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COMPARISON OF UV- AND SECOND DERIVATIVE SPECTROPHOTOMETRIC AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF LOSARTAN IN TABLETS

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Abstract

UV- and second derivative spectrophotometric and high-performance liquid chromatographic (HPLC) methods for the determination of losartan in tablets have been developed. For UV-spectrophotometric method, absorbances of the standard solutions were measured at 206.6 nm. For second derivative spectrophotometric method, the distance between two extremum values (peak-to-peak amplitudes), 219.6 nm and 228.8 nm, were measured in the second order derivative spectra of standard solutions. Calibration curves were constructed by plotting $d^2A/d\lambda^2$ values against concentrations. These wavelengths were selected depend on obtained the maximum values. HPLC method was carried out on C18 column using the mobile phase consisted of a mixture of acetonitrile and phosphate buffer (pH=3.8, 40:60, v/v) and column eluate was monitored at 225 nm. The three methods were applied to determine of losartan in tablets.

Keywords: *Losartan, UV-spectrophotometry, Derivative spectrophotometry, HPLC*

Tabletlerde Losartanın Miktar Tayini İçin UV ve 2. Türev Spektrofotometrik ve Yüksek Performanslı Sıvı Kromatografik Yöntemlerin Karşılaştırılması

Losartanın tabletlerde miktar tayini için UV, 2. türev spektrofotometrik ve yüksek performanslı sıvı kromatografik (HPLC) yöntemler geliştirildi. UV spektrofotometrik yöntem için standart çözeltilerin absorbansları 206.6 nm'de ölçüldü. 2. türev spekrofotometrik yöntem için iki ekstremum değer arasındaki aralık; 219.6 nm ve 228.8 nm'de standart çözeltilerin 2. türev spektrumları alındı. Kalibrasyon eğrileri konsantrasyona karşı $d^2A/d\lambda^2$ değerlerinin grafiğe geçirilmesiyle elde edildi. Bu dalga boyları elde edilen maksimum değerlere bağlı olarak seçildi. HPLC yöntemi asetonitril ve fosfat tamponu (pH=3.8, 40:60, h/h) karışımından oluşan bir mobil fazın kullanıldığı C18 kolonunda uygulandı ve kolon elüatı 225 nm'de gözlendi. Her 3 yöntem tabletlerde losartanın tayinine uygulandı.

Anahtar kelimeler: *Losartan, UV spektrofotometri, Türev spektrofotometri, HPLC*

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Introduction

Losartan (**LOS**), 2-butyl-4-chloro-1[p(o-1H-tetrazol-5-yl-phenyl)benzyl]imidazole-5-methanol monopotassium salt, is the first of a new class of antihypertensives and a nonpeptide angiotensin II receptor (type AT1) antagonist (Figure 1).

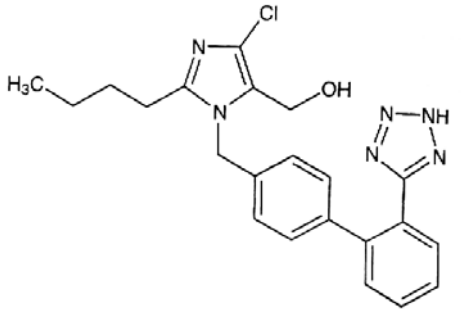

Figure 1. Chemical structure of losartan.

LOS has been determined, individually, by spectrophotometric (1, 2), high performance thin layer chromatographic (3), and high-performance liquid chromatographic (4) methods in pharmaceutical preparations and by colorimetric method (5) in bulk form. HPLC methods with ultraviolet detection (6,7) and liquid chromatography-tandem mass spectrometry (8) have also been reported for the determination of **LOS** in biological fluids.

LOS and hydrochlorothiazide have been simultaneously determined in pharmaceutical preparations by liquid chromatographic (9-14) and capillary electrophoretic (15) methods.

In this study UV- and second derivative spectrophotometric and HPLC methods for the determination of losartan have been developed. The methods were applied in the quality control of commercial tablets and proved to be suitable for rapid and reliable quality control. The proposed methods were sensitive and rapid, because at no heating and no organic solvent extraction are needed. They can be applied for the routine pharmaceutical analysis confidently.

Experimental

Materials

LOS potassium and its tablets (Cozaar® 50 mg) were kindly supplied from Merck Sharp Dohme (İstanbul, Turkey). Valsartan (**VAL**), the internal standard, was a gene-rous gift of Novartis (İstanbul, Turkey). Solvents and other chemicals were of analytical reagent and HPLC grade (Merck, Darmstadt, Germany).

Apparatus

A Shimadzu UV-160A UV-Visible spectrophotometer with 1 cm quartz cells was used under the following operating conditions: Scan speed 2400 nm min^{-1}, scan range 200-400 nm, slit width 2 nm and derivation interval ($\Delta\lambda$) 2.8 nm. A Shimadzu LC 10 high performance liquid chromatograph with SPD-10A spectrophotometric detector and the automation system software was used for the chromatographic analyses.

HPLC conditions

Chromatographic separation was achieved isocratically on a Shim-pack C18 column (250 mm x 4.6 mm, i.d. 10 µm, Shimadzu). Detection was carried out at 225 nm with a UV-detector. The mobile phase was acetonitrile-phosphate buffer (pH=3.8) (40:60, v/v). After filtration, this mixture was degassed and delivered at a flow rate 1.1 mL min^{-1}.

Solutions

Stock solutions of **LOS** was prepared by dissolving 10 mg, accurately weighed, in 100 mL of methanol. The first standard series containing a constant concentration of **LOS** (2.0, 3.0, 4.0, 5.0, 6.0 µg mL^{-1}) for UV-spectrophotometric method. The second standard series containing a constant concentration of **LOS** (1.0, 2.0, 3.0, 4.0, 5.0 µg mL^{-1}) for UV-second derivative spectrophotometric method.

For HPLC analysis, a stock solution containing **LOS** (0.1 mg mL^{-1}) was prepared by dissolving a weighed amount of substance in acetonitrile. Standard solutions were prepared by dilution of the above stock solutions with mobile phase (2.5, 3.5, 4.5, 5.5, 6.5 µg mL^{-1}). The standard solution of internal standard (**VAL**, 0.1 mg mL^{-1}) was prepared in mobile phase and appropriate dilutions were made to obtain a working solution of 4.5 µg mL^{-1}.

Assay validation

The inter-day and intra-day precision and accuracy were determined by analysing losartan samples. The mean percentage recoveries, relative standard deviations (RSD) and relative mean error (RME) were calculated.

Assay procedure

Ten tablets were weighed and powdered. An accurately weighed portion of the powdered tablets, equivalent to about 10 mg of **LOS** was transfered to 100 mL volumetric flask, 50 mL methanol was added, after shaking for 25 min diluted to the volume with methanol. The solutions were mixed and filtered before analysis. For HPLC analysis, acetonitrile was used instead of methanol.

Appropriate dilutions were made as 4.0 and 3.0 μg mL^{-1} with methanol from the stock solution for UV- and second derivative spectrophotometric analysis, respectively. Meanwhile the final concentration was 5.5 μg mL^{-1} for HPLC method, internal standard was added as 4.5 μg mL^{-1} and dilution was made with mobile phase.

Results and discussion

LOS is freely soluble in water, soluble in alcohols, and slightly soluble in common organic solvents, such as acetonitrile and methyl ethyl ketone. In developing methods such as UV- and derivative spectrophotometric the best results were obtained by using methanol as an organic solvent. The stock solutions could be stored at 4°C for over one month with decomposition.

UV absorption spectra of **LOS** at five different concentrations showed a maximum absorbance at 206.6 nm in methanol (Figure 2). Linear correlation was observed between absorbance and concentration of **LOS** over the range of 2.0-6.0 μg mL^{-1}. The regression equation was $A=1.29x10^{-1}C+5.2x10^{-3}$ with r=0.9999 (where A=Absorbans, C=Concentration; μg mL^{-1}). The limit of quantitation (LOQ) is the lowest concentration on the calibration curve was found as 2.0 μg mL^{-1}. The limit of detection (LOD) was 0.25 μg mL^{-1} at a signal to noise ratio of 20.

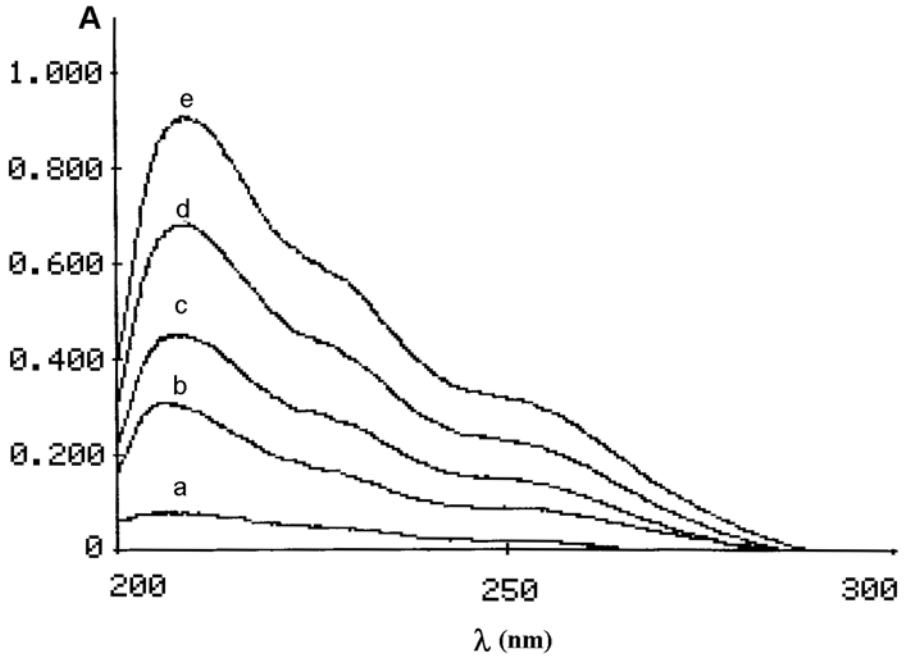

Figure 2. Absorption spectra of (a) 1 μg mL^{-1}, (b) 2 μg mL^{-1}, (c) 4 μg mL^{-1}, (d) 6 μg mL^{-1}, e) 7.5 μg mL^{-1} solutions of losartan in methanol.

In Table 1, the statistical parameters are given for the regression equations calculated from the calibration graphs, along with the standard deviations of the slope (Sb) and intercept (Sa) on the ordinate.

TABLE 1. Analytical data for the calibration graphs (n=6) for the determination of losartan by the proposed methods

Parameters	UV-spectrophotometric method	Second-derivative method	HPLC method
Range (μg mL^{-1})	2.0-6.0	1.0-5.0	2.5-6.5
Regression equation (Y)			
Slope (b)	$1.29x10^{-1}$	$2.94x10^{-2}$	$2.19x10^{-1}$
Std. Dev.on slope (Sb)	$1.1x10^{-4}$	$7.1x10^{-5}$	$7.0x10^{-5}$
Intercept (a)	$5.2x10^{-3}$	$7.76x10^{-2}$	$4.2x10^{-4}$
Std. Dev.on intercept (Sa)	$1.0x10^{-5}$	$1.4x10^{-4}$	$7.07x10^{-6}$
Correlation coefficient (r)	0.9999	0.9997	0.9999

LOS was determined by using peak-to-peak method be measured the distances between two extremum wavelengths, 219.6 nm and 228.8 nm for second derivative spectrophotometric method. Figure 3 shows the peak-to-peak spectra of **LOS** at two different concentrations in methanol. These wavelengths were selected depend on obtained the maximum values. Linear relationships were observed over the concentration ranges of 1.0-5.0 μg mL^{-1} for **LOS**. The equations of the calibration curves were obtained by the least-squares linear regression analysis and calculated as; $D=2.94x10^{-2}C+7.76x10^{-2}$ with 0.9997 (where D=Derivative value). LOQ was found as 1.0 μg mL^{-1} and LOD was 0.125 μg mL^{-1} at a signal to noise ratio of 3.

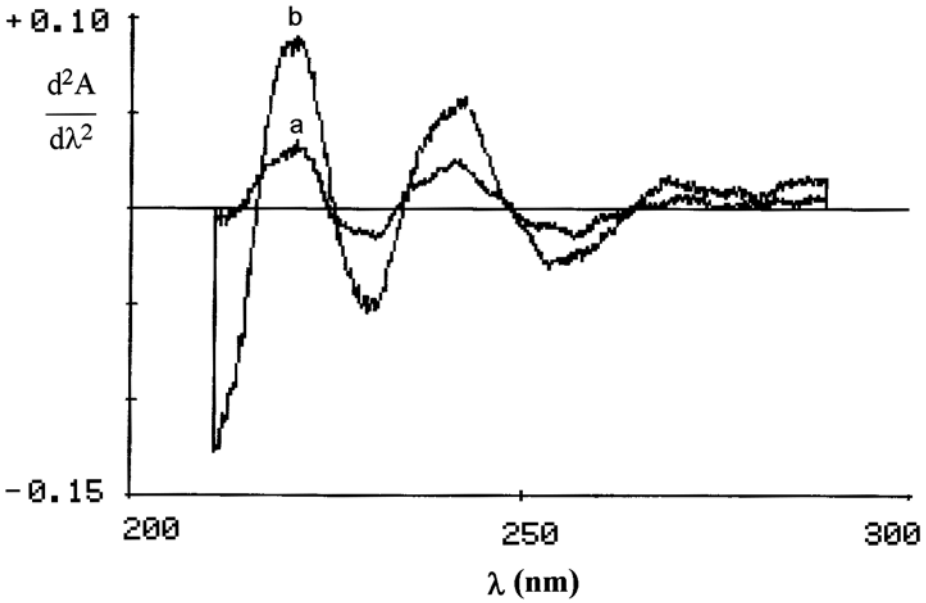

Figure 3. Second derivative spectra of (a) 4 μg mL^{-1} and (b) 6 μg mL^{-1} solutions of losartan in methanol.

LOS was also determined by using HPLC. **VAL** was selected as an internal standard. The composition of the mobile phase was varied to optimise the chromatographic conditions. The mobile phase used was consisted of acetonitrile-phosphate buffer (pH=3.8) (40:60, v/v). Figure 4 shows a typical chromatogram for **LOS** and **VAL** with retention times of 4.19 min and 7.82 min, respectively. For assay peak area ratios (**LOS** area / **VAL** area) were calculated. Linear relationships were observed over the concentration ranges of 2.5-6.5 μg mL^{-1} for **LOS**. The regression equation was found to be $R=2.19x10^{-1}C+4.2x10^{-4}$ with r=0.9999 (R= **LOS** area / **VAL** area) LOQ was found as 2.5 μg mL^{-1} and LOD was 0.5 μg mL^{-1} at a signal to noise ratio of 10.

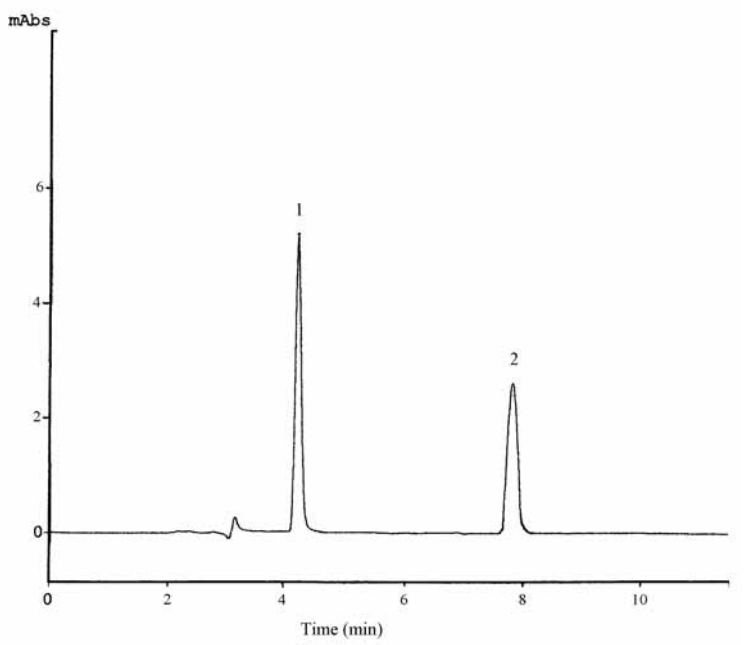

Figure 4. HPLC chromatogram of losartan (1) and valsartan (2) (5.5 μg mL^{-1} of 1 and 4.5 μg mL^{-1} of 2 in mobile phase, sample volume is 20 μL)

TABLE 2. Recovery results for losartan solutions (n=6)

Methods	Concentration (μg mL^{-1})		Mean recovery %	RSD %
	Added	Found (mean ± SD)		
UV-spectrophotometric method	2.0	2.01 ± 0.01	100.5	0.50
	4.0	4.10 ± 0.10	102.5	2.44
	6.0	6.02 ± 0.02	100.3	0.33
Second-derivative method	1.0	1.02 ± 0.02	102.0	1.96
	3.0	3.00 ± 0.07	100.0	2.33
	5.0	5.01 ± 0.11	100.2	2.20
HPLC method	2.5	2.52 ± 0.02	100.8	0.79
	4.5	4.54 ± 0.04	100.9	0.88
	6.5	6.60 ± 0.03	101.5	0.45

TABLE 3. Intra-day and inter-day precision and accuracy of losartan solutions (n=6)

Methods	Concentration (μg mL^{-1})		RSD %	RME %
	Added	Found (mean ± SD)		
UV-spectrophotometric method				
Intra-day	2.0	1.99 ± 0.01	0.50	-0.50
	4.0	3.93 ± 0.08	2.04	-1.75
	6.0	6.10 ± 0.02	0.33	1.67
Inter-day	2.0	2.02 ± 0.04	1.98	1.00
	4.0	3.93 ± 0.08	2.04	-1.75
	6.0	5.98 ± 0.02	0.33	-0.33
Second-derivative method				
Intra-day	1.0	1.01 ± 0.02	1.98	1.00
	3.0	2.98 ± 0.07	2.35	-0.67
	5.0	4.98 ± 0.02	0.40	-0.40
Inter-day	1.0	1.01 ± 0.02	1.98	1.00
	3.0	2.95 ± 0.06	2.03	-1.67
	5.0	4.97 ± 0.02	0.40	-0.60
HPLC method				
Intra-day	2.5	2.52 ± 0.07	2.78	0.80
	4.5	4.58 ± 0.04	0.87	1.78
	6.5	6.53 ± 0.03	0.46	0.46
Inter-day	2.5	2.48 ± 0.01	0.40	-0.80
	4.5	4.57 ± 0.04	0.88	1.56
	6.5	6.53 ± 0.02	0.31	0.46

TABLE 4. Determination of losartan potassium tablets labelled to contain 50 mg of losartan per tablet (n=6)

Statistical value	UV-spectrophotometric method	Second-derivative method	HPLC method
$\bar{x}$	49.75	49.83	49.90
SD	0.27	0.26	0.34
RSD(%)	0.54	0.52	0.68

TABLE 5. Statistical comparison of the results obtained by proposed methods*

Methods	*t*	*F*
UV-spectrophotometric method Second derivative method	0.52	1.08
UV-spectrophotometric method HPLC method	0.84	1.59
Second derivative method HPLC method	0.39	1.71

*n=6; P=0.05; t=2.57; F=5.05

The recoveries of losartan were in the range 100.3-102.5, 100.0-102.0 and 100.8-101.5 for UV-, second derivative spectrophotometric and HPLC methods, respectively (Table 2).

The intra-day and inter-day relative standard deviation (RSD) values were found to be within 0.31-2.78% and the relative mean error (RME) was below 1.78% (Table 3).

The proposed methods were applied to the assay of **LOS** in tablets (Table 4) and the results were compared with each other using t- and F-tests. As shown in Table 5, the calculated t- and F- values were less than the theoretical values indicating that the proposed methods have the same accuracy.

These methods were more sensitive than the literature methods in terms of the limit of quantitation and the range of linearity (2,4,9,12).

Conclusion

The proposed methods are simple, reliable and rapid methods that could be used for routine analysis in quality control laboratories. They are recommended for the determination of **LOS** in pharmaceutical preparations.

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