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# Isocratic RP-HPLC method validation and verification of losartan potassium in pharmaceutical formulations with stress test stability for drug substance

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## ABSTRACT

*This paper describes the validation of an isocratic HPLC method for the assay of Losartan Potassium Tablets and the evaluation of the stability of drug substance after stress test by Photodiode array detection. The HPLC separation was achieved on a liquid chromatogram is equipped with a 254 nm detector and a 4.6 × 25 mm column that contains 5 µm packing $L_1$ (Hypersil BDS $C_{18}$ 4.6× 250 mm, 5 µm column is suitable). A mixture of ammonium dihydrogen phosphate buffer pH 3.0 and acetonitrile (65:35) as a mobile phase, the flow rate is about 1.5 ml per minute. Chromatograph the Standard preparation, and record the peak response as directed under procedure: the tailing factor for the analyte peak is not more than 3.0 and the relative standard deviation of replicated injections is not more than 2%.*

**Keywords**: Losartan Potassium Tablets, Validation, RP-HPLC, Stability.

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## INTRODUCTION

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 No.1** ) , represents the first of a new class of orally active non-peptide angiotensin II (Type $AT_1$) receptor antagonists employed in the management of essential hypertension.[1,2]

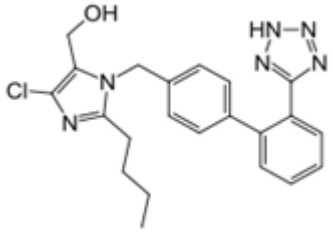



**Figure No. 1: Chemical Structure of Losartan.**

The individual determination of losartan has been carried out in tablets by capillary electrophoresis and super- critical fluid chromatography [3, 4], in bulk [5], by HPLC & LC-MS/MS [6-8] and UV-Spectrophotometric method [9], HPTLC [3,10]and with its active metabolite in biological fluid by HPLC.[11-14] , HPLC method for determination of Losartan potassium in combination with other drugs [15-19].

## MATERIALS AND METHODS

### Reagents and Chemicals

HPLC grade anhydrous ammonium hydrogen orthophosphate, Orthophosphoric acid, Acetonitrile from Merck. Water was deionised and purified by a Milli- Q water purification system was used to prepare the mobile phase and sample and standard solutions. A reference standard of Losartan Potassium was procured from Hetero Pharma.

### Chromatographic equipment and conditions

The development and validation of the assay is performed on Waters Alliance HPLC system with UV-VIS Detector which include following parts: Fluidics management system (Model: 2695) includes seal wash, degasser, sample heater/cooler and Column heater .UV-VIS Detector (Model No: 2487)-Dual wavelength absorbance detector. Empower chromatography data system (Software). The analytical column 4.6 × 25 mm column that contains 5 µm packing $L_1$ (hypersil BDS $C_{18}$ 4.6× 250 mm, 5 µm column is suitable). The mobile phase consist of ammonium dihydrogen phosphate buffer pH 3.0 and acetonitrile (65:35) as a mobile phase. The flow rate was 1.5ml/min and detection was performed at 254 nm

### Procedure for determination of Losartan Potassium in Reference Standard

Dissolve about 100 mg, accurately weighed, of Losartan potassium Working Standard in 100 ml of diluent, dilute 10 ml to 100 ml with the diluent.A chromatograms obtained from reference solution is presented in **Fig 2**.

### Procedure for determination of Losartan Potassium in Sample Solutions

**Intermediate Products:**

Weigh and powder 20 tablet, transfer a portion of powder equivalent to 50 mg Losartan potassium accurately weighed, into 50 ml volumetric flask, complete to volume with the diluent, shake for 30 minutes, centrifuge at 4000 rpm for 10 minutes, and dilute 5 ml to 50 ml with the diluent.

**Finished Product:**

Transfer 5 tablets into 250 ml volumetric flask, add 20 ml diluent and sonicate to disintegrate the tablets, complete to volume with diluent, shake for 30 minutes, centrifuge at 4000 rpm for 10 minutes, and dilute 5 ml to 50 ml with the diluent. A chromatograms obtained from reference solution is presented in **Fig 3.**

### Procedure for Injecting Reference and Standard Sample:

Separately inject equal volumes (about 20µl) of the Standard preparation and the Assay preparation, Calculate the quantity, in mg, of $C_{22}H_{22}ClKN_6O$ in the portion of powder or tablets taken by the formula:

(CL/D) ($r_u/r_s$) *Intermediate*

5 (CL/D)($r_u/r_s$) *Finished Product*

Where C is the concentration, in mg per ml, of Losartan potassium in the Standard preparation, calculated on anhydrous basis, L is the labelled quantity, in mg, of Losartan potassium in each tablet, D is the concentration, in mg per ml, of losartan potassium in Assay preparation based on the labelled quantity per tablet and the extent of dilution. $r_u$ and $r_s$ are the responses of Losartan potassium obtained from the Assay preparation and the Standard preparation, respectively

**Fig 2: Chromatogram of Losartan potassium Reference standard**

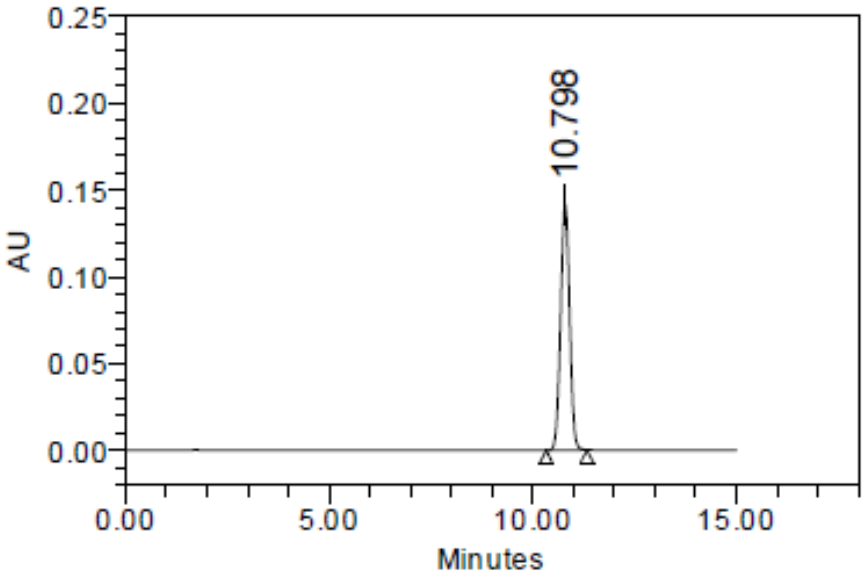
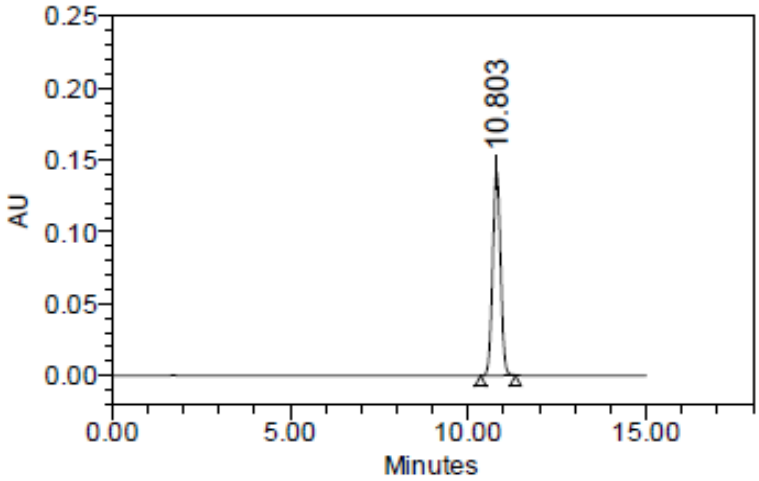



**Fig 3: Chromatogram of Losartan potassium in Zargo Tablet**




**Method validation**

The Method was validated for Specificity, precision (repeatability and intermediate precision), accuracy.

## RESULTS AND DICUSSION

**System Suitability solution**

Having optimised the efficiency of a chromatographic separation the quality of chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤ 2.0 and theoretical plates <2000. In all cases the

relative standard deviation (R.S.D) for the analyte peak area for two consecutive injections was <2.0 %. A chromatograms obtained from reference substance solution is presented in **Fig 4**

**Figure No.4: Resolution solution of Losartan Potassium and Releated C**

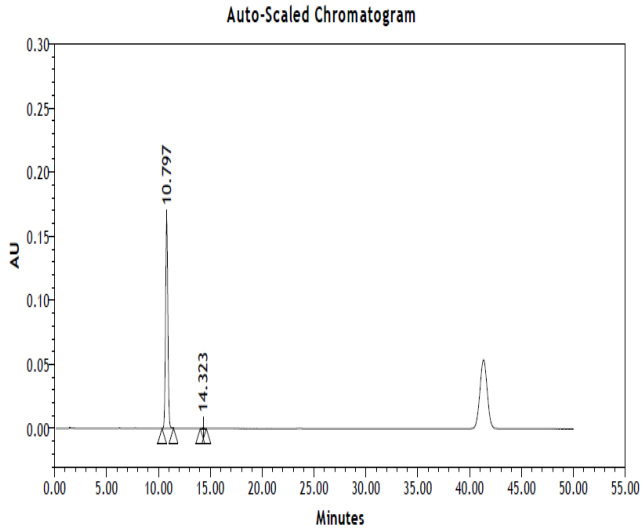
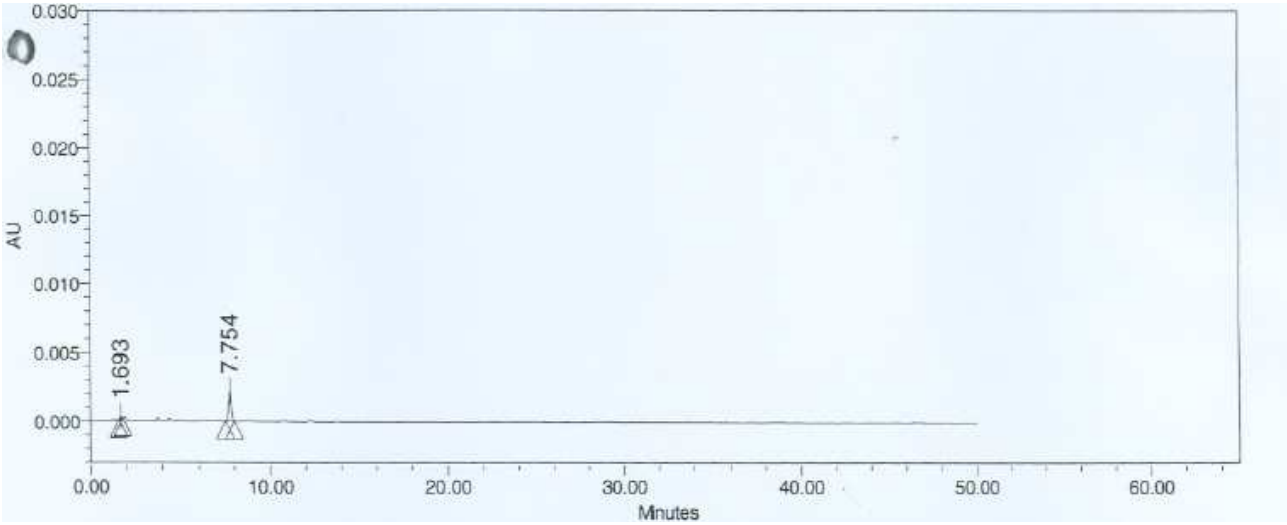



**Figure No. 5: Chromatograms of Placebo Preparation**




**Specificity**

The excipients in the Tablets contained the following in active ingredients: Microcrystalline cellulose (180 micro meter), Lactose DC, Pregelatinized maize starch , Magnesium stearate , Hydroxypropyl methyl cellulose type 2910 (methocel E5) , Polyethylene Glycol 400, Titanium dioxide as excipients Chromatograms of Placebo Tablets solution showed no interference with the main Peak. Chromatograms showed indicated that no excipients or impurities interfered with Losartan Potassium peak. (Refer Figure No. 5 for placebo and Figure No. 3 Sample ).

**Range of linearity:**

The linearity of the method was evaluated by a calibration curve in the range of (5.08-15.24mg/ml) which include (5.08, 7.62, 10.16, 12.70, 15.24 mg/ml) of the day (n=5). A calibration curve was constructed by plotting the absorbance versus final concentration of Losartan Potassium, which showed a linear response showing the linear dynamic range over the concentration range 0.5-15µg/ml.

**Precision (Repeatability and Intermediate Precision)**

Result of Precision was carried out for a series of six samples for strength of Losartan Potassium Tablets 50 mg , preparation is done by transferring Losartan Potassium standard with placebo preparation described above , on three consecutive days , the repeatability , Mean , CV and recovery were calculated (**Table No : 1**)

**Table 1 Results of Precision for Zargo tablet**

| Analyst | Sample No. | Conc. mg/100ml | Response Peak area ($\times 10^{-6}$) | % Recovery ≤ 5% | CV (≤ 2%) | CL (≤ 5.0%) |
|---|---|---|---|---|---|---|
| 1 | 1 | 10.52 | 2.320 | 102.2 | 1.20 | 101.4 ± 1.3 |
| | 2 | 10.18 | 2.255 | 102.6 | | |
| | 3 | 10.96 | 2.351 | 99.4 | | |
| | 4 | 10.60 | 2.341 | 102.3 | | |
| | 5 | 9.72 | 2.113 | 100.7 | | |
| | 6 | 10.22 | 2.234 | 101.3 | | |
| 2 | 1 | 10.02 | 2.200 | 100.5 | 0.55 | 100.8 ± 0.6 |
| | 2 | 10.18 | 2.250 | 101.2 | | |
| | 3 | 10.10 | 2.210 | 100.2 | | |
| | 4 | 10.10 | 2.230 | 101.6 | | |
| | 5 | 10.14 | 2.238 | 101.0 | | |
| | 6 | 10.06 | 2.204 | 100.3 | | |
| Overall CV | | | | | 0.95 | |

**Table 2 Results of Accuracy**

| Analyst | Conc. mg/100ml | Response Peak area ($\times 10^{-6}$) | % Recovery | % Bias (≤5%) |
|---|---|---|---|---|
| Analyst 1 | 5.74 | 1.270 | 101.0 | +1.0 |
| | 10.52 | 2.306 | 101.5 | +1.5 |
| | 14.80 | 3.200 | 98.9 | -1.1 |
| Analyst 2 | 4.94 | 1.103 | 102.1 | +2.1 |
| | 10.06 | 2.206 | 100.4 | +0.4 |
| | 15.02 | 3.288 | 100.2 | +0.2 |
| Analyst 3 | 5.08 | 1.110 | 100.2 | +0.2 |
| | 10.22 | 2.220 | 99.5 | -0.5 |
| | 14.94 | 3.276 | 100.5 | +0.5 |

**Accuracy:**

Separately transfer either 25, 50 or 75 mg Losartan potassium, accurately weighed, and 150 mg of placebo preparation into a 50 ml volumetric flask, complete to volume with the diluent, shake for 30 minutes, centrifuge at 4000 rpm for 10 minutes, and dilute 5 ml to 50 ml with the diluent to 75% to 125 % the result showed good recoveries ranging from 98.9% to 101.5. The mean recovery data obtained for each level as well as for all levels combined (**Table No: 2**) were within the limit.

## RESULTS AND DISCUSSION

The Table below showed the characteristics used during the work with their limits and values obtained. (**Table No. 3**) Characteristics, their limits and values obtained.

**Stress test for Stability**

Accelerated degradation studies were performed to provide an indication of the stability of the drug and specificity of the method. Losartan Potassium reference standard was stressed under conditions that cause degradation included the following Placebo Degradation Preparation,

Degradation Products Preparations, Drug Substances Degradation Preparations which involved (Decomposition in solid state and Photolysis in solid state)

**Table 3**

| Characteristics | Limit | Value obtained |
|---|---|---|
| -%Bias | ≤5% | Max. 2.1% |
| Precision:<br>-Coefficient of Variance (CV)<br>-Confidence limit (CL) (95%) | <br>≤2%<br>≤5% | <br>0.95%<br>1.28% |
| Specificity:<br>-% Interference<br>-Resolution (R)<br>-%Recovery | <br>≤2%<br>$\geq 5^{+}$<br>≥10% (Drop) | <br>-<br>Min. 5.8 for impurities<br>12.1% to 25.2% drop |
| Linearity and Range:<br>-Range<br>-Correlation coefficient (r)<br>-CV for $RF_{st}$ | <br>±50% of the target conc.<br>≥0.98<br>≤3% | <br>5.08-15.24 mg/100ml<br>0.9999<br>0.5% |
| Robustness:<br>-Solution stability (%D)<br>-Tailing Factor (T)<br>-Recovery<br>-CV | <br>≤3%<br>≤ 3<br>97%-103%<br>≤3% | <br>2.2%<br>Max. 1.8<br>101.1% - 103.8%<br>Max. 1.4% |

**Stress test and its Evaluation:**
**Stability of the Analytical Solutions (Robustness):**
**Standard Preparations:**
Prepare 3 solutions of one analyst as directed and keep at ambient conditions for 24 hours.

**Synthetic Preparation:**
Keep 3 solutions of the precision test of one analyst at ambient conditions for 24 hours

## RESULTS AND CALCULATION

Calculate the % recovery for each analyte in Standard preparations and Synthetic preparations before and after storage, use freshly prepared Standard preparation having target concentration for calculations of the % recovery Determine whether the solutions can be used within 24 hours without the results being affected or for a less period of time. Results of Stability of Solutions (Table No. 4) and Results of Placebo and Placebo Degradation Preparation Specificity Test (Table no. 5)

**Placebo and Drug Substances Degradation**
**Placebo Degradation**
No degradation products of Losartan Potassium reference substance was observed after refluxing for 48 hours at 65ºC, both wet and dry at 90°C for 6 hours.

**Decomposition in Solid State:**
Expose a quantity of drug substance on a hot plate to achieve decomposition.

**Table 4 Results of Stability of Solutions**

| Sample | Conc. (mg/100ml) | Response Peak area ($\times 10^{-6}$) | | Found Conc. (mg/100ml) | | % D (≤3.0%) |
|---|---|---|---|---|---|---|
| Standard | 11.34 | 2.468 | 2.470 | 11.34 | 11.27 | -0.6 |
| | 9.80 | 2.099 | 2.100 | 9.80 | 9.58 | -2.2 |
| | 10.12 | 2.215 | 2.246 | 10.12 | 10.25 | 1.3 |
| Synthetic | 10.52 | 2.320 | 2.330 | 10.75 | 10.64 | -1.0 |
| | 10.18 | 2.255 | 2.280 | 10.45 | 10.41 | -0.4 |
| | 10.96 | 2.351 | 2.380 | 10.89 | 10.86 | -0.3 |

**Thermal Decomposition in Solutions:**

Losartan working standard, accurately weighed, in a Petri dish add 5 ml of either 0.5M hydrochloric acid, 0.5M sodium hydroxide or 5% hydrogen peroxide, and incubate at 65°C for 1hours

**Temperature Stress**

Decomposition in Solid State:

Expose a quantity of drug substance on a hot plate to achieve decomposition,

**Acid Stress**

No degradation products of Losartan Potassium reference substance was observed after refluxing for 12 hours at 65ºC.

**Base Stress**.

No degradation products of Losartan Potassium reference substance was observed after refluxing for 12 hours at 65ºC.

Peroxide stress

No degradation of Losartan Potassium was observed for reference substance stressed in 5 % $H_2O_2$. Table No. 5: Results of Placebo and Placebo Degradation Preparation Specificity Test & Table No.6: Results of Specificity Test for Drug Substances Degradation Preparations (Stability Indicating)

**Table No. 5: Results of Placebo and Placebo Degradation Preparation Specificity Test:**

| Sample/Storage | Concentration (mg/100 ml) | Response (peak area) | % Interference (≤ 2%) |
|---|---|---|---|
| Placebo/25°C | - | 0.0 | 0.0 |
| Placebo/90°C/ dry 6 hours | - | 0.0 | 0.0 |
| Placebo/90°C/wet/6 hours | - | 0.0 | 0.0 |

**Table 6: Results of Specificity Test for Drug Substances Degradation Preparations (Stability Indicating)**

| Degradation Condition/ Time | Conc. (mg/100ml) | % Recovery | % Drop (≥ 10%) |
|---|---|---|---|
| Melting/60 minutes | 10.16 | 82.0 | -18.0 |
| 1 M HCl/ 12 hours/ 65°C | 10.46 | 74.8 | -25.2 |
| 1 M NaOH/ 12 hours/ 65°C | 10.48 | 102.0 | - |
| 5% $H_2O_2$/ 12 hours/ 65°C | 9.97 | 87.9 | -12.1 |

## CONCLUSION

The Proposed method for the assay of Losartan Potassium Tablets is very simple and rapid. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drugs from its degradation products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulation

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