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Research Article

VALIDATION OF UV – SPECTROPHOTOMETRIC METHOD FOR IDENTIFICATION AND DETERMINATION OF ANGIOTENSIN II RECEPTOR ANTAGONIST LOSARTAN POTASSIUM

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ABSTRACT

The aim of current study is to validate spectrophotometric method with UV – detection for identification and determination of Losartan Potassium in respect of analytical parameters accuracy, precision, linearity. For accuracy the degree of recovery R (%) ± RSD (%) correspond to the relevant confidence interval: RC_{L40}: 99.52 ± 1.71; RC_{L50}: 99.64 ± 1.54; $RC_{L62.5}$: 102.56 ± 3.05. All data for the obtained quantity of Losartan Potassium suit the respective requirements: C_{L40}: 39.53 ÷ 40.35; C_{L50}: 49.42 ÷ 50.4; $C_{L62.5}$: 61.08 ÷ 66.45. The method can be applied for quality control of Losartan Potassium in tablets.

Keywords: Losartan Potassium, UV – Spectrophotometry, Validation, Accuracy, Precision, Linearity.

INTRODUCTION

Losartan ([2 – butyl – 5 – chloro – 3 – [[4 – [2 – (2 H – tetrazol – 5 – yl) phenyl] phenyl] methyl] imidazol – 4 – yl] methanol) is non – peptide drug with gradual and long – lasting antihypertensive effect 1, 2, exerts it's action by specific blockade of angiotensin II receptors [1, 3] and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – alone [3, 4] and in combination with diuretic Hydrochlorothiazide (HCTZ) [5] or both with calcuim antagonist Amlodipine besilate (Aml) and HCTZ [6]; 2) hypertension with type 2 diabetes mellitus with proteinuria [7]; 3) stroke prevention 4.

For quantity analysis of Losartan Potassium (Los) are described the following spectrophotometric methods (SMF), by measurement of the absorption: I) first derivative (1D) UV SFM at λ = 232.5 nm – in Cozaar® tablets (25 mg Los) [8]; II) second derivative (2D) UV SFM at λ = 219.6 nm and λ = 228.8 nm – in Cozaar® tablets (50 mg Los) [9]; III) vis – charge transfer SFM, by using of microwell – plate reader for measuring the absorption at λ = 460 nm of complex of Los and 2.3 – dichloro – 5.6 – dicyano – 1.4 – benzoquinone 10.

For simultaneous determination of Los in combination with HCTZ and Aml in tablets are developed differend SMF methods, by measurement of the absorption: I) 1D UV SFM at: 1) λ_{Los} = 271.6 nm, λ_{HCTZ} = 335.0 nm [11]; 2) λ_{Aml} = 236.5 nm, λ_{Los} = 254 nm, λ_{HCTZ} = 271 nm – in Trilopace® tabl. [12]; II) 1D of the ratio UV – spectrum for 50 mg Los and 12.5 mg HCTZ in Hyzaar® filmtabl. [13]; III) simultaneous UV – equation method: at λ = 208 nm (Los, Aml); λ = 237.5 nm (Aml) [14]; IV) AUC – method: at 266 nm – 276 nm (Los), 231.5 nm – 241.5 nm (Aml), 249 nm – 259 nm (HCTZ) – in Trilopace® tabl. [12]; V) chemometric UV algorithms: PLS (partial least squares), multiple linear regression (MLR) by measurement of the absorption in the range of 230.5 nm – 350.4 nm of zero order spectra of Los, Aml and HCTZ 6.

HPLC is olso very often applied method for simultaneous analysis of sartans and other drugs: Olmesartan medoxomil and Amlodipine besylate 15.

The aim of current study is to validate spectrophotometric method with UV – detection for identification and determination of Losartan Potassium in respect of analytical parameters accuracy, precision, linearity.

MATERIALS AND METHODS

I. Reference standard (RS) of Losartan Potassium (Los), distilled water.

UV – spectrophotometry.

I) Accuracy and precision (repeatability)

1) Preparation of solutions of reference standard Losartan Potassium

An accurately weighed quantity of reference standard Losartan Potassium: 40 mg, 50 mg, 62.5 mg was dissolved in distilled water to 100.0 ml and 1.0 ml was diluted to 100.0 ml with the same solvent. From all of the resulted preparations, aliquot part of 1.0 ml was diluted with distilled water to 10.0 ml, to obtain samples with concentration of Losartan Potassium respectively: 4.10^{-7} g/ml, 5.10^{-7} g/ml, $6.25.10^{-7}$ g/ml. The absorbance of last solutions was measured at λ = 208 nm, using distilled water as blank solution.

2) Preparation of model mixtures with Losartan Potassium

Three equal homogenous model mixtures were prepared from all respective supplements in tablets (colloidalle anhydrous silica dioxide, crospovidone, magnesia stearate, mannitol, microctyctalline cellulosae, starch, talk), adding of reference standard Losartan Potassium, equivalent to 40 mg (80 %) (L40); 50 mg (100 %) (L50) and 62.5 mg (125 %) (L62.5) of theoretical concentration of Losartan Potassium in tablets (50 mg). An average weights of model mixtures were: 0.16 g (L40), 0.2 g (L50), 0.25 g (L62.5). From every model mixture were prepared three solutions as follows: an accurately weighed quantity, containing reference standard Losartan Potassium: 40 mg, 50 mg, 62.5 mg was dissolved in distilled water to 100.0 ml and aliquot part of 1.0 ml of all preparations was diluted to 100.0 ml with the same solvent. An aliquot part of 1.0 ml of all samples was diluted with distilled water to 10.0 ml. The absorbance of last solutions was measured at λ = 208 nm, using distilled water as blank solution.

II) Preparation of solutions of reference standard Losartan Potassium for linearity

An accurately weighed quantity of reference standard Losartan Potassium: 67.5 mg, 62.5 mg, 60 mg, 55 mg, 50 mg, 45 mg, 40 mg, 37 mg, 30 mg, 10 mg, 7 mg, 4 mg, 3,5 mg was dissolved in distilled water to 100.0 ml and 1.0 ml of all of the resulted preparations was diluted to 100.0 ml with the same solvent. From all of the obtained samples, 1.0 ml was diluted with distilled water to 10.0 ml, to obtain solutions with concentrations of Los respectively: $6.75.10^{-4}$ g/µl; $6.25.10^{-4}$ g/µl; 6.10^{-4} g/µl; $5.5.10^{-4}$ g/µl; 5.10^{-4} g/µl; $4.5.10^{-4}$ g/µl; 4.10^{-4} g/µl; $3.7.10^{-4}$ g/µl; 3.10^{-4} g/µl, which are analysed by UV – spectrophotometry, by measuring the absorbance at λ = 208 nm, using distilled water as blank solution.

RESULTS AND DISSCUSSION

I) Validation of spectrophotometric method for analytical parameter selectivity.

At the same manner like solutions of reference standard Losartan Potassium is prepared "placebo" solution, containing all labeled in tablets supplements (colloidalle anhydrous silica dioxide, crospovidone, magnesia stearate, talk, mannitol, microctyctalline cellulosae, starch, talk) in dosage formulations (tablets) without active ingredient Losartan Potassium. The selectivity of the applied UV – spectrophotometric method is proved by the lack of measured absorption of "placebo" solution at specific for Losartan Potassium wavelength λ = 208 nm.

II) Validation of spectrophotometric method for analytical parameters accuracy and precision (repeatability)

On Table 1. are summarized data for: 1) added quantity of reference standard Losartan Potassium in model mixtures: L40, L50, L62.5; 2) weighed quantity (W) of model mixtures for analysis: WL40, WL50, WL62.5; 3) values for absorbance (A) of solutions of model mixtures with Losartan Potassium in distilled water at λ = 208 nm: A_{L40}, A_{L50}, $A_{L62.5}$; 4) Chauvenet's criterion for absorbance (UA): UA_{L40}, UA_{L50}, $UA_{L62.5}$.

Absorbances of solutions of reference standard Losartan Potassium are correspondingly: L40 (A_{RS} = 0.50270); L50 (A_{RS} = 0.66003); L62.5 (A_{RS} = 0.79494).

Table 1: Absorbance of model mixtures of reference standard Losartan Potassium and Chauvenet's criterion for absorbance (U A)

Added content of RS of Losartan Potassium in model mixtures and weighed quantity of model mixtures.						
N :	L40 [mg]	W L40 [g]	L50 [mg]	W L50 [g]	L 62.5 [mg]	W L62.5 [g]
1.	39.7	0.1588	49.6	0.1984	61.9	0.2476
2.	40.2	0.1608	50.3	0.2012	62.1	0.2484
3.	40.5	0.1620	50.4	0.2016	62.5	0.25
Absorbanse of model mixtures of RS of Losartan Potassium and Chauvenet's criterion for absorbanse (U A).						
N :	A_{L40}	U A_{L40}	A_{L50}	U A_{L50}	$A_{L62.5}$	U $A_{L62.5}$
1.	0.50188	0.825	0.65852	1.67	0.79305	0.67
2.	0.50372	0.095	0.66069	0.66	0.79512	0.57
3.	0.50499	0.73	0.66135	1.16	0.83142	1.24
$\overline{X}$	0.50353		0.66019		0.80653	
SD	0.002		0.001		0.02	
RSD [%]	0.40		0.15		2.48	

For all of the obtained by UV – method results for the absorption in every sample is necessary to estimate the Chauvenet's criterion (U), because when U for one value is higher than the relevant statistical requirements for Chauvenet's criterion: UA < 1.68 (N = 3), the data must be removed as unexpected. The results for UA on Table 1. show the relations: $UA_{L40} < 1.68$; $UA_{L50} < 1.68$.; $UA_{L62.5} < 1,68$, which confirm that it isn't necessary to remove any of values as unexpected.

Table 2: Obtained quantity (C) of Losartan Potassium in MM, recovery R (%) for C (RC) and Chauvenet's criterion for C (UC)

N:	**C_{L40} [mg]**	**RC_{L40} [%]**	**UC_{L40}**	**C_{L50} [mg]**	**RC_{L50} [%]**	**UC_{L50}**	**$C_{L62.5}$ [mg]**	**$RC_{L62.5}$ [%]**	**$UC_{L62.5}$**
1.	40.24	101.36	1.07	50.29	101.39	1.15	62.35	99.76	0.78
2.	39.88	99.2	0.21	49.75	98.91	0.48	63.12	101.97	0.35
3.	39.69	98.0	0.89	49.7	98.61	0.64	65.79	105.94	1.13
$\overline{X}$ ± SD	39.94± 0.28			49.91± 0.33			63.75± 1.8		
$\overline{R}$ [%] ± RSD [%]		99.52± 1.71			99.64± 1.54			102.56± 3.05	
SD	0.28	1.7		0.33	1.53		1.8	3.13	
RSD [%]	0.7	1.71		0.66	1.54		2.82	3.05	
S $\overline{X}$	0.16	0.98		0.19	0.88		1.04	1.81	
P [%]	95.0	95.0		95.0	95.0		95.0	95.0	
t	4.3	4.3		4.3	4.3		4.3	4.3	
t.S $\overline{X}$	0.69	4.21		0.82	3.78		4.47	7.78	
$\overline{X}$ – t.S $\overline{X}$ ± $\overline{X}$ + t.S $\overline{X}$	39.25÷ 40.63	95.31÷ 103.73		49.09÷ 50.73	95.86÷ 103.42		59.28÷ 68.22	94.78÷ 110.34	
E [%]	0.40	0.98		0.38	0.88		1.63	1.76	

The content of Losartan Potassium is obtained by method of reference standard (RS). On Table 2. are indicated:

N – number of the individual measurements (1 ÷ 3); C – obtained quantity of Losartan Potassium (C_{L40}, C_{L50}, $C_{L62.5}$), after application of UV – spectrophoto – metric method; UC – Schöveneou's criterion for obtained quantity (UC_{L40}, UC_{L50}, $UC_{L62.5}$); R (%) – degree of recovery (RC_{L40}, RC_{L50}, $RC_{L62.5}$); $\overline{X}$ – arithmetical mean; SD – standard deviation; RSD – relative standard deviation (%); S $\overline{X}$ – mean quadratic error; P – confidence possibility (%); t – coefficient of Student; $\overline{X}$ ± t.S $\overline{X}$ = $\overline{X}$ – t.S $\overline{X}$ ÷ $\overline{X}$ + t.S $\overline{X}$ – confidence interval); E (%) – relative error [16].

For all values for UC (Table 2.) are shown relations: $UC_{L40} < 1.68$; $UC_{L50} < 1.68$; $UC_{L62.5} < 1.68$, which confirm, that all experimental data suit standard requirement [16].

For the assessment of accuracy and precision is calculated sample standard deviation (SD), by the applying of the Bessel's correction, in which the denominator N – 1 (degrees of freedom) is used instead of N and in this case $(S_{\overline{x}})^2$ is an unbiased estimator for $(SD)^2$.

1) Accuracy

Analytical parameter accuracy is presented by the degree recovery R (%) ± RSD (%) [16]: RC_{L40}; 95.31 ÷ 103.73; RC_{L50}: 95.86 ÷ 103.42; $RC_{L62.5}$: 94.78 ÷ 110.34. For all model mixtures mean quadratic error and relarive error are lower than 2.0.

2) Precision

For the estimation of an analytical parameter precision (repeatability) is used the uncertainty of the result, which is determined by: SD, RSD and $\overline{X} \pm t.S_{\overline{X}} = \overline{X} - t.S_{\overline{X}} \div \overline{X} + t.S_{\overline{X}}$) 15. At confidence possibility P = 95 % (t = 4.3) all data for the obtained quantity of Losartan Potassium correspond to the relevant confidence interval: 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).

All values for SD are lower than 2.0 and for RSD are lower than 3.0.

III) Validation of analytical parameter linearity: application of method of linear regression analysis.

The prepared solutions with decreasing concentration of RS Los were analyzed by the written UV – spectrophotometric method. For every concentration (C) in g/µl was measured the respective value of the absorption (A) in absorption units (AU) at λ = 208 nm. The experimental results are putted into linearity regression analysis. The regression calibration curve is built. The obtained regression equation: y = 1275.x – 0.008, shows the proportional accordance A = f (C) in linear concentration range: $6.75.10^{-4}$ g/µl ÷ 3.10^{-4} g/µl, where the Buge – Lambert – Beere Law is valid. Coefficient of regression (R) is calculated: $R^2 = 0.991$. On Table 3. are pointed out data for concentrations and absorbances for linearity and the calibration curve for A > 0.2 at λ = 208 nm is illustrated on Fig. 1.

Table 3: Concentrations and absorbances for linearity for Losartan Potassium

N :	Concentration [g / µl]	Absorbance
1.	$6.75.10^{-4}$	0.84961
2.	$6.25.10^{-4}$	0.79494
3.	$6.0.10^{-4}$	0.73882
4.	$5.5.10^{-4}$	0.67903
5.	$5.0.10^{-4}$	0.66003
6.	$4.5.10^{-4}$	0.57045
7.	$4.3.10^{-4}$	0.54944
8.	$4.0.10^{-4}$	0.5027
9.	$3.7.10^{-4}$	0.47047
10.	$3.5.10^{-4}$	0.42397
11.	$3.0.10^{-4}$	0.36386

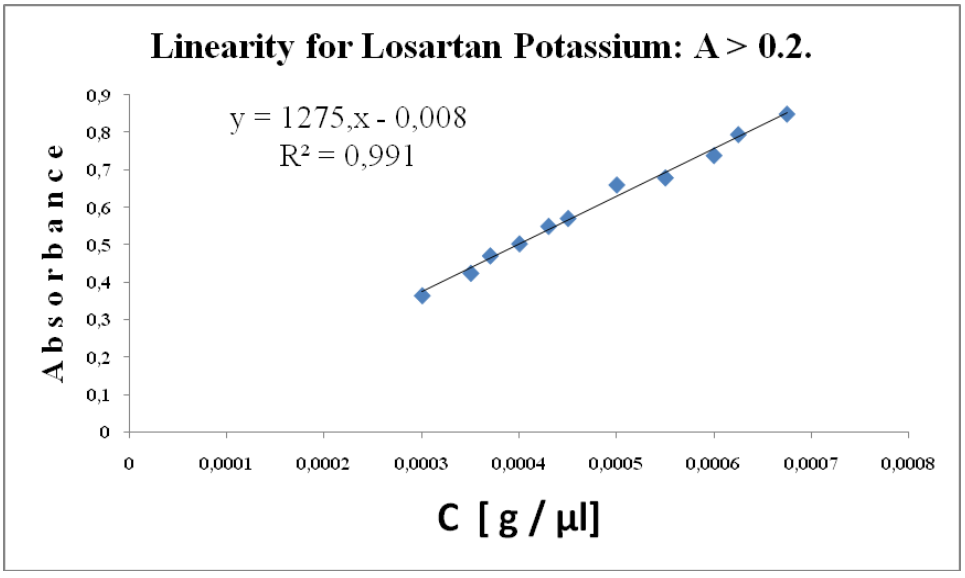

Fig. 1: Calibration curve for linearity for Losartan Potassium at λ = 208 nm (A > 0.2)

CONCLUSION

All data for UA and UC suit the standard requirement: U < 1.68. At confidence possibility P = 95 % all results for R correspond to the relevant CI: RC_{L40}: 99.52 ± 1.71; RC_{L50}: 99.64 ± 1.54; $RC_{L62.5}$: 102.56 ± 3.05. Precision is estimated by data, which suit the respective confidence interval: C_{L40}: 39.94 ± 0.28; C_{L50}: 49.91 ± 0.33; $C_{L62.5}$: 63.75 ± 1.8. Linearity is estimated by $R^2 > 0.99$. The applied UV – spectrophotometric method is appropriate for determination of Losartan Potassium in dosage pharmaceutical products – tablets.

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