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# A VALIDATED STABILITY INDICATING LIQUID CHROMATOGRAPHIC METHOD DEVELOPMENT FOR THE SIMULTANEOUS ESTIMATION OF VALSARTAN AND HYDROCHLOROTHIAZIDE IN BULK AND TABLET DOSAGE FORMS

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## ABSTRACT

A validated, reproducible, accurate, selective, quick and eco-friendly stability- indicating isocratic reverse phase liquid chromatography method for the quantitative estimation of Valsartan and Hydrochlorothiazide in bulk and tablet dosage forms was developed. Determination of selected drugs in this combination was achieved with a C18 column [Kromasil 100-5C18 column, $P_{134}$ 250×4.6mm], isocratic mode. For the best resolution mobile phase of composition Acetonitrile and phosphate buffer (48:52 v/v, pH 4) was used. The flow rate was 1ml/min and the eluents were quantified at 235 nm, using VWD detector. The retention times were 2.6 and 3.9 for Valsartan and Hydrochlorothiazide respectively. Established analytical parameters were validated as per ICH Q2b guidelines. The established method was found to be more stable even after the samples were exposed to different stress conditions as there is no ghost peaks were identified.

**KEYWORDS:** Valsartan, Hydrochlorothiazide, Method validation and Stability.

## INTRODUCTION

Valsartan is chemically designated as N- (1-oxopentyl)-N-[[2-(1H-tetrazol-5yl)[1,1-biphenyl]-4-yl]methyl]-L-valine.[1,2] Is an angiotensin II receptor antagonist, Valsartan is used to treat high blood pressure, congestive heart failure, and to reduce death for people with left ventricular dysfunction after having had a heart attack. There is contradictory evidence with regard to treating people with heart failure with a combination of an angiotensin receptor blocker like Valsartan and an angiotensin-converting enzyme inhibitor, with two major clinical trials (CHARM-additive and ValHeFt) showing a reduction in death, and two others (VALIANT and ONTARGET) showing no benefits, and more adverse effects including heart attacks. It is official drug in Indian Pharmacopoeia and United States Pharmacopoeia.[3,4] Hydrochlorothiazide is chemically designated as 6-chloro-3, 4dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide.[1,2] Hydrochlorothiazide is a prototype drug of thiazide diuretics. Hydrochlorothiazide is antihypertensive agent. It increases the urination and reduces the amount of water and sodium retained by the body. The drugs in this class are formally called benzothiazide, usually shortened to thiazides. The nature of the heterocyclic rings and the substitution on these rings may vary among the congeners but all of them retain an unsubstituted sulfonamide group. It is official drug in Indian Pharmacopoeia and United States Pharmacopoeia.[3,4] An attempt was made to develop and validate a stable liquid chromatographic method as little work was reported in the survey of literature for the investigated drugs of interest by RP-HPLC.[5-13]

## MATERIALS AND METHODS

### Equipment used

The chromatographic separation was performed on Agilent 1120 compact isocratic liquid chromatographic system integrated with VWD detector and a Rheodyne injector equipped with 20µl fixed loop. A reverse phase C18 [Kromasil ODS UG 5 column, 250mm × 4.5mm] was used. Elico SL-218 double beam UV visible spectrophotometer was used for wavelength detection Axis AGN204-PO electronic balances were used for weighing purpose.

### Reagents and chemicals

HPLC grade Methanol and Water were procured from Merck specialties private limited, Mumbai. Pharmaceutical grade pure Valsartan and Hydrochlorothiazide gift samples were procured from Mylan Laboratories, Hyderabad. Marketed formulation Tablets with dose of 80mg of Valsartan and 12.5mg of Hydrochlorothiazide were procured from local market. (Mfd.by DIOVAN Pharmaceuticals ltd).

**Chromatographic conditions**
Kromasil ODS-$C_{18}$ column 5µm [250mm x 4.6mm] was used for the chromatographic separation at a detection wave length of 235 nm, under ambient temperature. Mobile phase of composition Acetonitrile and Phosphate buffer pH-4 in a ratio of 48:52 v/v which was degassed under ultra-sonication was selected for elution and same mixture was used in the preparation of standard and sample solutions. Flow rate was optimized to 1.0 ml/min and the injection volume was 20µl.

**Preparation of Mobile phase**
Phosphate buffer pH 4 was prepared by dissolve 0.504gm of disodium hydrogen phosphate and 0.301gm of Potassium dihydrogen phosphate of HPLC grade water and adjusts the pH to 4.0 with glacial acetic acid and sufficient water was added to produce100 ml filtered through 0.45µ membrane filter and sonicated for 10 minutes.

**Preparation of Stock solutions**
25mg each of Valsartan and Hydrochlorothiazide were accurately weighed and transferred into two 25ml volumetric flasks, dissolved using mobile phase and the volume was made up with the same solvent to obtain primary stock solutions A (Valsartan) and B (Hydrochlorothiazide) of concentration 1000µg/ml of each drug. From the primary stock solutions, 2.5ml of each was pipette out from A and B respectively, transferred to a 10ml volumetric flask and the volume was made up with the mobile phase to obtain final concentrations of 250µg/ml of each was Valsartan and Hydrochlorothiazide respectively and this solution is (working stock solution A).

**Preparation of Pharmaceutical Dosage Sample**
Twenty tablets of Valsartan and Hydrochlorothiazide were weighed and crushed. Tablet powder equivalent to 80mg of Valsartan and 12.5mg of Hydrochlorothiazide was weighed accurately and transferred to a 25ml volumetric flask. The content was dissolved with 10ml of mobile phase and then sonicated for 15min. The volume was made up with the mobile phase and filtered with 0.45µmembrane filter and sonicated for 20min. 0.1ml of this solution was pipette out and transferred to a 10ml volumetric flask and the volume was made up with the mobile phase to obtain a concentration of 250µg/ml of Valsartan and 250µg/ml of Hydrochlorothiazide (working stock solution B).

**Optimization of RP-HPLC method**
The HPLC method was optimized with an aim to develop a simultaneous estimation procedure for the assay of Valsartan and Hydrochlorothiazide. For the method optimization, different mobile phases were tried, but acceptable retention times, theoretical plates and good resolution were observed with Acetonitrile, Phosphate buffer pH 4 (48:52 v/v) using Kromasil 100-5C18 column, $P_{134}$ [250×4.6mm]

**Validation of the RP-HPLC method**
Validation of the optimized method was performed according to the ICH Q2 (B) guidelines.

**System suitability**
System suitability was carried out with five injections of solution of 100% concentration having 250µg/ml of Valsartan and 250µg/ml of Hydrochlorothiazide in to the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factors (T) were reported in table 1.

**Specificity**
Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected and no interferences were found because of the presence of excipients. The optimized chromatogram of Valsartan and Hydrochlorothiazide without any interference was shown in figure 2.

**Calibration and Linearity**
A Calibration curve is the relationship between a response by instrument and to the known concentration of the sample. This will be drawn for each analyte from the stock solution. For the determination of linearity, appropriate aliquots were pipette out from working stock solution A to a series of 10ml volumetric flasks and volume was made up with the solvent to obtain concentration ranging from 50-250µg/ml of Valsartan and 50-250µg/ml of Hydrochlorothiazide. Each solution was injected in triplicate. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients. The calibration curves for Valsartan and Hydrochlorothiazide were shown in figure 3 and figure 4 their corresponding linearity parameters were given in table 2.

**Precision**
The repeatability of the method was verified by calculating the %RSD of six replicate injections of 100% concentration (250µg/ml of Valsartan and 250µg/ml of Hydrochlorothiazide) on the same day and for intermediate precision % RSD was calculated from repeated studies on different days. The results were given in table 3.

**Accuracy**
Accuracy of an analytical method is the degree of closeness of the true value to the mean of the observed results. To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analyzed sample and contents were reanalyzed by the proposed method and the percent recovery was reported. The results were given in table 4.

**Detection and Quantitation Limits**
The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas. The results were given in table 2.

**Robustness**
Robustness of the method was verified by altering the chromatographic conditions like mobile phase composition, wavelength detection, and flow rate to evaluate its reliability and to know small variations in the method conditions. A deviation of ±2nm in the detection wave length and ±0.2ml/min in the flow rate, were tried individually. A solution of highest concentration with the specified changes in the operational conditions was injected to the instrument in triplicate. The obtained values were reported in the table 5.

**Analysis of Marketed Formulations**
20µl of sample solution of concentration 250µg/ml of Valsartan and 250µg/ml of Hydrochlorothiazide was injected into chromatographic system and the peak responses were measured. The solution was injected three times in to the column. The amount of drug present and percentage purity was calculated by comparing the peak areas of the standards with that of test samples. A typical chromatogram for assay of marketed formulation was shown in figure 5 and the obtained values were reported in the table 6.

**Stability Studies**
**Acid degradation studies**
Valsartan and Hydrochlorothiazide stock solutions were prepared by using mobile phase as solvent, and then filtered through 0.45µm membrane filter paper. Stock solutions of 2.5 ml and 2.5ml of each were transferred into 10ml volumetric flask and added 1 ml of 0.1N HCL and diluted to volume with solvent. The obtained solution was injected into the system; there was no acid degradation products were found and the obtained chromatogram was shown in figure 6.

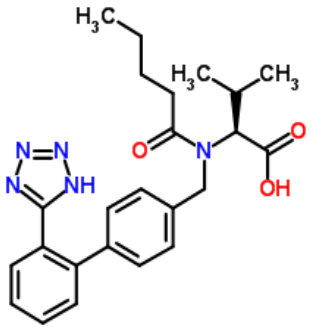
**Alkaline degradation studies**
Valsartan and Hydrochlorothiazide highest concentration of linearity range and premixed with 1ml of 0.1N NaOH solution then filtered through 0.45mm membrane filter paper. 20µl of Each stock solution was injected. The obtained non interfered chromatogram was represented in figure 7.

**Oxide degradation studies**
Valsartan and Hydrochlorothiazide were prepared by to have the primary stock solution of 1mg/ml and then filtered through 0.45µm membrane filter paper added 1 ml of $H_2O_2$ and diluted to volume with mobile phase. In this investigation no identifiable oxidative degradants were found and the chromatogram was shown in figure 8.

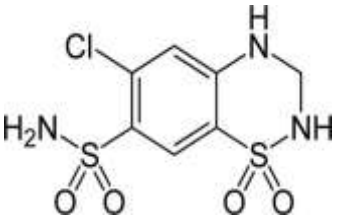
**Thermal degradation studies**
From the primary stock solutions of 1mg/ml 250µg/ml Valsartan and Hydrochlorothiazide was prepared in 10ml volumetric flask and diluted to volume with mobile phase and kept for 60min at $60^0$c in hot air oven. 20µl of above solution was injected in to system and from the obtained chromatogram it was proved that the selected samples were stable against thermal degradation. The obtained chromatogram was shown in figure 8.

## RESULTS AND DISCUSSION

After a number of trials with mobile phases of different composition, Acetonitrile, Phosphate buffer pH 4.0 in the ratio 48:52v/v was selected as mobile phase because of better resolution more no. of Theoretical plates and symmetric peaks. Valsartan and Hydrochlorothiazide were found to show appreciable absorbance at 235nm when determined spectrophotometrically and hence it was selected as the detection wavelength. An optimized chromatogram showing the separation of Valsartan and Hydrochlorothiazide at different $R_T$s was shown in figure 2.




**a. Valsartan**




**b. Hydrochlorothiazide**

**Figure 1: Chemical Structures of a) Valsartan and b) Hydrochlorothiazide.**

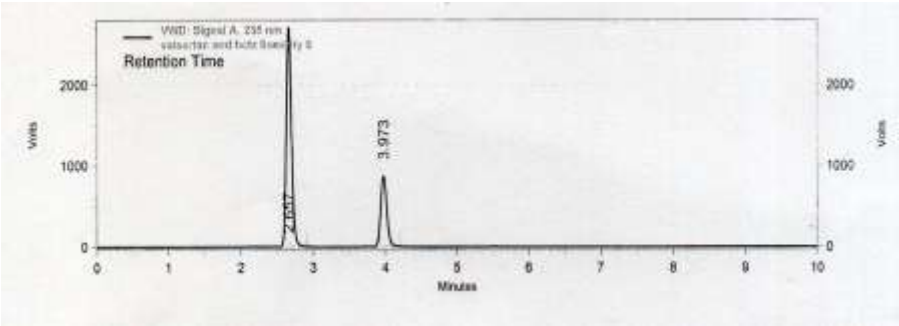



**Figure 2: Optimized chromatogram of Valsartan and Hydrochlorothiazide.**

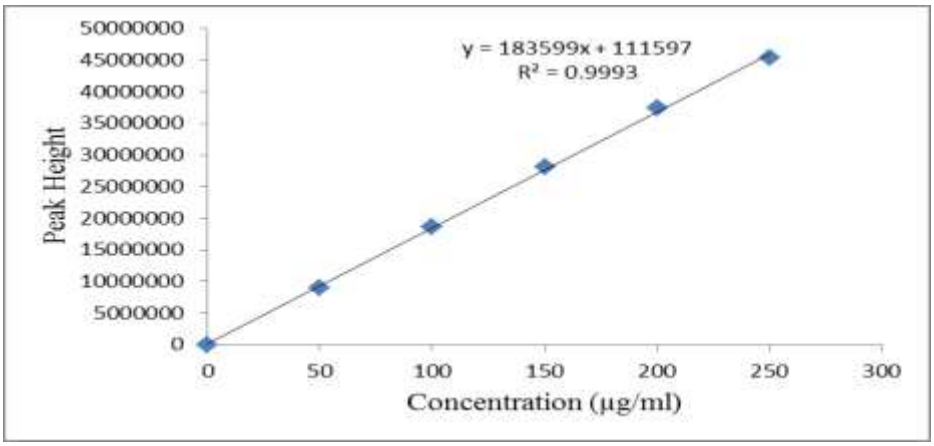



**Figure 3: Calibration plot of Valsartan.**

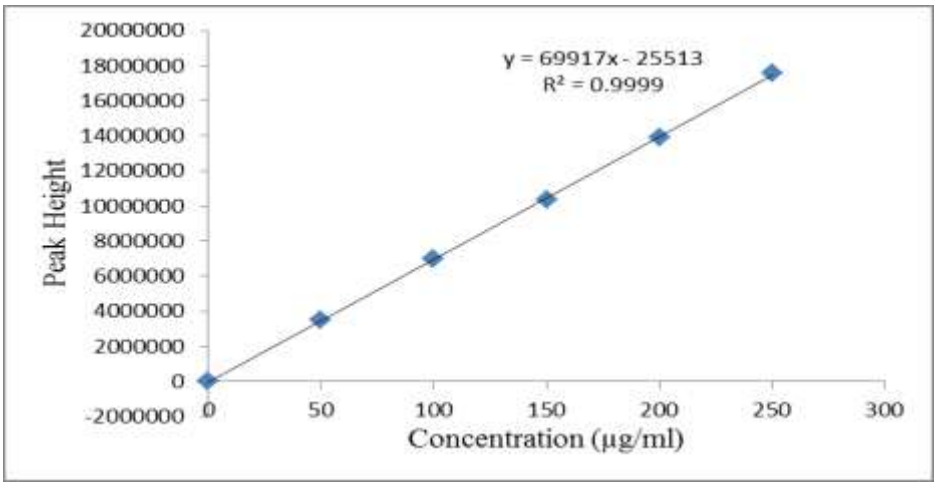



**Figure 4: Calibration plot of Hydrochlorothiazide.**

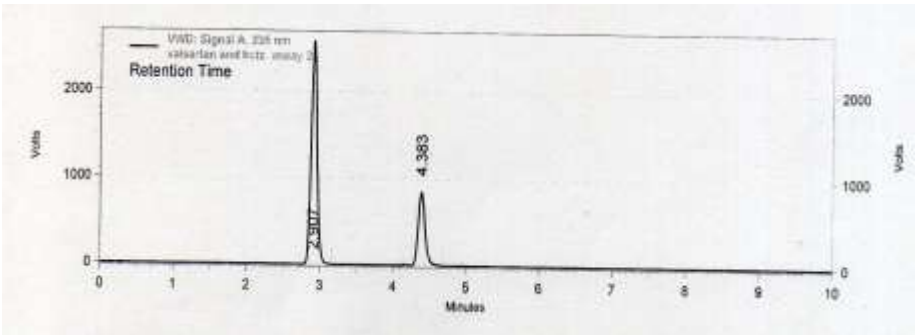



**Figure 5: A typical chromatogram for assay of marketed formulation containing 250µg/ml of Valsartan and 250 µg/ml Hydrochlorothiazide.**

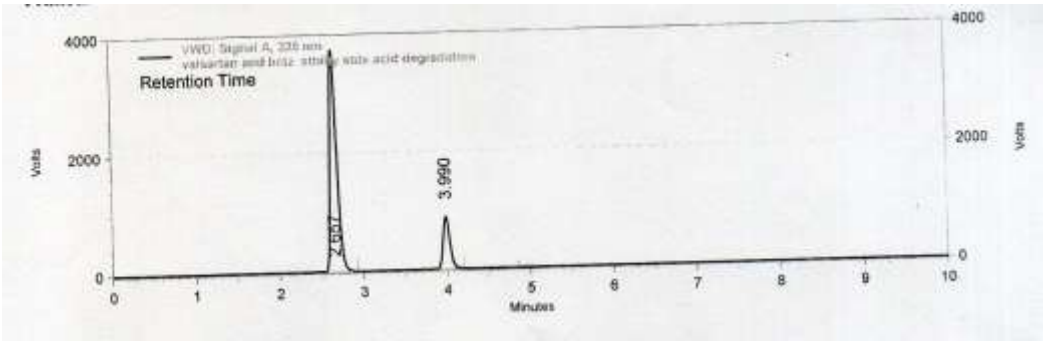



**Figure 6: Chromatogram of acid degradation.**

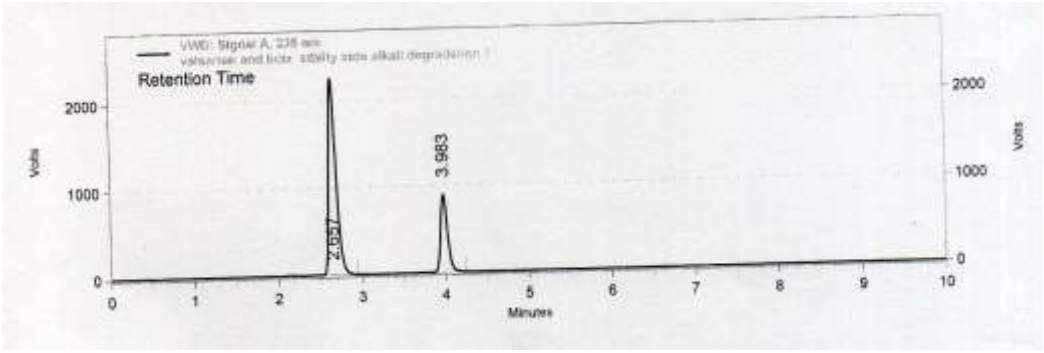



**Figure 7: Chromatogram of alkaline degradation.**

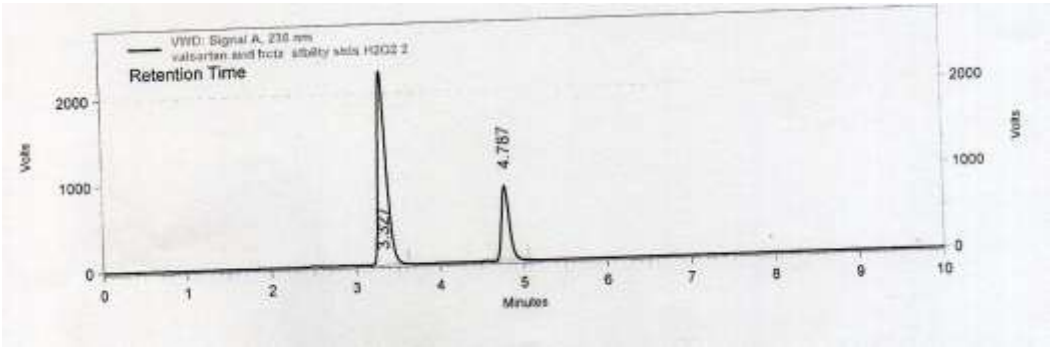



**Figure 8: Chromatogram of Hydrogen peroxide degradation.**

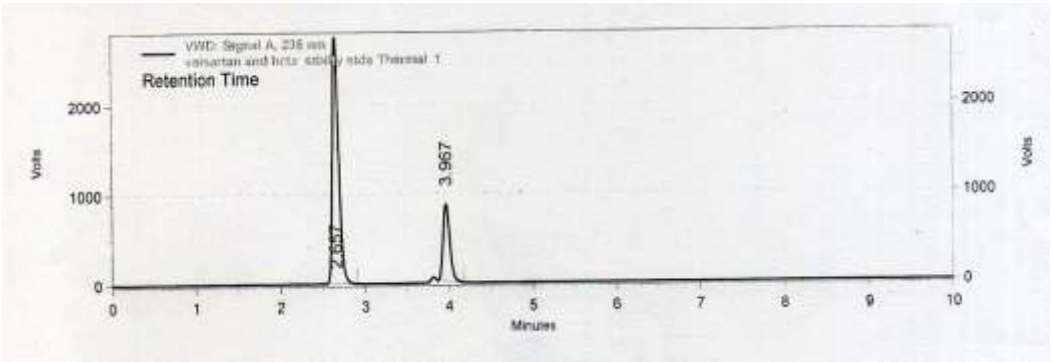



**Figure 9: Chromatogram of thermal degradation.**

**Table 1: System Suitability Parameters (n=5).**

| Parameters | Valsartan | Hydrochlorothiazide |
|---|---|---|
| Retention time (min) | 2.6 | 3.9 |
| Theoretical plates (N) | 10741 | 12586 |
| Tailing factor (T) | 1.30 | 1.05 |
| Resolution ($R_s$) | 1.7 | |

**Table 2: Results for Linearity (n=3).**

| Parameters | Valsartan | Hydrochlorothiazide |
|---|---|---|
| Slope | 183599 | 69917 |
| y intercept | 111597 | 25513 |
| Correlation coefficient $r^2$ | 0.9993 | 0.9999 |
| Regression Equation | y = 183599x + 111597 | y = 69917x – 25513 |
| Linearity range | 50-250µg/ml | 50-250µg/ml |
| LOD | 2.0µg/ml | 1.19µg/ml |
| LOQ | 6.6µg/ml | 3.92µg/ml |

**∗n= No. of determinants**

**Table 3: Results of Precision (n=6).**

| Drug | Intraday Precision (%RSD) | Interday Precision (%RSD) |
|---|---|---|
| Valsartan | 0.64 | 0.75 |
| Hydrochlorothiazide | 0.79 | 0.56 |

**∗n= No. of determinants**

**Table 4: Results for Accuracy (n=3).**

| Recovery level | Valsartan | | | | Hydrochlorothiazide | | | |
|---|---|---|---|---|---|---|---|---|
| | Amount Added (µg/ml) | | Amount Found (µg/ml) | % Recovery | Amount Added (µg/ml) | | Amount Found (µg/ml) | % Recovery |
| | std | test | | | Std | Test | | |
| 50% | 100 | 100 | 198.12 | 99.06 | 100 | 100 | 199.1 | 99.55 |
| 100% | 100 | 150 | 246.3 | 98.52 | 100 | 150 | 249.2 | 99.6 |
| 150% | 100 | 200 | 298.5 | 99.5 | 100 | 200 | 298.9 | 99.6 |
| Mean recovery | 99.02 | | | | 99.58 | | | |

**∗n= No. of determinant**

**Table 5: Results for Robustness (n=3).**

| Parameters (n=3) | %RSD | |
|---|---|---|
| | Valsartan | Hydrochlorothiazide |
| Detection wavelength at 233nm | 0.62 | 0.29 |
| Detection wavelength at 237nm | 0.71 | 0.67 |
| Flow rate 0.8ml/min | 0.83 | 0.81 |
| Flow rate 1.2ml/min | 0.54 | 0.78 |

**∗n= No. of determinants**

**Table 6: Results for Assay (n=3) of Marketed formulation.**

| Drug | Label claim (mg/tab) | Amount recovered | % Amount found in drug |
|---|---|---|---|
| Valsartan | 80 | 78.92 | 98.65% |
| Hydrochlorothiazide | 12.5 | 12.38 | 99.04% |

**∗n= No. of determinants**

**Table 7: Results for Stability studies of Valsartan and Hydrochlorothiazide combined form (n=3).**

| Parameters | Peak area | | % of degradation | |
|---|---|---|---|---|
| | Valsartan | Hydrochlorothiazide | Valsartan | Hydrochlorothiazide |
| Acid degradation | 235789215 | 84585264 | 0.122 | 0.187 |
| Alkali degradation | 235741694 | 85478962 | 0.214 | 0.145 |
| Peroxide degradation | 254187965 | 95487521 | 0.228 | 0.244 |
| Thermal degradation | 256489722 | 75895434 | 0.125 | 0.158 |

**∗n= No. of determinants**

System suitability was carried out by injecting 5 replicate injections of 100% test concentration, number of theoretical plates, HETP and resolution were satisfactory. The chromatograms confirm the presence of

Valsartan and Hydrochlorothiazide at 2.6min and 3.9min respectively without any interference. The parameters were given in table 1.

Concentration range of 50-250µg/ml for Valsartan and 50-250µg/ml of Hydrochlorothiazide were found to be linear with correlation coefficients 0.9993 and 0.9999 for Valsartan and Hydrochlorothiazide respectively. The results were given in table 2.

The proposed method was found to be precise and reproducible with %RSD of 0.64 and 0.79 for Valsartan and Hydrochlorothiazide respectively. %RSD was reported in table 3.

Accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery of the standard added to the pre-analysed sample was calculated and it was found to be for 99.02% Valsartan and 99.58% for Hydrochlorothiazide. This indicates that the method was accurate. Values obtained were given in table 4.

The limits of detection for Valsartan and Hydrochlorothiazide were found to be 2.0µg/ml and 1.19µg/ml respectively and the limit of Quantitation were 6.6µg/ml and 3.92µg/ml respectively. Values were represented in table 2.

The method was found to be robust after changing the conditions like detection wavelength (± 2nm) and flow rate (± 0.2 ml). %RSD was calculated for each variation and reported. Values obtained were given in table 5.

The method was found to be specific for the combination of interest after verifying the chromatograms showing no interference of the excipients present. Hence, the method was well suitable for the estimation of the commercial formulations of the selected combination with a percentage purity of 98.65% for Valsartan and 99.04% for Hydrochlorothiazide. The typical chromatogram for assay of marketed formulations was shown in figure.5 and Values obtained were given in table 6.

## FORCED DEGRADATION STUDY

Degradation studies indicated the specificity of developed method in presence of degradation products. Degradation was carried out in combination of two drugs and purity of drug peaks was confirmed by purity angles. Their combination drug products were exposed to acid, alkali, oxidative and thermal stress conditions .Then found to be no degradable substances presence and proved that the proposed method was stable towards acid, alkali, peroxide and thermal conditions. The obtained values were reported in table 7.

## CONCLUSION

The developed and validated high performance liquid chromatographic method was sensitive, specific and most robust, rapid for the estimation of selected drugs in combination as pure and in tablet dosage form. Validation parameters were proven to be according to the specified limits. There was no interference found indicates the selectivity and specificity. The forced degradation was conducted under stress conditions. So the developed method can be employed in the regular analysis of the marketed formulations with hundred percent confidence by modern analyst.

## ACKNOWLEDGMENTS

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