A NEW RP-HPLC METHOD FOR THE DETERMINATION OF VALSARTAN IN BULK AND ITS PHARMACEUTICAL FORMULATIONS WITH IT’S STABILITY INDICATIVE STUDIES

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ABSTRACT
A simple, precise, accurate, economical and reproducible HPLC method for estimation of Valsartan in tablet dosage form has been developed. Quantitative HPLC was performed with SHIMADZU LC2010c HT with class-10vp Software with UV-Visible Detector (SPD-IOA), PDA Detector (PDA-10A) PUMP (LC-IOAT) and (LC-IOATvp). Microbondapak, C18, 5μm, 25cmx4.6mm i.d column was used in the study. The mobile phase of Methanol: phosphate buffer of pH3 (65:35) were used in this study. The conditions optimized were: flow rate (1 ml/minute), wavelength (210 nm) and run time was 20 min. Retention time was found to be 6.22 min. The linearity was found to be in the concentration range of 10-100 μg/ml. The developed method was evaluated in the assay of commercially available tablets, Valzar and Valtan tablet containing 40 and 80 mg of Valsartan respectively. The amount of drug in tablet was found to be 40.35 and 79.34 mg/tab for the two brand. Results of analysis were validated statistically and by recovery studies. The recovery studies 99.35 % was indicative of the accuracy of proposed method. The precision was calculated as repeatability, inter and intraday variation (%RSD) for the drug. By using the method, stability of the drug has been studied.

Key words: Valsartan, RP-HPLC, PDA Detector, Assay

INTRODUCTION
It is necessary to find the content of each drug either in bulk or single or combined dosage forms for purity testing. It is also essential to know the concentration of the drug and it’s metabolites in biological fluids after taking the dosage form for treatment.
The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise\[1\]. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation.

According to the literature survey\[2-5\] it was found that few analytical methods such as (RP-HPLC\[5\], HPLC\[6\], UV-Visible analysis\[3,7\] and LC-MS\[4\]) were reported for the estimation of Valsartan. The objective of the proposed method is to develop simple and accurate methods for the determination of Valsartan by RP-HPLC\[5\] method in pharmaceutical dosage forms & it’s stability indicative studies.

Valsartan is chemically, \(N\)-(1-oxopentyl)-\(N\)-\{[2''-(1\text{H}-tetrazol-5-yl)][1,1''-biphenyl]-4-yl]methyl\}-L-valine\[8\] is a antihypertensive drug\[9\], which is non-peptide potent highly selective, orally active\[10\]. It is also used in Congestive Heart failure (CHF), & Post Myocardial Infarction (MI). It acts as antagonist at the angiotensin II AT\(_1\)-receptors\[11\].

MATERIALS AND METHOD

Instruments and Reagents

The chromatographic separation was performed on SCHIMADZU LC2010c HT (Autosampler) with Winchrom Software with Isocratic--Gradient with UV-Visible Detector (SPD-IOA), PDA Detector (PDA-10A), PUMP (LC-IOAT). Phenomenex ODS (C\(_{18}\)) RP Column, 250 mm x 4.6 mm has been used as a stationary phase. P\(_\text{H}\) Analyzer (ELICO), Electronic Balance (AFCOSET), Ultra Sonicator (ENERTECH) has been used in the work. Valsartan Active Pharmaceutical Ingredient (API) & marketed product under brand name Valtan-80 was collected from Cipla pharmaceutical ltd. & Valzar-40 Torrent pharmaceutical limited. Methanol & water of HPLC grade were taken from Standard reagents, Hyderabad. Ammonium dihydrogen phosphate from S.D Fine chemicals, Mumbai. Another commercial formulation of valzar-40 Torrent pharmaceutical limited.

Optimised Chromatographic conditions

The mobile phase of Methanol: potassium dihydrogen phosphate buffer (0.1M, pH 3.0) (65:35) were used in this study. The conditions optimized were: flow rate (1 ml/minute), wavelength (210 nm, selected after using PDA detector), injection volume 20
μl and run time was 20 min. Retention time was found to be 6.22 min. Microbondapak, C_{18}, 5μm, 25cmx4.6mm i.d column was used in the study.

**Preparation of Standard Drug Solutions**

Standard stock solution of a concentration of 100 μg/ml of Valsartan was prepared by using methanol.

**Preparation of mobile phase**

The mobile phase used in this analysis consists of a mixture of Buffer (ammonium dihydrogen phosphate, 0.1M) and methanol in a ratio of 35:65 pH of the solution adjusted to 3.0

A typical chromatogram obtained by using the optimal chromatographic conditions as shown below.

![Figure 1](image)

**HPLC spectrum of Valsartan (100 ppm) in optimized conditions (RT 6.22 min.)**

**Preparation of Calibration Curves**

Calibration curve was prepared by taking appropriate aliquots of standard valsartan stock solution in different 10 ml volumetric flask and diluted up to the mark with methanol to obtain the final concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μg/ml of valsartan. The calibration curve has been shown below. Standard solutions (n=6) were injected, the sample volume was 20 μl with a flow rate of 1.0 ml/min.
Degradation/ Stability studies

This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after a long time storage, within a very short time as compare to the real time or long term stability testing.

The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation

1. Acid hydrolysis:
An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1 M Hydrochloric acid was added & make up to the mark & kept for 24 hrs. from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with methanol, than injected into the HPLC system against a blank of HCl & methanol ( after all optimized conditions )

Figure 3
Chromatogram showing degradation in 0.1 M HCl

2. Basic hydrolysis

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1 M Sodium hydroxide was added & make up to the mark & kept for 24 hrs. from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with methanol, than injected into the HPLC system against a blank of NaOH and methanol ( after all optimized conditions )

Figure 4
Chromatogram showing degradation in 0.1 M NaOH
3. Oxidation with (3%) $\text{H}_2\text{O}_2$:

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 3% Hydrogen Peroxide was added, make up to the mark & kept for 24 hrs. from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with methanol, than injected into the HPLC system against a blank of $\text{H}_2\text{O}_2$ and methanol ( after all optimized conditions )

![Chromatogram showing degradation in 3% $\text{H}_2\text{O}_2$](image)

Figure 5
Chromatogram showing degradation in 3% $\text{H}_2\text{O}_2$

4. Thermal degradation

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 100 ml volumetric flask, make up to the mark with methanol & was maintained at $50^\circ\text{C}$. for 24 hrs. than injected into the HPLC system against a blank of methanol ( after all optimized conditions )

![Chromatogram showing thermal degradation](image)

Figure 6
Chromatogram showing thermal degradation

5. Photolytic Degradation:

Approximately 10 mg. of pure drug was taken in a clean & dry Petridis. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately
weighed 1 mg. of the UV exposed drug was transferred to a clean & dry 10 ml. volumetric flask. First the UV exposed drug was dissolved in methanol & make up to the mark. than injected into the HPLC system against a blank of methanol ( after all optimized conditions )

![Figure 7](image)

**Figure 7**
Chromatogram showing photolytic degradation

**RESULTS & DISCUSSION**

**Results of degradation studies:**

The results of the stress studies indicated the **specificity** of the method that has been developed. Valsartan was degraded only in 3% H₂O₂ & temperature stress conditions. The result of forced degradation studies are given in the following table.

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>Time</th>
<th>Assay of active substance</th>
<th>Assay of degraded products</th>
<th>Mass Balance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Hydrolysis (0.1 M HCl)</td>
<td>24Hrs.</td>
<td>98.36</td>
<td>---------------------------</td>
<td>98.36</td>
</tr>
<tr>
<td>Basic Hydrolysis (0.1 M NaOH)</td>
<td>24Hrs.</td>
<td>98.32</td>
<td>---------------------------</td>
<td>98.32</td>
</tr>
<tr>
<td>Oxidation(3% H₂O₂)</td>
<td>24Hrs.</td>
<td>77.15</td>
<td>22.05</td>
<td>99.20</td>
</tr>
<tr>
<td>Thermal Degradation (50 °C)</td>
<td>24Hrs.</td>
<td>81.36</td>
<td>16.93</td>
<td>98.29</td>
</tr>
<tr>
<td>UV (254nm)</td>
<td>24Hrs.</td>
<td>98.79</td>
<td>---------------------------</td>
<td>98.78</td>
</tr>
</tbody>
</table>
Method validation

Accuracy: Recovery study:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of valsartan were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated.

Precision:

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug. Valsartan (API) The percent relative standard deviation were calculated for valsartan

Intra-assay & inter-assay:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for valsartan revealed that the proposed method is precise

Linearity & Range:

The calibration curve showed good linearity in the range of 10 – 100 µg/ml, for valsartan (API) with correlation coefficient ($r^2$) of 0.999 (Fig. 02). A typical calibration curve has the regression equation of $y = 83907x - 78449$ for valsartan.

Method Robustness:

Influence of small changes in chromatographic conditions such as change in flow rate (+ 0.1ml/min), Temperature (+2°C), Wavelength of detection (+2nm) & methanol content in mobile phase (+2%) studied to determine the robustness of the method are also in favour of (Table-4, % RSD < 2%) the developed RP-HPLC method for the analysis of valsartan (API).

LOD & LOQ:

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.02 & 0.06 µg/ml respectively.

Specificity & stability in analytical solution:
The results of specificity indicated that the peak was pure in presence of degraded sample. It is important to mention here that the valsartan (API) was stable in solution form up to 24 hrs at 25°C.

The results of linearity, precision, inter & intraday assays, method robustness, LOD, LOQ, specificity and stability in analytical solution established the validation of the developed RP-HPLC method for analysis of valsartan.

**Table 3: Summary of validation parameters by rp-Hplc method**

<table>
<thead>
<tr>
<th>Validation parameters</th>
<th>VALSARTAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>% interference &lt;0.5 %</td>
</tr>
<tr>
<td>Range (µg/ml)</td>
<td>Linear range 10-100 µg/ml</td>
</tr>
<tr>
<td></td>
<td>Working range 0.02-100 µg/ml</td>
</tr>
<tr>
<td></td>
<td>Target range 44,55,60.5 µg/ml</td>
</tr>
<tr>
<td></td>
<td>Target concentration 55 µg/ml</td>
</tr>
<tr>
<td>Accuracy (% Recovery)</td>
<td>80, 100, 120 98.94667, 99.76, 99.37667</td>
</tr>
<tr>
<td>Precision (% RSD)</td>
<td>Repeatability 0.134</td>
</tr>
<tr>
<td></td>
<td>Intra day 0.86</td>
</tr>
<tr>
<td></td>
<td>Inter day 0.87</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.02</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

**ASSAY OF VALSARTAN IN DOSAGE FORM:**

Assay was performed as described in previous chapter. Results obtained are tabulated below:

**Table 4: Assay of Valsartan tablets**

<table>
<thead>
<tr>
<th>Brand name of tablets</th>
<th>Labeled amount of Drug (mg)</th>
<th>Mean (±SD) amount (mg) found by the proposed method (n=6)</th>
<th>Mean (± SD) Assay (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valzar</td>
<td>40</td>
<td>40.35 (±0.06)</td>
<td>100.88 (±0.48)</td>
</tr>
<tr>
<td>Valtan</td>
<td>80</td>
<td>79.34 (±0.04)</td>
<td>99.18 (±0.04)</td>
</tr>
</tbody>
</table>
The assay of valzar and valtan tablets containing valsartan was found to be 100.88\% and 98.18\% as per the method.

CONCLUSION

The proposed method is simple, sensitive and reproducible and hence can be used in routine for determination valsartan in bulk as well as in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision.

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