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Research Article

TABLET FORMULATION AND DEVELOPMENT OF A VALIDATED STABILITY INDICATING HPLC METHOD FOR QUANTIFICATION OF VALSARTAN AND HYDROCHLORTHIAZIDE COMBINATION

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ABSTRACT

This study was aimed to develop valsartan/ hydrochlorothiazide tablet formulation and to develop a stability indicating HPLC method for their analysis in raw materials and in its final dosage form according to the ICH guidelines. Film coating tablets containing valsartan and hydrochlorothiazide were developed. A gradient HPLC method was performed; the flow rate was 1.5 ml/min, injected volume 20µL, the mobile phases consist of two solvent: Solvent A (0.20 M ammonium acetate, adjusted to pH 5.6 with glacial acetic acid) and Solvent B (acetonitrile) and UV detection was carried out at 265nm. Valsartan and hydrochlorothiazide and their combined dosage form were exposed to thermal, oxidative, acid-base hydrolytic stress conditions, the stressed samples were analyzed. The method was validated with respect to linearity, precision, accuracy, system suitability, and robustness. The used method is specific for the estimation of valsartan and hydrochlorothiazide in presence of their degradation products and impurities. The method was linear over the range of 2.5–32µg/mL and 17.5-224µg/mL for valsartan and hydrochlorothiazide respectively. The mean recoveries were 100±2% for valsartan and hydrochlorothiazide respectively. The percentage of relative standard deviation (%RSD) was found to be less than critical value**.** Our developed analytical method is a stability indicating, economical and easy method which is useful in the quality control of valsartan and hydrochlorothiazide in tablet dosage forms.

Keywords: Valsartan, Hydrochlorothiazide, Formulation, Stability, Stress conditions, HPLC, Validation.

INTRODUCTION

Valsartan is (S)-3-methyl-2-(N-{[2'-(2H-1,2,3,4-tetrazol-5-yl)biphenyl-4-yl] methyl} pentanamido) butanoic acid. It is a nonpeptide orally active and specific angiotensin II antagonist acting on the AT_1 receptor subtype present in many tissue such as vascular smooth muscle and the adrenal gland[1]. A Palcebo controlled trial have found that valsartan to be both safe and effective for the treatment of hypertension[2] other studies have also shown that valsartan is as effective as enalpril, lisinopril and amlodipine in the treatment of mild to moderate hypertention[3-5]. Hydrochlorothiazide (HCT) is 6-chloro-3,4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide-1,1-, It is a diuretic of the class of benzothiadiazines mainly used as antihypertensive agent either alone or in combination with other drugs, which decreases active sodium reabsorption and reduces peripheral vascular resistance [6, 7]. Valsartan and hydrochlorothiazide in a combination was evaluated and showed to be effective in the treatment of hypertension[8]. HPLC method for the determination of valsartan and hydrochlorothiazide alone for assay and bioavailability is available in the literature [9-11]. However, few methods appeared in the literature for simultaneous determination of valsartan and hydrochlorothiazide in tablets, these methods used capillary electrophoresis, HPTLC and UV-derivative spectrophotometry and HPLC, most of these procedures were inconvenient for determination of the combination and their run time was rather long [12-17]. Accordingly, the development of a stability indicating HPLC method, as a part of the development of valsartan/hydrochlorothiazide tablet, would be a useful tool for the evaluation of the stability of these agents since this method is economical and stability indicating. This study was aim to develop valsartan/hydrochlorothiazide tablets and to develop a stability indicating HPLC method for their analysis in raw materials and tablet formulation according to the ICH guidelines [18].

MATERIALS AND METHODS

Instrumentation

HPLC system (Merck Hitachi Lachrom Elite HPLC system, Japan) with an L-2130 pump, an L-2200 autosampler, L-2300 oven, and L-2490 UV detector was employed. The Ezchrom Elite software was employed. The chromatographic analysis was performed on a phenyl group bonded to porous silica particles column Xterra, the particle size is 5 µm, the length is 25 cm and the internal diameter is 4.6 mm, the column part number is 186001147 and the lot number is0111360671 from water. Precisa 205A Analytical balance, Elma ultrasonic water bath, and Millipore filtration assembly were used in this study.

Materials and Reagents

Valsartan and Hydrochlorothiazide were purchased from (Ipca Laboratories, India); USP Reference standards were used as working reference standard. USP benzothiadiazine related compound A, USP Chlorothiazide and USP Valsartan related compound B were used in the validation. The tablet dosage form (containing Valsartan 80mg and hydrochlorothiazide 12.5mg) was formulated in our laboratory the formulation is detailed in section 2.3. All the excipients used for the development of formulations were obtained from commercial sources and were used as such. The acetonitrile used was of HPLC grade and water was obtained by reverse osmosis.

Other reagents such as ammonium acetate, glacial acetic acid, hydrochloric acid (2M), sodium hydroxide (2M), potassium dihydrogen phosphate and 35% hydrogen peroxide were used in the additions and reactions. Colloidal Silicon Dioxide was achieved from (Aerosil; Evonik, Germany), Croscarmellose Sodium (AcDiSol; FMC-Ireland), Magnesium Stearate, (Magnesia, Germany), Microcrystalline Cellulose (Avicel pH 102; FMC Corp, Ireland), Polyvinylpyrrolidone (PVP K-30; Zhongbao Chemicals, China), Sodium Benzoate (DSM, Netherlands) and Sodium Lauryl Sulphate was achieved from Cognis, Germany. Hydroxy propyl methyl cellulose aqueous polymer (Opadry White) was purchased from Colorcon, England.

Formulation of Valsartan/hydrochlorothiazide tablets

Core tablets containing the above combination were prepared using the dry granulation method using the following excipients: Colloidal Silicon Dioxide, Croscarmellose Sodium, Magnesium Stearate, Microcrystalline Cellulose, Polyvinylpyrrolidone, Sodium Benzoate and Sodium Lauryl Sulphate. All components were blended in a

double cone mixer (use only 50% of Magnesium Stearate and 50% of AcDiSol). The blended powder mixture was sieved through mesh # 30, and compacted using a roller compactor. The compacted material was crushed through an oscillator equipped with sieve mesh # 20, and blended with the remaining portions of AcDiSol and Magnesium Stearate. The tablets were compressed on 8-station rotary tablet press. Film coating was carried out by using aqueous dispersion of Opadry white and conventional coating pan at a temperature not exceeding 55 Celsius degrees.

Chromatographic conditions

Chromatographic separation was operated at room temperature on a reversed phase phenyl column. The diluents used to attain the final concentration consist of a mixture of water: acetonitrile (1:1). Flow rate was 1.5 ml/min, injected volume 20µL, wavelength of detection is 265nm. The mobile phases consist of two solvent: Solvent A (0.20 M ammonium acetate, adjusted to pH 5.6 with glacial acetic acid) and Solvent B (acetonitrile).

Gradient elution system was used which consist of solvent A and Solvent B. The program of gradient elution is shown in **Table 1** below.

Preparation of Stock Standard Solutions

An accurately weighed amount of USP hydrochlorothiazide RS (approximately12.5 mg) added into 200ml volumetric flask. The sample was dissolved in the diluents and sonicated for 15 minutes. A series of dilution was done to obtain a final concentration of 0.0125 mg/ml. For the valsartan stock solution the same procedure was followed in order to obtain a final concentration of 0.16mg/ml.

Table 1: Gradient elution (solvent programming) runs

Time(minutes)	Solution A (%)	Solution B (%)
0	88	12
4	65	35
7	88	12
8	88	12

Preparation of Sample

An accurately weighed 5 tablets were crushed to a fine powder and an amount of the powder was taken in order to get 62.5 mg of hydrochlorothiazide and 300mg of valsartan. 100mls of the diluents were added to the powder, the mixture was sonicated for 30 minutes, and then it was centrifuged at a high speed (3000 rpm). An accurate quantity of supernatant was pipetted and a series of dilution was made to give final concentration of 0.012 and 0.16mg/ml for hydrochlorothiazide and Valsartan respectively.

Method validation

The method was validated for the parameters like, range and linearity, accuracy, precision, limit of detection (LOD) and quantification (LOQ). Concerning the specificity of the method it was assessed by performing a stability indicating study.

To evaluate the linearity of the method, nine different dilutions were made from the standard stock solutions in the working range of 20–140%.

In order to determine the accuracy of the method, working standards of the combination were prepared in five replicates of two nominal concentration (0.125 and 0.08 mg/ml) and (0.125 and 0.16 mg/ml) for the hydrochlorothiazide and valsartan respectively, and the relative standard deviation (%RSD) was calculated. Three different concentrations (80%, 100% and 120%) of active ingredient combination were spiked in the tablet formulation and their recovery was calculated. Regarding the determination of the precision (repeatability) five replicate injections of the working standard at the two nominal concentrations for Valsartan & Hydrochlorothiazide Tablets and the relative standard deviation (RSD) of the peak areas were calculated for the replicate injections. To determine the LOD and LOQ, serial dilutions of the combination were made from the standard stock solution in the range of 0.008-1.92µg/ml for Valsartan 0.00625-0.15 µg/ml for hydrochlorothiazide. The signal from the samples was compared with those of blank samples. LOD and LOQ values were identified as signal-to-noise ratio (S/N) of 3:1 and 10:1, respectively.

To determine the specificity of method in the presence of pharmacopoeial impurities and formulation impurity; the valsartan and hydrochlorothiazide and the pharmacopeial impurity were spiked in the presence of excipients used in formulation development, the observed chromatograms were checked for the purity using a photo-diode array (PDA) detector. The detailed procedure mentioned in section 2.8.

Data from replicate injections at the nominal concentration was utilized for calculating various system suitability parameters using Merck-Hitachi Lachrom Elite HPLC system.

Specificity and Stability indicating studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Intentional degradation was carried out by exposing of samples to three stability condition (0.1 N HCl, at 65 ^{0}C), (2 N NaOH, at 65 ^{0}C) and (35% H_2O_2 at 65 ^{0}C) and tested after 1, 2 and 3 hours consequently. They were then analyzed against control samples (lacking of degradation treatment).

RESULT & DISCUSSION

Linearity and range

The linearity of the method was observed in the expected concentration range (20% to 140%) demonstrating its suitability for analysis. The goodness-of-fit (R^2) was found to be 0.9999 indicating functional linear relationship between the concentration of analyte and area under the peak. The regression equation for both the hydrochlorothiazide and valsartan is shown in **Figure 1 & 2** respectively.

Accuracy

The results of accuracy studies (**Table 3**) show that the method is accurate within the desired range.

The RSD was calculated for each recovery solution and all the results are within limits (100±2%) and as shown in **Figure 3 & 4** a plot of area under peak versus concentration for each level of Valsartan and Hydrochlorothiazide were plotted The goodness-of-fit (R^2) for Valsartan equal to (0.9999) and for Hydrochlorothiazide equal to (0.9999).

Precision

The precision (repeatability) of an analytical method refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment and is expressed as the %RSD. The precision study (**Table 4**) showed that method has a good reproducibility which was approved by the analysis of five replicate injections of the working standard solution at the two nominal concentrations the %RSD was 0.1% for Valsartan and Hydrochlorothiazide of the high nominal concentration and the RSD was 0.2% for Valsartan and Hydrochlorothiazide of the low nominal concentration, thus the system was repeatable.

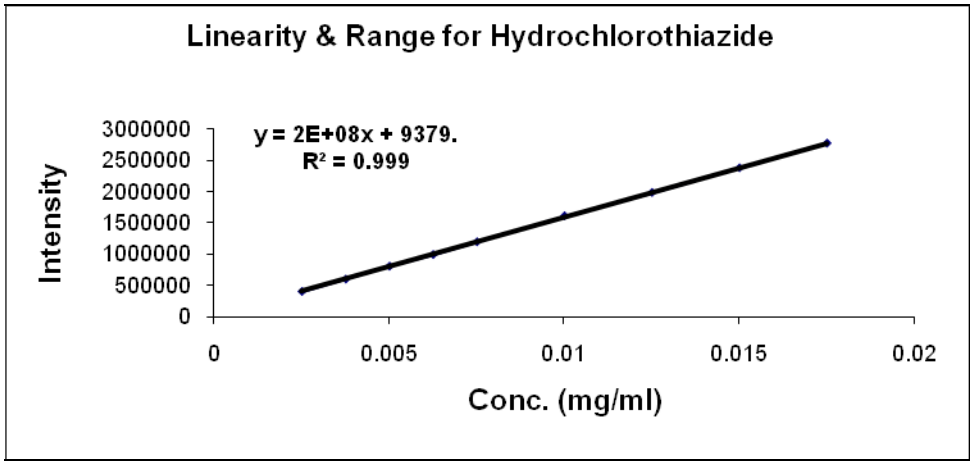


Fig. 1: Linearity and rang of hydrochlorothiazide, R^2=0.9999.

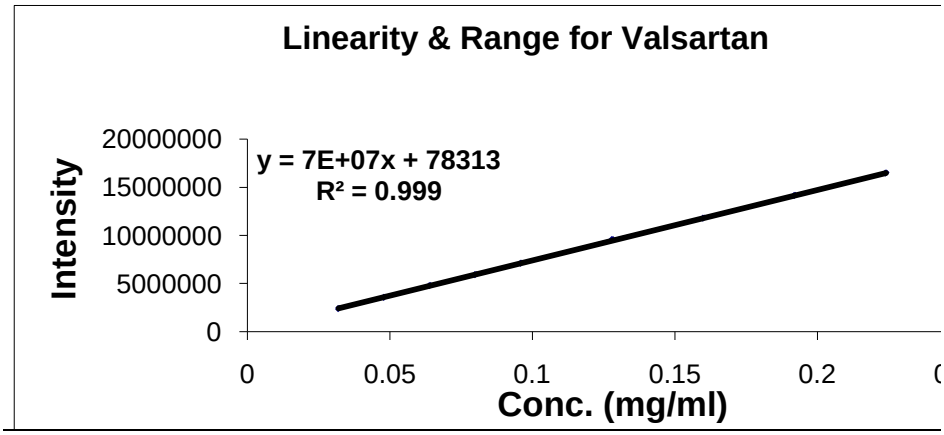


Fig. 2: Linearity and rang of Valsartan, R^2=0.9999

Table 3: (A) Accuracy of Drug Products Data for hydrochlorothiazide:

Conc. %	Conc. HCT (mg/ml)	Area Sa	% Accuracy (Recovery)	Average & %RSD
80%				
80%	0.01	1596162	100.6	100.7%
80%	0.01	1597927	100.7	&
80%	0.01	1598073	100.7	0.07%
100%				
100%	0.0125	1970267	99.4	99.4%
100%	0.0125	1969541	99.3	&
100%	0.0125	1971760	99.4	0.06%
120%				
120%	0.015	2359903	99.2	99.1%
120%	0.015	2359050	99.1	&
120%	0.015	2359166	99.1	0.02%

Table 3: (B) Accuracy of Drug Products Data for Valsartan

Conc.%	Conc. Valsartan (mg/ml)	Area Sa	% Accuracy (Recovery)	Average & %RSD
80%				
80%	0.128	9640866	102.3	102.0% & 0.2%
80%	0.128	9621415	102.1	
80%	0.128	9599046	101.8	
100%				
100%	0.16	11842204	100.5	100.6% & 0.2%
100%	0.16	11849328	100.6	
100%	0.16	11887146	100.9	
120%				
120%	0.192	14172564	100.2	100.2% & 0.1%
120%	0.192	14154596	100.1	
120%	0.192	14162613	100.2	

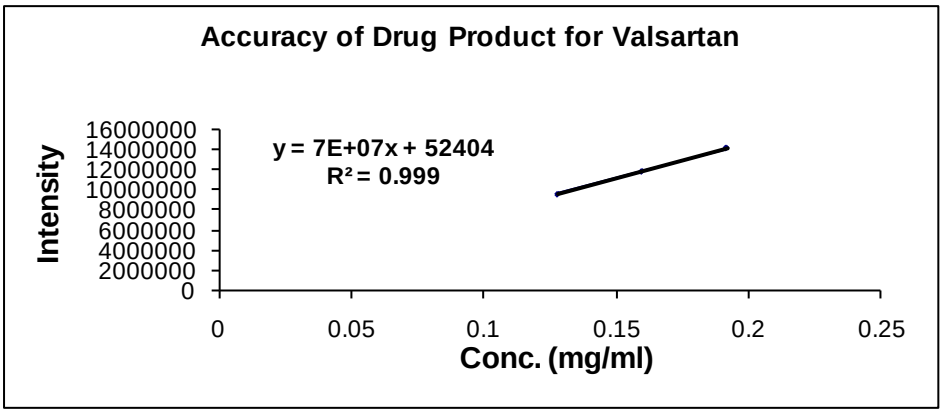


Fig. 3: Linearity of recovery results for each leve of Valsartan

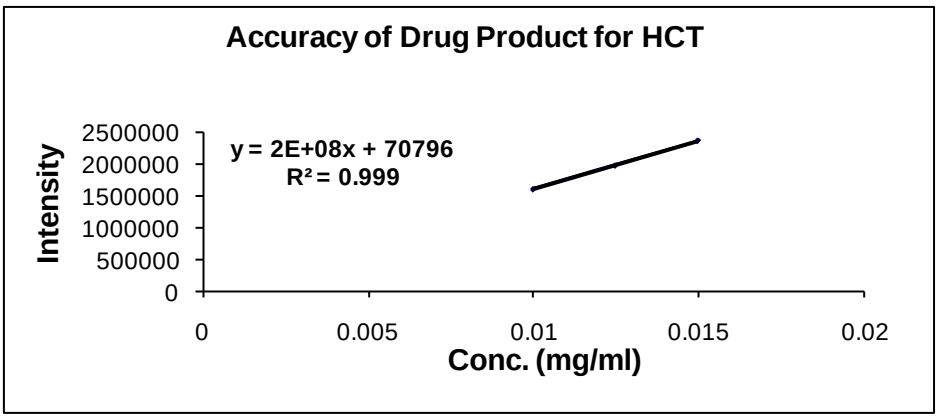


Fig. 4: Linearity of recovery results for each level of hydrochlorothiazide

Table 4: Precision of Standard Injection Data:

Injection No.	Nominal Concentration-1		Nominal Concentration-2	
	Valsartan	HCT	Valsartan	HCT
I	11771753	1981234	5907327	1976382
II	11798757	1986000	5931527	1985052
III	11778824	1981530	5935778	1985874
IV	11786028	1983579	5930076	1985410
V	11778766	1980904	5923565	1981678
Average	11782826	1982649.4	5925655	1982879.2
SD	10236.7	2144.4	11144.3	3992.2
RSD	0.1%	0.1%	0.2%	0.2%

Note: Nominal Concentration-1: 0.0125 mg/ml of HCT & 0.16 mg/ml of Valsartan; Nominal Concentration-2: 0.0125 mg/ml of HCT & 0.08 mg/ml of Valsartan

Detection and quantification limit (LOD &LOQ)

The detection limit or LOD is the lowest amount of analyte in a sample that can be detected. It may be expressed as a concentration that gives a signal to noise ratio of approximately 3:1. While the Quantification limit or LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy with a signal to noise ratio of approximately 10:1 can be taken as LOQ of the. Our method (**Table 5**) showed the (LOD) for Hydrochlothiazide and Valsartan were found to be 0.008µg/ml and 0.0375 µg/ml, respectively and The LOQ values for Hydrochlothiazide and Valsartan were found 0.075 µg/ml and 0.064 µg/ml respectively.

Table 5: (A) Detection and Quantification Limit Data for Valsartan

Concentration (mg/ml)	Area 1st Injection	Area 2nd Injection	Area 3rd Injection	Average Area	SD	Av. S/N
0.00192	174990	181731	174073	176931	4181.8	152.3
0.00096	98077	97546	97779	97801	266.2	84.9
0.00048	50302	61626	60809	57579	6315.3	47.8
0.000128	38242	64242	36623	46369	15499.6	15.7
0.000032	34149	35604	29757	33170	3044	17.7
0.000064	29757	27788	28385	28643	1009.6	11.5
0.000008	7468	5899	5820	6396	929.5	5.2

Table 5: (B) Detection and Quantification Limit Data for hydrochlorothiazide

Concentration (mg/ml)	Area 1st Injection	Area 2nd Injection	Area 3rd Injection	Average Area	SD	Av. S/N
0.00015	24847	25529	24350	24908.7	591.9	22.2
0.000075	11845	12124	11845	11938	161.1	12.3
0.0000375	5702	6009	6089	5933.3	204.3	4.3
0.00001	Undetectable					
0.0000025	Undetectable					
0.000005	Undetectable					
0.00000625	Undetectable					

Specificity and stability indicating study

Stress testing of the drug substance can help identify the likely degradation products, the stability of the molecule and also validate the stability and selectivity of the analytical procedures. Stability indicating study was performed under various stress conditions mentioned in section 2.8. The results of specificity studies (**Table 6**) indicated no interference from excipients, impurities and degraded products due to various stress conditions and assured that the peak response was due to a single component only.

The chromatogram of samples degraded with acid, base and hydrogen peroxide showed peak of USP related compounds and are well resolved from the drug peak. The Valsartan and Hydrochlorothiazide was affected in the presence of Hydrogen Peroxide and Hydrochloric Acid at the three period of digestion (After one, two and three hours), Hydrochlorothiazide was also affected when treated with temperature. Hydrochlorothiazide was degraded to Benzothiadiazine Related Compound A and Valsartan was degraded to Valsartan Related Compound B. Hydrochlorothiazide was not affected in the presence of Sodium Hydroxide at both conditions and also in the presence of Hydrogen Peroxide at room temperature. Valsartan was affected in the presence of Sodium Hydroxide at both conditions.

In all cases of study the peak of Hydrochlorothiazide and Valsartan is clearly separated from each other and from their related degradant (Benzothiadiazine Related Compound A, chlorothiazide and Valsartan Related Compound B, respectively) and from all other peaks with a resolution greater than 1.5 **(Figure 5**). The excepiet peak (Sodium benzoate) of the tablet formulation did not interfere with the active ingredient and its related degradative compounds as shown in (**Figure 6**) Thus the method is specific and stability indicating for determination of Hydrochlorothiazide and Valsartan and their related degradants in Hydrochlorothiazide and Valsartan Tablets.

Table 6: System Suitability Data

Injection No.	Benzo Thiadiazine R.C.A	HCT	R_1	Valsartan R.C.B	Valsartan	R_2
I	1399625	2440437	5.1	3714765	1497654	3.5
II	1397373	2447324	5.1	3713686	1517524	3.5
III	1397420	2444827	5.1	3704092	1501012	3.5
IV	1397748	2437231	5.1	3711011	1525622	3.5
V	1401041	2442007	5.1	3717381	1502139	3.5
AV	1398641	2442365	5.1	3712187	1508790	3.5
SD	1629.8	3900.5		5069.1	12127.9	
RSD	0.1%	0.2%		0.1%	0.8%	

R_1: Resolution between Benzothiadiazine Related Compound A & Hydrochlorothiazide

R_2: Resolution between Valsartan Related Compound B & Vasartan

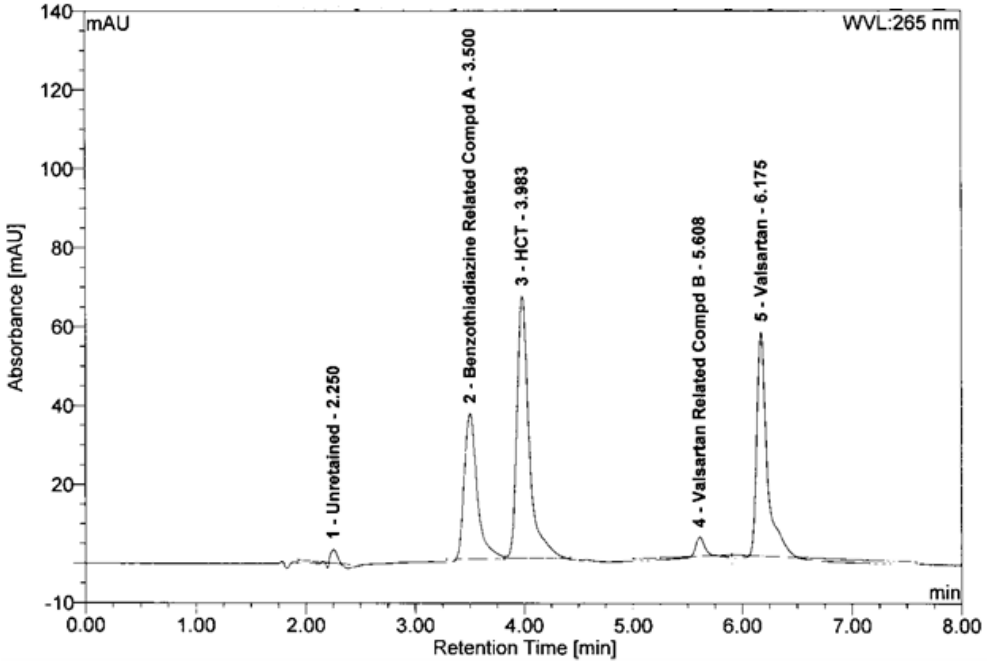


Fig. 5: Stability indicating chromatogram for determination of Hydrochlorothiazide and Valsartan.

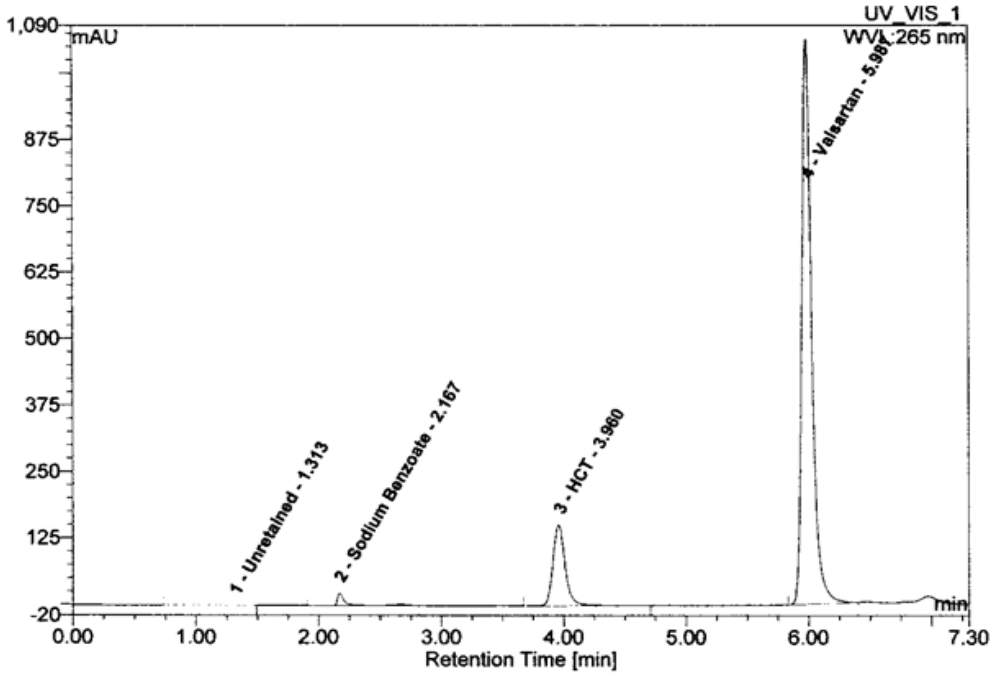


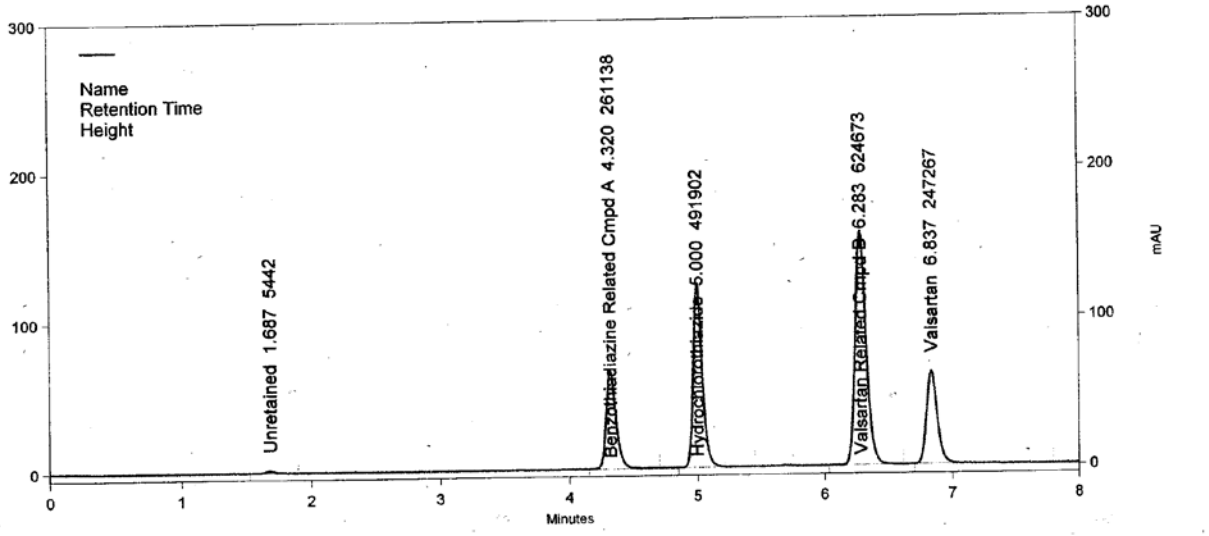
Fig. 6: Formulation excepient (Na Benzoate) early elute and do not interfere with the active ingredients or its related compounds.

System suitability testing

System suitability is used to verify that the system is adequate for the analysis to be performed.

Our method shows all the values for the system suitability parameters are within limits (**Table 7**).

The column efficiency is about 24940 and 29799 theoretical plates for hydrochlorothiazide and valsartan, respectively. The tailing factors are about 1.3 and 1.2 for hydrochlorothiazide and valsartan, respectively. The resolution between benzothiadiazine related compound A and hydrochlorothiazide is about 5.1, while for valsartan and related compound B is about 3.5 (**Figure 7**).



UV Results

Name	Retention Time	Area	Height	Theoretical plates (USP)	Asymmetry	Resolution (USP)
Unretained	1.687	31537	5442	2383	1.17505	0.00000
Benzothiadiazine Related Cmpd A	4.320	1399625	261138	15281	1.25576	18.94606
Hydrochlorothiazide	5.000	2440437	491902	24335	1.27621	5.07470
Valsartan Related Cmpd B	6.283	3714765	624673	25916	1.16765	9.02711
Valsartan	6.837	1497654	247267	30864	1.17454	3.54948

Fig.7: System parameters for valsartan and hydrochlorothiazide and its related compounds

Table 7: Specificity and stability indicating study

Stress Condition	HCT		Benzothiadiazine R.C.A.		Valsartan		Valsartan R.C.B.		Total Degradation	
	Area	%	Area	%	Area	%	Area	%	Area	%
A.24Hr.@ RT	1964644	99.1	57839	2.9	11811271	100.2	0	0.0	0	0.0
A.24Hr.+A.1Hr. at 65°C	1708710	86.2	336160	17.0	11730315	99.6	0	0.0	0	0.0
A.24Hr.+A.2Hr. at 65°C	1447350	73.0	648427	32.7	11796274	100.1	0	0.0	0	0.0
A.24Hr.+A.3Hr. at 65oC	1252015	63.1	873358	44.0	11805892	100.2	0	0.0	0	0.0
10ml of 2N NaOH	1983211	100.0	0	0	11733302	99.6	0	0.0	0	0.0
NaOH+A.1Hr. at 70°C	1982836	100.0	35591	1.8	11808261	100.2	0	0.0	0	0.0
10ml of 35% H_2O_2	1981457	99.9	11661	0.6	11650106	98.9	0	0.0	610109	4.4
H_2O_2+A.1Hr. at 65°C	917076	46.2	191379	9.7	8790370	74.6	772128	6.6	433293	3.1
H_2O_2+A.2Hr. at 65°C	474794	23.9	241443	12.2	6960153	59.1	1187290	10.1	516054	3.7
H_2O_2+A.3Hr. at 65°C	0	0	292123	14.7	5208048	44.2	1520483	12.9	625892	4.5
HCl+A.1Hr. at 65°C	1916826	96.7	86745	4.4	10706266	90.9	0	0	30237	0.2
HCl+A.1Hr. at 65°C	1866502	94.1	148857	7.5	10614487	90.1	0	0	51471	0.4
HCl+A.2Hr. at 65°C	1771598	89.3	256261	12.9	6811046	57.8	0	0	96905	0.7

HCT: Hydrochlorothiazide; R.C.A.: Related Compound A; R.C.B.: Related Compound B; A.: After; Hr.: Hour

A. 1Hr. at 65°C: Means after one hour of digestion at 65°C.

10ml of reagents (NaOH, HCl & H_2O_2) added to the test solution separately.

CONCLUSION

The valsartan/hydrochorthiazide tablets were successfully prepared. The developed stability indicating HPLC method was successfully developed and it showed several advantages over other known methods for the analysis of these agents since it is an economical, single method that can be used for assay of the two ingredients. In addition, the method has been successfully used for analysis of drug–excipient compatibility samples by performing a stability indicating study. The method was validated in accordance to the ICH guidelines showing linearity, accuracy, precision, selectivity, stability and system suitability. The method can also be used for purity and degradation evaluation.

Conflict of Interest

The authors declare no conflict of interests.

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