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Development and Validation of Analytical Method for Quantification of Losartan Potassium in Solid Dosage Form

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Abstract

The aim of this study was to develop and validate a simple, robust, reliable and an accurate isocratic reverse phase high-performance liquid chromatography (RP-HPLC) method for quantification of Losartan potassium in solid dosage form using DAD Detector. Elution was carried out with mobile phase comprising of 0.01 M monobasic potassium dihydrogen phosphate buffer (adjusted at pH 3.0 ± 0.05 with ortho-phosphoric acid) and methanol (40: 60), through octadecyl silyl (C18) column (15 cm x 4.6 mm x 5 µl), at flow rate of 1 ml/min. The detection was carried out at 230 nm. The developed method was validated according to International Conference on Harmonization (ICH) guidelines (ICH 2005). The assay was linear in concentration range of 1-3 μgmL^{-1} with Correlation coefficient of 0.999. The limit of detection was 0.036 μgmL^{-1} and limit of quantification was 0.110 µg/ml. Similarly, method accuracy was asses by comparing the %RSD of BP method with the %RSD of the method developed which shows RSD for standard method was 1.012% while for developed method it was 1.516% and combined RSD of both two methods was found 1.823% that was as per the precision criteria of accuracy i.e. <2%. The result of intraday study was 0.129% and Intermediate precision among inter day and brand to brand was 0.332%. Moreover, the devised method seems to be linear over broad range of LK concentration (1-3 μgmL^{-1}) with appreciable repeatability and reproducibility (RSD <2.00).

The results of present study indicate that the method is efficient, specific, sensitive and suitable to be used for the determination of losartan potassium in solid dosage forms using isocratic mode in comparison to gradient mode used by United States pharmacopoeia (USP 2016).

Keywords: RP-HPLC-DAD; ICH guidelines; Losartan potassium; Method development; Validation

Introduction

Losartan potassium (LK) is an orally active, non-peptide angiotensin II receptor antagonist. Because of effect of consequently reduced pressure of angiotensin II and Angiotensin-1 receptors selectively blockade, losartan is introduced for clinical usage in "Hypertension" as a new class drug [[1,2].

In clinical trials, dizziness was the only drug-related event reported more frequently with LK monotherapy. Combined dosage form of hydrochlorothiazide (diuretic) and Losartan is mostly used due to its highly anti-hypertensive action. To maintain plasma drug concentration, tablet should be taken 3-4 times. For the treatment of hypertension, LK is considered as most desirable drug as it developed a sustained release drug delivery system. Hence, current study reflects an attempt to develop and validate an appropriate quantification method for losartan potassium in dosage form.

LK is a potassium salt of biphenyl tetrazole is widely used for the treatment of hypertension and diabetic nephropathy [3]. Chemically polar nature of LK renders it very reactive i.e. easily protonated even under mild reaction or stress conditions and resultant molecules usually undergo addition reactions to form dimers while eliminating side chains of alkyl or halogen group. Although much work had been done for the determination of LK using state-of-the art analytical techniques however, literature lacks simple and pragmatic method to study the behavior of LK towards storage and processing conditions.

United States Pharmacopoeia (USP 2016) used a gradient method consisting of solution A (acetonitrile: pH 7.0 buffer of monobasic potassium phosphate and dibasic sodium phosphate) (15:85) and Solution B (acetonitrile). Chromatographic conditions are 1.0 ml/min flow rate, L7 column packing (5 µm,15 cm x 3.9 mm), UV detector wavelength 250 nm, injection volume 10 µl, 0.25 mg/ml of Standard solution and sample solutions are prepared by using Solution A as diluent. USP used gradient elution mode whereas the authors of this study developed analytical method on isocratic mode. Schellinger suggested avoiding gradient elution over isocratic mode because gradient mode requires more re-evaluation and isocratic mode is preferred in case of separation of single component or maximum for the separation of less than ten components.

Dos Passos et al. used an isocratic HPLC method for LK tablets assay. In this method he used L7 column packing (5 µm, 15 cm x 4.6 mm), triethylamine solution (0.5%) having pH of 2.4 and acetonitrile 60: 40 (v/v) as mobile phase having flow rate was 1 ml / min, sample injection volume of 20 µl and the peak response was determined at the wavelength of 225 nm through photo diode array (PDA) detector in the concentration range of 15-45 µg/ml with R-value of 0.999. The pH 2.4 used by them was highly dangerous for stability of stationary phase of the column. Therefore, authors of the current study used the pH 3.0 buffer in mobile phase and methanol (60: 40 v/v) which is comparatively safer pH for column stationary phase. Further, they performed forced degradation study by acid, base and peroxides for very short duration of 2 hours but authors of this study performed it for 24 hours [4].

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Kathiresan et al. used a method for analysis using C-18 Spherisorb ODS column (5 µl, 25 cm × 4.6 mm), 254 nm wavelength, 20 µl injection volume, 1.5 ml/min flow rate and 20 min run-time. The mobile phase used was premixed ammonium dihydrogen acetonitrile and phosphate buffer having pH 3.0 (35: 65). They reported that LK peak was eluted at 7.5 min whereas, in current study, the peak of LK was eluted at 5 ± 1 min. Therefore, the authors reported method is faster under the current method developed and which can save time as well as cost of analysis [5].

A simultaneous determination method for LK and ramipril was developed and validated by Rao and Srinivas. They used hypersil ODS C18 column (4.6 × 250 mm, 5 µm) and mobile phase acetonitrile: methanol: 10 mM tetra butyl ammonium hydrogen sulphate in water (30: 30:40% v/v/v) in isocratic elution mode. The flow rate was and effluent were set at 1.0 ml/min and 210 nm wavelength, respectively. They reported the retention times for LK and ramipril as 4.7 and 3.3 min, respectively. The drawback of their study was the use of ion-pairing agent (tetra butyl ammonium hydrogen sulphate). The ion-pairing agents interferer with stationary phase and cannot be completely washed from the column even with extensive column flushing. Therefore, such column cannot be used for other methods for analysis [6].

Therefore, there is an inevitable necessity to develop and validate a method for analysis of LK that will be simple, sensitive, fast and robust. The outcomes of the current study will provide an expedient and pragmatic high-performance liquid chromatography (HPLC) method for determination of LK in solid dosage form.

Materials and Method

Standards, samples, reagents and chemicals

The certified reference material of Losartan potassium (LK) was procured from United States Pharmacopoeia (USP Catalogue #1370462, Batch # H1M331). Two commercial brands of LK (25 mg per tablet) marketed under brand names A2A (Wilson's Pharmaceuticals, Islamabad-Pakistan) and Eziday (Werrick Pharmaceuticals, Karachi-Pakistan) were purchased from local market of Lahore-Pakistan. High-Performance Liquid Chromatography (HPLC) grade methanol was purchased from VWR Chemicals while ortho-phosphoric acid and monobasic potassium phosphate (Lot # C1790) was supplied by Honeywell (Seelze, Germany).

Preparation of mobile phase and diluent

Mobile phase contained monobasic potassium phosphate buffer (1.36 mg/ml) pH 3 and methanol (40: 60 v/v). The pH of buffer was adjusted to 3.0 with phosphoric acid. Diluent was prepared using methanol and phosphate buffer of pH 3.0 (50: 50 v/v).

Preparation of standard stock solution of LK (0.1 mg/mL)

Weighed accurately 10 mg of LK standard and transferred it to a 100 ml volumetric flask. Dissolved the contents in small amount of diluent with sonication. Final volume was made up to the mark using diluent. Filtered it through 0.45 µm syringe filter.

Preparation of test sample solution

Powdered tablets containing 10 mg of LK was accurately weighed and powder transferred it to a 100 ml volumetric flask. Dissolved the contents in small amount of diluent with sonication. Final volume was made up to the mark using diluent. Filtered it through 0.45 µm syringe filter.

Chromatographic conditions

The samples and standards were analyzed using HPLC (Waters, Alliance e2695 separation module) equipped with Photo Diode Array (PDA) detector (waters 2998) and Spherisorb column of packing ODS-2 (5 µm, 150 mm x 4.6 mm). Mobile phase comprising ratio (60: 40 v/v) of methanol and KH_2PO_4 buffer adjusted to pH 3.0 using orthophosphoric acid. Flow rate was isocratic at 1 ml/min. Column temperature was set at ambient. Injection volume was set at 20 µl and eluted compounds were seen at 230 nm fixed wavelength as well as scanned over range of 190 nm-400 nm to characterize impurities or degradants by using diode array detector. The chromatographic data obtained thus was processed using Empower 3 software.

Method Validation

Authors of this manuscript validated the developed method in accordance with International conference of Harmonization validation guidelines [7].

Linearity

Linearity was determined using five concentration levels. Each level was run in duplicate for five days. The linearity of developed HPLC method was acquired over wide range of concentration of 1-3 µg/mL as most of the pharmaceutical dosage forms are found in this range. Pharmaceutical formulations are not intended to have very high concentration of active ingredient, while this method is used to design the analyte LK in solid dosage forms.

Detection limit (DL) and quantification limit (QL)

LOD is typically the three times of the noise level and LOQ gives signal to noise ratio of ten times. Response was checked at different concentrations (1, 1.5, 2, 2.5 and 3 µg/ mL). A calibration curve between concentrations and response was then drawn in order to find out the y-intercept value using Pearson coefficient of correlation (r), LOD and LOQ by using linear regression equation.

LOD=3 SD/S

LOQ=10 SD/S

Precision

The degree of closeness of outputs among successive attempts to measure amount in analysis obtained under the constant conditions from different sampling of the same stock solution which is very homogeneous showed the precision of our analytical method under test.

Selectivity

To determine the selectivity of developed method for LK in standard as well as in pharmaceutical products, purity angle and threshold value was compared and further assured through 3D spectra acquired from PDA (190-400 nm).

Forced degradation

The forced degradation was performed following a previously documented method of Blessy, Patel et al. 10 mg of LK standard and an equal amount of LK samples taken from pooled tablets of both brands (A2A and Eziday) were subjected to thermal hydrolysis, photo-degradation, acid hydrolysis, base hydrolysis and forced oxidation observing conditions as shown in Table 1. All the samples were processed for the data generation after 24 hours incubation under above mentioned conditions. All tests and controls (except thermal hydrolysis and UV test) were processed in dark chamber [8].

Robustness

It is a measure of capacity of an analytical procedure to remain unaffected by small deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Accuracy

Accuracy is a crucial part of the method validation. It is the closeness of agreement between observed results through selected analytical procedure and true value.

Results and Discussion

The present study was designed to develop a validated, simple and reliable HPLC method using PDA detector for the quantitative analysis of LK. Initially, the analytical and instrumental parameters were optimized to establish a well resolved peaks of LK standard.

Linearity

In order to assess the linearity of devised assay, the detector response was plotted versus concentrations and was found to be linear over 1-3 µg/mL (Figure 1). The observed values of regression coefficient (R^2) i.e. 0.999 fits well over the ICH Guideline (2005). Although, a large number of studies has been conducted to analyze LK in various pharmaceutical formulations but linearity of these assays was with relatively smaller coefficient of determination (R^2).

Limit of detection and quantification

The results for LOD and LOQ determination are shown in Table 2. The LOD and LOQ determined for LK using the current method was 0.0364 and 0.110 respectively. Therefore, the current developed method is sensitive as compared to previously reported methods.

Precision

Hossen, Haque et al. calculated % RSD for precision as <0.5. The purpose of current study is to offer authentication that results obtained from different successive measurements are very close to each other under the same conditions [9].

This study is conducted by preparing six fresh replicates of 100% concentration of homogenized mixture of sample and proceeds the intermediate precision among day to day (inter-day and intra-day) and brand to brand. Results are shown in Tables 3 and 4.

SL. No.	Condition	Procedure
1	Thermal Hydrolysis	80°C and 75% relative humidity for 24 hours
2	Photo Degradation	254 nm UV lamp on closed chamber for 24 hours
3	Acid Hydrolysis	Treated with N/10 H_2SO_4 for 24 hours
4	Base Hydrolysis	Treated with N/10 NaOH for 24 hours
5	Induced Oxidation	Treated with 3% H_2O_2 for 24 hours

Table 1: Conditions followed for forced degradation of Losartan potassium (LK).

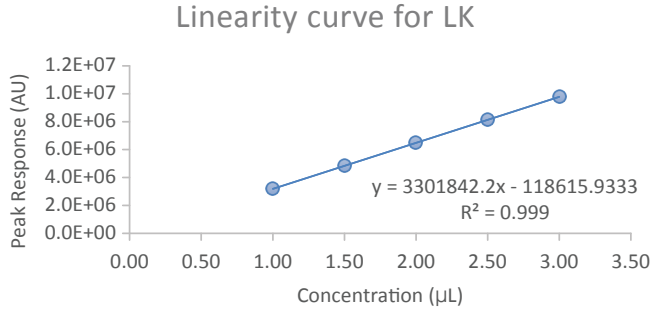

Figure 1: Linearity Curve for LK.

Repeatability and Reproducibility of Developed HPLC Based Stability Indicating Assay

Selectivity, specificity and degradation study

Selectivity and specificity of the method for losartan Potassium was evaluated through PDA (photodiode array detector) by comparing purity angle with threshold value. Lower the purity angle from threshold value confirms the selectivity of method for our specified analyte present in different brands (Eziday and A2A) [10-13]. Effect of various stress factors on standard that caused degradation was also evaluated on selectivity of the method through purity angle threshold value comparison. All these values confirmed the selectivity of the method even in the presence of degradation or impurities as given in Table 5 and their purity plots are given in Figures 2-7.

Robustness of method

Robustness of method is evaluated through minor but deliberate changes in method and measured the ability of method remained unaffected from these changes. These deliberate changes were in flow rate, mobile phase and temperature variations. In the validated method flow rate was varied from 0.8 ml/min to 1.2 ml/min, mobile phase pH up to 3.2

SL. No.	Conc. (µg/mL)	Linear regression Equation	R^2	Y-Intercept	Slope
Std.01	1-3	y=3300918.8x -115803	R^2=0.99999898	3300918.8	115803
Std.02	1-3	y=3300417.6x -117774.6	R^2=0.99999541	3300417.6	117774.6
Std.03	1-3	y=3304190.2x -122270.2	R^2=0.99999782	3304190.2	122270.2
Std.04	1-3	y=3302329.2x -121679.2	R^2=0.99999038	3302329.2	121679.2
Std.05	1-3	y=3301659.8x -118893.8	R^2=0.99999827	3301659.8	118893.8
Mean					119284.16
SD				1315.077	

Table 2: LOD and LOQ determination for LK.

Days	Brands	%RSD of retention time	%RSD of peak area	%RSD of assay
Day – 01	Brand-01	0.132	0.308	0.233
	Brand-02	0.033	0.140	0.128
Day – 02	Brand-01	0.042	0.131	0.129
	Brand-02	0.043	0.156	0.151
RSD		**0.149**	**0.395**	**0.332**

Table 3: Inter-day and Brand to Brand-Intermediate Precision.

Conc.	Intraday (n=6)		Inter-day (n=6)	
(mg/ml)	%Assay	Precision	%Assay	Precision
0.1	99.115	0.129	98.780	0.160

Table 4: Inter-day & Intra-day Precision.

Sample	RT	Area	Purity angle	Threshold value	Peak purity
THR	5.497	3018283	0.036	0.238	Pure
Photo	5.689	2908292	0.036	0.237	Pure
Acidic	5.670	2759760	0.033	0.235	Pure
Basic	5.702	2579636	0.031	0.238	Pure
Oxidation	5.708	2748721	0.038	0.235	Pure

Table 5: Detection of Selectivity.

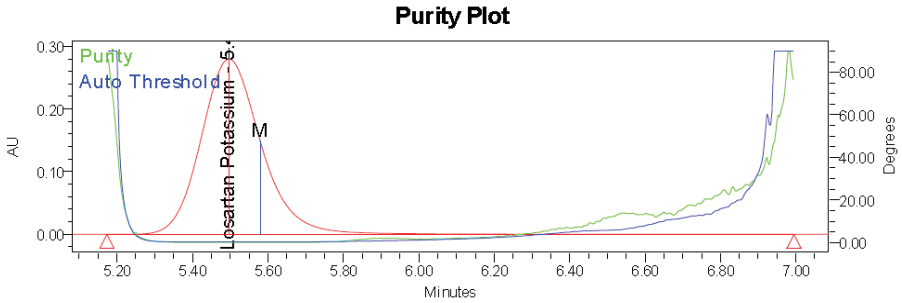

Figure 2: Thermal Stress–Purity plot (Green line showed purity angle, Blue line showed purity threshold, Purity angle<Purity threshold means peak is pure).

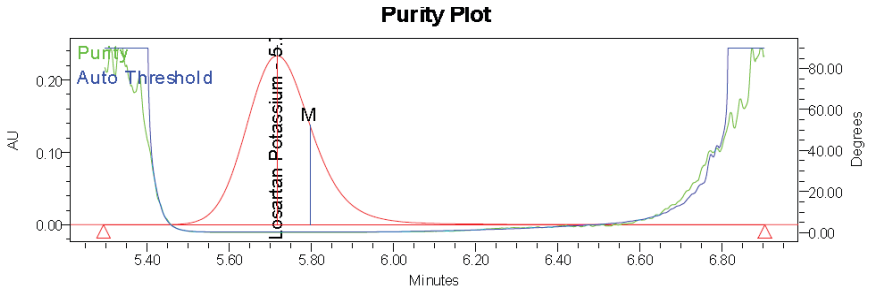

Figure 3: UV Stress-Purity plot (Green line showed purity angle, Blue line showed purity threshold, Purity angle<Purity threshold means peak is pure).

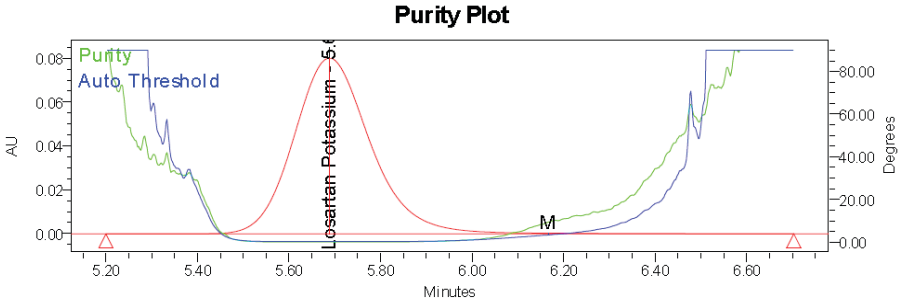

Figure 4: Acid Stress-Purity plot (Green line showed purity angle, Blue line showed purity threshold, Purity angle<Purity threshold means peak is pure).

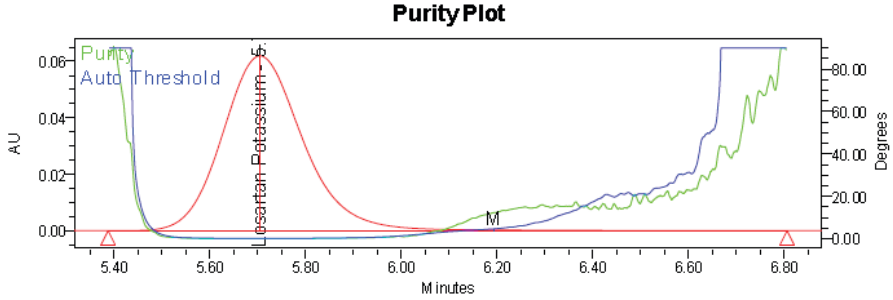

Figure 5: Base Stress-Purity plot(Green line showed purity angle, Blue line showed purity threshold, Purity angle < Purity threshold means peak is pure).

and effect of temperature was observed from ambient to 27°C. On all these deliberate changes, very minor effect on system suitability was observed and method found robust against these minor changes (Table 6).

Accuracy

Method accuracy was assessed by comparing the %RSD of BP method (standard method) with the percentage RSD of the method developed (non-standard method). The RSD for standard method was 1.012%, while for non-standard method it was 1.516% and combined RSD of both two methods was found 1.823% (Table 7) that is as per the criteria of precision of bias which should be less than 2%. In order to meet the criteria for practical bias, the mean should be smaller than permissible variation for the respective analyte quantity, which is ideally considered as zero. The results of the study as summarized in the (Table 8) shows that the mean is 0.021 that clearly indicated that the method developed was accurate as well as precise [14,15].

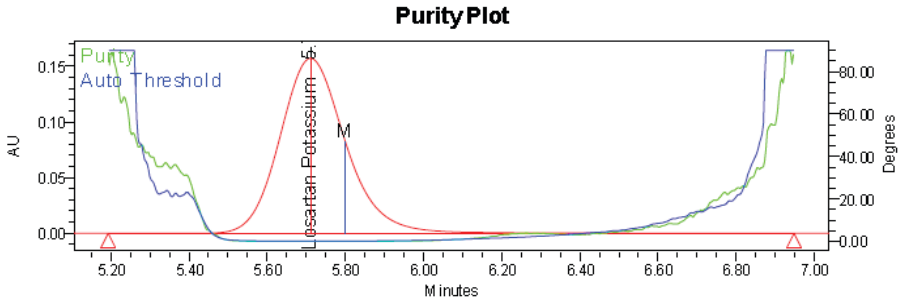

Figure 6: Oxidation Stress-Purity plot (Green line showed purity angle, Blue line showed purity threshold, Purity angle<Purity threshold means peak is pure).

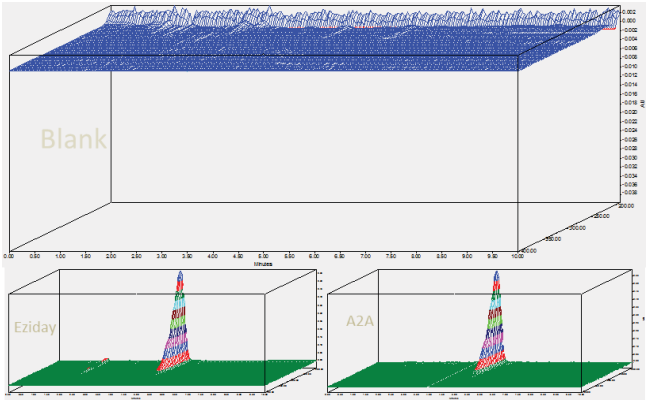

Figure 7: 3D Purity plot.

Variations	Changes	Retention time	Peak response	Tailing factor
pH	3.2	4.888	3071376	1.287
Temperature	27°C	4.734	3077245	1.199
Flow rate	0.8 ml/min	5.599	3064843	1.303
Injection volume	5 ul	4.879	1500837	1.280
	30 ul	4.911	10902988	1.302
Without variation		4.877	3071376	1.200

Table 6: Robustness of Method.

Parameters	IH method		BP method	
	Standard area	Sample area	Standard Area	Sample Area
Average	7106168.66	7211049	6085330.33	6304084
Std. Deviation	124640.19	92282.89	45385.98	80554.14
	1.753	1.279	0.746	1.278
%RSD	1.516		1.012	
Combined %RSD	1.823			

Table 7: Precision for Bias.

Parameters	Assay of IH	Assay of BP	Practical bias
Average	1.0148	1.036	0.021
Std. Deviation	0.013	0.013	
RSD	1.280	1.278	

Table 8: Practical Bias.

Conclusion

Authors of this study developed and validated an isocratic method for the determination of Losartan Potassium which is accurate, reproducible, simple and suitable for LK in solid dosage form (Tablets). Authors used PDA detector in order to determine the peak purity of losartan potassium as well as it proved that degradants and related impurities were also well separated. The present study revealed that RP-HPLC method is more sensitive, stable, rapid and reliable. This method can be successfully employed in pharmaceutical industry in order to minimize the cost and time of analysis.

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