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# SIMULTANEOUS ESTIMATION AND VALIDATION OF ATENOLOL, HYDROCHLORO THIAZIDE AND LOSARTAN K IN TABLET DOSAGE FORM BY RP-HPLC METHOD.

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**Abstract**

A simple RP-HPLC method was developed and validated for simultaneous estimation of Atenolol, Hydrochloro thiazide and Losartan K from pharmaceutical dosage forms. A sensitive chromatographic separation was accomplished on thermo scientific $C_{18}$ column (20 cm×4.6 mm, 5µ) with mobile phase consisting of Acetonitrile : phosphate buffer (pH 3.6 adjusted with anhydrous disodium hydrogen phosphate) in the ratio of 70:30 v/v, at a flow rate of 1.2 ml/min and eluents monitored at 229 nm. The developed method was validated in terms of accuracy, precision, linearity and limit of detection, limit of quantification, robustness and solution stability. The proposed method can be used for the estimation of these drugs in combined pharmaceutical dosage forms.

**Key words:** Simultaneous estimation, Atenolol, Hydrochloro thiazide, Losartan K.

## Introduction

Atenolol is (*RS*)-2-{4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl} acetamide. Atenolol is a selective $\beta_1$ receptor antagonist, a drug belonging to the group of beta blockers. Hydrochlorothiazide is 6-chloro-1, 1-dioxo-3, 4-dihydro-2*H*-1, 2, 4 benzothia-diazine-7-sulfonamide[1-3]. Hydrochlorothiazide belongs to the thiazide class of diuretics. It reduces blood volume by acting on kidneys to reduce sodium reabsorption in the distal convoluted tubule. The major site of action in the nephron appears on an electroneutral Na+-Cl- co-transporter by competing for the chloride site on the transporter. By impairing Na transport in the distal convoluted tubule, hydrochlorothiazide induces a natriuresis and concomitant water loss[1-3]. Losartan is 2-Butyl-4-chloro-1-[[2-(1H-tetrazol-5-yl) [1,1-biphenyl] -4-yl]methyl]-1H-imidazole-5-methanol monopotassium salt.

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Losartan is in a group of drugs called angiotensin II receptor antagonists. Losartan is used to treat high blood pressure (hypertension). It is also used to lower the risk of stroke in certain people with heart disease. The simultaneous determination of Losartan potassium and Atenolol in tablets by HPLC[5]. And the Simultaneous determination of valsartan and hydrochlorothiazide in tablets by first derivative UV spectrophotometry and LC[6]. And the determined Losartan potassium and Hydrochlorothiazide in tablet dosage form by simultaneous spectrophotometric estimation[9]. Literature surveys reveal that no method has been reported for these combinations. The present manuscript describes a novel LC method which is simple, rapid, precise, sensitive, selective and accurate isocratic reverse phase HPLC method for simultaneous estimation of Atenolol, Hydrochlorothiazide and Losartan K in tablet dosage form.

## Experimental Reagents and Materials

A tablet Losar-Beta-H (Unichem) contains 50mg of Atenolol (ATN), 12.5 mg of Hydrochloro thiazide (HCT) and 50mg of Losartan K (LSK). Methanol HPLC grade was procured from Merck Ltd, Mumbai. Citric acid and

anhydrous disodium hydrogen phosphate AR grade were procured from Qualigens Fine Chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system. Reference standards of Atenolol, Hydrochlorothiazide and Losartan K were gift samples from Ranbaxy Laboratories Limited.

**Apparatus and Chromatographic conditions**

The HPLC system consisted of a separation module (Water Alliance 2695) and Photo Diode Array (PDA) detector. An isocratic elution was performed on thermo scientific C18 column (20cm x 4.6 mm, 5µ). The mobile phase was degassed and filtered (0.45 µ, Millipore) mixture of Acetonitrile and phosphate buffer ($p^H$ 3.6) adjusted with anhydrous disodium hydrogen phosphate (70:30 v/v). Injection volume was 20µl and run time was 12min and flow rate was 1.2ml/min. The column was maintained ambient temperature and the eluents were detected at 229nm. Quantification was achieved by peak area-ratio method with reference to the standards.

**Preparation of stock and standard solutions of Atenolol, Hydrochlorothiazide and Losartan K**

Standard stock solution (1000µg/ml) of ATN, HCT and LSK were prepared separately in Acetonitrile. The working standard solutions were prepared and further diluted in mobile phase to contain a mixture of ATN, HCT and LSK in over the linearity range from 10-90µg/ml, 5-25µg/ml and 10-90µg/ml respectively.

**Estimation of drugs from marketed formulations**

Twenty tablets, each containing 50mg of ATN, 12.5 mg of HCT and 50mg of LSK were weighed and tablet contents are finely powdered. A quantity of powder equivalent to 50mg of ATN, 12.5 mg of HCT and 50mg of LSK were weighed and transferred into 100ml of standard volumetric flask containing 50 ml of Acetonitrile. The sample was kept in an ultrasonic bath for 20 min and further diluted to 100ml by using mobile phase. Then it is filtered through 0.2µ membrane filter paper. 10 ml of this solution was further diluted 100ml to get a concentration of 50 µg/ml of ATN, 12.5 µg/ml of HCT and 50 µg/ml of LSK. 20µl of this solution was injected into HPLC system and chromatograms were recorded. The amount of ATN, HCT and LSK present in each tablet was calculated by comparing the peak area of the standard solution and sample. The amount of the drugs were calculated and tabulated in table 01.

## Results and Discussion

The HPLC procedure was optimized with a view to develop precise and accurate assay method. Various mobile phase systems were prepared and used to provide an appropriate chromatographic separation, but the proposed mobile phase comprising of Acetonitrile, phosphate buffer ($p^H$ 3.6) (70:30 v/v) gave a better resolution. Using UV-visible PDA detector at 229nm carried out the detection. Amongst the several flow rates tested (0.8 - 2.0 ml/min), the flow rate of 1.2 ml/min was the best for all the drugs with respect to location and resolution of peaks. The retention time of ATN, HCT and LSK was found to be 2.6, 5.6 and 9.2 min respectively. The chromatograms of standard and sample solution of ATN, HCT and LSK were shown in figure 01 and 02 .The asymmetry factor of ATN, HCT and LSK was found to be 0.72, 0.54 and 0.81 respectively, which indicates symmetrical nature of the peak. The percentage label claim of individual drugs found in formulations were calculated and presented in table 01.The results of analysis shows that the amounts of drugs estimated were in good agreement with the label claim of the formulations.

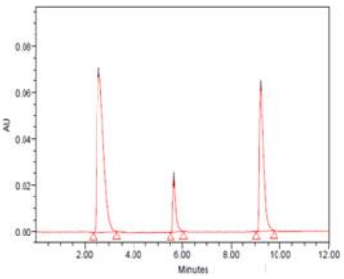


**Figure 01: Chromatogram of standard**

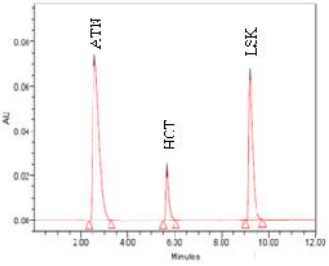


**Figure 02: Chromatogram of sample**

**Table 01: Table for Assay**

| S.No. | Tablet sample | Label claim (mg/tablet) | *Amount Present (mg/tablet) | *Percentage Label claim (%w/w) |
|---|---|---|---|---|
| 1 | **Atenolol** | 50 | 49.72 | 99.44 |
| 2 | **Hydrochloro thiazide** | 12.5 | 12.38 | 99.04 |
| 3 | **Losartan K** | 50 | 50.20 | 100.40 |

**Method Validation**

The proposed method was validated with respect to accuracy, precision, linearity and range, limit of detection, limit of quantification, robustness and stability of analytical solutions following the guidelines of International Conference on Harmonization.

**Accuracy and Precision**

The accuracy of the method was determined by recovery experiments. It was confirmed by studying the recovery at three different concentrations 50%, 100%, and 150 % of those expected by spiking a previously analyzed test solution with additional standard drug solutions, the analysis being done in replicate. The %RSD was within the acceptable limit ($\leq$ 2%). It is evident from the results of accuracy study, reported in table 02, that the proposed method enables very accurate quantitative simultaneous estimation of ATN, HCT and LSK.

The Precision of the method was demonstrated by system precision and method precision studies. In the system precision studies, six replicate injections of the working standard solution prepared as per the proposed method and chromatograms were recorded. Standard deviation and relative standard deviation for the area was calculated and presented in [Table 3]. In the method precision studies, six replicate injections of the analyte solution prepared as per the proposed method and chromatograms were recorded. Standard deviation and relative standard deviation for the area was calculated and presented in table 04. From the data obtained, the developed RP-HPLC method was found to be precise.

**Table 02: Accuracy of the method**

| Drug | %Concentration (at specification Level) | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean % Recovery |
|---|---|---|---|---|---|
| | 50% | 25 | 24.82 | 99.28 | |
| **Atenolol** | 100% | 50 | 50.16 | 100.32 | 99.69 |
| | 150% | 75 | 74.62 | 99.49 | |
| | 50% | 7.5 | 7.42 | 98.93 | |
| **Hydrochlorothiazide** | 100% | 15 | 14.92 | 99.46 | 99.58 |
| | 150% | 22.5 | 22.58 | 100.35 | |
| | 50% | 25 | 25.24 | 100.96 | |
| **Losartan K** | 100% | 50 | 50.19 | 100.38 | 100.21 |
| | 150% | 75 | 74.47 | 99.29 | |

**Table 03: System precision Report**

| Parameters | Area of Atenolol | Area of Hydrochloro thiazide | Area of Losartan K |
|---|---|---|---|
| **Trial 1** | 237653 | 69334 | 219243 |
| **Trial 2** | 235497 | 68948 | 221435 |
| **Trial 3** | 238129 | 68974 | 222857 |
| **Trial 4** | 239258 | 69163 | 221984 |
| **Trial 5** | 236385 | 69356 | 223124 |
| **Trial 6** | 234781 | 69034 | 221678 |
| **Average** | 236950.5 | 69134.83 | 221720.2 |
| **Standard deviation** | 1693.878 | 179.0625 | 1382.187 |
| **% Relative standard deviation** | 0.714866 | 0.259005 | 0.623392 |

**Linearity and Range**

A linear relationship was observed between the absorbance and concentration over the range from 10-90µg/ml for Atenolol, 5-25µg/ml for Hydrochloro thiazide and 10-90µg/ml for Losartan K Table 05. The linearity was expressed as $R^2$, which was 0.999 for Atenolol, 0.999 for Hydrochloro thiazide and 0.999 for Losartan K. Values of $R^2$ shown in figures 3, 4 & 5.

**Table 04: Method precision Report**

| Parameters | Area of Atenolol | Area of Hydrochloro thiazide | Area of Losartan K |
|---|---|---|---|
| **Trial 1** | 235423 | 68358 | 225823 |
| **Trial 2** | 239345 | 68698 | 225628 |
| **Trial 3** | 236534 | 68936 | 223649 |
| **Trial 4** | 237629 | 68269 | 223517 |
| **Trial 5** | 237459 | 69065 | 225372 |
| **Trial 6** | 233613 | 68703 | 224812 |
| **Average** | 236667.2 | 68671.5 | 224800.2 |
| **Standard deviation** | 1980.734 | 312.021 | 1003.028 |
| **% Relative standard deviation** | 0.836928 | 0.454368 | 0.446187 |

**Table 5: Linearity Report**

| Parameters | Results | | |
|---|---|---|---|
| | Atenolol | Hydrochloro thiazide | Losartan K |
| Linearity($R^2$) | 0.999 | 0.999 | 0.999 |
| RSD,% | ≤ 2 | ≤ 2 | ≤ 2 |
| Mean Recovery,% | 99.69 | 99.58 | 100.21 |

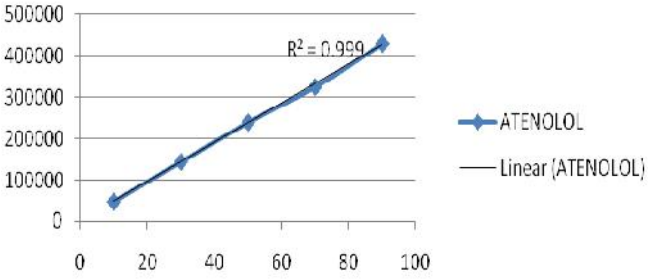


**Figure 03: Linearity of Atenolol**

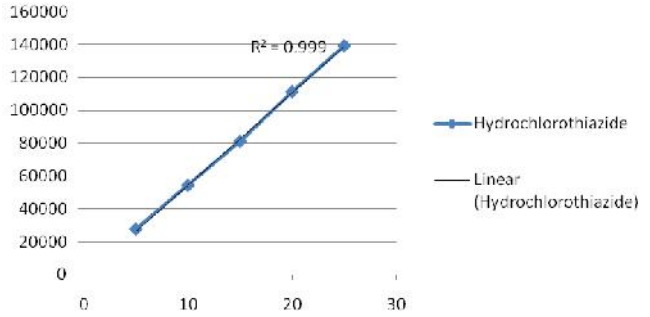


**Figure 04: Linearity of Hydrochloro thiazide**

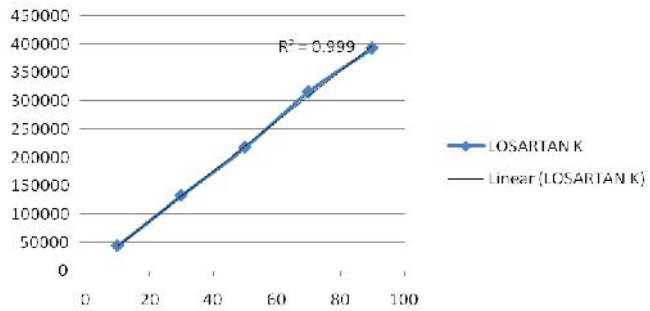


**Figure 05: Linearity of Losartan K**

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 $\partial/S$ and 10 $\partial/S$, respectively as per ICH guidelines, where $\partial$ is the standard deviation of the response (*y*-intercept) and *S* is the slope of the calibration plot. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3).The LOD for ATN, HCT and LSK was found to be 0.50µg/ml, 0.20µg/ml and 0.54µg/ml, respectively. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10).The LOQ of ATN, HCT and LSK was found to be 4.50µg/ml, 3.20µg/ml and 5.60µg/ml respectively.

**Ruggedness and Robustness**

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC 10 AT), Water Alliance 2695 by different operators using different columns. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP- HPLC method developed is rugged and robust.

**Solution Stability**

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 6 h at room temperature. The results show that for both solutions the retention time and peak area of ATN, HCT and LSK remained unchanged (percentage RSD less than 2.0), thus indicated that both solutions were stable for 24h,

which was sufficient to complete the whole analytical process.

**System suitability studies**

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table No.6). The values obtained, demonstrated the suitability of the system for the analysis of this drug combinations.

**Table 06: System suitability Report**

| Parameters | Atenolol | Hydrochloro thiazide | Losartan K |
|---|---|---|---|
| Retention time, min | 2.6 | 5.6 | 9.2 |
| Tailing factor | 0.56 | 0.38 | 0.72 |
| Number of theoretical plates | 12672 | 3941 | 11927 |
| **Resolution** | | 6.92 | 8.43 |

## Conclusion

The proposed RP-HPLC method is precise, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the simultaneous estimation of Atenolol, Hydrochloro thiazide and Losartan K in tablet dosage form.

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