

## TABLET FORMULATION AND DEVELOPMENT OF A VALIDATED STABILITY INDICATING HPLC METHOD FOR QUANTIFICATION OF VALSARTAN AND HYDROCHLORTHIAZIDE COMBINATION

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### ABSTRACT

This study was aimed to develop valsartan/ hydrochlorothiazide tablet formulation and to develop a stability indicating HPLC method for their analysis in raw materials and in its final dosage form according to the ICH guidelines. Film coating tablets containing valsartan and hydrochlorothiazide were developed. A gradient HPLC method was performed; the flow rate was 1.5 ml/min, injected volume 20 $\mu$ L, the mobile phases consist of two solvent: Solvent A (0.20 M ammonium acetate, adjusted to pH 5.6 with glacial acetic acid) and Solvent B (acetonitrile) and UV detection was carried out at 265nm. Valsartan and hydrochlorothiazide and their combined dosage form were exposed to thermal, oxidative, acid-base hydrolytic stress conditions, the stressed samples were analyzed. The method was validated with respect to linearity, precision, accuracy, system suitability, and robustness. The used method is specific for the estimation of valsartan and hydrochlorothiazide in presence of their degradation products and impurities. The method was linear over the range of 2.5–32 $\mu$ g/mL and 17.5–224 $\mu$ g/mL for valsartan and hydrochlorothiazide respectively. The mean recoveries were 100 $\pm$ 2% for valsartan and hydrochlorothiazide respectively. The percentage of relative standard deviation (%RSD) was found to be less than critical value. Our developed analytical method is a stability indicating, economical and easy method which is useful in the quality control of valsartan and hydrochlorothiazide in tablet dosage forms.

**Keywords:** Valsartan, Hydrochlorothiazide, Formulation, Stability, Stress conditions, HPLC, Validation.

### INTRODUCTION

Valsartan is (S)-3-methyl-2-(N-([2'-(2H-1,2,3,4-tetrazol-5-yl)biphenyl-4-yl] methyl) pentanamido) butanoic acid. It is a nonpeptide orally active and specific angiotensin II antagonist acting on the AT<sub>1</sub> receptor subtype present in many tissue such as vascular smooth muscle and the adrenal gland<sup>1</sup>. A Palcebo controlled trial have found that valsartan to be both safe and effective for the treatment of hypertension<sup>2</sup> other studies have also shown that valsartan is as effective as enalapril, lisinopril and amlodipine in the treatment of mild to moderate hypertension<sup>3-5</sup>. Hydrochlorothiazide (HCT) is 6-chloro-3,4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide-1,1-, It is a diuretic of the class of benzothiadiazines mainly used as antihypertensive agent either alone or in combination with other drugs, which decreases active sodium reabsorption and reduces peripheral vascular resistance<sup>6, 7</sup>. Valsartan and hydrochlorothiazide in a combination was evaluated and showed to be effective in the treatment of hypertension<sup>8</sup>. HPLC method for the determination of valsartan and hydrochlorothiazide alone for assay and bioavailability is available in the literature<sup>9-11</sup>. However, few methods appeared in the literature for simultaneous determination of valsartan and hydrochlorothiazide in tablets, these methods used capillary electrophoresis, HPTLC and UV-derivative spectrophotometry and HPLC, most of these procedures were inconvenient for determination of the combination and their run time was rather long<sup>12-17</sup>. Accordingly, the development of a stability indicating HPLC method, as a part of the development of valsartan/hydrochlorothiazide tablet, would be a useful tool for the evaluation of the stability of these agents since this method is economical and stability indicating. This study was aim to develop valsartan/hydrochlorothiazide tablets and to develop a stability indicating HPLC method for their analysis in raw materials and tablet formulation according to the ICH guidelines<sup>18</sup>.

### MATERIALS AND METHODS

#### Instrumentation

HPLC system (Merck Hitachi Lachrom Elite HPLC system, Japan) with an L-2130 pump, an L-2200 autosampler, L-2300 oven, and L-2490 UV detector was employed. The Ezchrom Elite software was employed. The chromatographic analysis was performed on a

phenyl group bonded to porous silica particles column Xterra, the particle size is 5  $\mu$ m, the length is 25 cm and the internal diameter is 4.6 mm, the column part number is 186001147 and the lot number is 0111360671 from water. Precisa 205A Analytical balance, Elma ultrasonic water bath, and Millipore filtration assembly were used in this study.

#### Materials and Reagents

Valsartan and Hydrochlorothiazide were purchased from (Ipca Laboratories, India); USP Reference standards were used as working reference standard. USP benzothiadiazine related compound A, USP Chlorothiazide and USP Valsartan related compound B were used in the validation. The tablet dosage form (containing Valsartan 80mg and hydrochlorothiazide 12.5mg) was formulated in our laboratory the formulation is detailed in section 2.3. All the excipients used for the development of formulations were obtained from commercial sources and were used as such. The acetonitrile used was of HPLC grade and water was obtained by reverse osmosis.

Other reagents such as ammonium acetate, glacial acetic acid, hydrochloric acid (2M), sodium hydroxide (2M), potassium dihydrogen phosphate and 35% hydrogen peroxide were used in the additions and reactions. Colloidal Silicon Dioxide was achieved from (Aerosil; Evonik, Germany), Croscarmellose Sodium (AcDiSol; FMC-Ireland), Magnesium Stearate, (Magnesia, Germany), Microcrystalline Cellulose (Avicel pH 102; FMC Corp, Ireland), Polyvinylpyrrolidone (PVP K-30; Zhongbao Chemicals, China), Sodium Benzoate (DSM, Netherlands) and Sodium Lauryl Sulphate was achieved from Cognis, Germany. Hydroxy propyl methyl cellulose aqueous polymer (Opadry White) was purchased from Colorcon, England.

#### Formulation of Valsartan/hydrochlorothiazide tablets

Core tablets containing the above combination were prepared using the dry granulation method using the following excipients: Colloidal Silicon Dioxide, Croscarmellose Sodium, Magnesium Stearate, Microcrystalline Cellulose, Polyvinylpyrrolidone, Sodium Benzoate and Sodium Lauryl Sulphate. All components were blended in a double cone mixer (use only 50% of Magnesium Stearate and 50% of AcDiSol). The blended powder mixture was sieved through mesh #

30, and compacted using a roller compactor. The compacted material was crushed through an oscillator equipped with sieve mesh # 20, and blended with the remaining portions of AcDiSol and Magnesium Stearate. The tablets were compressed on 8-station rotary tablet press. Film coating was carried out by using aqueous dispersion of Opadry white and conventional coating pan at a temperature not exceeding 55 Celsius degrees.

#### Chromatographic conditions

Chromatographic separation was operated at room temperature on a reversed phase phenyl column. The diluents used to attain the final concentration consist of a mixture of water: acetonitrile (1:1). Flow rate was 1.5 ml/min, injected volume 20 $\mu$ L, wavelength of detection is 265nm. The mobile phases consist of two solvent: Solvent A (0.20 M ammonium acetate, adjusted to pH 5.6 with glacial acetic acid) and Solvent B (acetonitrile).

Gradient elution system was used which consist of solvent A and Solvent B. The program of gradient elution is shown in **Table 1** below.

**Table 1: Gradient elution (solvent programming) runs**

| Time(minutes) | Solution A (%) | Solution B(%) |
|---------------|----------------|---------------|
| 0             | 88             | 12            |
| 4             | 65             | 35            |
| 7             | 88             | 12            |
| 8             | 88             | 12            |

#### Preparation of Stock Standard Solutions

An accurately weighed amount of USP hydrochlorothiazide RS (approximately 12.5 mg) added into 200ml volumetric flask. The sample was dissolved in the diluents and sonicated for 15 minutes. A series of dilution was done to obtain a final concentration of 0.0125 mg/ml. For the valsartan stock solution the same procedure was followed in order to obtain a final concentration of 0.16mg/ml.

#### Preparation of Sample

An accurately weighed 5 tablets were crushed to a fine powder and an amount of the powder was taken in order to get 62.5 mg of hydrochlorothiazide and 300mg of valsartan. 100mls of the diluents were added to the powder, the mixture was sonicated for 30 minutes, and then it was centrifuged at a high speed (3000 rpm). An accurate quantity of supernatant was pipetted and a series of dilution was made to give final concentration of 0.012 and 0.16mg/ml for hydrochlorothiazide and Valsartan respectively.

#### Method validation

The method was validated for the parameters like, range and linearity, accuracy, precision, limit of detection (LOD) and quantification (LOQ). Concerning the specificity of the method it was assessed by performing a stability indicating study.

To evaluate the linearity of the method, nine different dilutions were made from the standard stock solutions in the working range of 20–140%.

In order to determine the accuracy of the method, working standards of the combination were prepared in five replicates of two nominal concentration (0.125 and 0.08 mg/ml) and (0.125 and 0.16 mg/ml) for the hydrochlorothiazide and valsartan respectively, and the relative standard deviation (%RSD) was calculated. Three different concentrations (80%, 100% and 120%) of active ingredient combination were spiked in the tablet formulation and their recovery was calculated. Regarding the determination of the precision (repeatability) five replicate injections of the working standard at the two nominal concentrations for Valsartan & Hydrochlorothiazide Tablets and the relative standard deviation (RSD) of the peak areas were calculated for the replicate injections. To determine the LOD and LOQ, serial dilutions of the combination were made from the standard stock solution in the range of 0.008-1.92 $\mu$ g/ml for Valsartan 0.00625-0.15  $\mu$ g/ml for hydrochlorothiazide. The signal from the samples was compared with those of blank samples. LOD and LOQ values were identified as signal-to-noise ratio (S/N) of 3:1 and 10:1, respectively.

To determine the specificity of method in the presence of pharmacopoeial impurities and formulation impurity; the valsartan and hydrochlorothiazide and the pharmacopoeial impurity were spiked in the presence of excipients used in formulation development, the observed chromatograms were checked for the purity using a photo-diode array (PDA) detector. The detailed procedure mentioned in section 2.8.

Data from replicate injections at the nominal concentration was utilized for calculating various system suitability parameters using Merck-Hitachi Lachrom Elite HPLC system.

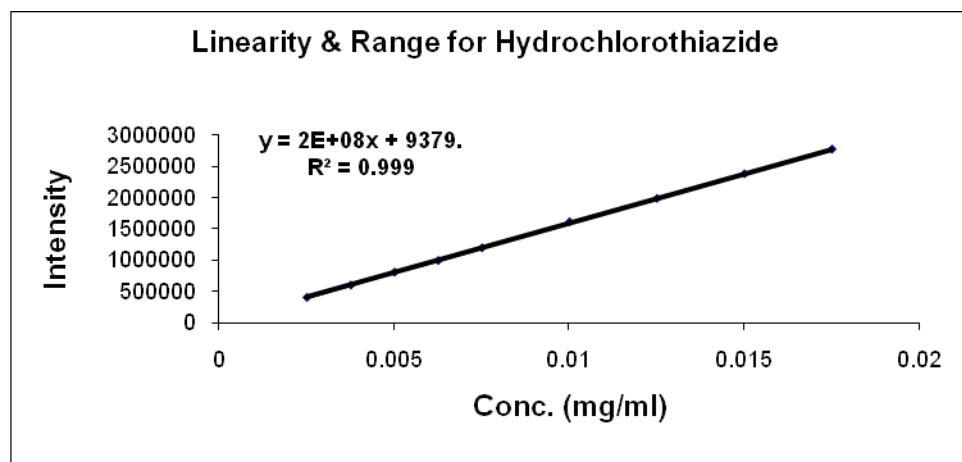
#### Specificity and Stability indicating studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Intentional degradation was carried out by exposing of samples to three stability condition (0.1 N HCl, at 65 °C), (2 N NaOH, at 65 °C) and (35% H<sub>2</sub>O<sub>2</sub> at 65 °C) and tested after 1, 2 and 3 hours consequently. They were then analyzed against control samples (lacking of degradation treatment).

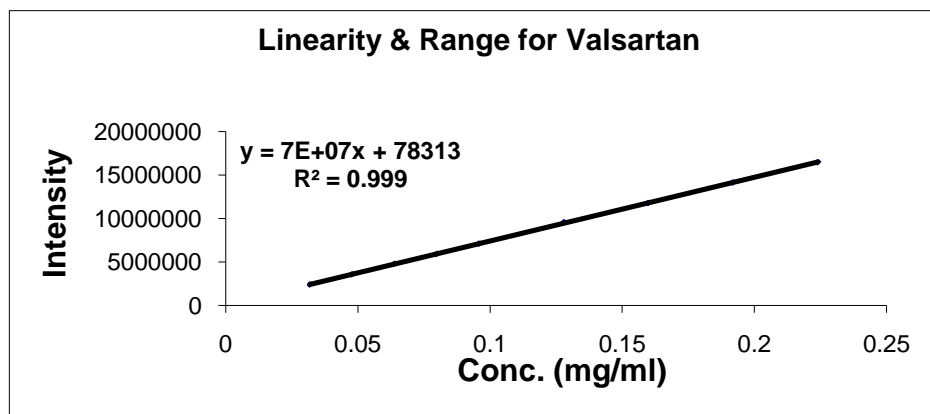
## RESULT & DISCUSSION

#### Linearity and range

The linearity of the method was observed in the expected concentration range (20% to 140%) demonstrating its suitability for analysis. The goodness-of-fit (R<sup>2</sup>) was found to be 0.9999 indicating functional linear relationship between the concentration of analyte and area under the peak. The regression equation for both the hydrochlorothiazide and valsartan is shown in **Figure 1 & 2** respectively.



**Fig. 1: Linearity and rang of hydrochlorothiazide, R<sup>2</sup>=0.9999.**

Fig. 2: Linearity and rang of Valsartan,  $R^2=0.9999$ 

### Accuracy

The results of accuracy studies (Table 3) show that the method is accurate within the desired range.

The RSD was calculated for each recovery solution and all the results are within limits ( $100 \pm 2\%$ ) and as shown in Figure 3 & 4 a plot of area under peak versus concentration for each level of Valsartan and Hydrochlorothiazide were plotted. The goodness-of-fit ( $R^2$ ) for Valsartan equal to (0.9999) and for Hydrochlorothiazide equal to (0.9999).

### Precision

The precision (repeatability) of an analytical method refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment and is expressed as the %RSD. The precision study (Table 4) showed that method has a good reproducibility which was approved by the analysis of five replicate injections of the working standard solution

at the two nominal concentrations the %RSD was 0.1% for Valsartan and Hydrochlorothiazide of the high nominal concentration and the RSD was 0.2% for Valsartan and Hydrochlorothiazide of the low nominal concentration, thus the system was repeatable.

### Detection and quantification limit (LOD & LOQ)

The detection limit or LOD is the lowest amount of analyte in a sample that can be detected. It may be expressed as a concentration that gives a signal to noise ratio of approximately 3:1. While the Quantification limit or LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy with a signal to noise ratio of approximately 10:1 can be taken as LOQ of the. Our method (Table 5) showed the (LOD) for Hydrochlorothiazide and Valsartan were found to be  $0.008 \mu\text{g/ml}$  and  $0.0375 \mu\text{g/ml}$ , respectively and The LOQ values for Hydrochlorothiazide and Valsartan were found  $0.075 \mu\text{g/ml}$  and  $0.064 \mu\text{g/ml}$  respectively.

Table 3: (A) Accuracy of Drug Products Data for hydrochlorothiazide:

| Conc. %     | Conc. HCT (mg/ml) | Area Sa | % Accuracy (Recovery) | Average & %RSD |
|-------------|-------------------|---------|-----------------------|----------------|
| <b>80%</b>  |                   |         |                       |                |
| 80%         | 0.01              | 1596162 | 100.6                 | 100.7%         |
| 80%         | 0.01              | 1597927 | 100.7                 | &              |
| 80%         | 0.01              | 1598073 | 100.7                 | 0.07%          |
| <b>100%</b> |                   |         |                       |                |
| 100%        | 0.0125            | 1970267 | 99.4                  | 99.4%          |
| 100%        | 0.0125            | 1969541 | 99.3                  | &              |
| 100%        | 0.0125            | 1971760 | 99.4                  | 0.06%          |
| <b>120%</b> |                   |         |                       |                |
| 120%        | 0.015             | 2359903 | 99.2                  | 99.1%          |
| 120%        | 0.015             | 2359050 | 99.1                  | &              |
| 120%        | 0.015             | 2359166 | 99.1                  | 0.02%          |

Table 3: (B) Accuracy of Drug Products Data for Valsartan

| Conc. %     | Conc. Valsartan (mg/ml) | Area Sa  | % Accuracy (Recovery) | Average & %RSD |
|-------------|-------------------------|----------|-----------------------|----------------|
| <b>80%</b>  |                         |          |                       |                |
| 80%         | 0.128                   | 9640866  | 102.3                 | 102.0%         |
| 80%         | 0.128                   | 9621415  | 102.1                 | &              |
| 80%         | 0.128                   | 9599046  | 101.8                 | 0.2%           |
| <b>100%</b> |                         |          |                       |                |
| 100%        | 0.16                    | 11842204 | 100.5                 | 100.6%         |
| 100%        | 0.16                    | 11849328 | 100.6                 | &              |
| 100%        | 0.16                    | 11887146 | 100.9                 | 0.2%           |
| <b>120%</b> |                         |          |                       |                |
| 120%        | 0.192                   | 14172564 | 100.2                 | 100.2%         |
| 120%        | 0.192                   | 14154596 | 100.1                 | &              |
| 120%        | 0.192                   | 14162613 | 100.2                 | 0.1%           |

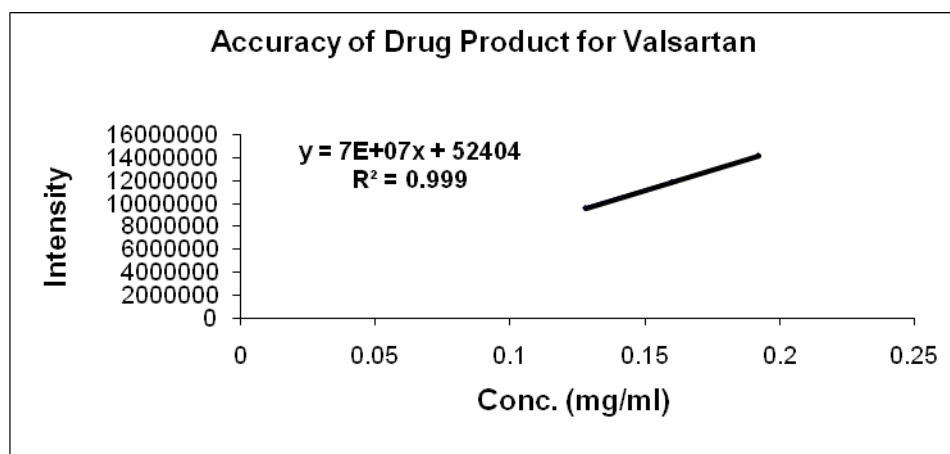


Fig. 3: Linearity of recovery results for each level of Valsartan

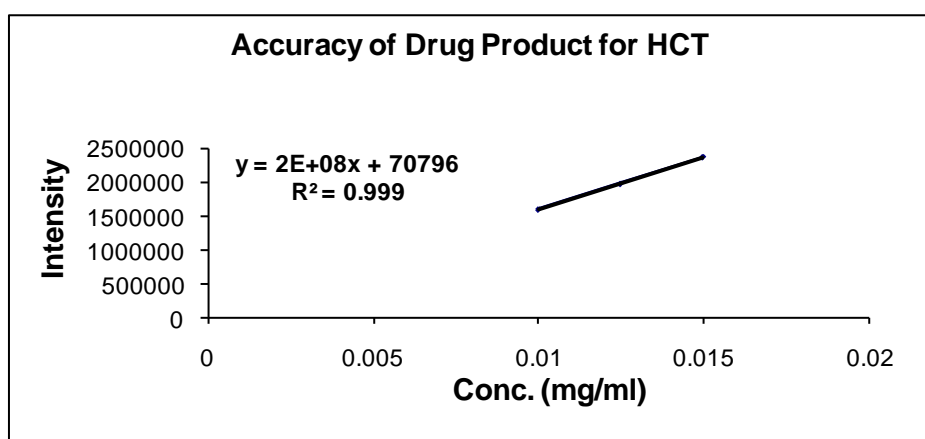


Fig. 4: Linearity of recovery results for each level of hydrochlorothiazide

Table 4: Precision of Standard Injection Data:

| Injection No.  | Nominal Concentration-1 |           | Nominal Concentration-2 |           |
|----------------|-------------------------|-----------|-------------------------|-----------|
|                | Valsartan               | HCT       | Valsartan               | HCT       |
| I              | 11771753                | 1981234   | 5907327                 | 1976382   |
| II             | 11798757                | 1986000   | 5931527                 | 1985052   |
| III            | 11778824                | 1981530   | 5935778                 | 1985874   |
| IV             | 11786028                | 1983579   | 5930076                 | 1985410   |
| V              | 11778766                | 1980904   | 5923565                 | 1981678   |
| <b>Average</b> | 11782826                | 1982649.4 | 5925655                 | 1982879.2 |
| <b>SD</b>      | 10236.7                 | 2144.4    | 11144.3                 | 3992.2    |
| <b>RSD</b>     | 0.1%                    | 0.1%      | 0.2%                    | 0.2%      |

Note: Nominal Concentration-1: 0.0125 mg/ml of HCT & 0.16 mg/ml of Valsartan

Nominal Concentration-2: 0.0125 mg/ml of HCT & 0.08 mg/ml of Valsartan

Table 5: (A) Detection and Quantification Limit Data for Valsartan

| Concentration (mg/ml) | Area 1 <sup>st</sup> Injection | Area 2 <sup>nd</sup> Injection | Area 3 <sup>rd</sup> Injection | Average Area | SD      | Av. S/N |
|-----------------------|--------------------------------|--------------------------------|--------------------------------|--------------|---------|---------|
| 0.00192               | 174990                         | 181731                         | 174073                         | 176931       | 4181.8  | 152.3   |
| 0.00096               | 98077                          | 97546                          | 97779                          | 97801        | 266.2   | 84.9    |
| 0.00048               | 50302                          | 61626                          | 60809                          | 57579        | 6315.3  | 47.8    |
| 0.000128              | 38242                          | 64242                          | 36623                          | 46369        | 15499.6 | 15.7    |
| 0.000032              | 34149                          | 35604                          | 29757                          | 33170        | 3044    | 17.7    |
| 0.000064              | 29757                          | 27788                          | 28385                          | 28643        | 1009.6  | 11.5    |
| 0.000008              | 7468                           | 5899                           | 5820                           | 6396         | 929.5   | 5.2     |

Table 5: (B) Detection and Quantification Limit Data for hydrochlorothiazide

| Concentration (mg/ml) | Area 1 <sup>st</sup> Injection | Area 2 <sup>nd</sup> Injection | Area 3 <sup>rd</sup> Injection | Average Area | SD    | Av. S/N |
|-----------------------|--------------------------------|--------------------------------|--------------------------------|--------------|-------|---------|
| 0.00015               | 24847                          | 25529                          | 24350                          | 24908.7      | 591.9 | 22.2    |
| 0.000075              | 11845                          | 12124                          | 11845                          | 11938        | 161.1 | 12.3    |
| 0.0000375             | 5702                           | 6009                           | 6089                           | 5933.3       | 204.3 | 4.3     |
| 0.00001               | Undetectable                   |                                |                                |              |       |         |
| 0.0000025             | Undetectable                   |                                |                                |              |       |         |
| 0.000005              | Undetectable                   |                                |                                |              |       |         |
| 0.00000625            | Undetectable                   |                                |                                |              |       |         |

Table 6: System Suitability Data

| Injection No. | BenzoThiadiazine R.C.A | HCT     | R <sub>1</sub> | Valsartan R.C.B | Valsartan | R <sub>2</sub> |
|---------------|------------------------|---------|----------------|-----------------|-----------|----------------|
| I             | 1399625                | 2440437 | 5.1            | 3714765         | 1497654   | 3.5            |
| II            | 1397373                | 2447324 | 5.1            | 3713686         | 1517524   | 3.5            |
| III           | 1397420                | 2444827 | 5.1            | 3704092         | 1501012   | 3.5            |
| IV            | 1397748                | 2437231 | 5.1            | 3711011         | 1525622   | 3.5            |
| V             | 1401041                | 2442007 | 5.1            | 3717381         | 1502139   | 3.5            |
| AV            | 1398641                | 2442365 | 5.1            | 3712187         | 1508790   | 3.5            |
| SD            | 1629.8                 | 3900.5  |                | 5069.1          | 12127.9   |                |
| RSD           | 0.1%                   | 0.2%    |                | 0.1%            | 0.8%      |                |

R<sub>1</sub>: Resolution between Benzothiadiazine Related Compound A & Hydrochlorothiazide; R<sub>2</sub>: Resolution between Valsartan Related Compound B & Valsartan

#### Specificity and stability indicating study

Stress testing of the drug substance can help identify the likely degradation products, the stability of the molecule and also validate the stability and selectivity of the analytical procedures. Stability indicating study was performed under various stress conditions mentioned in section 2.8. The results of specificity studies (Table 6) indicated no interference from excipients, impurities and degraded products due to various stress conditions and assured that the peak response was due to a single component only.

The chromatogram of samples degraded with acid, base and hydrogen peroxide showed peak of USP related compounds and are well resolved from the drug peak. The Valsartan and Hydrochlorothiazide was affected in the presence of Hydrogen Peroxide and Hydrochloric Acid at the three period of digestion (After one, two and three hours), Hydrochlorothiazide was also affected when treated with temperature. Hydrochlorothiazide was

degraded to Benzothiadiazine Related Compound A and Valsartan was degraded to Valsartan Related Compound B. Hydrochlorothiazide was not affected in the presence of Sodium Hydroxide at both conditions and also in the presence of Hydrogen Peroxide at room temperature. Valsartan was affected in the presence of Sodium Hydroxide at both conditions.

In all cases of study the peak of Hydrochlorothiazide and Valsartan is clearly separated from each other and from their related degradant (Benzothiadiazine Related Compound A, chlorothiazide and Valsartan Related Compound B, respectively) and from all other peaks with a resolution greater than 1.5 (Figure 5). The excipient peak (Sodium benzoate) of the tablet formulation did not interfere with the active ingredient and its related degradative compounds as shown in (Figure 6) Thus the method is specific and stability indicating for determination of Hydrochlorothiazide and Valsartan and their related degradants in Hydrochlorothiazide and Valsartan Tablets.

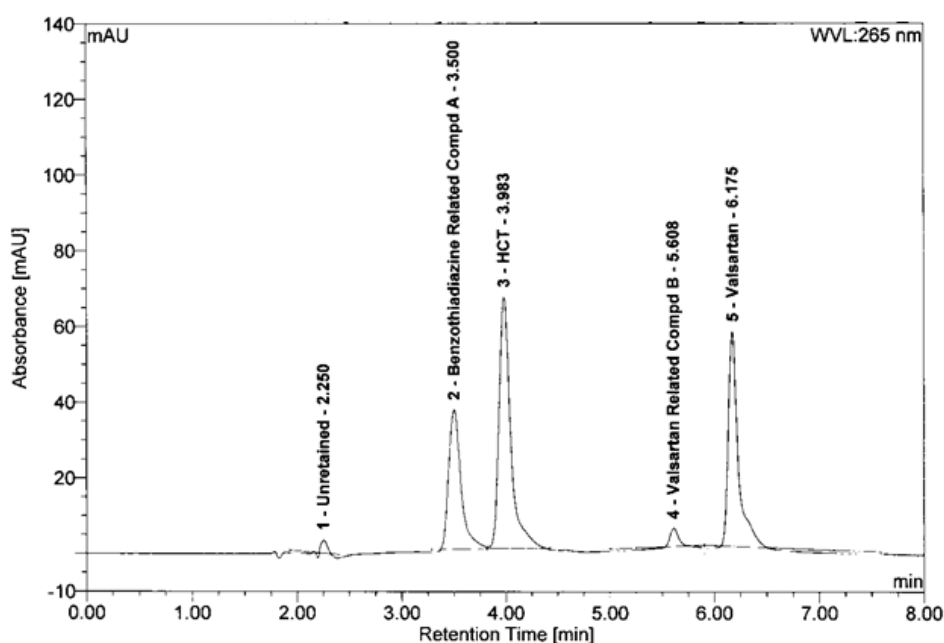


Fig. 5: Stability indicating chromatogram for determination of Hydrochlorothiazide and Valsartan.

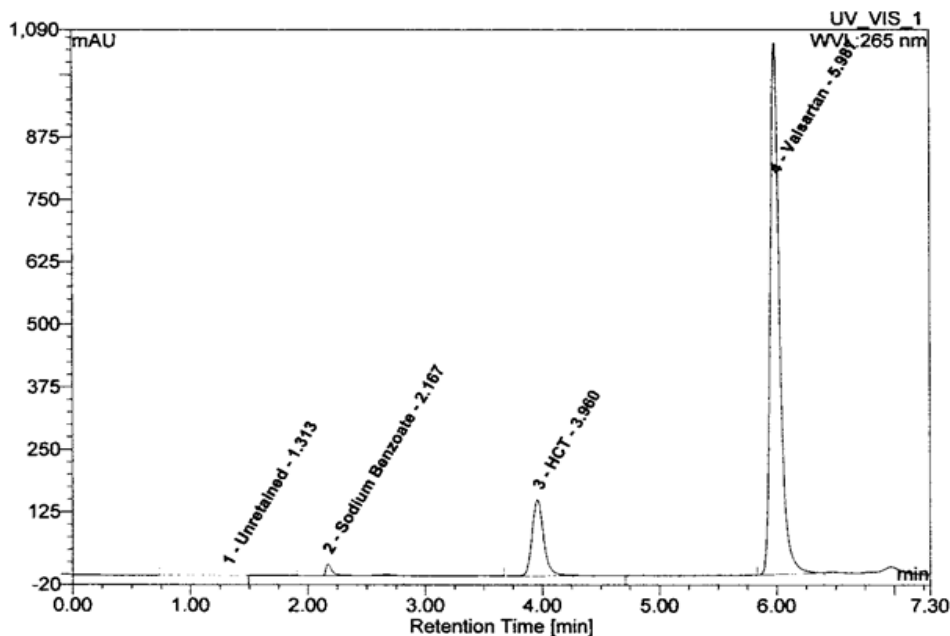


Fig. 6: Formulation except (Na Benzoate) early elute and do not interfere with the active ingredients or its related compounds.

**System suitability testing**

System suitability is used to verify that the system is adequate for the analysis to be performed.

Our method shows all the values for the system suitability parameters are within limits (Table 7).

The column efficiency is about 24940 and 29799 theoretical plates for hydrochlorothiazide and valsartan, respectively. The tailing factors are about 1.3 and 1.2 for hydrochlorothiazide and valsartan, respectively. The resolution between benzothiadiazine related compound A and hydrochlorothiazide is about 5.1, while for valsartan and related compound B is about 3.5 (Figure 7).

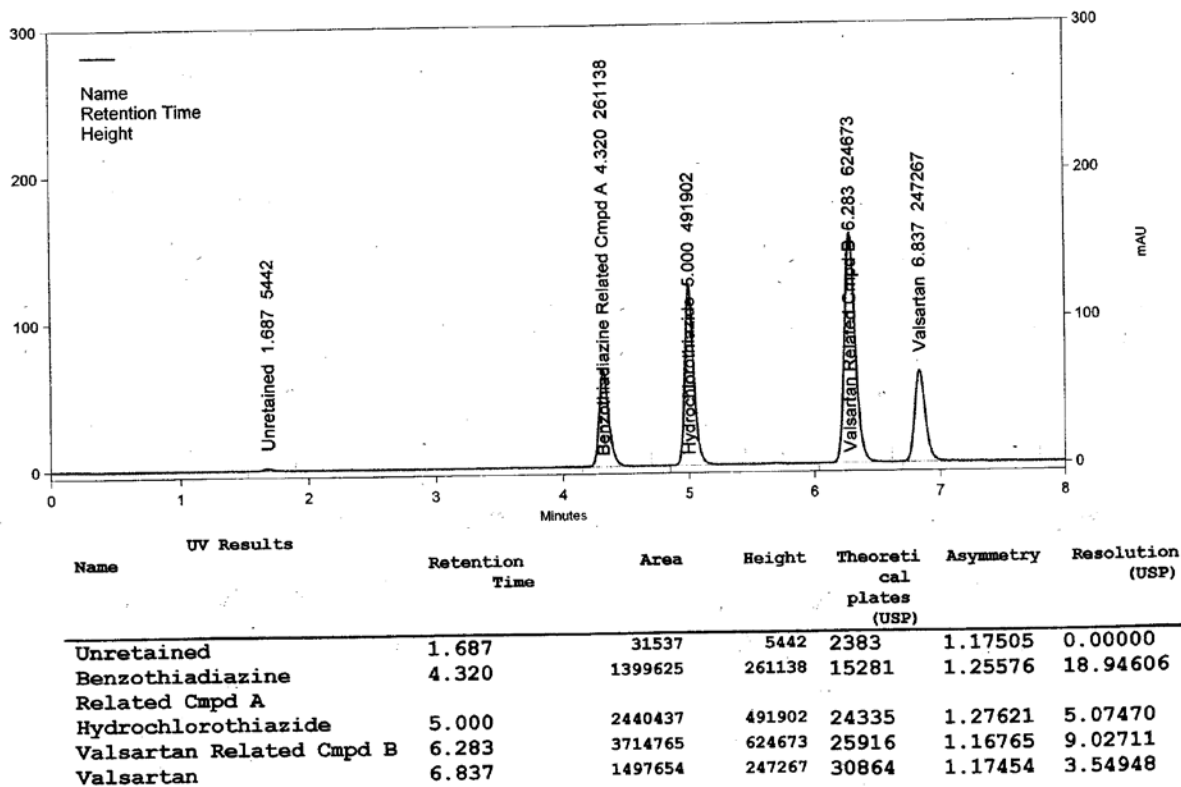


Fig. 7: System parameters for valsartan and hydrochlorothiazide and its related compounds

Table 7: Specificity and stability indicating study

| Stress Condition                              | HCT     |       | Benzothiadiazine R.C.A. |      | Valsartan |       | Valsartan R.C.B. |      | Total Degradation |     |
|---|---------|-------|-------------------------|------|-----------|-------|------------------|------|-------------------|-----|
|   | Area    | %     | Area                    | %    | Area      | %     | Area             | %    | Area              | %   |
| A.24Hr.@ RT                                   | 1964644 | 99.1  | 57839                   | 2.9  | 11811271  | 100.2 | 0                | 0.0  | 0                 | 0.0 |
| A.24Hr.+A.1Hr. at 65°C                        | 1708710 | 86.2  | 336160                  | 17.0 | 11730315  | 99.6  | 0                | 0.0  | 0                 | 0.0 |
| A.24Hr.+A.2Hr. at 65°C                        | 1447350 | 73.0  | 648427                  | 32.7 | 11796274  | 100.1 | 0                | 0.0  | 0                 | 0.0 |
| A.24Hr.+A.3Hr. at 65°C                        | 1252015 | 63.1  | 873358                  | 44.0 | 11805892  | 100.2 | 0                | 0.0  | 0                 | 0.0 |
| 10ml of 2N NaOH                               | 1983211 | 100.0 | 0                       | 0    | 11733302  | 99.6  | 0                | 0.0  | 0                 | 0.0 |
| NaOH+A.1Hr. at 70°C                           | 1982836 | 100.0 | 35591                   | 1.8  | 11808261  | 100.2 | 0                | 0.0  | 0                 | 0.0 |
| 10ml of 35% H <sub>2</sub> O <sub>2</sub>     | 1981457 | 99.9  | 11661                   | 0.6  | 11650106  | 98.9  | 0                | 0.0  | 610109            | 4.4 |
| H <sub>2</sub> O <sub>2</sub> +A.1Hr. at 65°C | 917076  | 46.2  | 191379                  | 9.7  | 8790370   | 74.6  | 772128           | 6.6  | 433293            | 3.1 |
| H <sub>2</sub> O <sub>2</sub> +A.2Hr. at 65°C | 474794  | 23.9  | 241443                  | 12.2 | 6960153   | 59.1  | 1187290          | 10.1 | 516054            | 3.7 |
| H <sub>2</sub> O <sub>2</sub> +A.3Hr. at 65°C | 0       | 0     | 292123                  | 14.7 | 5208048   | 44.2  | 1520483          | 12.9 | 625892            | 4.5 |
| HCl+A.1Hr. at 65°C                            | 1916826 | 96.7  | 86745                   | 4.4  | 10706266  | 90.9  | 0                | 0    | 30237             | 0.2 |
| HCl+A.1Hr. at 65°C                            | 1866502 | 94.1  | 148857                  | 7.5  | 10614487  | 90.1  | 0                | 0    | 51471             | 0.4 |
| HCl+A.2Hr. at 65°C                            | 1771598 | 89.3  | 256261                  | 12.9 | 6811046   | 57.8  | 0                | 0    | 96905             | 0.7 |

HCT: Hydrochlorothiazide; R.C.A.: Related Compound A; R.C.B.: Related Compound B; A.: After; Hr.: Hour; A. 1Hr. at 65°C: Means after one hour of digestion at 65°C.

10ml of reagents (NaOH, HCl & H<sub>2</sub>O<sub>2</sub>) added to the test solution separately.

### CONCLUSION

The valsartan/hydrochlorothiazide tablets were successfully prepared. The developed stability indicating HPLC method was successfully developed and it showed several advantages over other known methods for the analysis of these agents since it is an economical, single method that can be used for assay of the two ingredients. In addition, the method has been successfully used for analysis of drug-excipient compatibility samples by performing a stability indicating study. The method was validated in accordance to the ICH guidelines showing linearity, accuracy, precision, selectivity, stability and system suitability. The method can also be used for purity and degradation evaluation.

### Conflict of Interest

The authors declare no conflict of interests.

### REFERENCES

- Weber, M. A., Angiotensin II receptor blockers and cardiovascular outcomes: what does the future hold? *Journal of the Renin-Angiotensin-Aldosterone System* 2003, 4 (2), 62-73.
- Oparil, S.; Dyke, S.; Harris, F.; Kief, J.; James, D.; Hester, A.; Fitzsimmons, S., The efficacy and safety of valsartan compared with placebo in the treatment of patients with essential hypertension. *Clinical Therapeutics* 1996, 18 (5), 797-810.
- Holwerda, N. J.; Fogari, R.; Angeli, P.; Porcellati, C.; Hereng, C.; Oddou-Stock, P.; Heath, R.; Bodin, F. Valsartan, a new angiotensin II antagonist for the treatment of essential hypertension: efficacy and safety compared with placebo and enalapril. *Journal of Hypertension* 1996, 14 (9), 1147-51.
- Black, H. R.; Graff, A.; Shute, D.; Stoltz, R.; Ruff, D.; Levine, J.; Shi, Y.; Mallovs, S., Valsartan, a new angiotensin II antagonist for the treatment of essential hypertension: efficacy, tolerability and safety compared to an angiotensin-converting enzyme inhibitor, lisinopril. *Journal of Human Hypertension* 1997, 11 (8), 483-9.
- Corea, L.; Cardoni, O.; Fogari, R.; Innocenti, P.; Porcellati, C.; Provvidenza, M.; Meilenbrock, S.; Sullivan, J.; Bodin, F., Valsartan, a new angiotensin II antagonist for the treatment of essential hypertension: a comparative study of the efficacy and safety against amlodipine. *Clinical Pharmacology & Therapeutics* 1996, 60 (3), 341-6.
- Beermann, B.; Groschinsky-Grind, M.; Rosen, A., Absorption, metabolism, and excretion of hydrochlorothiazide. *Clinical Pharmacology & Therapeutics* 1976, 19 (5 Pt 1), 531-7.
- Carlucci, G.; Palumbo, G.; Mazzeo, P.; Quaglia, M. G., Simultaneous determination of losartan and hydrochlorothiazide in tablets by high-performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis* 2000, 23 (1), 185-9.
- Wellington, K.; Faulds, D. M., Valsartan/hydrochlorothiazide: a review of its pharmacology, therapeutic efficacy and place in the management of hypertension. *Drugs* 2002, 62 (13), 1983-2005.
- Zaid, A. N.; Cortes, R.; Qaddomi, A.; Khamash, S., Formulation and Bioequivalence of Two Valsartan Tablets After a Single Oral administration. *Scientia Pharmaceutica* 2010.
- Grace, D.; Parambi, T.; Mathew, M.; V.Ganesan, A validated stability indicating HPLC method for the determination of Valsartan in tablet dosage forms. *Journal of Applied Pharmaceutical Science* 2011, 01 (04), 9.99-7
- Priya, S. P.; Rajendran, N. N.; Lakshmi, P. K.; Umadevi, S. K.; Vijanthi, V.; Kausalya, J.; Ravichandran, V., A novel captopril hydrochlorothiazide solid dispersion *International Journal of Pharmacy and Pharmaceutical Sciences* 2010, 2 (2), 30-32.
- Hillaert, S.; Van den Bossche, W., Simultaneous determination of hydrochlorothiazide and several angiotensin-II-receptor antagonists by capillary electrophoresis. *Journal of Pharmaceutical and Biomedical Analysis* 2003, 31 (2), 329-39.
- Kadam, B. R.; Bari, S. B., Quantitative analysis of Valsartan and hydrochlorothiazide in tablets by high performance thin layer chromatography with ultraviolet absorption densitometry. *Acta Chromatographica* 2007, 18.
- Tian, D. F.; Tian, X. L.; Tian, T.; Wang, Z. Y.; Mo, F. K., Simultaneous determination of valsartan and hydrochlorothiazide in tablets by RP-HPLC. *Indian Journal of Pharmaceutical Science* 2008, 70 (3), 372-374.
- Satana, E.; Altınay, S.; Göger, N. G.; Ozkan, S. A.; Sentürk, Z., Simultaneous determination of valsartan and hydrochlorothiazide in tablets by first-derivative ultraviolet spectrophotometry and LC. *Journal of Pharmaceutical and Biomedical Analysis* 2001, 25 (5-6), 1009-1013.
- Rao, K. S.; Jena, N.; Rao, M., Development and validation of a specific stability indicating high performance liquid chromatographic method for valsartan. *Journal of chromatography* 2010, 2 (2), 183-189.
- Jothieswari, D.; Anandkumar, K., Absorption and correction method for estimation of amlodipine besylate, valsartan and hydrochlorothiazide in bulk and combined tablet dosage form. *International Journal of Pharmacy and Pharmaceutical Sciences* 2011, Supp 3, 30-34.
- Ghulam A, S., Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *Journal of Chromatography A* 2003, 987 (1-2), 57-66.