Original Article

Stability indicating reproducible high performance liquid chromatographic method for determination of losartan potassium and atenolol in bulk and tablet dosage forms

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ABSTRACT

An accurate, precise and reproducible high-performance liquid chromatographic (RP-HPLC) method was developed for quantitative estimation of losartan potassium and atenolol simultaneously in tablet dosage forms. Young Lin (S.K.) gradient system ultraviolet detector and RP C18 (Thermo) with 250 mm × 4.6 mm i.d. and 5 µm particle size. Methanol 0.1% O-phosphoric acid (65:35) was used as the mobile phase for the method. The detection wavelength was 274 nm, and flow rate was 0.9 ml/min. In the developed method, the retention time of losartan potassium and atenolol was found to be 7.76 min and 4.05 min, respectively. The drug was subjected to oxidation, acid hydrolysis, alkaline hydrolysis, and heat to apply stress condition for degradation. The method was validated for specificity, linearity, precision, accuracy, robustness, and solution stability. The linearity, precision, range, and robustness were within the limits as specified by the International Conference on Harmonization Guidelines. Hence, the method was found to be simple, accurate, precise, economic, and reproducible. Hence, the proposed methods can be used for the routine quality control analysis of losartan potassium and atenolol in bulk drug as well as in formulations.

Keywords: Atenolol, degradation, losartan potassium, reproducible high performance liquid chromatographic, stress condition

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Introduction

Losartan potassium (LK) is monopotassium salt of 4-butyl-4- chloro-1-[[2′-(1H-tetrazol-5-yl) [1, 1 ′-biphenyl]-4-yl] methyl]- 1H-imidazole-5-methanol (Figure 1)[1,2] and shows angiotensin II receptor antagonist used as an antihypertensive.[3] Atenolol (ATN) is (RS)-4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetamide (Figure 2),[4] which shows beta adrenergic blocker used for hypertension. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance. Literature survey reveals the availability of few methods for estimation of both losartan K and atenolol includes ultraviolet (UV), HPLC as alone or in combination with other drugs.[5-10] No method has been reported for stability indicating method development for simultaneous estimation of both drugs in binary dosage form. This work emphasizes on the stability indicating assay method development for simultaneous estimation of losartan K and atenolol in their combined dosage form by RP-HPLC.

The purpose of this study was to develop a stability indicating method for the simultaneous determination of atenolol and losartan K in bulk drugs and to apply the developed method for the quantitative determination of these drugs from tablets. The HPLC technique was chosen because of its previously mentioned advantages. The proposed method was able to separate the compounds of interest and their degradation products within 10 min. Thereafter, this method was validated as per International Conference on Harmonization (ICH) Guidelines.[11,12] A literature survey has shown that a stability indicating HPLC method for the simultaneous determination of atenolol and losartan K has not been developed. The previously developed methods have been able to separate both the drugs during a minimum run time, but they were not stability indicating, i.e., the separation of various degradation products, employing ICH prescribed stress conditions, was not achieved. In this research article, we report the development and validation of a stability indicating LC method for the simultaneous

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determination of atenolol and losartan K in pharmaceutical dosage form. It separates drug components from degradation products under ICH suggested stress conditions (acid and base hydrolysis, oxidation, photolysis, and thermal stress).[13-15]

Materials and Methods

Instruments

The analysis of the drug was carried out on Young Lin (S.K.) gradient system UV detector. Equipped with RP C_{18} (Thermo), (4.6 mm × 250 mm; 5 µm), SP930 D pump, a 20 µl injection loop and UV730D absorbance detector and running autochro-3000 software.

Materials and reagents

Atenolol and losartan K were obtained as gift samples from Cipla Ltd., Mumbai and Micro Labs Ltd Bangalore, respectively. O-phosphoric acid and methanol were HPLC grade procured from Space Lab. Nashik, hydrochloric acid, sodium hydroxide, and hydrogen peroxide (AR) grade procured from Loba Chemie. Nashik. The pharmaceutical preparations of binary combination of atenolol and losartan K that is Losar Beta tablet (Unichem Laboratories Ltd.). The commercial formulation of atenolol and losartan K is available in the ratio of 1:1 (50/50 mg) in tablet.

Chromatographic conditions

RP C_{18} (Thermo), (4.6 mm × 250 mm) particle size packing 5 µm; detection wavelength 274 nm; flow rate 0.9 ml/min; temperature ambient; sample size 20 µl; mobile phase methanol: Water (OPA 0.05%) (65:35).

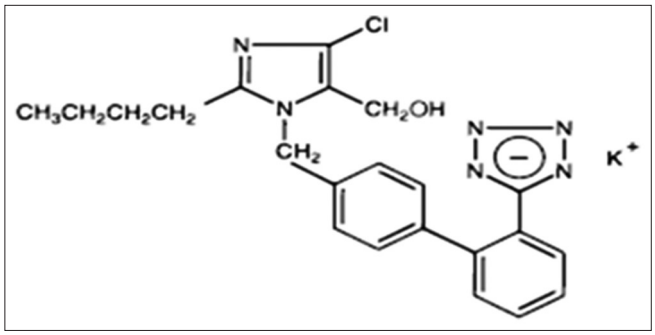

Figure 1: Structure of losartan K

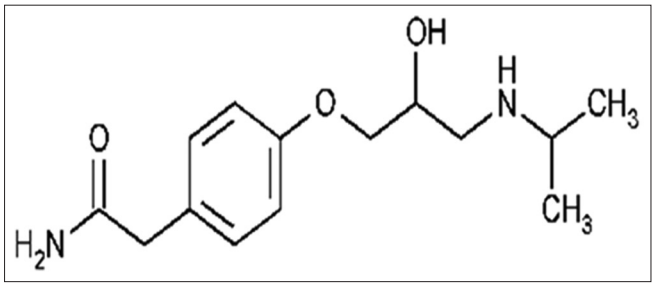

Figure 2: Structure of atenolol

Preparation of standard stock solution

Stock solution (1000 µg/ml)

Stock solutions were prepared by weighing 10 mg each of LK and Ate. The weighed drugs were transferred to two separate 10 ml volumetric flasks and adjust the volume with mobile phase. The stock solutions were sonicated for 10 min. Then, filtered through Whatman's filter paper.

Standard solutions (100 µg/ml)

Take 1 ml solution from stock solution and transfer separately in 10 ml volumetric flask and dilute volume up to 10 ml with mobile phase.

The HPLC analysis was performed on reversed phase high performance liquid chromatographic system with isocratic elution mode using a mobile phase of methanol: O-phosphoric acid (65:35 v/v) on RP C-18 column (250×4.6 mm, 5 µm particle size) with 0.9 ml/min flow rate at 274 nm using UV detector (Figure 3).

Forced degradation study

According to ICH Guideline, the limit of degradation approximately 0-20%. The amount of degradation given in percentage (Table 1).

Acid hydrolysis

These samples were prepared by weighing the drugs 10 mg each and transfer in 10 ml volumetric flask. Then, addition of 2 ml HPLC grade methanol in it and dissolve completely. After complete dissolution add hydrochloric acid (0.1 N) and adjust volume 10 ml with it. After complete preparation of the solution, store it at 80^{0}c about 3 h in a water bath (Figure 4).

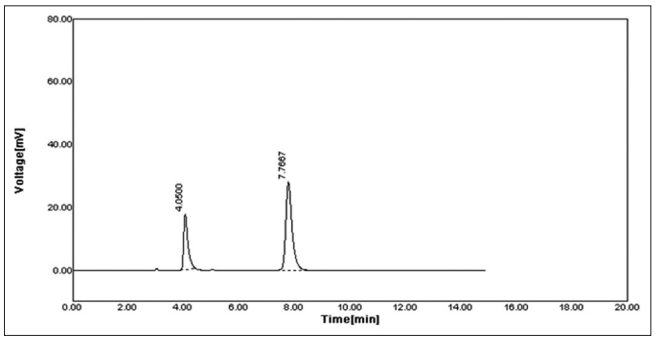

Figure 3: Optimized chromatographic conditions for losartan potassium and atenolol

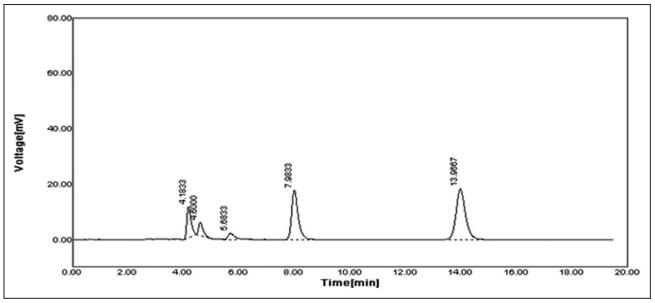

Figure 4: Acid degradation study

Alkaline hydrolysis

These samples were prepared by weighing the samples 10 mg each and transfer in 10 ml volumetric flask. Then, addition of 2 ml HPLC grade methanol in it and dissolve completely. After complete dissolution add sodium hydroxide (0.1 N) and adjust volume 10 ml with it. After complete preparation of the solution, store it at 80°C about 3 h in a water bath (Figure 5).

Oxidation

These samples were prepared by weighing the samples 10 mg each and transfer in 10 ml volumetric flask. Then, addition of 2 ml HPLC grade methanol in it and dissolve completely. After complete dissolution add hydrogen peroxide (3%) and adjust volume 10 ml with it. After complete preparation of the solution, store it at 80°C about 3 h in a water bath (Figure 6).

Thermal degradation

These samples were prepared by weighing the samples 10 mg each and transfer in 10 ml volumetric flask. Then, addition of 2 ml HPLC grade methanol in it and dissolve completely. After complete dissolution add HPLC grade water and adjust volume 10 ml with it. After complete preparation of the solution, store it at 80°C about 3 h in a water bath (Figure 7).

After degrading the samples, allow it to cool. Take 0.5 ml of solution from it and transfer to 10 ml volumetric flask. Adjust volume up to 10 ml with mobile phase. Each sample filtered through 0.22 µ Millipore filter paper for injecting into HPLC.

Table 1: Stress studies (amount of degradation in percentage)

Stress condition	Amount of degradation (%)
Acid hydrolysis	55.98
Alkaline hydrolysis	18.30
Oxidation	10.01
Thermal degradation	0.47

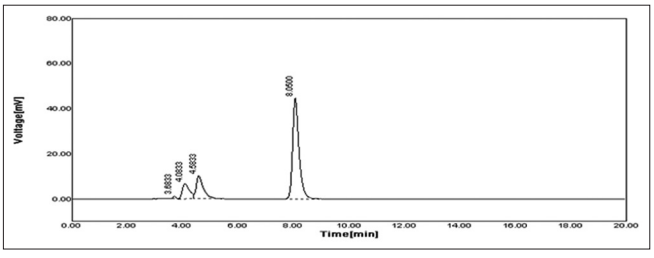

Figure 5: Alkaline degradation study

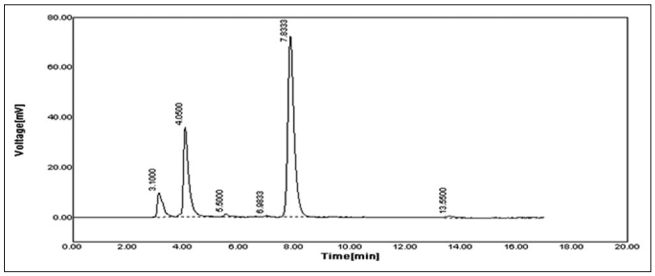

Figure 6: Oxidative degradation study

Method validation

Linearity

Linearity was performed by preparing stock (1000 µg/ml) first then prepare working standard (100 µg/ml) from the stock solution. The linear concentration was prepared and injected in HPLC and response was measured at 274 nm (Figures 8 and 9). The samples injected in triplicates and measure the average peak area to determine standard deviation and relative standard deviation. Furthermore, regression equation was calculated with the help of excel graph by plotting concentration versus peak area (Tables 2 and 3).

Range

The range can be determined as the upper limit of concentration and lower limit of concentration. The linearity range occurs in between 10 and 50 µg/ml. Therefore, the upper concentration limit is 50 µg/ml, and lower concentration limit is 10 µg/ml.

Table 2: Linearity study of losartan potassium

Concentration (µg/ml)	Area I	II	III	Mean±SD	%RSD
10	83.14	85.11	86.11	84.79±1.51	1.78
20	168.76	167.27	166.54	167.52±1.13	0.68
30	269.12	271.54	268.98	269.88±1.44	0.53
40	365.36	367.16	365.98	366.17±0.91	0.25
50	443.86	440.86	444.58	443.10±1.97	0.45
				Average (1.39)	0.738

Regression equation: y=9.152×−8.289, slope: 9.152, intercept: −8.289, correlation coefficient: 0.997, %RSD: 0.738, SD: Standard deviation

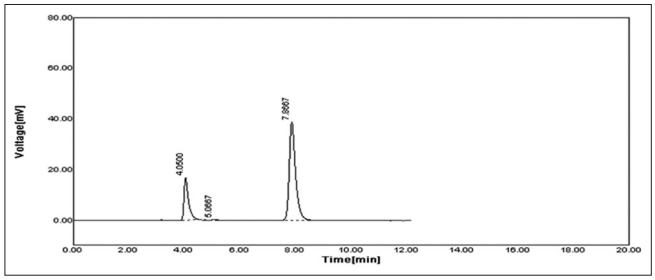

Figure 7: Thermal degradation study

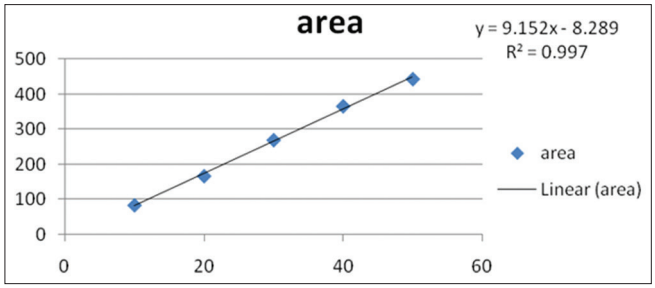

Figure 8: Linearity of LK

Accuracy

Accuracy was performed with the help of recovery method by standard addition of standard solution in pre-analyzed tablet solution in different levels (80%, 100%, and 120%). Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD at each level was calculated and presented in following Table 4. The result indicates that the proposed method was accurate (Tables 4 and 5).

Precision

The precision was performed by intraday (repeatability) and interday (reproducibility) study. The detailed observation is shown in Tables 6-9.

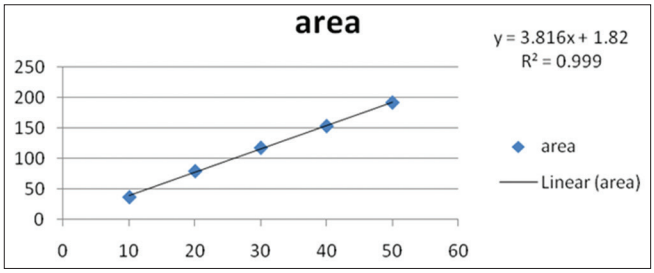

Figure 9: Linearity of ATN

Table 3: Linearity study of atenolol

Concentration (µg/ml)	Area I	II	III	Mean±SD	%RSD
10	37.37	38.21	37.87	37.82±0.42	1.12
20	79.88	77.84	80.11	79.28±1.25	1.58
30	117.54	120.6	116.21	118.12±2.25	1.91
40	154.72	153.18	152.63	153.51±1.08	0.71
50	192.33	190.18	193.25	191.92±1.58	0.82
				Average±1.32	1.22

Regression equation: y=3.816×+1.82, slope: 3.816, intercept: 1.82, correlation coefficient: 0.999, %RSD: 1.04, SD: Standard deviation

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions. LOQ is the lowest concentration of an analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions (Table 10).

Robustness

To determine robustness of the method, experimental conditions were purposely altered and chromatographic resolution between atenolol and losartan K were evaluated. The flow rate of the mobile phase was 0.9 ml/min. To study the effect of flow rate on resolution of atenolol and losartan K, it was changed to 0.1 units from 0.8 ml/min to 1 ml/min. While other mobile phase components were held constant. The effect of mobile phase composition on resolution of atenolol and losartan K was studied with methanol: 0.1% O-phosphoric acid at 64:36 (v/v) and 66:34 (v/v). The effect of wavelength on resolution of atenolol and losartan K was studied with applying wavelengths 273 nm and 275 nm. Detailed information about the robustness incorporated inside (Tables 11 and 12).

System suitability

The system suitability parameter such as capacity factor, asymmetry factor, tailing factor, HETP, and number of theoretical plates was also calculated. It was observed that all the values are within the limits and the results are shown in Table 12. The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of losartan potassium and atenolol in tablet formulation. The results are furnished in Table 13.

Specificity

The specificity of method was performed by comparing the chromatograms of blank, standard and sample. It was found that there is no interference due to excipients in the tablet formulation and also

Table 4: Accuracy study of losartan potassium

Level (%)	Average area	Amount recovered	% recovered	SD	%RSD	Average % RSD
80	322.573	15.94	99.63	1.46	0.45	0.96
100	360.99	20.13	100.65	5.47	1.52	
120	395.87	23.94	99.75	3.56	0.9	

SD: Standard deviation

Table 5: Accuracy study of atenolol

Level (%)	Average area	Amount recovered	% recovered	SD	%RSD	Average % RSD
80	139.13	16.04	100.31	1.41	1.02	0.62
100	153.83	19.92	99.6	0.76	0.49	
120	169.42	24.04	100.17	0.6	0.36	

SD: Standard deviation

found a good correlation between the retention time of standard and sample of losartan potassium and atenolol. The detail observation is shown in Table 14.

Table 6: Repeatability study of LK

Concentration	Average area	SD	%RSD
40 µg/ml	367.7617	3.12	0.85

SD: Standard deviation

Table 7: Reproducibility studies of LK

Concentration	Areas (average)			SD	Average %RSD
	Day 1	Day 2	Day 3		
40 µg/ml	367.7617	372.39	375.6733	2.93	0.90

SD: Standard deviation

Table 8: Repeatability studies of atenolol

Concentration	Average area	SD	%RSD
40 µg/ml	177.5	1.12	0.63

SD: Standard deviation

Table 9: Reproducibility studies of atenolol

Concentration	Areas (average)			SD	Average %RSD
	Day 1	Day 2	Day 3		
40 µg/ml	177.5	179.42	182.83	3.06	0.75

SD: Standard deviation

Table 10: LOD and LOQ studies for losartan potassium and atenolol

Parameters	Drug	
	LK	Atenolol
SD	1.39	1.32
SLOPE	9.152	3.816
LOD	0.5	1.14
LOQ	1.52	3.49

LOD: Limit of detection, LOQ: Limit of quantitation

Results and Discussion

This study was aimed at stability indicating RP-HPLC method development and validation for simultaneous estimation of losartan potassium, atenolol and their degradation products. A nonpolar C-18 analytical chromatographic column was chosen as the stationary phase for the separation and simultaneous determination of losartan potassium, atenolol and their degradation products. Mixtures of commonly used solvents such as water and methanol in different combinations were tested as mobile phases. The choice of the optimum composition is based on the chromatographic response factor, a good peak shape with minimum tailing. A mixture of methanol and 0.1% O-phosphoric acid in the ratio of 60:35 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was well defined, better resolved and almost free from tailing. The retention times of the losartan potassium and atenolol were found to be 4.05 and 7.76 min, respectively. The forced degradation study was conducted for determining the stability indicating power of an analytical procedure. The result of the stress studies shown in Table 1.

The linearity was found satisfactory for losartan potassium and atenolol in the range 10-50 µg/ml for both Tables 2 and 3. The regression equation of the linearity curve between concentrations of losartan potassium and atenolol over its peak areas were found to be y = 9.152×-8.289 (where "Y" is the peak area and X is the concentration of losartan potassium in µg/ml) and y = 3.816×+1.82 (where Y is the peak area and X is the concentration of atenolol in µg/ml), respectively. The percent recoveries of the drug solutions were studied at three different concentration levels. The percent individual recovery and the %RSD at each level were within the acceptable limits (Tables 4 and 5). This indicates that the method is accurate. The absence of additional peaks in the chromatogram indicates non-interference of the commonly used excipients in the tablets, and hence the method is specific. Precision of the method

Table 11: Robustness studies for LK

Parameter	Condition	Concentration	Area	SD	%RSD
Flow rate (ml/min)	0.8	40 µg/ml	204.36	2.194751	1.07
	1		159.59	1.496474	0.9
Wavelength (nm)	273		190.96	2.212811	1.16
	275		174.07	1.489262	0.84
Mobile phase composition	64:36		179.6	0.966713	0.53
	66:34		177.56	2.368889	1.34

SD: Standard deviation

Table 12: Robustness studies for atenolol

Parameter	Condition	Concentration	Area	SD	%RSD
Flow rate (ml/min)	0.8	40 µg/ml	97.86223	1.74	1.8
	1		87.65667	1.49	1.7
Wavelength (nm)	273		86.39667	1.14	1.23
	275		94.54333	1.01	1.06
Mobile phase composition	64:36		93.12333	1.5	1.6
	66:34		87.64667	1.56	1.7

SD: Standard deviation

Table 13: Data for system suitability studies for losartan potassium and atenolol

Parameter	Experimental value	Limit as per USP
Retention time (min)	7.9 and 4.05	<10
Tailing factor	1.4 and 1.4	<2
Number of theoretical plates	138300 and 36347	More than 2000
Capacity factor		2-10
Asymmetric factor	1.4 and 1.3	<2
%RSD	0.73 and 1.04	<2

Table 14: Specificity study

Name of solution	RT	
	Losartan potassium	Atenolol
Blank	No peak	No peak
Standard	7.95	4.05
Sample	7.9	4.06

RT: Retention time

was studied by repeated and shown (Tables 6-9). This reveals that the method is quite precise. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive (Table 10). The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that this method is robust (Tables 11 and 12). The system suitability studies were conducted to check various parameters such as theoretical plates and tailing factor (Table 13). The specificity study is shown in Table 14.

Therefore, the proposed method was simple, specific and sensitive and can be used for simultaneous analysis of losartan potassium, atenolol and their degradation products in bulk samples and its tablet dosage forms.

Conclusion

A new analytical method was developed to be routinely applied to simultaneous determination of atenolol and losartan K in pharmaceutical dosage form. In this study, the stability of atenolol and losartan K in present dosage forms was established through employment of ICH recommended stress conditions. The developed procedure was evaluated for specificity, linearity, accuracy, precision, and robustness to ascertain the stability of the analytical method. The method was proved to be specific, linear, precise, accurate, robust, and stability indicating. Hence, the method is recommended for routine quality control analysis and stability sample analysis.

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