Development of RP-HPLC Method for Estimation of Valsartan and Hydrochlorothiazide in Tablets

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ABSTRACT
A simple, efficient and reproducible reversed phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of valsartan and hydrochlorothiazide in bulk and in tablets. A column having 250 x 4.6 mm i.d. (Kromasil C18) in isocratic mode with mobile phase containing 50 mM potassium dihydrogen o-phosphate buffer (triethylamine 0.2%), (pH 3.7 adjusted with o-phosphoric acid): acetonitrile (56:44 v/v) was used. The flow rate was 1.0 ml/min and effluent was monitored at 232 nm. The retention time of valsartan and hydrochlorothiazide was 10.15 and 3.78 min respectively. All calibration curves showed good linear correlation coefficients within the tested limits (r² > 0.9995). The linearity dynamic range was found to be 20-150 µg/ml and 5-45 µg/ml for valsartan and hydrochlorothiazide respectively. Percentage recoveries for valsartan and Hydrochlorothiazide were 100.45 % and 98.75 % respectively. All the analytical validation parameters were determined and found in the limit as per the International Conference on Harmonization (ICH) guidelines which indicates the validity of the method. The developed method was found to be accurate, precise and robust for the simultaneous estimation of valsartan and hydrochlorothiazide in bulk and in tablets.

KEYWORDS: Hydroclorothiazide; RP-HPLC; Quantitative Estimation; Valsartan; Method Validation.

Introduction
Valsartan (VAL) is an orally active angiotensin II receptor blocker effective in lowering blood pressure in hypertensive patients. Chemically it is known as S)-3-methyl-2-[N-(4-[2]([2H-1,2,3,4-tetrazol-5yl) phenyl] phenyl) methyl) pentanamido] butanoic acid. A number of methods are available for separation and quantitation of Valsartan from pharmaceutical dosage forms. Hydrochlorothiazide (HCTZ) is a diuretic of the class benzothiadiazines widely used as anti hypertensive agents, 6-chloro-7-sulfamoyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide (Indian Pharmacopoeia, 2010). It was successfully used as one content in association with other drugs in the treatment of hypertension. The chemical structure of Valsartan and Hydrochlorothiazide are shown in Fig. 1 and 2 respectively (Indian Pharmacopoeia, 2010).

Fig. 1. Chemical structure of valsartan (VAL).

Fig. 2. Chemical structure of hydrochlorothiazide (HCTZ).

In literature, few analytical methods like UV-Visible spectrophotometry (Chaudhary et al., 2010; Gupta et al., 2010; Raja et al., 2010; Satana et al., 2001), Capillary electrophoresis (Hillaert et al., 2003), RP-HPLC (Ramadan et al., 2010; Wankhede et al., 2010; Liu et al., 2007; Macek, 2006), UPLC (Krishnaiah, 2010) are reported for the estimation of Valsartan and Hydrochlorothiazide as individual drug or in combination with same or other drugs in tablet dosage form. Literature survey reveals that there are only few reported methods for simultaneous estimation of Valsartan and Hydrochlorothiazide in tablets.

The main objective of the present paper work was to develop and validate chromatographic method for simultaneous analysis of Valsartan and Hydrochlorothiazide in tablets.

Materials and Methods

Chemicals and Drug Materials
Pharmaceutical grade Valsartan and Hydrochlorothiazide were provided by Macleods Pharmaceuticals.
Ltd., Daman, India. Tablet dosage forms (Valzaar - H, Torrent) were procured from the local market, each tablet containing 160 mg of Valsartan and 12.5 mg of Hydrochlorothiazide. All reagents and solvents used for study were of analytical grade.

**Instrumentation**

JASCO HPLC system consisting of pump (model Jasco Plus) with manual injector was used. Loop used was of 20 µl fixed capacity (Rheodyne-7125). UV-Visible detector (UV-VIS 2075 PLUS) was used. Detection was carried out at 232 nm and software used was Borwin Software version 1.50. Kromasil KR-5 C18 (250 mm x 4.6 mm, 5 µm) column was used. Apart from this Digitial pH meter (Analytical Lab Scientific Instrument), Electronic weighing balance (Citizen CX200), Ultrasonicator (Trans-o-sonic) were used.

**Preparation of Mobile Phase**

Mobile phase was prepared by mixing 560 ml of potassium dihydrogen phosphate (KH2PO4) buffer (pH 3.7 adjusted with ortho phosphoric acid), 0.2% triethyl amine and 440 ml of acetonitrile (ACN) and filtered through 0.2 µm Supor 200 membrane filter using vacuum pump and ultrasonicated for 15 min for degassing.

**Preparation of stock solution of Valsartan (VAL)**

25 mg of standard VAL was accurately weighed and transferred to a 25 ml volumetric flask and dissolved in methanol and volume was made up to mark with methanol and labeled as Standard Stock VAL - A. Then 1 ml aliquot of above solution was diluted to 10 ml with methanol to get final concentration of 100 µg/ml of VAL and labeled as ‘Standard Stock VAL - B’.

**Preparation of stock solution of Hydrochlorothiazide (HCTZ)**

25 mg of standard HCTZ was accurately weighed and transferred to a 25 ml volumetric flask and dissolved in methanol and volume was made up to mark with methanol. Then 1 ml aliquot of above solution was diluted to 10 ml with methanol to get final concentration of 100 µg/ml of HCTZ and labeled as ‘Standard Stock HCTZ’.

**Preparation of stock solution of Mix Standard Solution**

25 mg of standard VAL and 25 mg of standard HCTZ were weighed and transferred to a 25 ml volumetric flask and dissolved in methanol and volume was made up to mark with methanol. Then 1 ml aliquot of above solution was diluted to 10 ml with methanol to get final concentration of 100 µg/ml of VAL and HCTZ. The solution was labeled as ‘Standard Stock MIX-A’.

**Calibration curves for VAL and HCTZ**

From the Standard Stock MIX-A, suitable aliquots were diluted with diluents ACN and millipore water (50:50, v/v) to obtain different concentration ranging from 20-150 µg/ml and 5-45 µg/ml for VAL and HCTZ respectively. With the optimized chromatographic conditions, a steady baseline was recorded. 20 µl of each mixed standard solution was injected six times and chromatograms were recorded. The retention time of VAL and HCTZ were 10.15 and 3.78 min respectively. Calibration curves were constructed by plotting the average peak areas against the respective concentrations (Fig. 3 and 4).

**Analysis of Marketed Formulation**

Amount equivalent to about 160 mg of VAL (equivalent to 12.5 mg of HCTZ) in Valzaar –H tablet was weighed accurately and transferred carefully to 100 ml volumetric flask, then the volume was made up to the mark with methanol. From this solution 5 ml aliquot was taken in 50 ml volumetric flask and diluted with diluent to obtain final concentration containing 160 µg/ml of VAL and 12.5 µg/ml of HCTZ. Similarly, from the ‘Standard Stock MIX-A’ (100 µg/ml of VAL and 100 µg/ml of HCTZ) solution suitable aliquots were taken and diluted with diluent to get final concentration containing 160 µg/ml of VAL and 12.5 µg/ml of HCTZ. Both solutions were filtered through 0.45 µm cellulose acetate filter using syringe and injected into the Rheodyne injector (20 µl) of HPLC system and their chromatograms were recorded (Fig. 5) under the finalized chromatographic conditions as described above after getting a stable baseline.
Method Validation

The method was validated for various parameters as per ICH Guidelines (International Conference on Harmonization, 2005; The United States Pharmacopoeia and National Formulary, 2005).

1. Accuracy

The accuracy of the method was determined by recovery experiment. Recovery studies were carried out by standard addition method by adding the known amount of VAL and HCTZ (reference standard) to the pre analyzed sample at three different concentration levels i.e. 80 %, 100 %, and 120 % of assay concentration and percent recoveries were calculated.

For accuracy study of VAL, 0.4 ml of solution was pipetted from the above ‘Sample Stock’ (160 µg/ml of VAL and 12.5 µg/ml of HCTZ) and transferred to three different 10 ml volumetric flasks separately along with 0.5, 0.6, 0.7 ml of aliquot from the Standard Stock VAL -A solution containing 1000 µg/ml of VAL solution. The volume was made up to the mark with methanol. For accuracy study of HCTZ, 0.4 ml of solution was pipetted from ‘Sample Stock’ and transferred to three different 10 ml volumetric flasks separately along with 0.4, 0.5, 0.6 ml of aliquot from the Standard Stock HCTZ. All the solutions were filtered through 0.45 µm cellulose acetate filter using syringe and injected into the Rheodyne injector (20 µl) of HPLC system and their chromatograms were recorded. The percentage recovery and standard deviation of the percentage recovery were calculated.

2. Precision

Precision was studied to find out intra and inter day variation in the proposed method at three different levels on the same day and on three different days, respectively. The % RSD was calculated for intra-day and inter-day precision.

3. Linearity and Range

The linearity of analytical method for VAL and HCTZ were determined by studying standard calibration curves. The range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the log curve.

4. Limit of Detection and Limit of Quantitation

Detection limit and quantitation limit were determined based on the standard deviation of y-intercepts of six calibration curves and average slope of six calibration curves.

\[
\text{LOD} = 3.3 \times \frac{\text{Standard Deviation of intercept}}{\text{Slope}} \\
\text{LOQ} = 10 \times \frac{\text{Standard Deviation of intercept}}{\text{Slope}}
\]

5. Robustness

Combined standard solutions of VAL (100 µg/ml), HCTZ (100 µg/ml) were prepared and analyzed at different pH (3.65, 3.75), at different flow rates (0.8, 1.2 ml/min) and at different wavelength (230, 234 nm) separately.

6. System Suitability

Sample solutions of VAL (100 µg/ml) and HCTZ (100 µg/ml) were prepared and analyzed. Chromatograms were studied for different parameters such as tailing factor, resolution and theoretical plates to see that whether they comply with the recommended limit or not.

7. Specificity

Separated chromatographic peaks of both drugs were analyzed for peak purity (specificity) by scanning in the range of 200-400 nm with the help of borwin PDA software. The specificity of the method was determined by analyzing standard drug and test samples. The peak purity of VAL and HCTZ was determined by comparing spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E) with that of standard peak purity determined by UV-FDA detector.

Results and Discussion

For chromatographic separation, solvent system using combinations of potassium dihydrogen phosphate (KH₂PO₄) and acetonitrile (ACN) at various proportions and pH were investigated. Changing the ratio of the mobile phase and its pH, a change in the retention time and peak area of the drugs were observed. Out of various combinations, mobile phase containing mixture of KH₂PO₄ buffer: ACN (56:44 v/v), Triethylamine (0.2%) at pH 3.7 adjusted with 1% orthophosphoric acid, at a flow rate of 1 ml/min with UV detection at 232 nm, using C18 column as a stationary phase was finalized. The retention time of VAL and HCTZ were 10.15 and 3.78 min respectively.

The overlain spectra of VAL and HCTZ showed absorbance at 232 nm hence detection was carried out at 232 nm. The linearity of analytical method at six concentration levels was ranging from 20-150 µg/ml and 5-45 µg/ml for VAL and HCTZ respectively and is presented in Table 3. The regression equation of
calibration curves were Y= 112822x - 83839 and Y= 135283x + 114584 for VAL and HCTZ respectively (Fig. 3, Fig. 4). The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range. The LOD was found to be 1.1 µg/ml for VAL and 0.48 µg/ml for HCTZ. LOQ was found to be 3.3 µg/ml for VAL and 1.47 µg/ml for HCTZ.

Assay results were satisfactorily obtained and were found to be 100.62 % for VAL and 99.87 % for HCTZ, as they were compared with the labeled amounts (Table 1). The % recovery values for accuracy study indicated that the developed method was found to be accurate. The results are shown in Table 2. In repeatability study, % RSD was found to be 0.77-1.01 for VAL and 0.24-0.36 for HCTZ. At all three concentration levels, precision showed satisfactory levels. Results of intermediate precision study, % RSD values for each set (all three levels) were found to be < 3 % indicating that these methods have excellent repeatability and intermediate precision. For, robustness it was observed that there were no marked changes in the retention time and the area of the chromatograms and the % RSD was less than 3 %, which demonstrated that RP-HPLC method developed was robust. For specificity, peak purity front and peak purity tail for both drug were determined using UV-PDA detector and peak purity was found specific. The results for validation and system suitability test parameters are summarized in Table 3. The method gives good resolution between the compounds with a short analysis time.

### TABLE 2

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Amount Present (mg)</th>
<th>Amount Found (mg)</th>
<th>% Assay</th>
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<tbody>
<tr>
<td></td>
<td>VAL</td>
<td>HCTZ</td>
<td>VAL</td>
</tr>
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<td>12.5</td>
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<tr>
<td>%RSD</td>
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### References


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