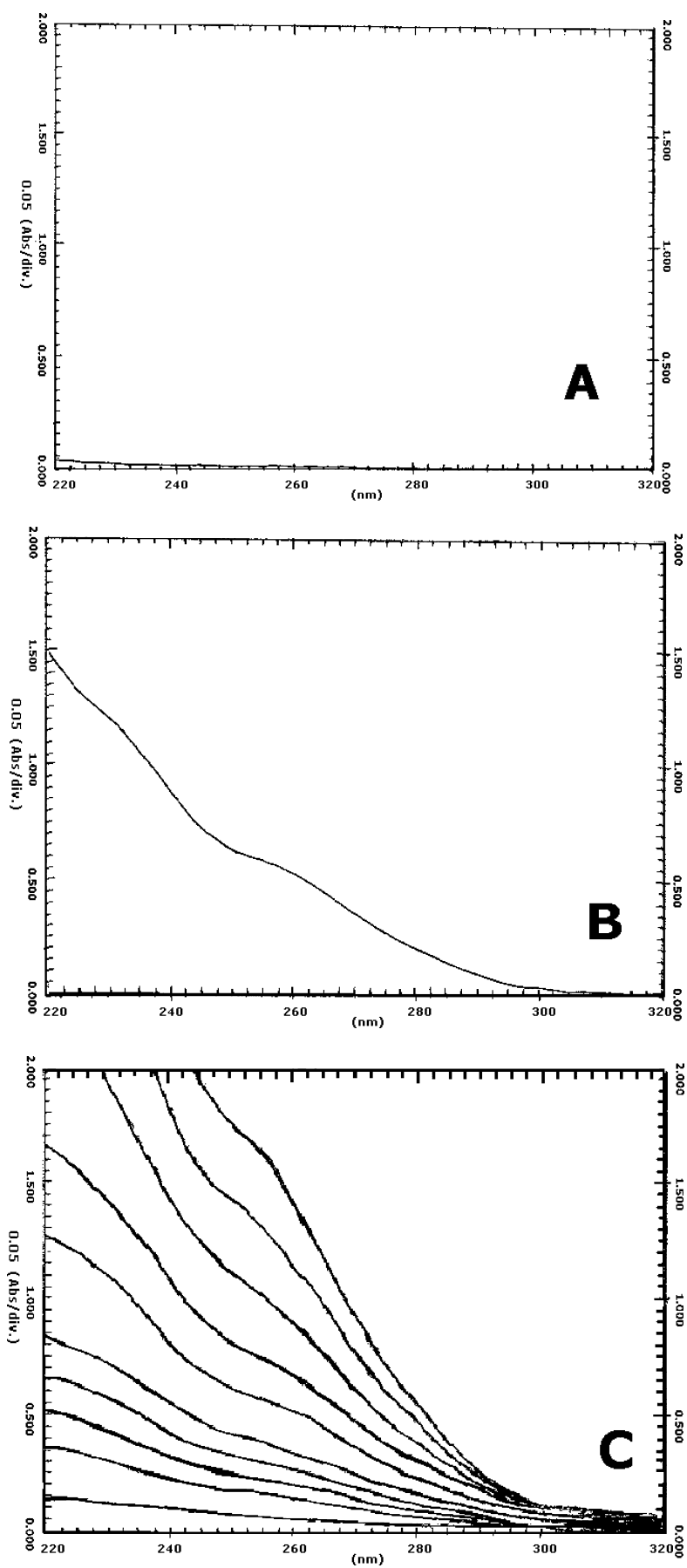


Fig. 2. Normal Spectra of (A) Placebo Tablet, (B) Pure Losartan Potassium 20 $\mu\text{g ml}^{-1}$, and (C) Different Concentration of Losartan (2 to 50 $\mu\text{g ml}^{-1}$)

tion volume at ambient temperature; the detector wavelength was set at 254 nm. Routine analyses were carried out isocratically on a Novapack 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⁻¹.

Methods. Preparation of Losartan Standard Solutions and Calibration A stock solution containing losartan 200 µg ml⁻¹ was prepared by dissolving 0.020 g of losartan in double-distilled water, then transferring it to a 100 ml calibrated flask and diluting to the mark with water. For calibration, a series of losartan solutions containing 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, 20.0, 30.0, 40.0, and 50.0 µg ml⁻¹ were prepared by diluting the stock losartan standard solution with water in volumetric flasks (10 ml) for the UV derivative-spectrophotometric method.

For HPLC analysis, a series of losartan solutions 2.0, 6.0, 10.0, 20.0, and 50.0 µg ml⁻¹ were also prepared from the stock losartan solution and diluted in the mobile phase.

Stability of Losartan Solution Losartan solution containing 20 µg ml⁻¹ were analyzed by UV derivative spectrophotometry and HPLC methods at 0, 1, 2, 3, 5, 24, 48, 72, 168, 336, 720, 1440, and 2160 h after preparation. The behavior of the analyte remained unchanged up to about 3 months from its preparation. Further tests of stability (*i.e.*, over 3 months) were found unnecessary and were not performed. All measurements were made at room temperature.

Procedures for Pharmaceutical Dosage Forms. Derivative Spectrophotometric Method One tablet was placed into each of ten 100 ml volumetric flasks. Fifty milliliters of water was added, sonicated for 10 min, completed to volume with water, and filtered (membrane filter 0.45 µm, Milipore, U.S.A.). The first few milliliters of the filtrate were discarded, and 2 ml of the filtrate were diluted to 25 ml with water. These samples claimed to contain 20 µg ml⁻¹ of losartan potassium. The first-derivative spectrum of sample solutions was recorded and D_1 values were determined at 232.5 nm. The content of losartan was calculated using the linear regression equation of the calibration curve.

HPLC Method One tablet was placed into each of ten 100 ml volumetric flasks. Fifty milliliters of the mobile phase was added, sonicated for 10 min, completed to volume with the mobile phase, and filtered. The first few milliliters of the filtrate were discarded and 2 ml of the filtrate was diluted to 25 ml with water. These samples claimed to contain 20 µg ml⁻¹ of losartan potassium.

Separate equal volumes (50 µl) of solutions were injected into the chromatograph, the chromatograms were recorded, and the *AUC* of the peaks at 2.79 min were calculated. The content of losartan was determined using the linear regression equation of the HPLC method calibration curve.

Possible Interference of Tablet Excipients To study the possible interference of excipients, excipients reported to be used in the tablets were analyzed using the spectrophotometric method in the concentration range that can be used in tablets separately and in combination.

Precision Assays Losartan standard solutions were analyzed six times within the same day to 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 determined using 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 in standard losartan solution prepared ranging from 2 to 50 µg ml⁻¹. The first-derivative spectra were recorded using the diluents as blanks and D_1 values were determined at 232.5 nm. Graphs were constructed by plotting D_1 against standard concentrations.

Limit of Detection and Limit of Quantitation Limit of detection (LOD) was determined by measuring the D_1 absorbances at 232.5 nm of at least 25 separate base placebo tablet samples. The mean and SD of blank responses were calculated, and LOD and limit of quantitation (LOQ) were estimated by calculating 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 D_1 spectra of losartan in the wavelength range of 220–320 nm are shown in Figs. 2 and 3. In

Fig. 2, there is no peak in the zero-order spectrum of losartan, and its breakthrough wavelength at 250 nm changes with different concentrations. The D_1 spectra (Fig. 3A) have a trough at 232.5 nm with a good sensitivity and linearity. Spectra with higher orders of derivation had lower sensitivity and linearity, and because of this only first-order derivative

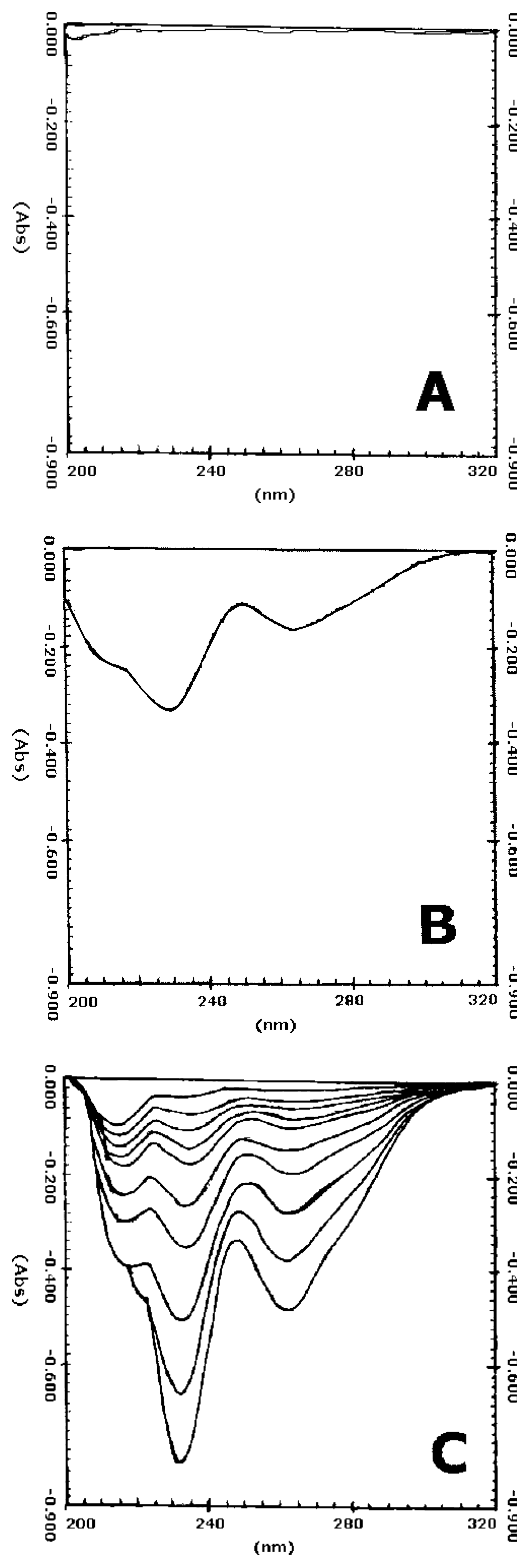


Fig. 3. First Order Derivative Spectra of (A) Placebo Tablet, (B) Pure Losartan Potassium 20 µg ml⁻¹, and (C) Different Concentration of Losartan (2 to 50 µg ml⁻¹)

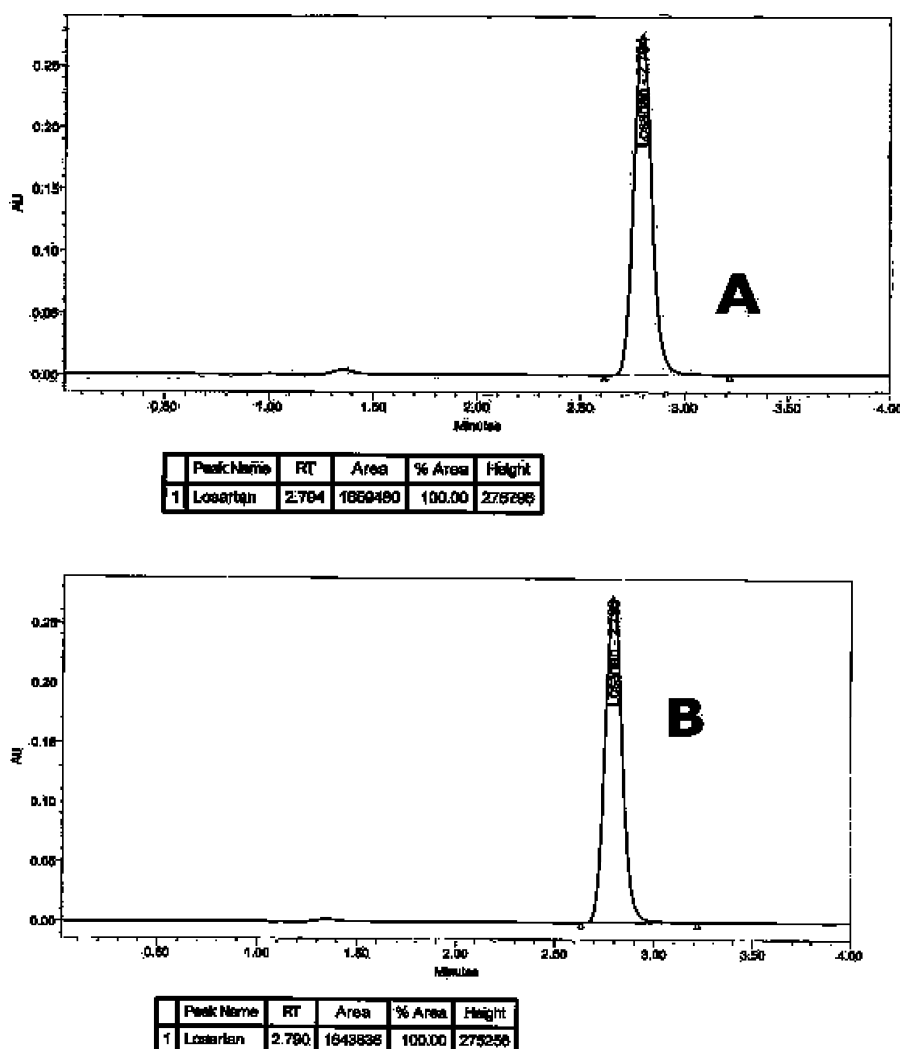


Fig. 4. Chromatograms of: (A) Losartan ($20 \mu\text{g ml}^{-1}$), and (B) Cozaar Tablet ($20 \mu\text{g ml}^{-1}$)

spectra were selected for quantitation analysis.

However, under most circumstances, pronounced interference from excipients was observed. A typical UV spectrum of a placebo tablet is 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 the above-mentioned materials. Losartan potassium is soluble in the mobile phase and was stable in solution for at least 2 weeks. As shown in Fig. 4, at a flow rate of 1 ml min^{-1} , the retention time of losartan was 2.79 min.

The reversed phase HPLC method was used for the rapid quality control analysis of losartan dosage forms. For quantitative analysis, the analytical data for the calibration graphs were obtained with a correlation coefficient of 0.999997. The 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 the tablets. The data shown in Table 1 indicate good accuracy and precision of the proposed procedure. The LOD and LOQ were $0.4 \mu\text{g/ml}$ and

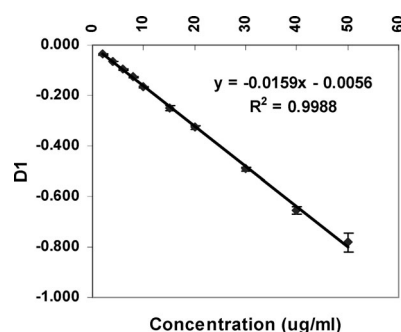


Fig. 5. Calibration Curve of Losartan Potassium in Water by First Order Derivative Spectrophotometry (2 to $50 \mu\text{g ml}^{-1}$)

$1.5 \mu\text{g/ml}$, respectively.

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

Method Validation Using regression analysis, the following equation was obtained for the standard calibration curve of losartan (Fig. 5):

$$D_1 = -0.0159C - 0.0056 \quad (r = 0.9994)$$

Table 1. First-Derivative Spectrophotometric Determination of Losartan Tablet

Parameters	Losartan ($\lambda=232.5$ nm)
Concentration range ($\mu\text{g ml}^{-1}$)	2—50
$Y=aX+b$	$D_1=-0.0159C-0.0056$
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 ($\mu\text{g ml}^{-1}$)	0.4
Limit of quantitation ($\mu\text{g ml}^{-1}$)	1.5
Losartan tablet (taken) (mg)	25
Losartan tablet (found) (%)	99.2—110.5
Recovery (%)	98.1

where D_1 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 $\mu\text{g/ml}$ ($r=0.9994$). The calibration curve was in agreement with Beer's law. The regression equations for losartan were calculated including the standard error of the slope, standard error of the intercept, correlation coefficient (r), and p value of the correlation coefficient are shown in Table 1.

The validation parameters (linearity, selectivity, recovery, precision, LOD, and LOQ) were also determined (Table 1). The derivative spectrophotometric method is selective because the excipients did not interfere during the determination of losartan. Relatively small CV% values (2.1%, 2.9%) confirm the precision of the method, and high recovery rate (greater than 98%) shows good accuracy. As demonstrated, interference does not occur between tablet excipients and losartan. Therefore first-derivative spectra can be used for quantitation of the drug. Meanwhile, the D_1 spectrum displayed a trough at 232.5 nm without any interference when the stability of samples prepared in water was studied, they were stable at least for 1 month, and changes during sample preparation and time of reading were negligible. For quantitative analysis of the losartan tablets, 10 solutions were prepared by dissolving 10 individual tablets in water or the mobile phase and analyzed using derivative UV and HPLC procedures. The amount of losartan was calculated by the standard method. All of the formulations analyzed using these methods were in the acceptable ranges (85—115% of label claim) (Table 2).

Conclusion

In conclusion, the proposed D_1 method provides a simple and sensitive method suitable for the quality control analysis of losartan in dosage forms. Furthermore, the proposed method does not require the elaborate treatment and procedures usually associated with chromatographic methods. It is efficient and offers high sample throughput in comparison with the HPLC method. Therefore, it undoubtedly renders

Table 2. Determination of Losartan in Its Tablet Formulation Form by Derivative UV Spectrophotometric, and HPLC Methods

Tablet No	Percent of label claimed	
	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
S.D.	3.4	1.6
R.S.D.	3.3	1.6

in-time data turnaround during formulation development. The first-derivative spectra of losartan are suitable for its determination in tablets. The results obtained are accurate and precise as confirmed by statistical parameters. There was no interference by the excipients in the tablets. The first-derivative spectrophotometric method is a simple, rapid, selective, accurate, precise and excellent alternative to the HPLC method for determination of losartan in tablets or a corresponding mixture.

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