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# Simultaneous estimation of Losartan and Atenolol by RP-High Performance Liquid Chromatography

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**Abstract- Losar-beta is available for the treatment of hypertension. It contains losartan potassium(LS; 50mg) and atenolol (AT; 50mg). In the present study, simple, rapid, precise and accurate methods for the simultaneous estimation of these drugs have been developed and validated by HPLC. The method was validated with respect to its linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy and robustness. HPLC was carried out with C-18 ODS column having 5µm, 250mm×4.60mm specifications along with a UV detector (235nm). The mobile phase used was acetonitrile: water: methanol (60:30:10 ) at a flow rate of 1ml/minute. Linearity was established by least square linear regression analysis and it was found to be linear over the concentration range of 5-50 µg/mL for LS and AT. LOQ was found to be 0.071 µg/mL for LS and 0.98 µg/ml for AT. The LOD was 0.002 µg/mL and 0.032 for LS and AT respectively. In precision studies, the % RSD was found to be 0.212 and 0.094 for LS and AT respectively. Percentage recovery was found to be 99.32 ± 0.23 for LS and 99.34± 0.28 for AT.**

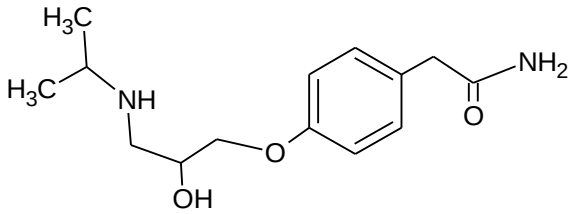
**Keyword – losartan, atenolol, HPLC, method validation, simultaneous estimation.**

## 1. INTRODUCTION

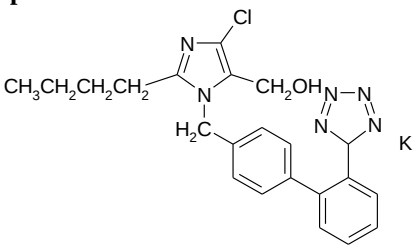
Losar-beta is available in the market for the treatment of hypertension. It contains losartan potassium (LS; 50mg) and atenolol (AT; 50mg). Losartan is a agiotensin II receptor antagonist and atenolol is β1 receptor antagonist. These drugs are more effective in combination therapy as compared to monotherapy **[1, 2]**. The combinations of these drugs are marketed under various brand names as tablet dosage form. Literature reveals that very few spectrophotometric and chromatographic methods are available for the simultaneous estimation for these combinations, which are also too expensive. Hence, it was thought that a simultaneous estimation for these combinations can be carried out to make the methods more cost effective **[3, 4]**.

High performance liquid chromatography is basically a highly improved form of column chromatography. HPLC is a method in which the compounds of a mixture are separated on an adsorbent column in a flowing system.

**Atenolol**




**Losartan potassium**




## 2. EXPERIMENTAL

A combination of atenolol and losartan was selected for analysis. These drugs are more effective in combination therapy as compared to monotherapy. Literature reveals that very few chromatographic methods are available for the simultaneous estimation for these combinations, which are also too expensive.Hence, it was thought that a simultaneous estimation for this combination can be carried out to make the method more cost effective.

**Method development**

**Table 1: Mobile phase selection for Atenolol - Losartan potassium**

| Mobile phase | Ratio V/V | Flow rate (mL/ min) | Retention Time in min. | | Conclusion |
|---|---|---|---|---|---|
| | | | ATN | LOS | |
| **ACN: MeOH** | 50:50 | 1.0 | 5 | 28 | Splitting of peak observed and elution time is more. |
| **ACN: MeOH :Water** | 33:2: 65 | 1.0 | 2.05 | 25 | Elution time is slightly decreased and splitting is increased. |
| **ACN: Water: MeOH** | 60:30 :10 | 1.0 | 2.18 | 5 | Peak was sharp and suitable |

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The mobile phase, most suitable for analysis was acetonitrile : water: methanol 60:30:10 flow rate was 1.0 mL/min and detection wavelength was 235 nm.

**Selection of separation variable**

Considering the theoretical information and after several trials for mobile phase selection and flow rate the following separation variables were selected which were constant during whole experiment are shown in Table 2

**Table 2: Selection of separation variable**

| Variable | Condition |
|---|---|
| **Column** | |
| Dimension. | 250mm x 4.60mm |
| Particle Size | 5µ |
| Bonded Phase | Octadecylsilane ($C_{18}$) |
| **Mobile Phase** | |
| Acetonitrile(HPLC) | 60% |
| Water | 30% |
| Methanol | 10% |
| **Flow Rate** | 1.0 mL/min |
| **Temperature** | Room Temperature(27-28°C) |
| **Sample Size** | 20µl |
| **Detection wavelength** | 235nm |
| **Retention time** | |
| Atenolol | 2.18 |
| Losartan | 5.00 |

**Preparation of standard solution**

**Atenolol:** Atenolol (100mg) was accurately weighed and transferred to a 100 mL volumetric flask and dissolved in mobile phase, 2.5mL was taken and further diluted to 25mL with mobile phase. Concentration of stock A is 100 µg/mL.

**Losartan:** Losartan (100mg) was accurately weighed and transferred to a 100 mL volumetric flask and dissolved in mobile phase, 2.5mL was taken and further diluted to 25mL with mobile phase. Concentration of stock B is 100 µg/mL.

**Mixed standard:** From the stock solutions A and B, aliquots were diluted up to 10 mL with a mobile phase to obtain the concentrations given in Table 3**.**

**Table 3: Preparation of mix standard**

| S.No. | Concentration of atenolol (µg/mL) | Concentration of losartan potassium(µg/mL) |
|---|---|---|
| 1 | 5 | 5 |
| 2 | 10 | 10 |
| 3 | 15 | 15 |
| 4 | 20 | 20 |

**Calibration graph**

To find the linearity, a series of dilution ranging from 2-200 µg/mL (2, 5-50, 100 and 200) for atenolol and losartan potassium were prepared in the same manner as described above. All the solutions were filtered through a 0.22µm membrane filter and injected. The chromatograms were recorded. A calibration graph was plotted between the area under curve Vs respective concentration. Regression equation was derived and results are as shown in Table 4 and 5; Fig.1 and 2.

**Table 4: Calibration curve data for atenolol**

| S.No. | Concentration (µg/mL) | AUC |
|---|---|---|
| 1 | 5 | 100562 |
| 2 | 10 | 209968 |
| 3 | 15 | 301376 |
| 4 | 20 | 410019 |
| 5 | 25 | 509214 |
| 6 | 30 | 612636 |

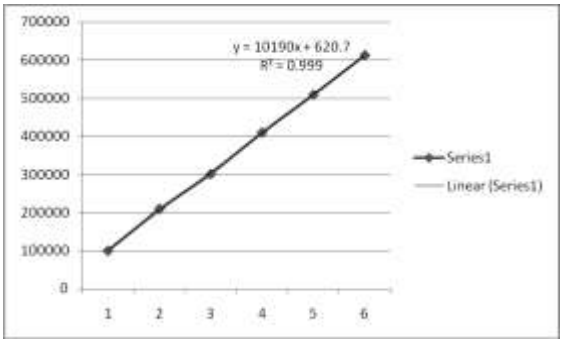


**Fig. 1 Calibration graph of Atenolol**

**Regression Equation**

**Y = mx + c,**

Where, Y = AUC,
m = Slope (10190)
X = Conc. in µg/mL,
C = Intercept (620.7)

**AUC =** 10190 conc. + 620.7

**Correlation coefficient ($r^2$)** = 0.999

**Table 5: Calibration curve data of losartan potassium**

| S.No. | Concentration (µg/mL) | AUC |
|---|---|---|
| 1 | 5 | 282900 |
| 2 | 10 | 561006 |
| 3 | 15 | 840988 |
| 4 | 20 | 1124548 |
| 5 | 25 | 1429556 |
| 6 | 30 | 1682122 |

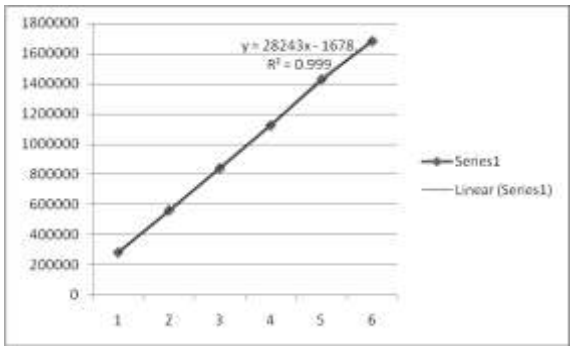


**Fig. 2: Calibration graph of Losartan potassium**

**Regression Equation**

Y = mx + c,

Where Y = AUC,

m = Slope (28243),

X = Conc. in µg/mL,

c = Intercept (-1678)

AUC = 28243 Conc.-1678

Correlation coefficient ($r^2$) = 0.999

**Chromatogram**

The retention time of atenolol and losartan potassium were observed at 2.177 min. and 5.0 min. The complete elution of both drugs was achieved in 7 min. at 235 nm. Chromatogram of atenolol and losartan potassium observed is shown in Fig. 3.

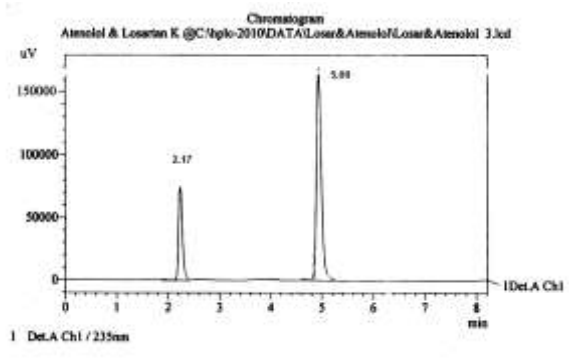


**Fig. 3: Chromatogram of Atenolol and Losartan potassium**

**HPLC Peak table of atenolol and losartan potassium**

| Name | Retention time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| AT | 2.177 | 419062 | 79982 | 26.216 | 33.10 |
| LS | 5.000 | 1136129 | 170002 | 73.784 | 66.90 |
| | | 1555191 | 249984 | 100.00 | 100.00 |

**Validation of developed method**

**Linearity**

Different dilutions of atenolol and losartan potassium ranging from 1µg/mL to 50 µg/mL of were prepared and detected by HPLC. Concentration of Atenolol and Losartan potassium at 235 nm found linear in the range of 5µg/mL-50µg/mL.

**Accuracy**

The recovery studies were performed to validate the accuracy of the developed method with different concentrations of preanalyzed sample solution of tablet. Varying concentration of the standard drug was added and then its recovery was analyzed and results are shown in Table 6 and 7.

**Intermediate precision**

Mixed standard dilutions of Atenolol and Losartan potassium were prepared and their analysis was carried out on different days in different concentrations and analyst to analyst intermediate precision was done. The results were validated statistically and shown in Table 8 and 9.

**Robustness**

As per ICH guidelines, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected.

The ratio of mobile phase was acetonitrile : water : methanol 60:30:10(v/v/v) Change in ratios of mobile phase from 60:30:10 (v/v/v) to 61:29:10 (v/v/v) and 59:31:10 (v/v/v) was done.

These variations did not cause any significant difference in resolution of HPLC method and the results are shown in Table 10**.**

**Limit of Detection (LOD)**

Based on the standard deviation of the response and slope The detection limit is express as:

$$LOD = \frac{3.3\ \sigma}{S}$$

Where $\sigma$ = the standard deviation of the response

S = the slope of the calibration curve.

For Atenolol it was found to be 0.032µg/mL and for losartan potassium, it was 0.002µg/mL.

**Limit of Quantitation (LOQ)**

Based on the standard deviation of the response and the slope

The quantization limit is expressed as:

$$LOQ = \frac{10\ \sigma}{S}$$

For atenolol it was found to be 0.98 µg/mL and for losartan it was 0.071µg/mL

**Stability**

Both the drug solution containing 10µg/mL was analyzed by HPLC method at 1, 2, 3, 5 and 24 hours after preparation. The behavior of the analyte remained unchanged up to 24 hours from its preparation. All the measurements were made at room temperature (27-28$^{\circ}$C).

**Analysis of marketed tablets**

Commercial formulations of atenolol and losartan potassium (Losar-beta) were selected for analysis. Twenty tablets were weighed, their coating was removed using ethyl acetate and powdered separately. Weight equivalent to 10mg of atenolol and losartan potassium was dissolved in 10mL mobile phase and then sonicated for 15min. the supernatant was filtered through 0.22µm membrane filter. Different concentration of solutions were prepared by serial dilution technique, as per

standard and each dilution was analyzed. The result of analysis of marketed formulation is shown in Table 11.

**Results and discussion**

The present work comprised of development of an analytical method for the simultaneous estimation of atenolol and losartan by reverse phase high performance liquid chromatography, as well as, validation of the developed method. The commercially available tablet dosage forms selected for the estimation is Losar beta of Unichem Ltd. containing Atenolol-50 mg and LosartanPotassium-50mg.

The calibration curve was plotted between concentration and AUC measured at the selected wavelength of 235 nm. The concentration of drugs in the tablet was found by using slope and 'Y' intercept of linearity curve. Validation challenges showed that the methods show reproducibility when carried out by different persons, in the same or different laboratories using different reagents, etc.

Considering the solubility, solvent triangle optimization, column performance, peak performance, the best wavelength and the mobile phase chosen was acetonitrile: water: methanol 60:30:10(v/v/v). The wavelength selected for analysis is 235 nm.

**Developed equations for estimation atenolol and losartan (Losar-beta)**

For Atenolol - AUC =10190 conc. + 620.7

For Losartan- AUC = 28243 conc.-1678

**Validation of the HPLC Method**

The method was validated with respect to its linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy and robustness**.**

**Table 6: Recovery data for accuracy of Atenolol**

| Level of standard addition (%) | Amount found in three replicate (mg) | | | %Recovery±SD |
|---|---|---|---|---|
| 90 | 49.82 | 49.98 | 49.86 | 99.88±0.12 |
| 100 | 49.48 | 49.76 | 49.88 | 99.34±0.28 |
| 110 | 49.28 | 49.98 | 49.94 | 99.65±0.32 |

**Table 7: Recovery data for accuracy of Losartan**

| Level of standard addition (%) | Amount found in three replicate (mg) | | | %Recovery±SD |
|---|---|---|---|---|
| 90 | 49.68 | 49.58 | 49.82 | 99.48±0.18 |
| 100 | 49.47 | 49.65 | 49.88 | 99.32±0.23 |
| 110 | 49.64 | 49.86 | 49.98 | 99.65±0.18 |

**Table 8: Intermediate precision: day-to-day**

| Atenolol | | | Losartan | | |
|---|---|---|---|---|---|
| **Conc. (µg/mL)** | **Day-1** | | **Conc. (µg/mL)** | **Day 1** | **Day-2** |
| 5 | 50.28 | 50.18 | 5 | 50.22 | 50.42 |
| 10 | 50.18 | 50.12 | 10 | 49.86 | 50.28 |
| 20 | 50.02 | 49.98 | 20 | 50.06 | 49.89 |
| Mean | 50.16 | 50.093 | Mean | 50.05 | 50.20 |
| **Mean** | | **50.13** | **Mean** | | **50.12** |
| **S.D.** | | **0.05** | **S.D.** | | **0.11** |
| **%RSD** | | **0.094** | **%RSD** | | **0.212** |

**Table 9: Intermediate precision: Analyst-to-Analyst**

| Atenolol | | | Losartan | | |
|---|---|---|---|---|---|
| **Conc. (µg/mL)** | **A-1** | **A-2** | **Conc. (µg/mL)** | **A-1** | **A-2** |
| 5 | 50.32 | 50.12 | 5 | 50.18 | 50.28 |
| 10 | 50.10 | 49.78 | 10 | 49.82 | 49.84 |
| 20 | 50.20 | 50.26 | 20 | 50.08 | 49.98 |
| Mean | 50.21 | 50.05 | Mean | 50.02 | 50.03 |
| **Mean** | | **50.13** | **Mean** | | **50.03** |
| **S.D.** | | **0.100** | **S.D.** | | **0.002** |
| **%RSD** | | **0.100** | **%RSD** | | **0.003** |

A-1 = Analyst 1, A-2 =Analyst 2

**Table 10: Determination of Robustness**

| Drug concentration found after mobile phase variation | | | | | |
|---|---|---|---|---|---|
| 60:30:10 | | 61:29:10 | | 59:31:10 | |
| **ATN** | **LOS** | **ATN** | **LOS** | **ATN** | **LOS** |
| 50.27 | 50.20 | 50.12 | 49.78 | 49.98 | 49.98 |
| 50.16 | 50.16 | 49.88 | 50.26 | 50.23 | 50.10 |
| 50.30 | 50.16 | 49.68 | 50.22 | 50.12 | 50.24 |

**Table 11: Result of analysis of Losar-beta tablet**

| Amount present (µg/mL) | | Conc. found (µg/mL) | | % Found | |
|---|---|---|---|---|---|
| **ATN** | **LOS** | **ATN** | **LOS** | **ATN** | **LOS** |
| 50 | 50 | 50.26 | 50.25 | 100.44 | 100.56 |
| 50 | 50 | 50.20 | 49.88 | 100.32 | 99.82 |
| 50 | 50 | 49.98 | 49.98 | 99.96 | 99.96 |
| | | | **Mean** | 100.24 | 100.11 |
| | | | **S.D.** | **0.177** | **0.278** |
| | | | **%RSD** | **0.177** | **0.278** |

**Table 12: Validation data for the developed RP-HPLC method**

| Validation Parameters | Losar-Beta | |
|---|---|---|
| | **Atenolol** | **Losartan** |
| **Linearity ($r^2$)** | 0.9994 | 0.9994 |
| **Accuracy (%SD)** | 0.1200 | 0.1000 |
| **Precision (%SD)** | | |
| Analyst variation | 0.0470 | 0.1060 |
| Inter day Variation | 0.1000 | 0.0020 |
| **LOD** | 0.0320 | 0.0021 |
| **LOQ** | 0.9800 | 0.0700 |

## 4. CONCLUSION

The HPLC method developed is simple, precise, rapid, selective and economical for the simultaneous estimation of atenolol and losartan potassium in solid dosage form. It can also be used for the analysis of these drugs in biological fluids and in quality control laboratories.

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## AUTHOR'S PROFILE

Dr. Asmita Gajbhiye has about 15 years experience of research and teaching experience at both UG and PG levels. She is a well renowned scientist who has published more then 25 papers in journals of international and national repute and presented more then 50 papers in the various conferences/ seminars and symposia at national and international level. She has successfully completed the various research projects at PG and Ph. D. level. She has also received the best presentation awards at national level. Her research projects have been appreciated at international level during presentation of research papers. She has delivered invited lectures and chaired many sessions in several National and International conferences and symposia in India and abroad. Presently, she is working as Associate Professor in Department of Pharmaceutical Sciences, Dr. H.S. Gour Central University, Sagar, MP.