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Development and Validation of Reverse Phase High Performance Liquid Chromatographic Method for Determination of Valsartan and Hydrocholorothiazide in Combined Dosage Forms

Gandhi Jimmy M.*[1], Patel Satish A[2]

1. Department of Quality Assurance, Zydus Cadila Health Care Limited, Ahmedabad.

2. Department of Pharmaceutical Quality Assurance, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar - 384012, Mehsana, Gujarat, India.

ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic (RP-HPLC) method was developed with high sensitivity for determination of Valsartan and Hydrochlorothiazide drugs in combined dosage forms. The separation was achieved on Zorbax CN (25 cm x 4.6 mm, 5 µm) at flow rate of 1.8 ml/min with 70: 30 mixture of phosphate buffer: acetonitrile (pH 6) as the mobile phase. The quantification was achieved with PDA detector at 265 nm. The injection volume was 20 µl. The retention times of Valsartan and Hydrochlorothiazide were 6.04 min and 2.27 min, respectively. The method was validated for linearity, precision, specificity, robustness and recovery according to the ICH guidelines. The linearity of response for Valsartan and Hydrochlorothiazide were assessed by analysis of five independent levels of calibration curve in range of 20-100 µg/ml and 2-10 µg/ml respectively. The recovery data was between 98.77-99.73% and 98.75-100.56% for Valsartan and Hydrochlorothiazide respectively. The limit of detection and quantification for Valsartan were 0.98 and 2.97µg/ml respectively and for Hydrochlorothiazide were 0.18 and 0.57µg/ml, respectively. The method was found to be simple and highly sensitive and can be useful in the routine quality control of Valsartan and Hydrochlorothiazide in bulk manufacturing and pharmaceutical dosage forms.

Keywords: Valsartan, Hydrochlorothiazide, RP-HPLC, validation, system suitability.

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*Corresponding Author Email: jimmygandhi1986@gmail.com

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INTRODUCTION

Valsartan (VLT) ((2S)-3-methyl-2-[N-({4-[2-(2H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl} methyl)pentanamido] butanoic acid) (Figure.: 1) is an angiotensin-receptor blocker that may be used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure[1-2]. VLT lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system (RAAS). Hydrochlorthiazide (HCTZ) (6-chloro-1,1-dioxo-3,4-dihydro-2H-1$l^{6},2,4-benzothiadiazine-7-sulfonamide) (Figure.: 2) is a thiazide diuretic that reduces the reabsorption of electrolytes from the renal tubules which results in increased excretion of water and electrolytes, including sodium, potassium, chloride, and magnesium. Moreover, it has been used in the treatment of several disorders including edema, hypertension, diabetes insipidus, and hyperparathyroidism[1-3].The combination of VLT and HCTZ has been shown to be more effective in the management of chronic hypertension[4]. On succinct amount of literature survey, it reveals various analytical techniques like spectrophotometric, fluorimetric, HPLC and HPTLC method for estimation of either VLT or HCTZ alone but no simple RP-HPLC method is available for simultaneous estimation of above two components in combined dosage form [5-11]. The research envisaged describes simple, sensitive, accurate, precise, specific and robust RP-HPLC method for simultaneous determination of VLT and HCTZ drugs in combined dosage form.

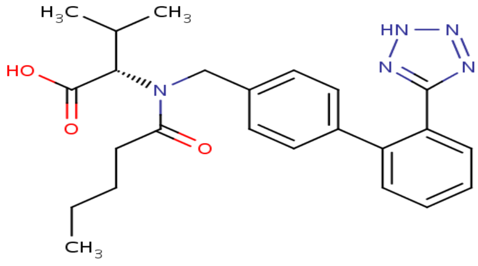

Figure 1: Chemical structure of Valsartan

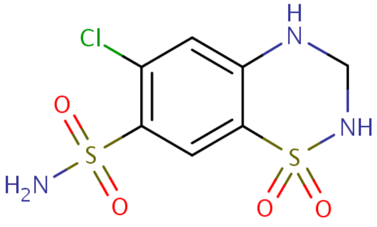

Figure 2: Chemical structure of Hydrochlorthiazide

MATERIALS AND METHODS

Reagents and Materials

VLT and HCTZ bulk powder were gifted by Zydus Cadila Healthcare Ltd, India. The commercial fixed dose combination product containing 80 mg VLT and 12.5 mg HCTZ were procured from the local pharmacy store. HPLC grade acetonitrile was purchased from S.D. Fine Chemicals Ltd., Mumbai, India. The water for HPLC was prepared by triple distillation and filtered through nylon

0.22 µm membrane filter (Gelman laboratory, Mumbai, India). Ortho-phosphoric acid was purchased from S.D. Fine Chemicals Ltd., Mumbai, India and was of analytical reagent grade.

Instrumentation

A Shimadzu RP-HPLC instrument (LC-20 AT) equipped with a photodiode array detector, manual injector with 20 µL loop, Zorbax CN (25 cm x 4.6 mm) 5 µm, LC-solution software, digital pH meter (LI 612 pH analyzer, Elico, Mumbai), analytical balance (Sartorius CP224S, Gottingen, Germany), ultra sonicator (Frontline FS 4, Mumbai, India) and filtration assembly were used in the study.

Preparation of mobile phase

Firstly, 10 mM of phosphate buffer was prepared as procedure mentioned in USP[12] which was subsequently filtered through 0.22µ filter. This filtered buffer was then mixed thoroughly with HPLC grade ACN in ratio of 70:30 and final pH was adjusted to 6.0 with HPLC grade orthophosphoric acid. The prepared mobile phase was degassed in ultrasonicator for 5 minutes and then further used.

Preparation of VLT and HCTZ standard stock solutions

VLT (100 mg) was dissolved in 50 ml of mobile phase employing sonication for 5 minutes and further volume was made up to 100 ml with mobile phase to obtain standard stock solution of 1000µg/ml. Similar procedure was employed for obtaining standard stock solution of HCTZ of 1000µg/ml.

Preparation of VLT and HCTZ working Standard solutions

An aliquot of 10 ml was accurately taken out from respective standard stock solutions of VLT and HCTZ with graduated calibrated pipette and further diluted upto 100 ml with mobile phase to obtain working standard solution of 100 µg/ml for VLT and HCTZ respectively.

Preparation of VLT and HCTZ mixed standard solution

An aliquot from VLT working standard solution (4.0 ml) and HCTZ working standard solution (0.4 ml) was diluted with mobile phase upto 10.0 ml to obtain a mixed standard solution of 40 µg/ml of VLT and 4 µg/ml of HCTZ respectively.

Determination of analytical wavelength

The standard solution of VLT and HCTZ were injected under the chromatographic condition described above. The elution showed reasonable good response at 265 nm with PDA detector.

Validation of the RP-HPLC method[13]

Calibration Curve (Linearity)

Calibration curves were constructed by plotting peak areas vs concentrations of VLT and HCTZ, and the regression equations were calculated. Accurately measured working standard solutions of VLT (2.0, 4.0, 6.0, 8.0 and10.0 ml) and HCTZ (0.2, 0.4, 0.6, 0.8 and 1.0 ml) were transferred to a series of 10.0 ml of volumetric flasks and diluted to the mark with mobile phase. Aliquots (20 µl) of each solution were injected under the operating chromatographic conditions described above.The linearity of response for VLT and HCTZ was assessed by analysis of five independent levels of calibration curve in range of 20-100 µg/ml and 2-10 µg/ml respectively in terms of slope, intercept and correlation coefficient values.

Precision

Repeatability

The precision of the instrument was checked by repeatedly injecting (n = 6) standard solutions of VLT (40µg/ml) and HCTZ (4µg/ml) under the same chromatographic condition and peak area, retention time and tailing factor was measured. The results were reported in terms of percent relative standard deviation (% RSD).

Intraday Precision

A variation of result within same day is called intraday variation. It was determined by repeating calibration curve 3 times on same day.

Interday Precision

Variation of result amongst day is called interday variation. It was determined by repeating calibration curve daily for 3 different days.

Accuracy

The accuracy of the methods was determined by calculating recoveries of VLT and HCTZ by the standard addition method. Known amounts of standard solutions of VLT and HCTZ were added at 80, 100 and 120 % levels to pre-quantified sample solutions of VLT and HCTZ (60 and 6 µg/ml respectively). The amounts of VLT and HCTZ were estimated by applying the obtained values to the regression equation.

Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by using the following equations as per International Conference on Harmonization (ICH) guidelines.

$$\mathrm{LOD} = 3.3 \times \sigma/S$$

$$\mathrm{LOQ} = 10 \times \sigma/S$$

Where σ = the standard deviation of the response

S = Slope of calibration curve.

RESULTS AND DISCUSSION

Method development

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for VLT and HCTZ was obtained with a mobile phase composition of phosphate buffer: ACN in ratio of 70:30 (pH 6.0) at a flow rate of 1.8 ml/min. Quantification was achieved with PDA detection at 265 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained. The retention time of VLT was found to be 6.04 min and that of HCTZ was found to be 2.27 min, respectively.(Figure.: 3). Peak purity of drugs was confirmed by comparing the spectra of standard and sample solutions. System suitability test parameters for VLT and HCTZ for the proposed method are reported in Table 1-2.

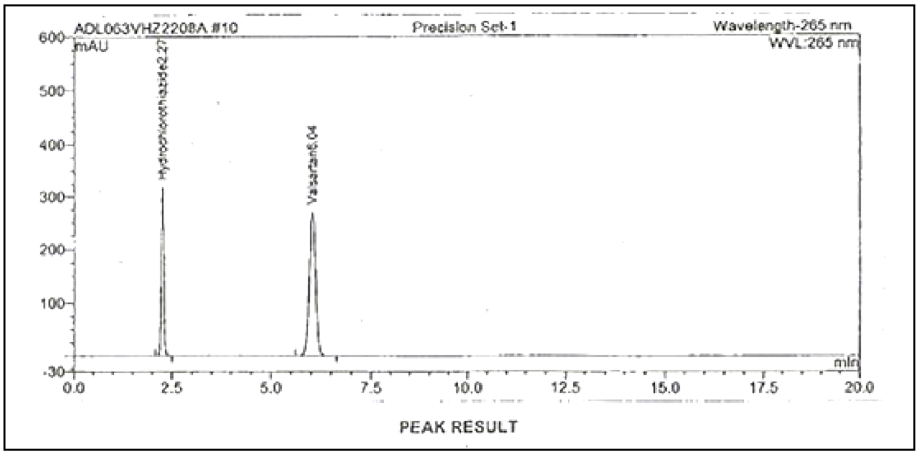

Figure 3: RP-HPLC chromatogram of standard VLT and HCTZ with corresponding retention time at 265 nm

Specificity

No peaks were found at retention time of 6.04 and 2.27 min, the proposed method was specific for detection of VLT and HCTZ (Figure: 4).

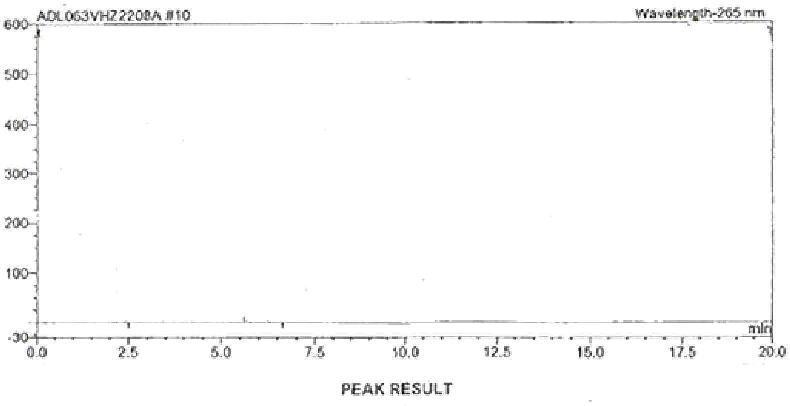

Figure 4: Chromatogram for specificity

Linearity and Range:

Was performed by plotting a calibration graph by taking different concentrations in the range of 20-100 µg/ml (Figure: 5) for VLT and 2-10 µg/ml (Figure: 7) for HCTZ. The linearity graph for both the drugs was satisfactory as observed from the correlation coefficient values which were 0.999 and 0.999 for VLT (Figure: 6) and HCTZ (Figure: 8) respectively.

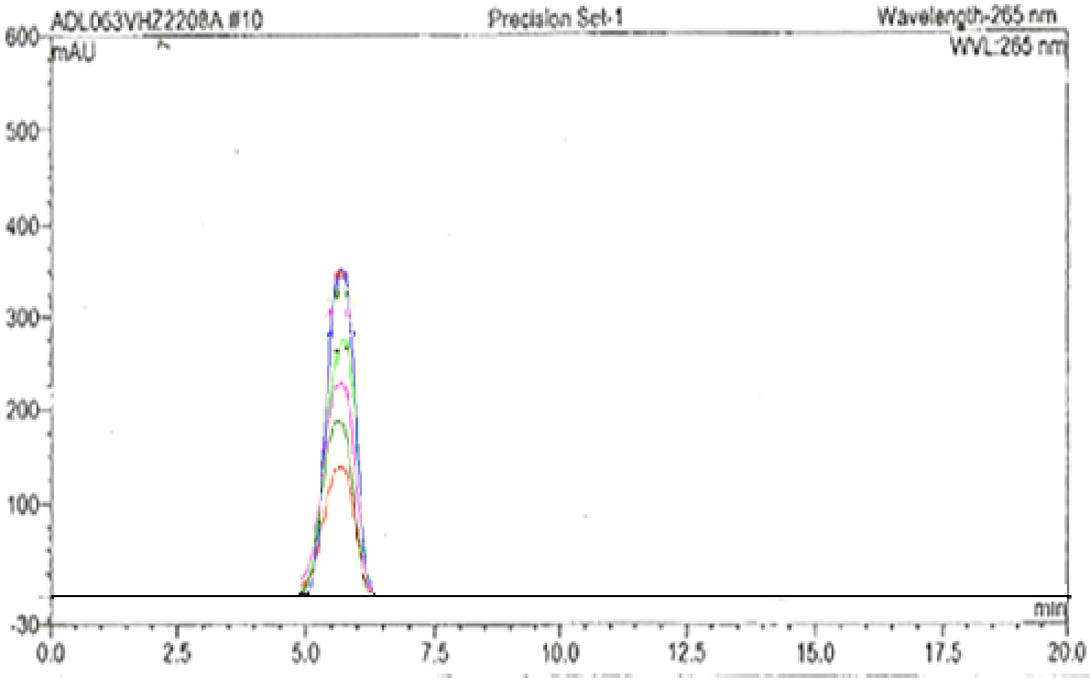

Figure 5: Chromatogram of calibration curve for VLT (20-100 µg/ml)

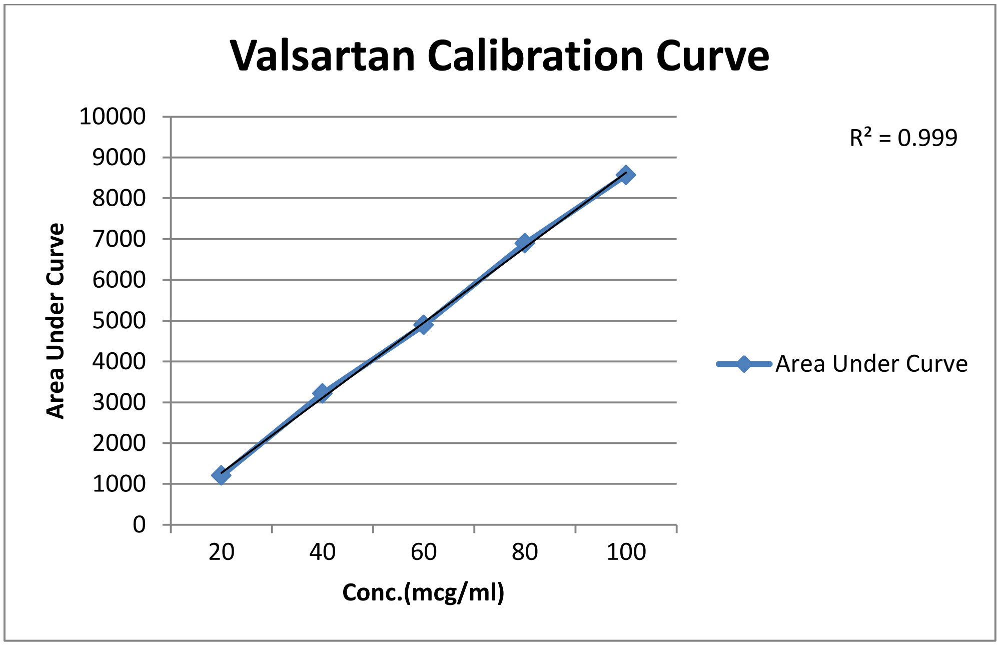

Figure 6: Linearity curve for VLT (20-100 µg/ml)

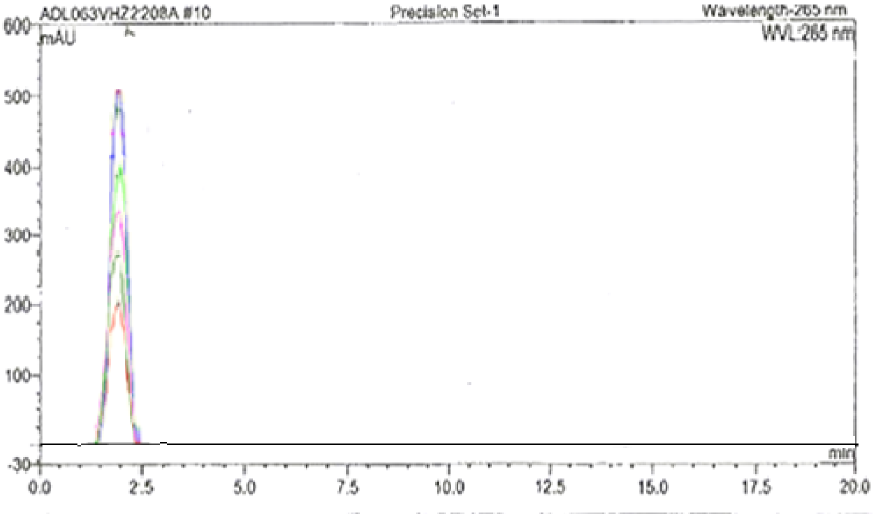

Figure 7: Chromatogram of calibration curve for HCTZ (2-10 µg/ml)

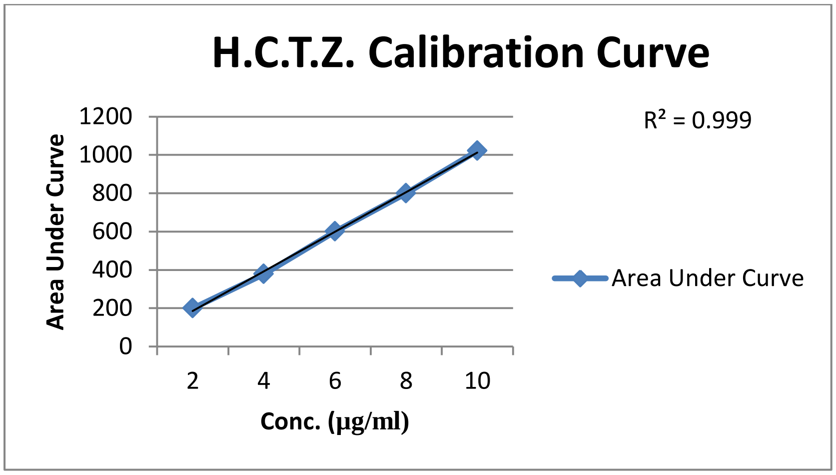

Figure 8: Linearity curve for HCTZ (2-10 µg/ml)

Limit of detection

The LOD was determined by visualization method and found to be at the concentration of 0.98 µg/ml and 0.18 µg/ml for VLT & HCTZ (Table: 3) respectively.

Limit of quntification

The LOQ was determined by visualization method and found to be at the concentration of 2.97 µg/ml and 0.57 µg/ml for VLT & HCTZ (Table: 3) respectively.

Precision

Six replicated injections were performed into the HPLC system from the stock solution within the same day and different days and for method precision and system precision. From the peak areas the %RSD was calculated for repeatability and inter day precision. The % RSD was found to be less than 2 (Table: 4).

Accuracy (mean % recovery)

The accuracy of the methods was determined by calculating recoveries of VLT and HCTZ by the standard addition method. Known amounts of standard solutions of VLT and HCTZ were added at 80, 100 and 120 % levels to pre-quantified sample solutions of VLT and HCTZ (60 and 6 µg/ml respectively). The percentage recovery was found to be 98.77% to 99.73% for VLT and 98.75% to 100.56% for HCTZ (Table: 4). The % recovery was in total agreement with acceptance criteria of 90% -110%.

Table 1: Data for system suitability test for VLT

System Suitability Test	Mean±SD
Retention time (min)	6.04±0.08
Number of plates (N)	6553±32.3
Symmatry factor (S)	1.05±0.03
% RSD of area	0.95

Table 2: Data for system suitability test for HCTZ

System Suitability Test	Observed Values
Retention time (min)	2.27±0.06
Number of plates (N)	8328±26.2
Symmatry factor (S)	1.08±0.05
% RSD of area	0.85
Resolution (Rs)	3.770

Table 3 LOD and LOQ data for VLT and HCTZ

Parameters	For VLT	For HCTZ
LOD = 3.3 × (SD/Slope) (µg/ml)	0.98	0.18
LOQ = 10 × (SD/Slope) (µg/ml)	2.97	0.57

Table 4 Summary of Validation parameters

Parameters	Result for VLT	Result for HCTZ
Linearity range	20-100 µg/ ml	2-10 µg/ ml
Correlation coefficient	0.999	0.999
Precision (% RSD)		
I) Repeatability	0.11	0.53
II) Intraday (n=3)	0.776-1.742	0.416-1.897
III) Interday (n=3)	0.445-1.650	0.953-2.426
Mean % recovery	98.77-99.73	98.75-100.56
Limit of Detection(µg/ ml)	0.98	0.18
Limit of Quantification (µg/ ml)	2.97	0.57

CONCLUSION

The proposed RP-HPLC method was found to be simple, sensitive, accurate, precise, specific and robust and can be used for the routine quality control analysis of VLT and HCTZ in combined dosage form without any interference of excipients.

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