

A new stability indicating RP-HPLC method for the analysis of Losartan and Hydrochlorothiazide Tablets

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ABSTRACT

A new stability indicating RP-HPLC method has been developed for the determination of Losartan and Hydrochlorothiazide in tablets (UV detection at 235 nm). Hydrochlorothiazide and Losartan obey Beer-Lambert's law over the concentration range 1.0-300.3 µg/ml and 4.4.0-240.24 µg/ml respectively with regression equations $y = 5980.5x + 535.64$ ($r^2 = 0.9999$) and $y = 17490x - 3093.1$ ($r^2 = 0.9997$) for Losartan and Hydrochlorothiazide respectively. The combined tablet formulation of Losartan and Hydrochlorothiazide was subjected to acidic, alkaline, oxidation, thermal, photolytic and humidity degradations and the method was validated. The LOQ and LOD were found to be 0.734 µg/ml and 0.232 µg/ml for Losartan whereas and 0.921 µg/ml and 0.304 µg/ml for Hydrochlorothiazide respectively.

KEY WORDS: Losartan, Hydrochlorothiazide, RP-HPLC, stability-indicating and validation.

1. INTRODUCTION

Chemically Losartan (Budavari, 2006) (LSR) is an angiotensin II receptor antagonist used mainly to treat hypertension. Hydrochlorothiazide (HCTZ) is chemically 6-chloro-1, 1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7 sulfonamide which is a first line diuretic drug of the thiazide class. It acts by lowering blood pressure initially by increasing sodium and water excretion. This causes a decrease in extracellular volume, resulting in a decrease in cardiac output and renal blood flow. With long-term treatment, plasma volume approaches a normal value, but peripheral resistance decreases. Combination of HCTZ and LSR is widely prescribed by the physicians due to simple dosing regimens, improved hypertension control, fewer dose-dependent side effects and low cost treatment of hypertension. Very few UV method (Lande, 2000; Charles, 2003) and HPLC (Carlucci, 2000; Ozkam, 2001; Arif Hossen, 2011; Dinc, 2005; Hertzog, 2002) methods are reported for simultaneous estimation of HCTZ and LSR in tablet dosage form therefore, there is still a need to develop a simple HPLC method for the estimation of HCTZ and LSR in combination dosage form. In the present study a simple, robust, precise and accurate RP-HPLC method (gradient mode) was developed for the simultaneous determination of Hydrochlorothiazide and Losartan and validated as per ICH guidelines (ICH guidelines 2005). The forced degradation studies were conducted as per ICH guidelines (ICH guidelines, 1995).

2. MATERIALS AND METHODS

Chemicals and reagents: Hydrochlorothiazide (Purity 99.8) and Losartan (Purity 99.4) were obtained from Ranbaxy Laboratories and Solvay (India). The combination of Hydrochlorothiazide and Losartan is available in as tablets with brand names ANGILOCK Plus (Square Pharmaceuticals Ltd.) and LOPO Plus (BIOPHARMA Laboratories Limited) with label claim Hydrochlorothiazide: 12.5 mg and Losartan: 50 mg. Acetonitrile, formic acid, sodium hydroxide (AR), hydrochloric acid (AR) and hydrogen peroxide (AR) were procured from Merck (India) and all chemicals are of HPLC grade.

Waters Model 2997 HPLC system with PDA detector and X Bridge Shield RP18 (150 x 3.0 mm, 3.5µm) column (Injection volume 5µL) was used for the chromatographic study. Gradient mode of elution was performed with column oven temperature 45°C. Losartan and Hydrochlorothiazide stock solutions were prepared in mobile phase containing acetonitrile and formic acid and working standard solutions were prepared on dilution.

Validation:

Linearity: A series of solutions were prepared from by diluting the stock solutions of Hydrochlorothiazide (1-300 µg/ml) and Losartan (0.8-250 µg/ml) with mobile phase. The representative chromatogram of Hydrochlorothiazide and Losartan was shown in Figure.1a, and the corresponding peak purity plots were shown in Figure.1b.

Precision: The intra-day precision of the assay method was evaluated by carrying out 6 independent assays of test samples of Losartan and Hydrochlorothiazide (Losartan 200 µg/ml and Hydrochlorothiazide 250 µg/ml) against a qualified reference standard and the % RSD was calculated. The inter-day precision study was performed on different days (n=6) (Losartan 200 µg/ml and Hydrochlorothiazide 250 µg/ml) on different system by different analyst and the % RSD was calculated.

Accuracy: The accuracy of the assay method was evaluated in triplicate by spiking individual standard solutions at three concentration levels (80, 100 and 120%), and the percentage recoveries were calculated. Standard addition and

recovery experiments were conducted to determine the accuracy of the method for the quantification of Hydrochlorothiazide & Losartan respectively in the drug product and the % RSD was calculated.

Robustness: The robustness of the assay method was established by introducing small deliberate changes in the HPLC conditions which included flow rate (0.72 and 0.88 mL/min), percentage of acetonitrile in the mobile phase (absolute $\pm 2\%$ composition) and column oven temperature ($\pm 5^\circ\text{C}$). Robustness of the method was studied using five replicates of Losartan (200 $\mu\text{g/ml}$) and Hydrochlorothiazide (250 $\mu\text{g/ml}$).

Forced degradation studies: Forced degradation studies were intended to ensure the effective separation of Losartan and Hydrochlorothiazide and their degradation peaks of formulation ingredients at the retention time of Losartan and Hydrochlorothiazide respectively. Forced degradation studies were performed with 200 $\mu\text{g/ml}$ of Losartan and 250 $\mu\text{g/ml}$ of Hydrochlorothiazide. Initially acidic degradation started with 0.1N HCl and acid concentration slowly increased to 1N HCl. The combined formulation of Losartan and of Hydrochlorothiazide was treated with 1N HCl and refluxed for 2 hours in thermostat maintained at 80°C . Alkaline degradation was started with 0.1N NaOH and alkali concentration slowly increased to 1N NaOH. The combined formulation of Losartan and of Hydrochlorothiazide was treated with 1N NaOH and refluxed for 2 hours in thermostat maintained at 80°C . The combined formulation of Losartan and of Hydrochlorothiazide was treated with 1 % H_2O_2 and refluxed for 2 hours in thermostat maintained at 80°C during the oxidation degradation study. During the oxidation degradation study the combined formulation of Losartan and of Hydrochlorothiazide was kept for thermal degradation at 105°C for 72 Hours in oven. During the photolytic degradation study the combined formulation of Losartan and of Hydrochlorothiazide was kept in photolytic chamber for photolytic degradation at 1289069 Lux Hours and 1026.66 Watt-Hour/ m^2 and kept in desiccator at 25°C , 95%RH for 120 hours during humidity degradation study.

Analysis of commercial formulations: Twenty tablets of two different brands containing Losartan and Hydrochlorothiazide were procured from the local pharmacy store and analyzed as per the method. The percentage recovery was calculated from the respective chromatograms.

3. RESULTS AND DISCUSSION

The present proposed method is simple, precise, accurate and robust. Figure 1 represents the typical chromatogram of Hydrochlorothiazide (Rt 2.894 mins) and Losartan (Rt 9.906 mins). Hydrochlorothiazide and Losartan obey Beer-Lambert's law over the concentration range 1.0-300.3 $\mu\text{g/ml}$ and 4.4.0-240.24 $\mu\text{g/ml}$ respectively with regression equations $y = 5980.5x + 535.64$ (HCTZ) ($R^2 = 0.9999$) and $y = 17490x - 3093.1$ (LSR) ($R^2 = 0.9997$) respectively (Figure 2). The LOQ and LOD were determined as described in International Conference on Harmonization guidelines Q2 (R1). The LOQ and LOD for Losartan were found to be 0.734 $\mu\text{g/ml}$ and 0.232 $\mu\text{g/ml}$ respectively whereas the LOQ and LOD for Hydrochlorothiazide were found to be 0.921 $\mu\text{g/ml}$ and 0.304 $\mu\text{g/ml}$ respectively. The method is more precise as the % RSD was found to be 0.18-0.33 and 0.11-0.21 for intra-day and inter-day precision studies respectively for HCTZ and the % RSD was found to be 0.11-0.26 and 0.21-0.36 for intra-day and inter-day precision studies respectively for LSR (RSD < 2). The % RSD in accuracy studies was found to be 0.24-0.61 (RSD < 2) with percentage recovery 99.55-100.67 for HCTZ and 0.19-1.22 (RSD < 2) with percentage recovery 98.44-100.48 for LSR (Table 1) respectively. The method is more robust as the % RSD was found to be 0.12-0.84 and 0.16-0.56 for HCTZ and LSR respectively (Table.2). The proposed method was applied for the determination of Hydrochlorothiazide and Losartan tablets and the percentage recovery was found to be 99.28-99.44 and 99.28-100.06 respectively (Table.3).

The system suitability tests were performed to ensure that the complete testing system was suitable for the intended application. The tailing factor was 1.19 (HCTZ) and 0.98 (LSR) which is $<1.5-2$ or <2 and the theoretical plates were found to be 2894 (HCTZ) and 90268 (LSR) which is >2000 . A brief summary of forced degradation studies was given in Table.4.

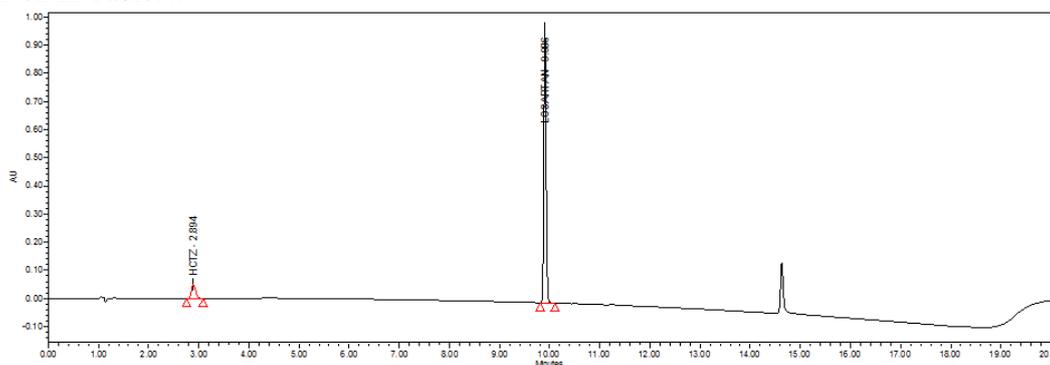


Figure.1a. Representative chromatogram of Hydrochlorothiazide (50 $\mu\text{g/ml}$) and Losartan (200 $\mu\text{g/ml}$)

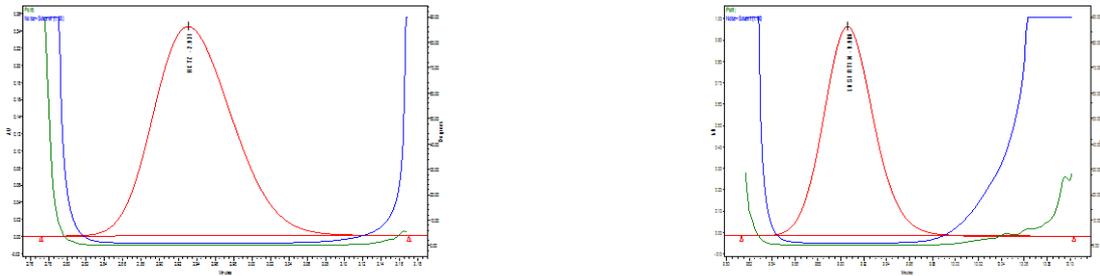


Figure.1b. Peak purity plots of Hydrochlorothiazide and Losartan



Figure.2. Calibration curves of and Losartan and Hydrochlorothiazide

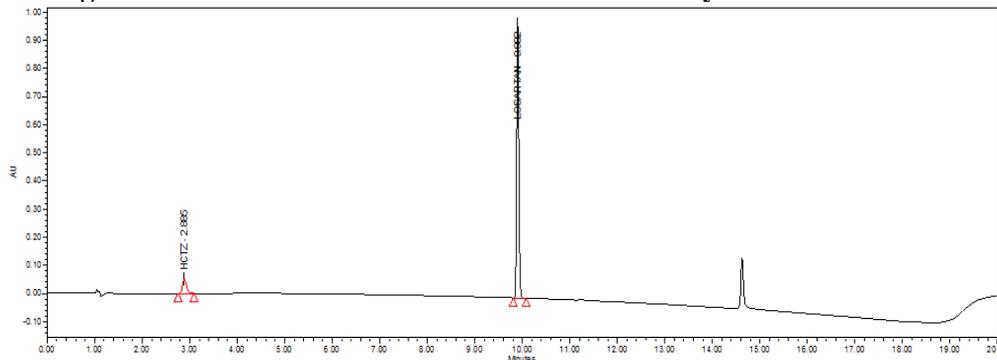


Figure.3a. Representative chromatogram of Hydrochlorothiazide (50 µg/ml) and Losartan (200 µg/ml) (Acidic degradation)

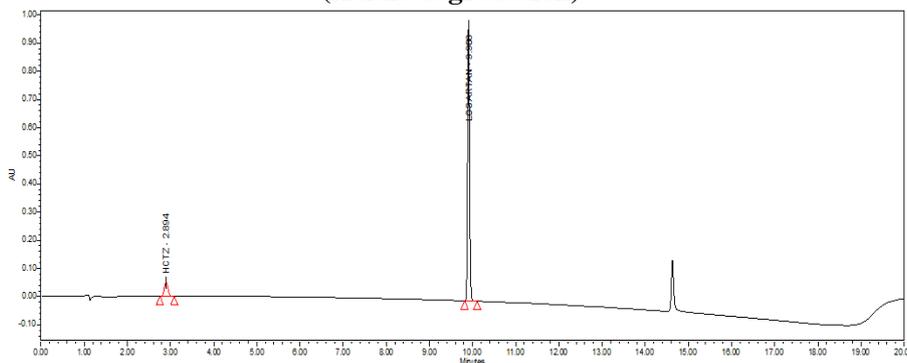


Figure.3b. Representative chromatogram of Hydrochlorothiazide (50 µg/ml) and Losartan (200 µg/ml) (Alkaline degradation)

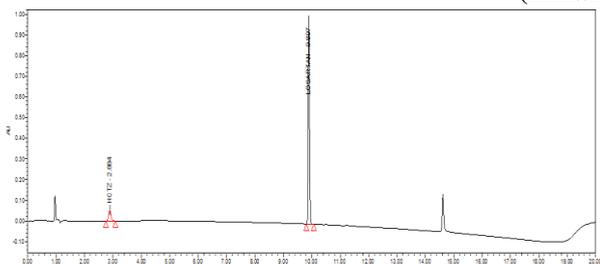


Figure.3c. Representative chromatogram of Hydrochlorothiazide (50 µg/ml) and Losartan (200 µg/ml) (Oxidative degradation)

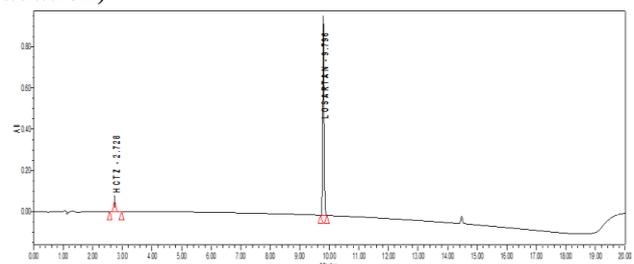


Figure.3d. Representative chromatogram of Hydrochlorothiazide (50 µg/ml) and Losartan (200 µg/ml) (Thermal degradation)

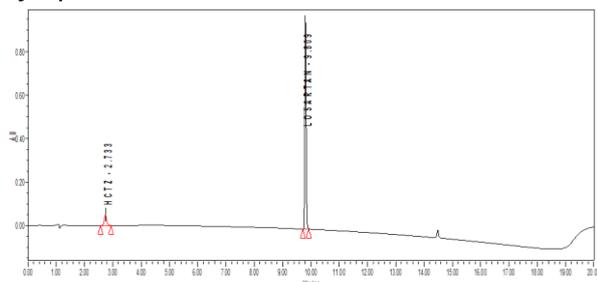


Figure.3e. Representative chromatogram of Hydrochlorothiazide (50 µg/ml) and Losartan (200 µg/ml) (Photolytic degradation)

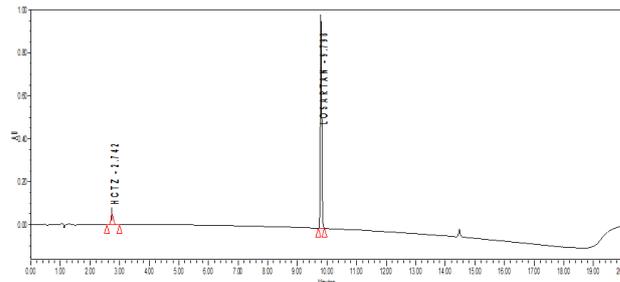


Figure.3f. Representative chromatogram of Hydrochlorothiazide (50 µg/ml) and Losartan (200 µg/ml) (Humidity degradation)

Table.1. Accuracy study

| Component | Level | Mean | % RSD |
|---------------------|-------|--------|-------|
| Losartan | 80% | 100.01 | 1.22 |
| | 100% | 98.44 | 0.19 |
| | 120% | 100.48 | 1.00 |
| Hydrochlorothiazide | 80% | 100.67 | 0.55 |
| | 100% | 99.55 | 0.61 |
| | 120% | 100.47 | 0.24 |

Table.2. Robustness Study

| Conditions | Parameter | System suitability | | | | | |
|---|-------------|---------------------|--------------------|------|----------|--------------------|------|
| | | Hydrochlorothiazide | | | Losartan | | |
| | | Tailing | Theoretical plates | %RSD | Tailing | Theoretical plates | %RSD |
| Flow rate (± 0.08 , mL/min) | 0.72 mL/min | 1.16 | 2677 | 0.34 | 1.04 | 97456 | 0.19 |
| | 0.88 mL/min | 1.11 | 2879 | 0.64 | 1.03 | 98974 | 0.56 |
| Mobile phase composition (Acetonitrile : 0.1% (v/v) formic acid) ($\pm 2\%$, v/v) | 58:42 | 1.06 | 2746 | 0.26 | 1.11 | 88465 | 0.17 |
| | 62:38 | 1.03 | 2844 | 0.84 | 1.09 | 89745 | 0.49 |
| Column oven temperature ($\pm 5^\circ\text{C}$) | 40°C | 1.11 | 2648 | 0.12 | 1.08 | 85794 | 0.16 |
| | 50°C | 1.09 | 2728 | 0.56 | 1.04 | 88926 | 0.42 |

Table.3. Analysis of commercial formulation (Tablets)

| Brand name | Labeled amount (mg) | | *Amount found (mg) | | % Assay (%) | |
|------------|---------------------|------|--------------------|-------|-------------|-------|
| | LSR | HCTZ | LSR | HCTZ | LSR | HCTZ |
| Brand I | 50 | 12.5 | 49.64 | 12.43 | 99.28 | 99.44 |
| Brand II | 50 | 12.5 | 50.03 | 12.41 | 100.06 | 99.28 |

Table.4. Forced degradation studies of Hydrochlorothiazide and Losartan

| Stress conditions | Peak area | | Drug Recovered (%) | | Drug decomposed (%) | | Purity angle | | Purity threshold | |
|------------------------|-----------|---------|--------------------|-------|---------------------|------|--------------|-------|------------------|-------|
| | HCTZ | LSR | HCTZ | LSR | HCTZ | LSR | HCTZ | LSR | HCTZ | LSR |
| Untreated | 2506645 | 3489964 | 100 | 100 | - | - | 0.049 | 0.087 | 1.013 | 1.044 |
| Acidic degradation | 2501642 | 3460195 | 99.80 | 99.15 | 0.20 | 0.85 | 0.101 | 0.067 | 1.029 | 1.022 |
| Alkaline degradation | 2501945 | 3476453 | 99.81 | 99.61 | 0.19 | 0.39 | 0.044 | 0.068 | 1.011 | 1.023 |
| Oxidative degradation | 2503041 | 3356984 | 99.86 | 96.19 | 0.14 | 3.81 | 0.086 | 0.102 | 1.018 | 1.053 |
| Thermal degradation | 2448944 | 3310440 | 97.70 | 94.86 | 2.30 | 5.14 | 0.094 | 0.455 | 1.042 | 1.073 |
| Photolytic degradation | 2454802 | 3389474 | 97.93 | 97.12 | 2.07 | 2.88 | 0.090 | 0.443 | 1.039 | 1.075 |
| Humidity degradation | 2438658 | 3398631 | 97.29 | 97.38 | 2.71 | 2.62 | 0.092 | 0.450 | 1.044 | 1.081 |

4. CONCLUSION

The present developed RP-HPLC method was stability indicating, simple, specific, precise, accurate and robust. It can be applied for the determination of Losartan and Hydrochlorothiazide in pharmaceutical dosage forms (tablets) as well as pharmacokinetic studies.

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