CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1411659

Available online at: http://www.iajps.com Research Article

APPLICATION OF UV-SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF LOSARTAN POTASSIUM IN TABLETS

Dobrina Doncheva Tsvetkova[1*] and Stefka Achkova Ivanova[1]

[1]Medical University-Sofia, Faculty of Pharmacy, Department of Pharmaceutical Chemistry
Dunav Str. 2, Sofia 1000, Bulgaria

Abstract:

Losartan is non-peptide drug with gradual and long-lasting antihypertensive effect and exerts it's action by specific blockage of angiotensin II receptors.

The aim of current study was the application of the validated UV-spectrophotometric method for determination of Losartan Potassium at λ = 208 nm in Lorista® tabl. and Lozap® tabl. UV-VIS diode array spectrophotometer was used.

The absorbances data at A < 0.2 were subjected to linear regression analysis and the linear correlation coefficient (R^2) was obtained. Limit of detection and limit of quantitation were determined from the regression equation $2.10^6.x - 0.0216$ by application of root mean square error method. The obtained data were: LOD = $3.1.10^{-8}$ g/ml; LOQ = $1.04.10^{-7}$ g/ml.

From the homogenized tablets, respectively of Lorista® tabl. 50 mg and Lozap® tabl. 50 mg were weighed 6 samples, containing an amount, equivalent to 50 mg Losartan Potassium and were dissolved to 100.0 ml with distilled water. An aliquot parts of 1.0 ml were diluted separatelly with the same solvent to 100.0 ml. From the obtained solutions, aliquots of 1.0 ml were diluted separatelly with distilled water to 10.0 ml. The final sample solutions of Lorista® tabl. 50 mg and Lozap® 50 mg tabl. and standard solutions of Losartan Potassium were analysed spectrophotometrically at λ = 208 nm by using as a compensatory solution distilled water.

Validated UV-spectrophotometric method for determination of Losartan Potassium in pharmaceutical dosage preparations (tablets) by the external standard method at λmax = 208 nm was applied. Data for Chauvenet's criterion are lower than maximum permissible value (U = 1.73; N = 6), which is applied for the assessment of the need for the removal of sharply different results. All of the experimental results suit the respective confidence intervals at the corresponding confidence probability: Lorista® tabl.: 49.61 ÷ 50.89 (SD = 0.38; RSD = 0.76); Lozap® tabl.: 49.6 ÷ 51.9 (SD = 0.83 ; RSD = 1.15).

The validated method can be applied for the determination of Valsartan in dosage drug preparations.

Key words: *Valsartan, UV-spectrophotometry, tablets, determination.*

*** Corresponding author:**

Dobrina Doncheva Tsvetkova,
Medical University-Sofia, Faculty of Pharmacy
Department of Pharmaceutical Chemistry
2 Dunav Str., Sofia 1000, Bulgaria
dobrinka30@mail.bg
Phone: +359 02 9236 566
Mobile: 0877757118

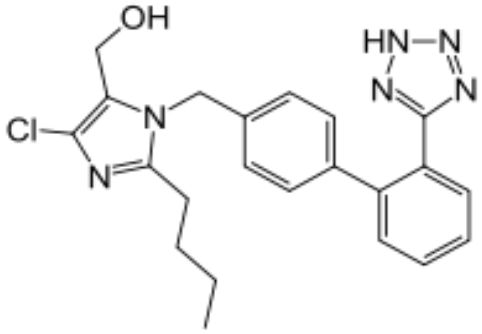
QR code

Please cite this article in press Dobrina Doncheva Tsvetkova and Stefka Achkova Ivanova., ***Application of UV-Spectrophotometric Method for Determination of Losartan Potassium in Tablets.,*** *Indo Am. J. P. Sci, 2018; 05(08).*

INTRODUCTION:

Losartan ([2-butyl-5-chloro-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]imidazol-4-yl]methanol) (Figure 1.) is non-peptide drug with gradual and long-lasting antihypertensive effect and exerts it's action by specific blockage of angiotensin II receptors [1]. Losartan Potassium is produsedin different trade products: Cozaar, Lortaan, Neo-Lotan (Merck & Co.); Losaprex (Sigma-Tau); Oscaar (Riesel); Lavestra (Hungary); Lorista (Bulgaria, Romenia); Losartan-Kalium TAD (Germany); Losartan Krka (Danemark, Greece, Italy, France); Lozitar (Pinewood Laboratories Ltd.).

Figure 1. Chemical structure of Losartan.



For the determination of sartans alone or in combinations with other drugs in dosage pharmaceutical preparations are reported the following different analytical methods: I) fourth-order UV derivative spectrophotometry: Irbesartan [2] and Hydrochlorothiazide [3]; 2) absorbance correction method: Telmisartan (λ = 325 nm), Chlorthalidon (λ = 225 nm) and Cilnidipine (λ = 350 nm) [4]; 3) Reversed Phase High Performance Liquid Chromatography (RP-HPLC): Azilsartan Medoxomil on C_{18} column Qualisil Gold (250 × 4.6 mm × 5 µm) at λ = 248 nm [5, 6]; Valsartan and Sacubitril on ODS C_{18} column (250 mm × 4.6 mm) × 5 µm) at λ= 241 nm [7]; Losartan Potassium in tablets [8] as Cozaar® tabl. [9] and in capsules [10]; Losartan Potassium and Hydrochlorothiazide in tablets [11-13]; Losartan Potassium, Ramipril and Hydrochlorothiazide in pharmaceutical preparations [14]; 4) capillary electrophoresis and super-critical fluid chromatography: Losartan Potassium in Cozaar® tabl. [9]; 5) High Performance Thin-Layer Chromatography: Losartan and its degradates in Cozaar® tabl. [15]; Losartan and Hydrochlorothiazide in combined dosage forms (chloroform : methanol : acetone : formic acid = 7.5 : 1.5 : 0.5 : 0.03 v/v; λ = 254 nm; [16]; Atenolol and Losartan Potassium: ethylacetate : methanol : 1.4 dioxane : ammonia = 10 : 2 : 1 : 2 v/v, λ = 225 nm [17].

For quantity analysis of Losartan Potassium in tablets are described the following spectrophotometric methods: I) first derivative UV spectrophotometry [18, 19] at λ = 232.5 nm in Cozaar® tabl. [20]; Losartan (λ = 271.6 nm) / Hydrochlorothiazide (λ = 335 nm) [16]; Losartan (λ = 254 nm) / Hydrochlorothiazide (λ = 271 nm) / Amlodipine (λ = 236.5 nm) in Trilopace® tabl. [21]; II) first derivative of the ratio UV-spectrum: Losartan / Hydrochlorothiazide in Hyzaar® filmtabl. [12]; III) second derivative UV spectrophotometry at λ = 219.6 nm and λ = 228.8 nm – in Cozaar® tabl. [22]; III) simultaneous UV-equation method: at λ = 208 nm (Losartan/Amlodipine); λ = 237.5 nm (Amlodipine) [23]; IV) AUC-method: Losartan (266 nm – 276 nm) / Hydrochlorothiazide (249 nm – 259 nm) / Amlodipine (231.5 nm – 241.5 nm) in Trilopace® tabl. [21]; V) chemometric UV algorithms: partial least squares, multiple linear regression by measurement of the absorbance in the range of 230.5 nm – 350.4 nm of zero order spectra of Losartan, Hydrochlorothiazide and Amlodipine [24].

The disadvantage of derivative spectrophotometry especially of the zero-crossing technique, is that little differences in the wavelength setting are the reason for method non-reproducibility. The advantage of the classical UV-spectrophotometry in comparison with UV-derivative method, is the low susceptibility towards changes in the apparatus parameters [25].

The aim of current study was the application of the validated UV-spectrophotometric method for determination of Losartan Potassium by conventional UV-spectrophotometric method at λ = 208 nm in Lorista® tabl. and Lozap® tabl. by application of method of external standard.

MATERIALS AND METHODS:

I. Drug products.

Lorista® film. tabl. 50 mg KRKA (Slovenija) N:67714

Losap® film. tabl. 50 mg Zentiva (Slovakia) N:22204008.

II. Reference standard:

Losartan Potassium (98 %) (Sigma Aldrich, N:61188).

III. Reagents with analytical grade of purity:

Distilled water.

METHOD:

UV-spectrophotometry was applied.

I. Equippment
UV-VIS diode array spectrophotometer (Hullett Packard N: 8452 A) was used.

II. Preparation of reference solutions of Losartan Potassium for validation of UV-spectrophotometric method for determination of analytical parameters limit of detection (LOD) and limit of quantitation (LOQ).
An accurately weighed quantity, equivalent respectively to 10 mg, 7 mg, 4 mg and 3.5 mg of reference standard Losartan Potassium were measured on analytical balance with an accuracy of 4 characters and were dissolved to 100.0 ml with distilled water in volumetric flask. From every solution an aliquot part of 1.0 ml was separately diluted with the same solvent to 100.0 ml. From the last solutions, an aliquot part of 1.0 ml was separately diluted with distilled water to 10.0 ml, to obtaining solutions with Losartan Potassium concentrations respectively: 1.10^{-7} g/ml; 7.10^{-8} g/ml; 4.10^{-8} g/ml; $3.5.10^{-8}$ g/ml, which were analysed at λ = 208 nm.

III. Preparation of sample solutions of Lorista® tabl. 50 mg and Lozap® 50 mg tabl.
From the homogenized tablets, respectively of Lorista® tabl. 50 mg and Lozap® tabl. 50 mg (with an average weight) on an analytical balance with an accuracy of 4 characters accurately were weighed 6 samples, containing an amount, equivalent to 50 mg Losartan Potassium and were dissolved to 100.0 ml with distilled water in volumetric flasks. An aliquot parts of 1.0 ml were diluted separatelly with the same solvent to 100.0 ml. From the obtained solutions, aliquots of 1.0 ml were diluted separatelly with distilled water to 10.0 ml.

IV. Preparation of reference solution of Losartan Potassium for quantity analysis of Lorista® tabl. 50 mg and Lozap® 50 mg tabl. by method of external standard.
An accurately weighed quantity, equivalent to 50 mg of reference standard Losartan Potassium was measured on analytical balance with an accuracy of 4 characters and was dissolved to 100.0 ml with distilled water in volumetric flask. From this solution an aliquot part of 1.0 ml was diluted with the same solvent to 100.0 ml. From the last solution, an aliquot part of 1.0 ml was diluted with distilled water to 10.0 ml, to obtaining solution with Losartan Potassium with concentration: 5.10^{-7} g/ml.

V. UV-spectrophotometric procedure.
The final sample solutions of Lorista® tabl. 50 mg and Lozap® 50 mg tabl. and standard solutions of Losartan Potassium were analysed spectrophotometrically at λ = 208 nm by using as a compensatory solution distilled water.

VI. Root limit mean square error method (RMSE) for the determination of limit of detection (LOD) and limit of quantitation (LOQ).
Calibration curves were constructed by analysis of solutions with absorbance A < 0.2. The data were subjected to linear regression analysis and the linear correlation coefficient (R^2) was obtained. From the regression equation: y = a.x + b were calculated the predictable absorbance value (Ap); the error E = |Ap - A|; E2 = $[|Ap - A|]^2$, E1= $\frac{\sum E2}{n-2}$; RMSE = $\sqrt{E1}$; LOD = 3.RMSE/a; LOQ = 10.RMSE/a [26].

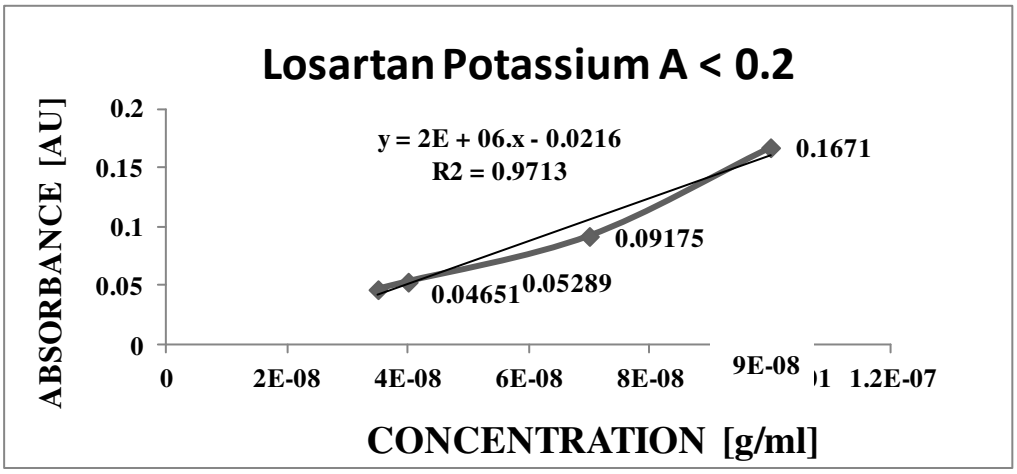
RESULTS AND DISCUSSION:

In our previous investigation [27] the UV-spectrophotometric method for determination of Losartan Potassium at λ = 208 nm in distilled water, was validated for analytical parameters selectivity, linearity, accuracy and precision in accordance with the basic validation concepts [28] and the International Conference on Harmonization Guidelines [29].

In our previous investigation [26] from regression equation: y = 1275.x – 0.008 (A > 2), was obtained the proportional accordance between absorbance A and concentration C: A = f (C) in linear concentration range: $6.75.10^{-4}$ g/µl ÷ 3.10^{-4} g/µl, where the Buge – Lambert – Beere Law was valid. Coefficient of regression was calculated: R^2 = 0.991. Analytical parameter accuracy was presented by the degree recovery R [%] ± RSD [%]: RC_{L40}: 99.52 % ± 1.71 %; RC_{L50}: 99.64 % ± 1.54 %; $RC_{L62.5}$: 102.56 % ± 3.05 %. For all model mixtures mean quadratic error and relarive error were lower than 2.0. For the estimation of an analytical parameter precision (repeatability) was used the uncertainty of the result, which is determined by: standard deviation SD, related standard deviation RSD and confidence interval ($\bar{\bar{X}} \pm t.S_{\bar{\bar{X}}}$). At confidence possibility P = 95 % (t = 4.3) all data for the obtained quantity (mg} of Losartan Potassium corresponded to the relevant confidence interval: Losartan Potassium: C_{L40}: 39.25 ÷ 40.63 (SD = 0.28; RSD = 0.7); C_{L50}: 49.09 ÷ 50.73 (SD = 0.33; RSD = 0.66); $C_{L62.5}$: 59.28 ÷ 68.22 (SD = 1.8; RSD = 2.82) [27]. In current study from reference subsastanse Losartan Potassium series of

solutions with decreasing concentrations were prepared. For A < 0.2 for every concentration the values of the absorbance (A) at the respective wavelenght were obtained and spectra for Losartan Potassium for A < 0.2 are illustrated on Figure 2.
In current study from reference subsastanse Losartan Potassium series of solutions with decreasing concentrations were prepared. For A < 0.2 for every concentration the values of the absorbance (A) at the respective wavelenght were obtained and spectra for Losartan Potassium for A < 0.2 are illustrated on Figure 2.

Figure 2. Spectra of reference solutions of Losartan Potassium.



Linearity is the range within the signal from the detector remains in linear dependency from the concentration of analyte [29]. The absorbances data at A < 0.2 were subjected to linear regression analysis and the linear correlation coefficient (R^2) was obtained. Parameters of regression equation are presented on Table 1., where: λ max [nm] – absorbance maximum.

Table 1: Characteristics of the UV-method by parameters of regression equations.

N:	Parameter	A < 0.2
1.	λ max [nm]	208
2.	Concentration range [g/ml]	$3.5.10^{-8} \div 1.10^{-7}$
3.	Regression equation	$2.10^{6}.x - 0.0216$
4.	Slope (a)	$2.10^{6}.x$
5.	Intersept (b)	– 0.0216
6.	Correlation coefficient (R^2)	0.9713

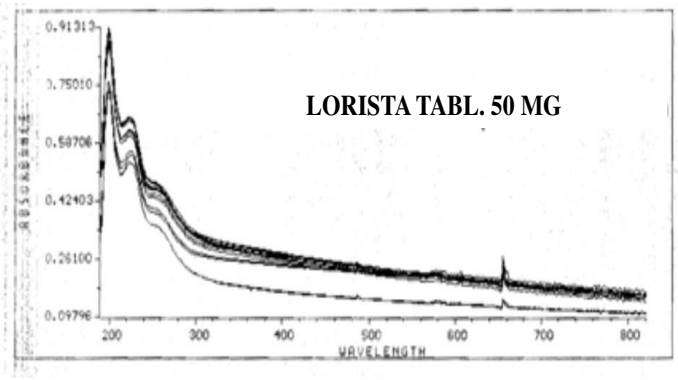
Limit of detection and limit of quantitation were determined from the regression equation $2.10^{6}.x - 0.0216$ by application of root mean square error method (Table. 2.). LOD = $3.1.10^{-8}$ g/ml; LOQ = $1.04.10^{-7}$ g/ml.

Table. 2: RMSE-method for LOD and LOQ for Losartan Potassium at A < 0.2.

C [g/ml]	A	Ap	| A – Ap|	$E^2 = [\|Ap - A\|]^2$	$E1 = \frac{\sum E^2}{n-2}$	RMSE = $\sqrt{E1}$
1.10^{-7}	0.16710	0.1784	0.0113	$1.28.10^{-4}$	$4.36.10^{-4}$	$2.09.10^{-2}$
7.10^{-8}	0.09175	0.1184	0.02665	$7.1.10^{-4}$		
4.10^{-8}	0.05289	0.0584	0.00551	$3.04.10^{-5}$		
$3.5.10^{-8}$	0.04651	0.0484	0.00189	$3.57.10^{-6}$		

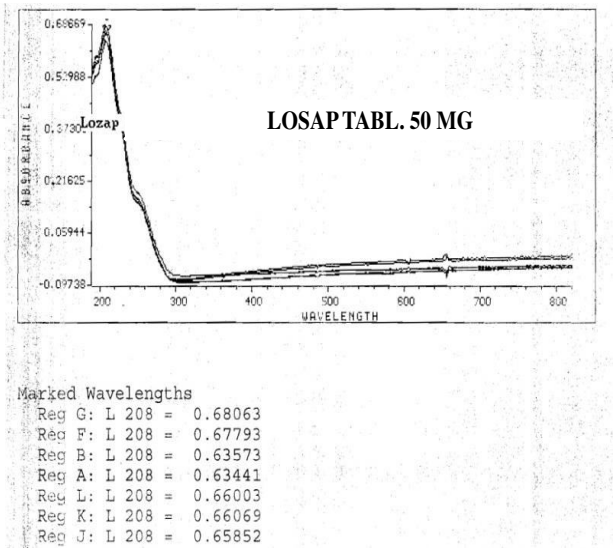
In current study the validated UV-spectrophotometric method for determination of Losartan Potassium at λ = 208 nm was apllied for its quantity analysis in tablets. Spectra of Losartan Potassium are illustrated on Figure. 3.(Lorista® tabl.) and Figure. 4. (Lozap® tabl.).

Figure 3. Spectra of Losartan Potassium in Lorista® tabl.



Marked Wavelengths
Reg A : L 208 = 0.66147
Reg B : L 208 = 0.66020
Reg C : L 208 = 0.66141
Reg D : L 208 = 0.65919
Reg E : L 208 = 0.65724
Reg F : L 208 = 0.65578

Figure 4. Spectra of Losartan Potassium in Lozap® tabl.



Marked Wavelengths
Reg G: L 208 = 0.68063
Reg F: L 208 = 0.67793
Reg B: L 208 = 0.63573
Reg A: L 208 = 0.63441
Reg L: L 208 = 0.66003
Reg K: L 208 = 0.66069
Reg J: L 208 = 0.65852

On Table 3. are presented results for: weighted quantities of Lorista® tabl. (average weight = 0.2009 g) and Lozap® tabl. (Average weight = 0.2094 g); absobances of sample solutions at λ = 208 nm ($A_{Lorista}$, A_{Lozap}), (Astandard = 0.66003) and Chauvenet's criteria for absobances (U $A_{Lorista}$, U A_{Lozap}).

Table 3: Absobances of sample solutions at λ = 208 nm of Lorista® tabl. and Lozap® tabl.

N :	Weighted Lorista®	$A_{Lorista}$	U $A_{Lorista}$	Weighted Lozap®	A_{Lozap}	U A_{Lozap}
1.	0.2018	0.66147	1.13	0.2095	0.68063	1.13
2.	0.2004	0.66020	0.49	0.2080	0.67793	1.00
3.	0.2015	0.66141	1.10	0.2015	0.63573	1.11
4.	0.2002	0.65919	0.02	0.2027	0.63441	1.18
5.	0.1980	0.65724	0.99	0.2050	0.66069	0.14
6.	0.1962	0.65578	1.72	0.2070	0.65852	0.03
$\overline{\overline{\overline{X}}}$		0.65922			0.65799	
SD		0.002			0.02	
RSD [%]		0.03			3.04	

On Table 4. are summarized results for: obtained by method of external standard content of Losartan Potassium in Lorista® tabl. ($C_{Lorista}$) and Lozap® tabl. (C_{Lozap}); degree of recovery [%] (R $C_{Lorista}$; R C_{Losap}); Chauvenet's criteria for obtained content of Losartan Potassium (U $C_{Lorista}$; U C_{Lozap}); N – number of individual measurements (1 ÷ 3); $\overline{\overline{\overline{X}}}$ – mean arithmetic error; S $\overline{\overline{\overline{X}}}$ – mean square error; E [%] – relative error; P – confidence possibility: 95 %, t – coefficient of Student: 2.57.

Data for Chauvenet's criteria for absobances and for obtained by method of external standard content of Losartan Potassium are lower than maximum permissible value (U = 1.73; N = 6), which is applied for the assessment of the need for the removal of sharply different results.

Accuracy is represented by the degree of recovery R [%] ± RSD [%] as per ICH guidelines. The analytical parameter repeatability for Lorista® tabl., Lozap® and tabl. is characterized by the uncertainty of the result, which includes standard deviation (SD), relative standard deviation (RSD) and confidential interval ($\overline{\overline{\overline{X}}}$ ± t.S $\overline{\overline{\overline{X}}}$) [28].

All of the experimental data correspond to the respective confidence intervals at the corresponding confidence probability. Relative error is lower than 0.7 %.

Table 4. Content of Losartan Potassium in Lorista® tabl. and Lozap® tabl., degree of recovery and Chauvenet's criteria.

N:	$C_{Lorista}$ [mg]	R $C_{Lorista}$ [%]	U $C_{Lorista}$	C_{Lozap} [mg]	R C_{Lozap} [%]	U C_{Lozap}
1.	49.89	99.78	0.95	51.54	103.08	0.95
2.	50.14	100.28	0.29	51.70	103.4	1.14
3.	49.96	99.92	0.76	50.05	100.1	0.84
4.	50.11	100.22	0.37	49.65	99.30	1.33
5.	50.52	101.04	0.71	51.12	102.24	0.45
6.	50.87	101.74	1.63	50.46	100.92	0.35
$\overline{\overline{\overline{X}}}$ ± SD	50.25 ±0.38			50.75±0.83		
$\overline{\overline{R}}$ [%] ± RSD [%]		100.5 ± 0.75			101.51 ±1.64	
SD	0.38	0.75		0.83	1.66	
RSD [%]	0.76	0.75		1.64	1.64	
$S_{\overline{\overline{\overline{X}}}}$	0.16	0.31		0.34	0.68	
P [%]	99.0	99.0		98.0	98.0	
t	4.03	4.03		3.37	3.37	
t. $S_{\overline{\overline{\overline{X}}}}$	0.64	1.25		1.15	2.29	
$\overline{\overline{\overline{X}}} - t.S_{\overline{\overline{\overline{X}}}}$ $\overline{\overline{\overline{X}}} + t.S_{\overline{\overline{\overline{X}}}}$	49.61÷50.89	99.25÷101.75		49.6 ÷ 51.9	99.22 ÷103.8	
E [%]	0.32	0.31		0.67	0.67	

CONCLUSION:

Limit of detection and limit of quantitation were determined from the regression equation $2.10^{6}.x - 0.0216$ by application of root mean square error method (Table. 2.). LOD = $3.1.10^{-8}$ g/ml; LOQ = $1.04.10^{-7}$ g/ml.

Validated UV-spectrophotometric methods for determination of Losartan Potassium in pharmaceutical dosage preparations (tablets) by the external standard method at λmax = 208 nm was applied. All of the experimental data correspond to the respective confidence intervals at the corresponding confidence probability: Lorista® tabl.: 49.61 ÷ 50.89 (SD = 0.38; RSD = 0.76); Lozap® tabl.: 49.6 ÷ 51.9 (SD = 0.83 ; RSD = 1.15). The validated method can be applied for the determination of Valsartan in dosage drug preparations.

List of symbols and abbreviations

A – Absorbance
C – concentration
C_{L40} – 40 mg Losartan Potassium
C_{L50} – 50 mg Losartan Potassium
$C_{L62.5}$ – 62.5 mg Losartan Potassium
E [%] – relative error
λ – Analytical wavelenght
N – number of individual measurements
P – confidence possibility
R – degree of recovery
RP-HPLC– Reversed Phase High Performance Liquid Chromatography
RSD – related standard deviation
SD – standard deviation
$S_{\overline{\overline{\overline{X}}}}$ – Mean square error;
t – Coefficient of Student
$\overline{\overline{\overline{X}}}$ – mean arithmetic error
$\overline{\overline{\overline{X}}} \pm t.S_{\overline{\overline{\overline{X}}}}$ – confidence interval
UV – ultraviolet

REFERENCES:

1. Devereux RB, Dahlof B. Potential mechanisms of stroke benefit favoring Losartan in the Losartan intervention for endpoint reduction in hypertension (LIFE) study. Curr Med Res Opin, 2007;23(2):443-457.
2. Paras V, Sojitra R, Raj H, Jain V. A review on new antihypertensive agent: Irbesartan. Indo Am J Pharm Sci, 2015;2(1):510-513.
3. Vetuschi C, Giannandrea A, Carlucci G, Mazzeo P. Determination of Hydrochlorothiazide and Irbesartan in pharmaceuticals by fourth-order UV derivative spectrophotometry. Il Farmaco, 2005;60(8):665-670.
4. Reddy BH, Spandana B, Mounika D, Devi S, Dacha A, Prakash KV. Simultaneous estimation of Telmisartan, Chlorthalidon and Cilnidipine by absorbance correction method using UV spectrophotometry. Indo Am J Pharm Sci, 2018;5(3):1998-2003.
5. Anuradha M. RP-HPLC method development and validation of Azilsartan Medoxomil API and its application to forced degradation studies. Indo Am J Pharm Sci, 2016;3(8):854-861.
6. Anuradha M, Naik SPB. A novel validated stability indicating RP-HPLC method development for determination of Azilsartan Medoxomil in its dosage form. Indo Am J Pharm Sci, 2016;3(9):945-952.
7. Jabeen A, Rizwan SH. Simultaneous estimation of Sacubitril and Valsartan in pharmaceutical dosage form in development and validation of stability indicating chromatographic method. Indo Am J Pharm Sci, 2018;5(7):7079-7087.
8. Kathiresan K, Gothandaraman S, Manickam MS, Kumar SM, Manavalan R. Analytical method development and validation of Losartan Potassium tablet by RP-HPLC. Rasayan J Chem. 2008;1(3): 521-525.
9. Williams RC, Alasandro MS, Fasone VL, Boucher RJ, Edwards JF. Comparison of liquid chromatography, capillary electrophoresis and super-critical fluid chromatography in the determination of Losartan Potassium drug substance in Cozaar® tablets. J Pharm Biomed Anal, 1996;14(11):1539-1546.
10. Bonfilio R, Tarley CRT, Pereira GR, Salgado HRN, de Araújo MB. Multivariate optimization and validation of an analytical methodology by RP-HPLC for the determination of Losartan Potassium in capsules.Talanta, 2009;80(1):236-241.
11. Carlucci G, Palumbo G, Mazzeo P, Quaglia MG. Simultaneous determination of Losartan and Hydrochlorothiazide in tablets by high-performance liquid chromatography. J Pharm Biomed Anal, 2000;23(1):185-189.
12. Erk N. Analysis of binary mixtures of Losartan Potassium and Hydrochlorothiazide by using high performance liquid chromatography, ratio derivative spectrophotometric and compensation technique. J Pharm Biomed Anal, 2001;24(4):603-611.
13. Hertzog DL, McCafferty JF, Fang X, Tyrrell RJ, Reed RA. Development and validation of a stability-indicating HPLC method for the simultaneous determination of Losartan Potassium, Hydrochlorothiazide, and their degradation products. J Pharm Biomed Anal, 2002;30(3):747-760.
14. Baing MM, Vaidya VV, Sane RT, Menon SN, Dalvi K. Simultaneous RP-LC determination of Losartan Potassium, Ramipril, and Hydrochlorothiazide in pharmaceutical preparations. J Chromatogr, 2006;64(5-6):293-296.
15. McCarthy KE, Wang Q, Tsai EW, Gilbert RE, Ip DP, Brooks MA. Determination of Losartan and its degradates in Cozaar® tablets by reversed-phase high-performance thin-layer chromatography. J Pharm Biomed Anal, 1998;17(4-5):671-677.
16. Shah SA, Rathod IS, Suhagia BN, Savale SS, Patel JB.Simultaneous determination of Losartan and Hydrochlorothiazide in combined dosage forms by first-derivative spectroscopy and high-performance thin-layer chromatography. JAOAC Int, 2001;84(6):1715-1723.
17. Keny SM, Nazareth C, Sawaikar L. Development and validation of a new sensitive method for the quantitative analysis of Atenolol-Losartan potassium in a tablet dosage form by using HPTLC. Sch Acad J Pharm, 2013;2(6):464-467.
18. Ansari AM, Kazemipour M, Khosravi F, Baradaran M. A comparative study of first-derivative spectrophotometry and high-performance liquid chromatography applied to the determination of Losartan Potassium in tablets. Chem Pharm Bull (Tokyo), 2004;52(10):1166-1170.
19. Lastra OC, Lemus IG, Sánchez HJ, Pérez RF. Development and validation of an UV derivative spectrophotometric determination of Losartan Potassium in tablets. J Pharm Biomed Anal, 2003;33(2):175-180.
20. Ansari M, Kazemipour M, Baradaran M, Jalalizadeh H. Derivative spectrophotometric method for determination of Losartan in

pharmaceutical formulations. Iran J Pharmacol Ther, 2004;3(1):21-25.
21. Wankhede SB, Raka KC, Wadkar SB, Chitlange SS. Spectrophotometric and HPLC methods for simultaneous estimation of Amlodipine Besilate, Losartan Potassium and Hydrochlorothiazide in tablets. Indian J Pharm Sci, 2010;72(1):136-140.
22. Ulu ST, Sağlik S. Comparison of UV and second derivative spectrophotometric and high – performance liquid chromatographic methods for the determination of Losartan in tablets. Turk J Pharm Sci, 2004;1(3):165-175.
23. Priyanka RP, Rakesh SU, Dhabale PN, Burade KB. Simultaneous UV spectrophotometric method for estimation of Losartan Potssium and Amlodipine Besylate in tablet dosage form. Asian J Res Chem, 2009;2(1):183-187.
24. Nagavalli D, Vaidhyalingam V, Santha A, Sankar ASK, Divya O. Simultaneous spectrophotometric determination of Losartan Potassium, Amlodipine Besilate and Hydro-chlorothiazide in pharmaceuticals by chemometric methods. Acta Pharm, 2010;60(1):141-452.
25. Kus S, Marczenko Z, Obarski N. Derivative UV-VIS Spectrophotometry in analytical chemistry. Chern Anal Warsaw 1996;41(1):899-927.
26. Shrivastava A., Gupta V.B. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chron. Young Sci. 2011;2(1): 21-25.
27. Tsvetkova DD, Obreshkova DP. Validation of UV-spectrophotometric method for identification and determination of angiotensin II receptor antagonist Losartan Potassium. Int J Pharm Pharm Sci, 2012;4(1):428-431.
28. Gurram I, Kavitha MVS, Nagabhushnam MV, Bonthagara B, Reddy DN, Overview of validation, basic concepts and analytival method process validation. Indo Am J Pharm Sci, 2017;4(6):1665-1680.
29. International Conference on Harmonization guidelines. Validation of analytical procedures: text and methodology Q2 (R1): FDA. Fed. Regist., 1995, 60, 11260.