UPLC Method for Simultaneous Determination of Valsartan & Hydrochlorothiazide in Drug Products

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Abstract

A new, simple, precise and stability-indicating UPLC (Ultra Performance Liquid Chromatography) method was developed and validated for the simultaneous determination of anti-hypertensive drug Valsartan (VAL) and Hydrochlorothiazide (HCTZ) in combined dosage forms. The method was developed using Kromasil eternity C-18 column (50 mm×2.1 mm, 3.5 μm) with isocratic elution. Triethylamine buffer (0.1% v/v) and methanol (75:25 v/v) were used as mobile phase with 0.6 mL min⁻¹ flow rate at room temperature. The detection wavelength was fixed at 225 nm; the run time was within 2 min. The method was validated in terms of linearity, accuracy and reproducibility. Calibration plots were linear over the range of 7-13 μg/ml for HCTZ and 56-104 μg/ml for VAL. Recovery was in the range of 98-102% with the relative standard deviation of less than 2% for both drugs. The limit of detection and the limit of quantification for the valsartan were found to be 0.8 and 2.4 μg/ml respectively and for hydrochlorothiazide 0.12 and 0.36 μg/ml respectively. The proposed method was also suitable for determination of VAL & HCTZ in bulk and pharmaceutical dosage forms.

Keywords: Valsartan; Hydrochlorothiazide; UPLC

Introduction

Ultra Performance Liquid Chromatography (UPLC) [1] system is an innovative product that brought revolution in high performance liquid chromatography by outperforming conventional high performance liquid chromatography (HPLC). UPLC decreases sample run times up to a factor of 10, uses up to 95 percent less solvent and significantly improves productivity in the laboratory. UPLC achieves the speed by using novel sub-two-micron particles that reduces chromatographic run times and improves resolution. UPLC was designed as a total system to leverage both ultra-high pressure and small particle separation attributes that result in unique superior performance with significant improvements in resolution, sensitivity and speed [2]. UPLC system eliminates significant time and cost per sample from analytical process while improving the quality of results, the system allows chromatographers to work at higher efficiencies, flow rates, and back pressures [3]. UPLC photodiode array (PDA) detector detects and quantifies lower concentrations of sample analyte, trace impurities with maximum sensitivity and compares spectra across wavelengths and broad concentration ranges [4]. The present study was conducted to separate and quantify valsartan and hydrochlorothiazide in combined tablet dosage form by using RP-UPLC technique.

Valsartan, (S)-N-(1-Oxopentyl)-N-[2’-(1H-tetrazol-5- yl)[1,1’-biphenyl]-4-yl[methyl]-L-valine, is an orally active specific angiotensin II receptor blocker effective in lowering blood pressure in hypertensive patients. It is a selective type-1 angiotensin II receptor antagonist which blocks the blood pressure increasing effects of angiotensin II via rennin-angiotensin-aldosterone system. It is used as a first line agent to treat uncomplicated hypertension, isolated systolic hypertension and left ventricular hypertrophy. Very few methods appeared in the literature for the determination of VAL individually based on HPLC. There has been some estimation of assays of analyte in human plasma including the use of liquid chromatography and some combination with other drugs using highpressure liquid chromatography and derivative spectrosopy [5-9].

Hydrochlorothiazide (6-chloro-3,4-dihydro-2H- 1,2,4- benzothiadiazine- 7-sulfonamide-1,1 -dioxide) is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical formulations, alone or in combination with other drugs, which decreases active sodium reabsorption and reduces peripheral vascular resistance. It was successfully used as one of the content in association with other drugs in the treatment of hypertension II. Numerous publications described the determination of HCTZ concentration in plasma or urine by HPLC with ultraviolet or electrochemical detection. Several chromatographic methods have been reported for the analysis of HCTZ individually or in combination, such methods have included: HPLC coupled with UV or diode array, electrochemical detection, LC–MS or with tandem LC–MS/MS [10-14]. There are a few HPLC methods appearing in the literature for the simultaneous determination of VAL and HCTZ in tablets, wherein longer run time (8 to 15 min) along with a complex mobile phase combinations has been used [13,14]. Since the available methods were based on HPLC, LC-MS, GC-MS, capillary electrophoresis [15] and UV-derivative spectrophotometry, the procedure was inconvenient for determination and the run times were rather long [16-19]. Simultaneous determination of both drugs is highly desirable as this would allow more efficient generation of clinical data and could be more cost-effective than separate assays. To the best of our knowledge, there are no reports available on RP-UPLC method for VAL & HCTZ in combined tablet dosage form with short run time. It is, therefore, felt necessary to develop a new method for simultaneous determination of both the drugs with shorter run time. We intend to opt for a faster chromatographic technique UPLC, for the said study.

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An attempt was made to determine whether UPLC can reduce analysis times without compromising the resolution and sensitivity.

Hence a rapid, simple and precise reverse-phase Ultra performance liquid chromatographic method was developed and validated for simultaneous estimation of valsartan and hydrochlorothiazide in tablet dosage form.

Material and Method

Materials

The reference sample of valsartan and hydrochlorothiazide was supplied by Ranbaxy laboratory limited, gurgaon. Tablet for analysis were of Lupin pharmaceuticals available in the market under brand name of Valent-H having combination of valsartan and hydrochlorothiazide 80 mg and 12.5 mg respectively. HPLC grade methanol and acetonitrile were purchased from S.D. Fine Chemicals Ltd., Mumbai, orthophosphoric acid of AR Grade from Qualigens whereas triethylamine of HPLC grade was obtained from Thermo Fischer scientific Ltd. Reagents as ammonium acetate, isopropyl alcohol, hydrochloric acid, sodium hydroxide, hydrogen peroxide were of analytical grade obtained from merck (India). Water was collected from Mill Q purification unit with 0.22 μ filter.

Chromatographic conditions: The analysis of the drug was carried out on a Waters Acquity UPLC system (Milford, MA, USA) equipped with a binary solvent (loop capacity 10 μl), column manager composed of column oven, pre column heater, (50 mm×2.1 mm) column with 3.5 μ particle size and a photo diode array detector. Data acquisition, data handling and instrumentation control were performed by Empower software version 2.0. Kromasil C-18 column was used to optimize the method.

Sample preparation: Standard solution was prepared by dissolving HCTZ and VAL in 100 ml diluents (methanol: water, 80:20 v/v) to obtain a concentration of 12.5 μg/ml and 80 μg/ml respectively. Ten tablet of Valent-H containing VAL and HCTZ as active ingredient were weighed and finely powdered. Equivalent amount to standard APIs of powder was weighed, transferred in 1000 ml volumetric flask, diluted with methanol: water 80:20 v/v, sonicated for 30 min and then same diluent was added to make up the volume up to 1000 ml.
Method development

A variety of mobile phases were investigated in the development of a stability-indicating UPLC method for the analysis of HCTZ and VAL. A mixture of methanol and 0.1% v/v triethylamine (pH 3.0 adjusted with orthophosphoric acid) (75:25, v/v) was found to be the best suitable mobile phase for ideal separation of HCTZ and VAL. The solvent mixture was filtered through a 0.22 μm PVDF filter and sonicated before use. It was pumped through the column at a flow rate of 0.6 ml/min. The column was maintained at an ambient temperature. The detection of the drug was monitored at 225 nm. The run time was set at 2 min. Under these optimized chromatographic conditions the retention time obtained for the drugs HCTZ and VAL was 0.817 min and 1.647 min respectively. A typical chromatogram showing the separation of the drugs is as shown in Figure 1.

Wavelength selection: The UV spectra of individual drug substances was recorded in methanol: water (80:20) solvent system as depicted in Figure 2. From the UV spectra wavelength 225 nm selected because at this combination both drugs contribute linear relationship between absorbance and concentration.

Validation of developed and optimized method: The validation of developed method was done as per ICH guidelines [20,21] which include System suitability (retention time, peak area), Precision (system precision, method precision), Accuracy, Linearity, Robustness (flow rate, wavelength, mobile phaseratio, column temperature, pH) and Solution stability at 25ºC.

Specificity: The specificity of the method was determined by checking the interference of placebo with analyte and the proposed method was evaluated by checking peak purity, USP tailing, plate count and resolution of HCTZ & VAL during study. The peak purity of both the drugs was found satisfactory under different conditions as shown in Table 1.

Precision: The precision of the proposed method was checked by carrying out six independent assays of test samples. Mean, SD and an RSD (%) value of six assays was calculated. Intermediate precision was carried out by analyzing the samples on a different day on another instrument. System precision and method precision both were under the permissible limit i.e. 1% and 2% respectively (Table 2).

Linearity: Standard stock solution of the drug was diluted to prepare linearity standard solutions in the concentration range of 70-130% of standard concentration. The calibration curve of analytical method was assessed by plotting concentration versus peak area and represented graphically in Figure 3a and 3b. It was showing suitable linearity in the range of 7-13 μg/ml for HCTZ and 56-104 μg/ml for VAL respectively.

Accuracy: To assess the accuracy both the samples were studied in three different concentrations of 80%, 100% & 120% and recovery was found within the acceptance criteria i.e. 95-105% as shown in Table 3.

LOD & LOQ: The limit of detection (LOD) & limit of quantitation (LOQ) was calculated on the basis of signal to noise ratio of 3:1 and 10:1 respectively. The lowest limit of quantitation for HCTZ and VAL was found to be 0.36 μg/ml & 2.4 μg/ml respectively. The lowest limit of detection for HCTZ and VAL was found to be 0.12 μg/ml & 0.8 μg/ml respectively. Chromatograms showing LOD & LOQ is as shown in Figure 4 and 5 respectively.

Robustness: The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (± 0.02 mL min⁻¹), wavelength (± 5 nm), column oven temperature (± 5.0°C), pH of buffer in mobile phase (± 0.2) and organic composition (± 2 from absolute value). % RSD for all the robustness studies is as shown in Table 4.

Solution stability: To assess the solution stability, standard and test solutions were kept at 25°C (laboratory temperature) for 24 hrs. They were analysed at 6 different time intervals. The cumulative RSD (%) at each time interval is shown in the Table 5 and 6.

Conclusion

The rapid isocratic RP-UPLC method developed for quantitative
analysis of HCTZ and VAL in pharmaceutical dosage form is precise, accurate, linear, robust and ultra fast. The shorter run time of 2 minute enables rapid determination of the drugs individually or in combination. The method was found to be specific and stability indicating as no interfering peaks of excipients was noticed. Satisfactory results were obtained from validation of the method. This method exhibited an excellent performance in terms of sensitivity and speed. The method is more economical and suitable for laboratory use as solvent consumption is very less. Conventional reported HPLC methods may be replaced by the proposed UPLC method because of it’s superiority in cost effectiveness, saving of analysis time per sample and better detection.

References


