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## Research Article

# Development and validation of a new sensitive method for the quantitative analysis of Atenolol-Losartan potassium in a Tablet dosage form by using HPTLC

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**Abstract:** The basic objective of the study was to develop and validate a new sensitive method using HPTLC for the quantitation of Atenolol and Losartan Potassium in an Antihypertensive combination. The chromatographic separation was done using a Camag HPTLC system consisting of Camag Linomat V automatic sample applicator, Camag Syringe, Camag TLC scanner 3, Camag WinCats software and Camag Twin Trough Chamber, with precoated Silica Gel 60 F-254 aluminum sheets as stationary phase and Ethyl acetate: Methanol: 1,4 Dioxane: Ammonia (10:2:1:2) as the mobile phase. The scanning was done at 225nm. The accuracy and reliability of the proposed method was ascertained by evaluating parameters like linearity Atenolol (400ng/µl to 1000 ng/ µl) and Losartan Potassium(200 ng/µl to 1000 ng/µl) with the coeffiecient of correlation ( r) of Atenolol and Losartan Potassium to be 0.99640 and 0.99736 respectively. The percentage recovery was found to be in the range of 95-105% (Atenolol) and 100 – 103% (Losartan Potassium). Intra day and Inter day precision for Atenolol was found to be 0.53 and 0.87respectively. Intra day and Inter day precision for Losartan Potassium was found to be 0.76 and 1.33 respectively. LOD for Atenolol and Losartan Potassium was found to be 120ng and 80ng respectively. LOQ for Atenolol and Losartan Potassium was found to be 400ng and 200ng respectively. The method was found to be robust and rugged. Assay of the formulated tablets was found to be 96.66% for Atenolol and 99.5% for Losartan Potassium. The content of Atenolol and Losartan Potassium in the tablet was found to be 49mg and 49.75mg respectively. The proposed method was found to be accurate, precise.
**Keywords**: Atenolol, Losartan Potassium, HPTLC, Accuracy, Precision, Percentage, Recovery and RSD

## INTRODUCTION

Atenolol, 4-[2-Hydroxy-3-[(1-methylethyl)amino]propoxy] benzeneacetamide (Figure-1 (a)) is a β1 selective adrenergic receptor antagonist which is used in the treatment of hypertension, angina pectoris, cardiac dysarhythmias and myocardial infarction [1]. It reduces blood pressure by adrenergic receptor blocking thereby decreasing the cardiac output by decreasing the sympathetic outflow from the CNS and by suppressing rennin [2-3].

Losartan Potassium, a potassium salt of 2-Butyl-4-chloro-1-[[2¢-(1H-tetrazol-5-yl) [1,1¢-biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol (Figure-1(b) is an effective orally acting non-peptide angiotensin II resoconstrictor and aldosterone secreting effects of Angiotensin II by selectively blocking the binding on angiotensin II to its angiotensin II (Type $AT_1$) receptor sites and employed in the management of essential hypertension [4-5].

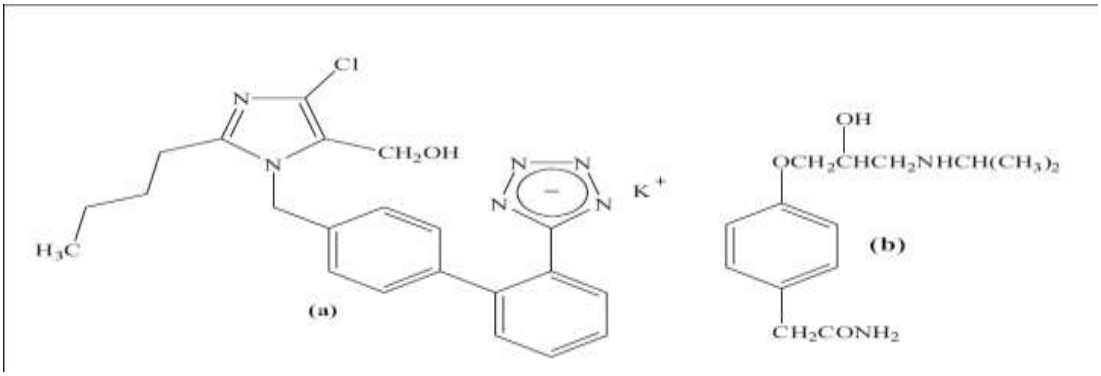

**Figure-1: Chemical Structure of (a)Atenolol, (b) Losartan Potassium**

Literature revealed different UV, HPLC, RP-HPLC, HPTLC etc methods [6-12] were used for Atenolol and Losartan Potassium alone or different combinations in pharmaceutical formulations.

The aim of the present study was to devise a newer, sensitive and simpler method for the separation and quantitative analysis of Atenolol and Losartan Potassium together in a combined tablet dosage form.

## MATERIAL AND METHODS

### Drugs, Chemicals and Instruments

Pharmaceutical grade Atenolol and Losartan Potassium was a generous gift from Kare Laboratories, Ciple Pvt Ltd and Microlabs, Verna,Goa. Fixed Dose tablets (COVANCE-$^{TM}$ - AT) containing 50mg Atenolol and Losartan Potassium each were procured from Ranbaxy CV. Precoated Silica Gel 60 F-254 aluminum sheets were used as stationary phase. All chemicals and reagents were of analytical grade and were purchased from Merck Chemicals Corporation Ltd, Mumbai. A Camag HPTLC system consisting of Camag Linomat V automatic sample applicator, Camag Syringe, Camag TLC scanner 3, Camag WinCats software and Camag Twin Trough Chamber was used to develop the method.

## EXPERIMENTAL SECTION

To confirm specificity, standard solutions of pure drug sample were prepared by dissolving 10mg of each in 10ml of methanol in separate volumetric flasks corresponding to 1µg/ µl of Atenolol and Losartan Potassium respectively. Tablet analysis was conducted by taking 10 tablets. Tablets on weighing were finely powdered and powder equivalent to 10mg (both drugs) was weighed and placed in the test tube and was extracted with methanol. Further 2ml was withdrawn and mixed with 8ml methanol to correspond to 0.2 µg/ µl of Atenolol and Losartan Potassium respectively.

HPTLC plates were prewashed and activated prior to use. The chromatographic estimation were performed using conditions: Precoated Silica Gel 60 F-254 aluminum sheets (10 x 10cm) and mobile phase – Ethyl acetate: Methanol: 1,4 Dioxane: Ammonia (10:2:1:2), chamber saturation – 10mins, wavelength of scanning 225nm. It was observed that developed method was specific and no interferences from excipients used by manufacturer for tablet was reported (Rf = 0.41 and 0.56 for Losartan Potassium and Atenolol respectively).

To determine Linearity same procedure was followed to get 0.2 µg/ µl of Atenolol and Losartan Potassium from the marketed sample and for pure drug sample stock solutions were also further diluted to get 0.2 µg/ µl of Atenolol and Losartan Potassium each in separate volumetric flasks.

The proposed method was validated according to ICH guidelines [13] interms of accuracy; inter day and intraday precision, repeatability and LOD/LOQ.

## RESULTS AND DISCUSSION

The linearity for Atenolol and Losartan Potassium was found to be in the range of 400ng/µl to 1000 ng/ µl and 200 ng/µl to 1000 ng/µl respectively with coeffiecient of correlation (r) of Atenolol and Losartan Potassium as 0.99640 and 0.99736 respectively.(Figure-3 and Figure-4).

**Table-1: HPTC results of Atenolol and Losartan Potassium**

| Rf | Atenolol (amount) | Area | Rf | Losartan Potassium (amount) | Area |
|---|---|---|---|---|---|
| 0.57 | 0.4 µg (400ng) | 1016.53 | 0.43 | 0.2 µg (200ng) | 1174.87 |
| 0.57 | 0.8 µg (800ng) | 1826.47 | 0.42 | 0.4 µg (400ng) | 1893.69 |
| 0.57 | 1.2 µg (1200ng) | 2561.12 | 0.41 | 0.6 µg (600ng) | 2637.92 |
| 0.57 | 1.6 µg (1600ng) | 3349.29 | 0.41 | 0.8 µg (800ng) | 3154.70 |
| 0.57 | 2.0 µg (2000ng) | 3822.03 | 0.41 | 1.0 µg (1000ng) | 3742.59 |

Accuracy of the analysis was evaluated by carrying out a recovery study at three different levels namely 80, 100 & 120%. The percentage recovery was found to be in the range of 95-105% (Atenolol) and 100 – 103% (Losartan Potassium). The percentage recovery obtained for Atenolol and Losartan Potassium was found to be 99.35 % and 101.47% respectively.

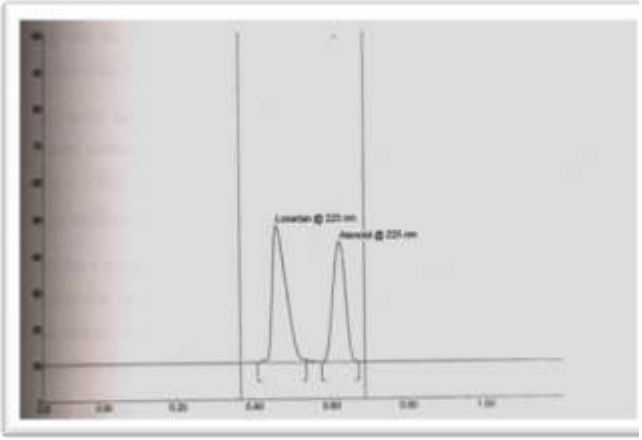

**Figure-2 – Chormatogram of Atenolol and Losartan Potassium.**

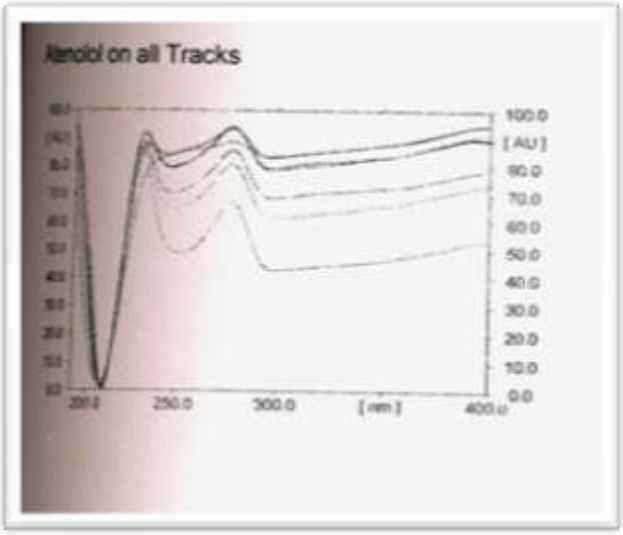


**Figure 3 – Linearity plot of Atenolol at all dilutions.**

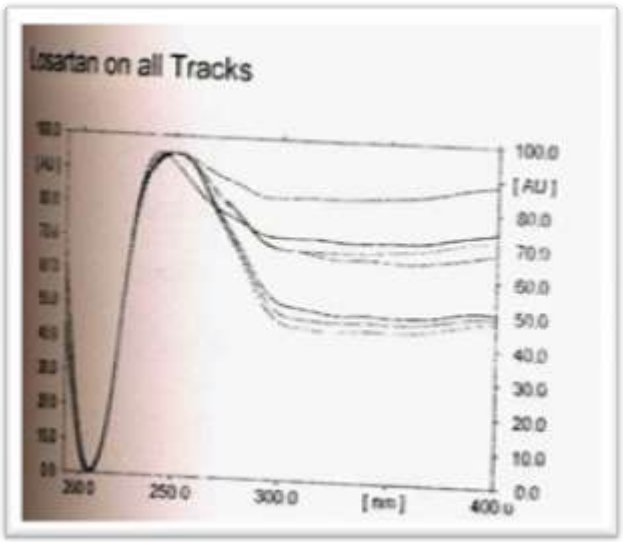


**Figure 4 – Linearity plot of Losartan Potassium at all dilutions.**

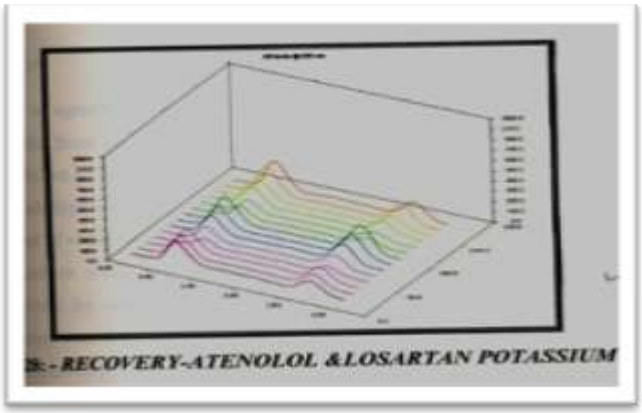


**Figure 5: Recovery –Atenolol and Losartan Potassium**

The intra day precision was determined by analyzing standard solutions of Atenolol and Losartan Potassium in the range of 400 – 1200ng / spot for three times on the same day and three different days (Inter-day) with relative standard deviations reported as 0.53-0.87(Atenolol) and 0.76 -1.33 (Losartan Potassium). The smaller values of intra-day and inter-day variation in the analysis indicated that the method is precise.

Marketed preparation containing Losartan Potassium and Atenolol 50mg each were analyzed and percentage purity for each drug was determined. Ten tablets were weighed, powdered and accurately 10 mg of it was diluted with methanol in a volumetric flask producing 10ml. 2ml from this stock was further diluted to get 0.2 µg/ µl of Atenolol and Losartan Potassium respectively.

The percentage purity was found to be 96.66% for Atenolol and 99.5% for Losartan Potassium and the content of Atenolol and Losartan Potassium in the tablet was found to be 49mg and 49.75mg respectively.

## CONCLUSION

An HPTLC method for simultaneous analysis of losartan potassium, atenolol, in pharmaceutical dosage forms has been
established . The method is very simple and rapid, and pro-vides accurate and precise results.

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