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METHOD DEVELOPMENT AND VALIDATION OF VALSARTAN AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid and precise method was developed for the simultaneous determination of Valsartan (VAL) and Hydrochlorothiazide (HTZ) in tablet dosage form. Analysis was performed on a C18 (125 x 4 mm, 5 µm) column with acetonitrile: phosphate buffer (pH 3) 45:55 (v/v) as mobile phase, a flow rate 1.0 mL/min, column temperature 30ºC and UV detection at 250 nm. Both the drugs were well resolved on the stationary phase and the retention time for hydrochlorothiazide was 3.124 min and for valsartan 6.469 min. The calibration curves were linear in the concentration range of 5.00-50.00 µg/mL for VAL and 1.0-10.0 µg/mL for HTC. Intra- and inter-day relative standard deviations for both the components were <2.0%. The percentage recoveries obtained for VAL and HCT ranges from 99.28 % to 100.3 %.

KEYWORDS: Liquid Chromatography, Validation, Valsartan, Hydrochlorothiazide, Tablet dosage form.

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INTRODUCTION

Fixed-dose combination of antihypertensive drugs can simplify dosing regimens, improve compliance, improve hypertension control, decrease dose-dependent side effects and reduce cost as the first-line treatment of hypertension[1]. These potential advantages make it recommendable for the combination antihypertensive therapy to be used as initial treatment, particularly in patients with target-organ damage or more severe initial hypertension[2, 3]. Valsartan (VAL) chemically, (S)-N-(1-Oxopentyl)-N-[[2'-(1*H*-tetrazol - 5 - yl) [1,1'-biphenyl]-4-yl]methyl]-L-valine (Figure 1), 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 HPLC, capillary electrophoresis and derivative spectroscopy[4-9].

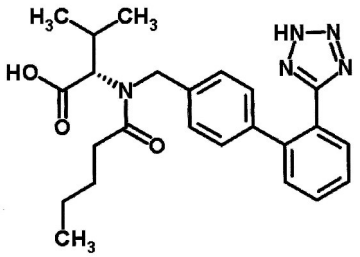

Figure 1
Structure of Valsartan

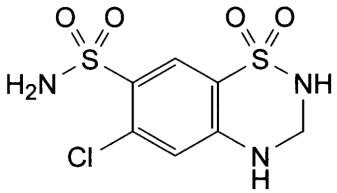

Figure 2
Structure of Hydrochlorothiazide

Hydrochlorothiazide (HCT), 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiazine-7-sulphonamide (Figure 2), is a thiazide diuretic that increases sodium and chloride excretion by distal convoluted tubule[10-11]. There are several reports of the determination of hydrochlorothiazide individually or in combination with other drugs, including use of HPLC, HPTLC, spectrophotometry, and non-aqueous potentiometric titration[12-20]. There are very few methods appearing in the literature for the simultaneous determination of valsartan and hydrochlorothiazide in tablets. Since these methods were based on HPLC, GC-MS and capillary electrophoresis[7, 21-23], the procedures was inconvenient for determination and the run times were very long. We have developed a simple, precise, accurate and specific RP – HPLC method for the simultaneous determination of VAL and HCT in pharmaceutical dosage forms. Because analytical methods must be validated before use by the pharmaceutical industry, the proposed HPLC – UV detection method was

validated in accordance with International Conference on Harmonization (ICH) guidelines[24, 25], by assessing its selectivity, linearity, accuracy, precision, limit of detection and limit of quantitation.

MATERIALS AND METHODS

Reagents and Chemicals

HPLC grade acetonitrile, potassium dihydrogen orthophosphate and orto-phosphoric acid (Analytical grade) were obtained from Merck, Germany. Tablets were purchased from local market each containing 80 mg of VAL and 12.5 mg of HCT. Valsartan RS and Hydrochlorothiazide RS were used as standards and were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Instrumentation

A Shimadzu HPLC system was utilized consisting of the following components: pump LC – 20 AD, vaccum degasser unit DGU – 20 A_5, column oven CTO-10AD, and a UV/VIS variable detector SPD – 20 A. Separation was carried out on a LiChrospher C 18 column (125 x 4 mm, particle size 5 µm) under reversed phase partition chromatographic conditions. The equipment was controlled by a PC installed properly with the chromatographic software. The mobile phase was a 45:55 % v/v mixture of acetonitrile: phosphate buffer (50 mM, pH 3 ± 0.1, adjusted with ortho-phosphoric acid). The flow rate was 1.0 mL/min and the run time was 7 min. Before analysis both the mobile phase and sample solutions were degassed by the use of a sonicator and filtered through a 0.4 µm filter. The identity of the compounds was established by comparing the retention times of compounds in the sample solution with those in the standard solutions. The column temperature was maintained at 30°C and injection volume was 20 µL. The detection of the drug was monitored at 250 nm.

Preparation of Standard Stock Solution of VAL and HCT

Standard solution was prepared by dissolving HCT and VAL in 100 mL diluents (mobile phase) to obtain a concentration of 12.5 µg/mL and 80 µg/mL respectively.

Sample Preparation

Twenty tablets were weighed and finely powdered. Quantity equivalent to 80 mg of VAL was transferred into a 100 mL volumetric flask with about 50 mL mobile phase. The contents were sonicated for 15 min and the mixture was made up to 100 mL with mobile phase. The solution was then centrifuged at 2000 rpm for 3 min and the clear supernatant was collected and filtered through 12 mm membrane syringe filter syringe filter (pore size 0.2 µm). From the clear solution, further dilutions were made by diluting 5.0 mL into 50 mL with mobile phase to obtain 80 µg/mL of VAL which is also contains 12.5 µg/mL of HCT theoretically. Each sample solution was injected and the peak areas were measured for the determination of VAL and HCT in tablet formulation.

RESULTS AND DISCUSSION

In this work an LC method with UV detection for analysis of VAL and HCT in a tablet formulation was developed and validated. From the chromatogram shown in Figure 3, it is evident that, under the proposed chromatographic conditions, VAL and HCT are well separated, that indicates that the method is applicable for their simultaneous quantification.

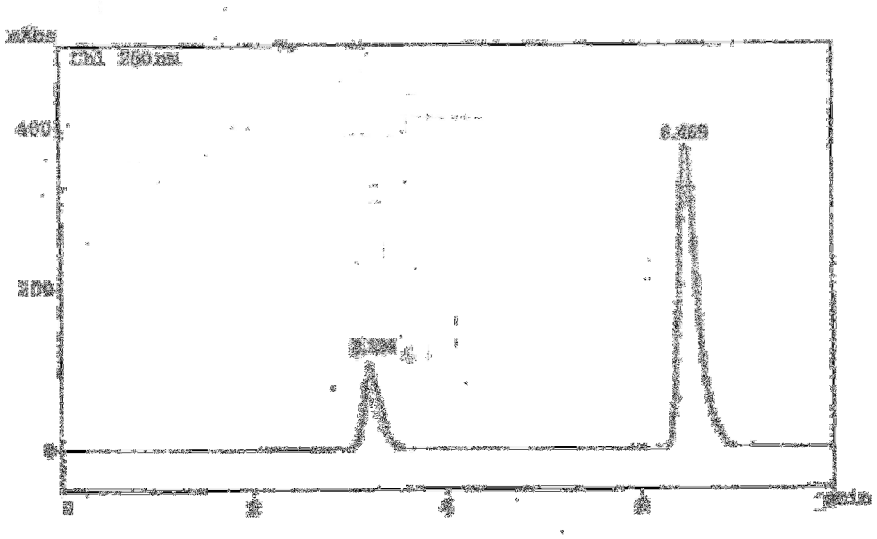

Figure 3
HPLC - Chromatogram of HCT and VAL

Method validation
The proposed method was validated as per ICH guidelines[24, 25] with respect to specificity, linearity, precision, accuracy, limit of quantitation (LOQ) and limit of detection (LOD).

Specificity
The specificity of the HPLC method was ascertained by analyzing standard drug and sample solutions. The retention time of VAL and HCT was confirmed by comparing the retention time with that of the standard.

Linearity
Under the experimental conditions described above, linear calibration curves for VAL and HCT were constructed with five concentration level each. The Table 1 presents the correlations coefficient, values of the slope and the intercept between the peak areas and concentrations of each drug substance.

Limits of quantitation and Limits of detection
The limit of detection (LOD) was calculated to be three times the standard deviation of baseline noise from analysis of each compound. The limit of quantitation (LOQ) was calculated to be the lowest amount of analyte in the sample, which was quantitatively determined with suitable precision. The results are given in Table 1.

Table 1
Linearity Results, Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Drugs	VAL	HCT
Concentration range (µg/mL)	5-50	1-10
Slope	404517.5	112611.7
Intercept	16120.5	753.1
Correlation coefficient (R^2)	0.99980	0.99984
LOQ (µg/mL)	0.718	0.499
LOD (µg/mL)	0.237	0.165

Accuracy
The accuracy of the method was determined by recovery studies. Three different levels of concentrations with triple injections for each sample were applied. were The recovery study results ranged from 99.32 to 100.3 % for VAL and from 99.28 to 100.2 for HCT, respectively. Recovery values were close to 100 % and % RSD not more than 2 which indicated that the method was accurate. Results of recovery investigations are reported in Table 2.

Table 2
Recovery studies of VAL and HCT

Valsartan			
Labelled claim (mg)	**Level of addition (%)**	**Amount of pure drug added (mg)**	**% Recovery***
80	50	40	100.3
80	100	80	99.54
80	150	120	99.32
Statistical analysis			Mean 99.72
			SD 0.510
			%RSD 0.508
Hydrochlorothiazide			
Labelled claim (mg)	**Level of addition (%)**	**Amount of pure drug added (mg)**	**% Recovery***
12.5	50	6.25	99.28
12.5	100	12.5	100.2
12.5	150	18.75	99.57
Statistical analysis			Mean 99.68
			SD 0.470
			%RSD 0.468

Average value of three determinations, RSD is relative standard deviation

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 3).

Table 3
Precision of the method

No	**Repeatability**		**Intermediate Precision**	
	HCT	**VAL**	**HCT**	**VAL**
1	98.55	100.03	99.05	99.76
2	98.67	100.05	98.89	100.11
3	98.68	99.39	99.45	102.04
4	98.46	99.81	98.97	100.79
5	98.23	99.47	99.03	102.11
6	98.97	99.86	99.18	100.74
Mean	98.59	99.77	99.09	100.9
SD	0.248	0.279	0.198	1.082
% RSD	0.251	0.280	0.200	1.071

CONCLUSION

A simple isocratic RP-HPLC method with UV detection has been developed for simultaneous determination of valsartan and hydrochlorothiazide. The method was validated for accuracy, precision, specificity and linearity. The run time is relatively short (7 min), which enables rapid quantification of many samples in routine and quality control analysis of tablets. This method was validated as per ICH guidelines.

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