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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF VALSARATN AND HYDROCHLOROTHAIZIDE IN BULK AND COMBINED TABLET DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

A Rapid and Precise RP-HPLC Method has been developed and validated for simultaneous estimation of Valsartan (VAL) and Hydrochlorothiazide (HCTZ) in bulk and combined Tablet dosage form. The method was carried out on the chromatographic separation was achieved by isocratic elution technique on (reverse phase) Primesil C18 column (250 mm x 4.6 mm ID, Particle size 5µm), using mobile phase composition of Methanol : Water (0.05%OPA) in the ratio of 80:20 v/v with a flow rate 0.7ml/min. The two wavelengths 246nm and 268 nm were selected for estimation of Valsartan and Hydrochlorothiazide respectively. The retention time of VAL and HCTZ were found to be 4.40 min and 7.88 min respectively. The VAL and HCTZ followed linearity in the concentration range of 32-60µg/ml and 5-25µg/ml respectively with r^2=0.999 for both VAL and HCTZ. The amount of both drugs estimated by the proposed method was found to be in good agreement with labelled claim. The developed analytical validation for precision, accuracy, sensitivity, robustness and ruggedness. The developed method can be used for routine analysis of titled drugs in bulk and combined dosage form.

KEYWORDS: Valsartan and Hydrochlorothiazide, RP-HPLC, Validation.

INTRODUCTION

VALSARTAN

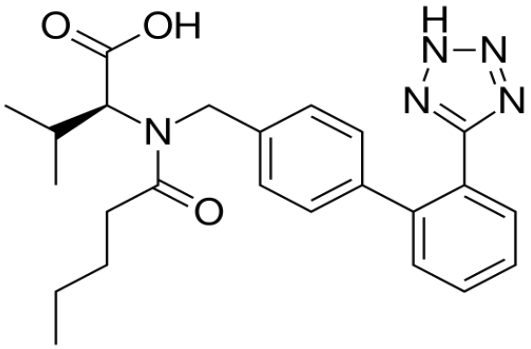


Fig No. 1(a): Chemical Structure Of Valsartan

Valsartan[1] [Figure 1(a)] chemically (*S*)-3-methyl-2-(*N*-{[2'-(2*H*-1,2,3,4-tetrazol-5-yl)biphenyl-4-yl]methyl}pentanamido)butanoic acid. Valsartan is an angiotensin II receptor antagonist used in management of hypertension. Valsartan It may also be used in patients with heart failure who are unable to tolerate ACE inhibitors [2]. Valsartan lowers blood pressure by antagonizing the Renin-Angiotensin-Aldosterone System (RAAS); it competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of angiotensin II [3]. Valsartan may be used to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabeticnephropathy. It may also be used as an alternative agent for the treatment of heart failure,systolic dysfunction, myocardial infarction and coronary artery disease [4-5].

HYDROCHLOROTHIAZIDE

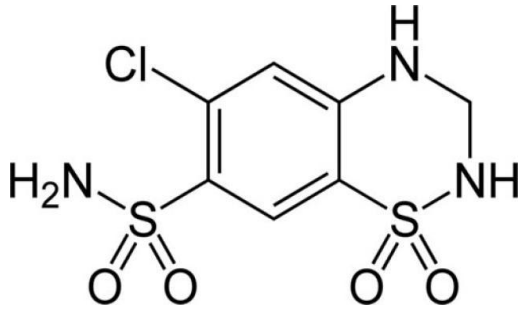


Fig No. 2(b): Chemical Structure Of Hydrochlorothiazide

Hydrochlorothiazide (HTZ) [Figure 2(a)] It is chemically 6 - Chloro - 3, 4 -dihydro - 7 - sulfamoyl - 2H - 1, 2, 4 - benzothia - diazine -1, 1 - dioxide, is a thiazide diuretic[6]. Hydrochlorothiazide 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 Hydrochlorothiazide (HCTZ), It increases sodium and chloride excretion in distilled convoluted tubule. Many analytical methods were reported for the analysis of HCTZ alone and combination with other drugs by stability indicating method[7], RP - HPLC methods[8,9],and Spectrophotometric methods[10,11] .

MATERIAL AND METHODS

Material

a) Selection and Procurement of Drug and Chemical

Pharmaceutical grade Valsartan and Hydrochlotrothiazide was kindly supplied as a gift sample by Mylan Labotatories Ltd in.Waluj, Aurangabad.

All chemical and reagents used were of HPLC grade, Formulation used for combined Tablet formulation Valsartan 80mg and Hydrochlorothiazide 12.5mg Manufactured by Lupin pharmaceuticals, Pinnacles. CVN were purchased from local market.\

b) Instrumentation

Table No.1: Instrument (HPLC) Details used during Method Development

	Name of Instrument	Company Name
1	HPLC Instrument	YounglineAcme9000 (Autochro-3000 software)
2	UV-Spectrophotometer	Analytical Technologies Limited
3	Column(C_{18})	Primesil C_{18} (250mmX 4.6mm,5µm)

c) Chromatographic Conditions

Primesil C18 column (250 mm x 4.6 mm ID, Particle size 5µm) was used for chromatographic separation using mobile phase composition of Methanol : Water (0.05% OPA) in the ratio of 80:20 v/v with a flow rate 0.7ml/min. with run time 15 min Mobile phase and sample solutions were filtered through a 0.45 µm membrane filter and degassed. The detection of both drugs was carried out at 259 nm.

EXPERIMENTAL SECTION

Study of Spectra and Selection of Wavelength

The aliquot portions of standard stock solutions of VAL and HCTZ were diluted appropriately with Methanol to obtain concentration 10 µg/mL of both drugs. The solutions of both drugs were scanned separately in the range of 200 – 400nm. and the two wavelength

246 nm (λ max of VAL) and 268 nm (λ max of HCTZ) were selected for further study .The overlain UV absorbance spectrum of VAL and HCTZ is shown in [**Fig No. 3(A),4(B),5(C)].**

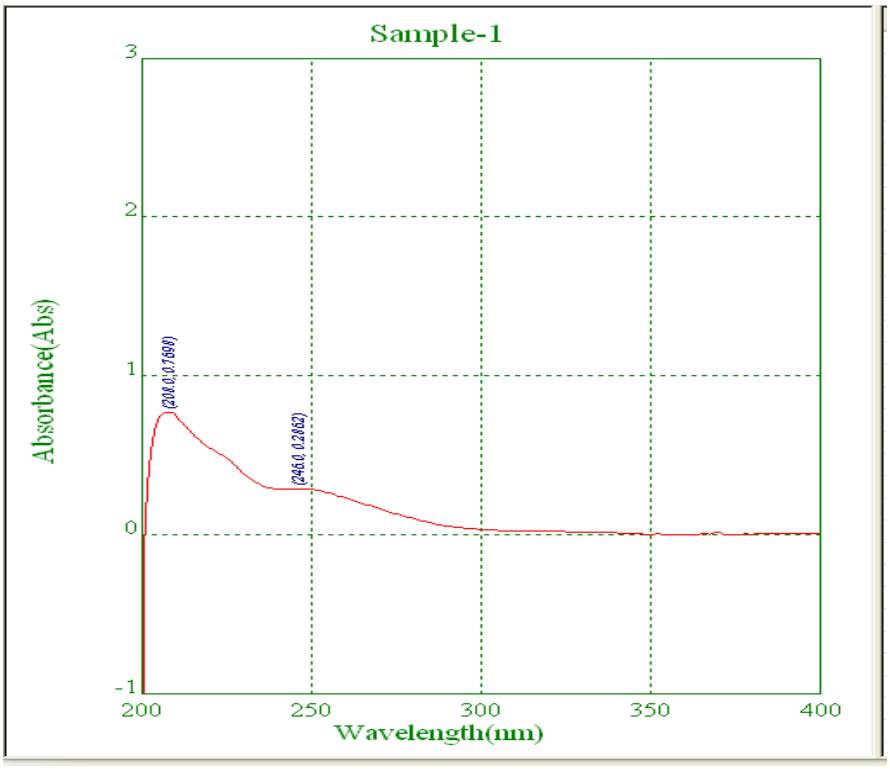


Fig No.3: UV Spectrum(A) of Valsartan

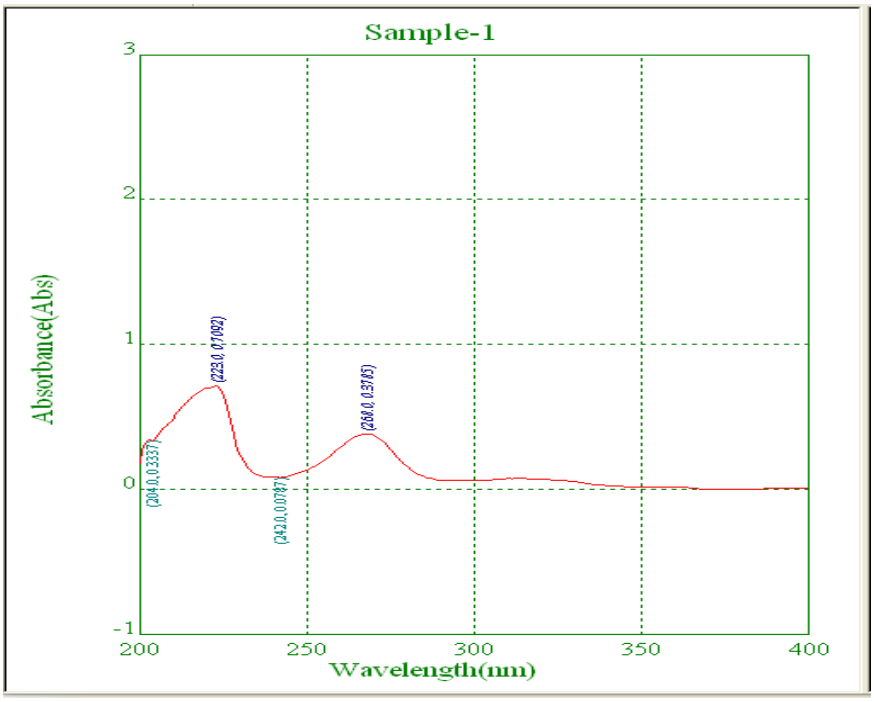


Fig No.4: UV Spectrum(B) of HCTZ

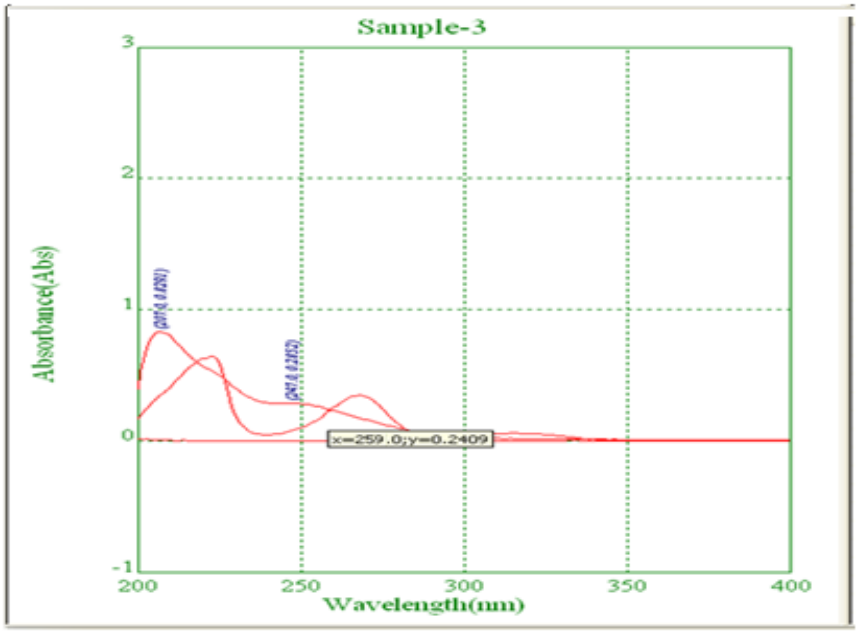
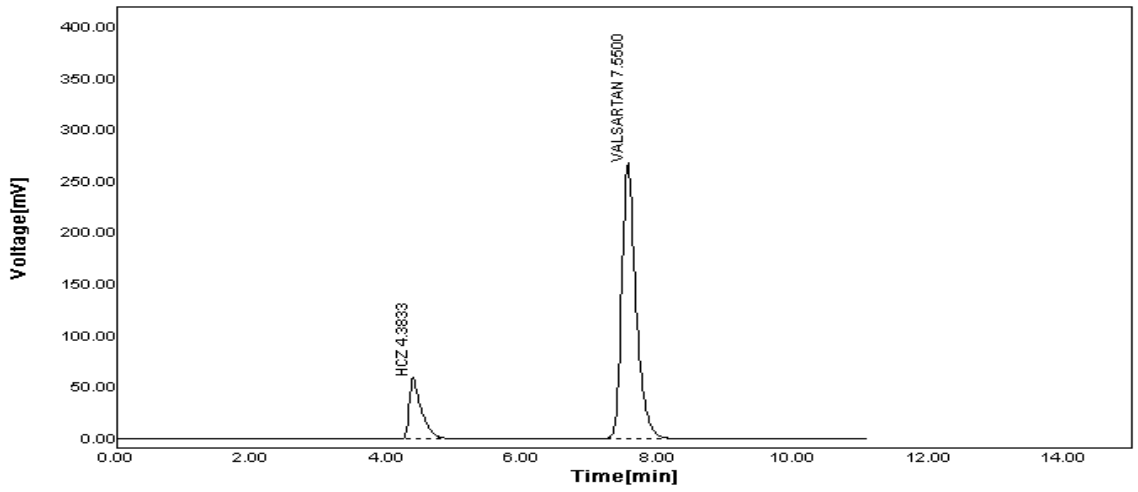


Fig No. 5: Overlain Spectra (C) of VAL and HCTZ

From the overlain spectrum the wavelengths selected for estimation of drugs were 246 nm as λ max of VAL and 268 nm as λ max of HCTZ.

Preparation of Standard stock solution

Standard stock solution prepared of accurate weigh of 64mg of Valsartan and 10 mg Hydrochlorothiazide transfer into clean dry 10 ml volumetric flask add about the 8 ml Methanol Diluent and sonicate to dissolve in it completely and make volume up to the mark with the same solvent. (Stock were prepared) Then from that stock solution pipette out 0.1ml solution & dilute to 10 ml in volumetric flask.add about the mobile phase methanol :Water(80:20 v/v) in diluents the make volume mark to the 10 ml volumetric flask.



FigNo.6: Chromatogram of standard Combination of Valsartan&Hydrochlorothiazide

Analytical Method Validation

Validation

To validate the developed method parameters like,Accuracy in term of % recovery, Precision, System suitability, Linearity and Range , in term of %RSD, Robustness were studied**.**

Linearity and Range

Valsartan and Hydrochlorothiazide Standard stock solution, The suitable aliquots were taken to obtain 32,64,96,128,160 µg/ml. from VAL stock solution. The suitable aliquots were taken to obtain 5,10,15,20,25 µg/ml from HCTZ stock solution. The results are shown in **[Table No: 2,3], [Figure No: 7 and Figure No: 8].**

Table No: 2. Linearity of Valsartan

Sr. No.	Concentration µg/ml	Area Valsartan
1	32	1795.22
2	64	3339.86
3	96	4843.22
4	128	6359.34
5	160	7744.64

Table No:3. Linearity of Hydrochlorothaizide

Sr. No.	Concentration µg/ml	Area Valsartan
1	5	469.57
2	10	749.22
3	15	991.52
4	20	1286.35
5	25	1541.51

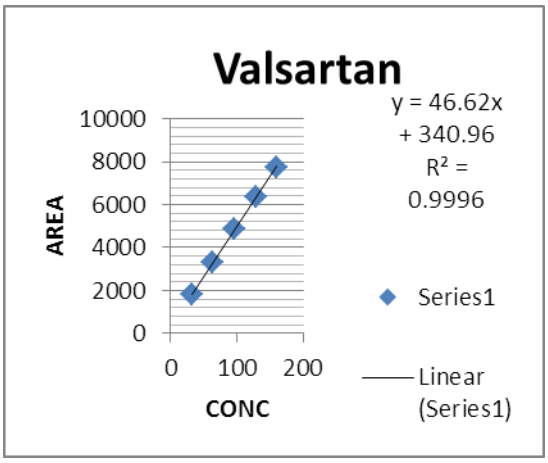


Fig.No.7: Calibration curve of Valsartan

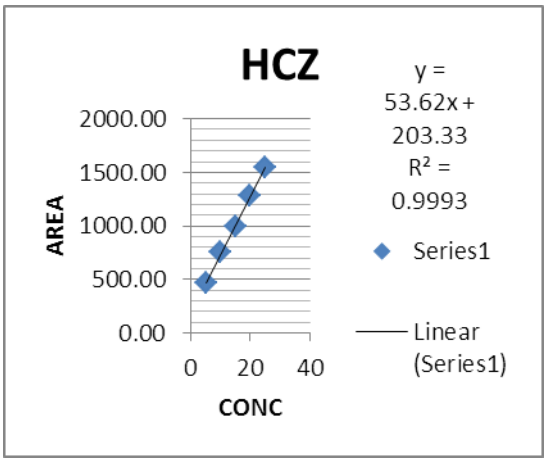


Fig.No.8: Calibration curve of HCTZ

Accuracy

Accuracy of each of the proposed method was ascertained on the basis of recovery studies performed by standard addition method as shown in the **[Table No.4]**

Table No .4: Recovery data for Valsartan & Hydrochlorothiazide

Drug	Sr No.	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
VAL	1	80	64	51.2	114.80 ±0.07	50.80 ±0.07	99.23± 0.13
	2	100	64	64	127.49±63.49	63.49 ±0.65	99.21± 1.03
	3	120	64	76.8	139.54± 0.38	75.54 ± 0.38	98.37± 0.50
HCTZ	1	80	10	8	18.04±0.04	8.04± 0.04	100.22± 0.21
	2	100	10	10	20.39± 0.13	10.39± 0.13	101.98 ±0.65
	3	120	10	12	22.18± 0.11	12.18 ±0.11	101.34±1.22

***mean of each 3 reading**

Precision

Precision of the analytical method is expressed as the series of the measurement. It was ascertained by replicate estimation of the drug by the proposed method as shown in **[Table No.5]**

Table No .5: Intra day and Inter day Precision for Valsartan & Hydrochlorothiazide

Drug	Conc^{n} (µg/ml)	Intraday Precision		Interday Precision	
		Mean± SD	%AmtFound	Mean± SD	%AmtFound
Valsartan	32	1835.90±2.07	100.4%	1827.90±2.57	99.6%
	96	485.23±20.4	100.6%	4843.23±28.04	100.5%
	160	7855.12±3.91	100.7%	7747.42±73.91	99.29%
HCTZ	5	470.56±5.32	99.6%	477.56±5.32	92.2%
	15	995.64±4.10	98.5%	997.54±2.62	95.3%
	25	1562.0±4.36	99.3%	1560.0±6.46	99.19%

***Mean of each 3 reading**

Repeatability

Repeatability was ascertained by getting the sample analyzed by different analyst and carrying out analysis for number of times. The results are shown in (**Table no. 6,7)**and Mean of Five Determination System Suitability Parameters **(Table no. 8)**

Table No.6: Reapetability studies on Valsartan

Sr.No.	Concentrationof Valsartan(mg/ml)	Peak area	Amount found (mg)	%Amountfound
1	15	4865.34	97.04	101.09
2	15	4863.06	96.99	101.04
3	15	4839.60	96.49	100.5
4	15	4833.43	96.36	100.3
5	15	4823.43	96.14	100.15
		Mean	96.60	100.6

		SD	0.39	0.49
		%RSD	1.09	0.81

Table No.7: Reapetability studies on Hydrochlorothiazide

Sr.No.	Concentrationof Valsartan(mg/ml)	Peak area	Amount found (mg)	% Amount found
1	96	999.18	14.84	98.95
2	96	999.46	14.84	98.95
3	96	1000.63	14.87	99.13
4	96	998.79	14.83	98.90
5	96	995.79	14.77	98.53
		Mean	14.83	98.89
		SD	0.10	0.66
		%RSD	1.02	0.86

Table No.8: System Suitability Parameters

System Suitability Parameters	Mean of Five Determination System Suitability Parameters	
	Valsartan	**HCZ**
Retention Time (min)	7.59	4.38
Area	4844.9	2797.5
Theoretical Plate Number	8199.5	3935.5
Taling Factor	1.54	1.13
Resolution	9.04	0.00

*** Mean of 5 Determination for SST**

Robustness

The mobile phase composition was changed in (±1 ml/ min^{-1}**)** proportion, the flow rate, and wavelength change was varied by optimized chromatographic condition. %RSD for peak area was calculated which should be less than 2%.[**Table No:9,10]**

Table No.9: Robustness Study of Valsartan

Parameters	Conc.(µg/ml)	Amount of detected(mean ±SD)	%RSD
Mobile phasecomposition(81ml+19ml) Methanol + 0.05% (OPA)water	96	5170.78 ± 0.54	0.01
Mobilephasecomposition(79ml+21ml) Methanol + 0.05% (OPA)water	96	5080.74 ± 60.61	1.19
Wavelength change258nm	96	4987.79 ± 4.04	0.08
Wavelength Change 260nm	96	5333.18 ± 3.59	0.07
Flow rate change(0.6ml)	96	5902.96 ± 3.36	0.06
Flow rate change(0.8ml)	96	4581.30 ± 43.18	0.94

Table No.10: Robustness Study of Hydrochlorothizide

Parameters	Conc.(µg /ml)	Amount of detected(mean ±SD)	%RSD
Mobile phase composition-(81ml+19ml)) Methanol + 0.05% (OPA)water	15	1153.22 ± 3.26	0.28
Mobile phase composition-(79ml+21ml)) Methanol + 0.05% (OPA)water	15	972.42 ± 10.30	1.06
Wavelength change258nm	15	895.24 ± 4.82	0.54
Wavelength Change 260nm	15	961.75 ± 5085	0.61
Flow rate change(0.6ml)	15	1175.96 ±12.33	1.05
Flow rate change(0.8ml)	15	917.50 ± 1.27	0.14

LOD AND LOQ

Calibration curve was repeated for five times and standard deviation (SD) of the intercept was calculated. [Table No:11,12]

Table No.11: LOD and LOQ of Valsartan

Parameter	Measured value (µg/mL)
Limit of detection	0.55 (µg/mL)
Limit of quantification	1.68 (µg/mL)

Table No: 12: LOD and LOQ of Hydrochlorothiazide

Parameter	Measured value (µg/mL)
Limit of detection	3.04 (µg/mL)
Limit of quantification	4.93 (µg/mL)

Rougudness

Table No: 13 Rougudness of Valsartan

Sr No.	Conc	Analyst 1	Analyst II	Mean	Amt Found	% Amt Fnd	SD	% RSD
1	64	3369.9	3309.81	3339.86	20.73	100.02	42.49	1.27

Table No: 14 Rougudness of Hydrochlorothiazide

Sr No.	Conc	Analyst 1	Analyst II	Mean	Amt Found	% Amt Fnd	SD	% RSD
1	10	755.85	742.58	749.22	24.85	100.50	9.38	1.25

Analysis of Marketed Formulation by Proposed Method

Procedure

Weigh 20Tablets of VAL and HCTZ combination and calculated the average weigh, accurately& weigh the sample equivalent to 0.148 mg VAL and HCTZ transfer in 10 ml

volumetric flask.Add about 8ml of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and The solution was filtered through Whatman filter paper no. 41. Further pipette 0.1ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents.(10 µg/ml). The simple chromatogram of test VAL and HCTZ Shown in **(Fig No:9)** The amounts of Valsartan & Hydrochlorothiazide per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for %Lable claim for %RSD Calculated, Result was shown in **(Table No. 15.)**

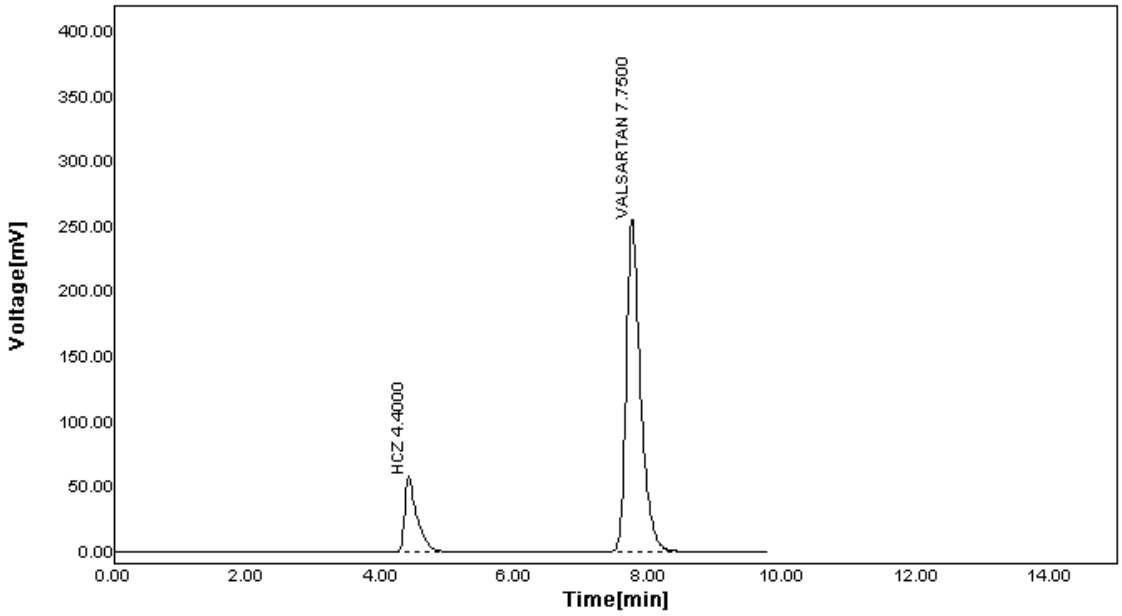


Fig No.9: Chromatogram for Marketed Formulation

Analysis of marketed formulation for %Lable claim

Table No.15: Tablet for %Lable claim

Sample	Label claimed	%Label claimed± SD	%RSD
VALENT-H	Valsartan=80mg Hydrochlorothiazide=12.5mg	100.02 ± 0.64 100.50 ± 0.35	0.61 0.37

Table no.16 Analytical Method Validation parameter

Sr No	Parameters	Valsartan	Hydrochlotothiazide
1	Range	32-160 µg/ml	5-25 µg/ml
2	Detection of Wavelength	248 nm	268 nm
3	Mean of R^2 Variation	0.999	0.999
4	Slope	46.62	53.62
5	Intercept	340.9	203.3

6	Retention Time	4.383 min	7.550 min
7	Run Time	15 min	15 min
8	Theroretical Plates	267.6	8611.6
9	Tiling Factor	1.9337	1.1771
10	%Recovery±RSD	98.93±0.56	101.18±0.35
11	Precision(Intraday) ±SD (Interday) ±SD	3392.08±8.79 4806.18±34.84	1009.4±4.59 1011.7±4.80
12	Repetability	100.6	98.89
13	Robustness(SD±RSD%)	19.22±0.39	63.05±0.61
14	Limit of Detection	0.55	3.04
15	Limit of Quantitation	1.68	4.93
16	Raggudness(mean±%RSD)	3339.86± 1.27	749.22± 1.25

RESULTS AND DISCUSSION

Analytical method validation parameter in HPLC method the two wavelengths 246nm and 268 nm were selected for estimation of Valsartan and Hydrochlorothiazide.The linearity was determined in coefficient regression value was found to be (r^2 0.999). precision was studied as repeatability (% RSD < 2) and inter and intra-day variations (%RSD < 2) for both drugs. The accuracy of method was determined by calculating mean percentage recovery. It was determined at 80%, 100% and 120 % level. & The ruggedness of the methods was studied by two different analysts using the same operational and environmental conditions. The % recovery, repeatability data, robustness, LOD,LOQ,& System suitability parameter data were presented were also satisfactory concluded that result shown in [**Table-16].**

CONCLUSION

The developed method was successfully applied for simultaneous estimation of Valsartan and hydrochlorothiazide in compound tablet formulation. The proposed method was found to be, rapid, accurate and precise. The method was free from interferences due to excipients present in formulation. Therefore, this method may be useful for routine analysis of Valsartan and hydrochlorothiazide in bulk drugs and combined Tablet dosage forms.

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