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DEVELOPMENT AND VALIDATION OF BIOANALYTICAL RP-HPLC METHOD FOR DETERMINATION OF VALSARTAN AND DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF VALSARTAN IN BULK DRUG AND FORMULATION

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ABSTRACT

New bioanalytical method developed for determination of valsartan is highly accurate, sensitive, precise, robust. This analytical method developed can also be applied for determination of valsartan in bulk drug and in formulation also. Validation is performed as per ICH guidelines. A new method was established for estimation of Valsartan by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Valsartan by using Kromosil C_{18} 4.5×150 mm 5.0 µm, flow rate was 0.8ml/min, and mobile phase ratio was 75:25% v/v ACE: di-potassium hydrogen phosphate, detection wavelength was 247 nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 6.323 mins. The % purity of Valsartan was found to be 99.87%. The system suitability parameters for Valsartan such as theoretical plates and tailing factor were found to be 4146, 1.23. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Valsartan was found in concentration range of 30µg-150µg and correlation coefficient (r^2) was found to be 0.997, % recovery was found to be 100.4%, % RSD for repeatability was 0.5, % RSD for intermediate precision was 1.0. The precision study was precision, robustness and repeatability. LOD value was 2.97 and LOQ value was 9.92. Hence the suggested RP-HPLC method can be used for routine analysis of Valsartan in API and Pharmaceutical dosage form.

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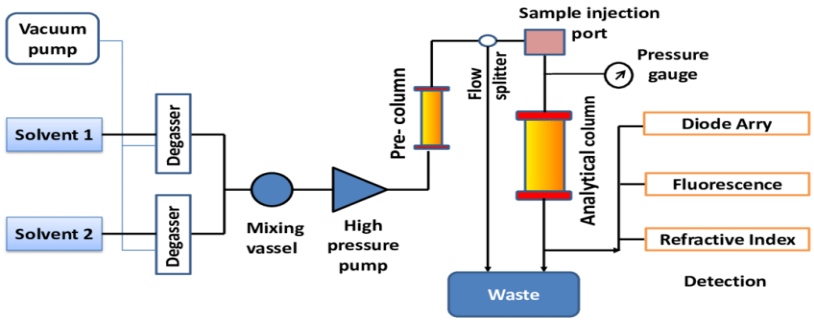
INTRODUCTION

The aim of present study is to develop and validate simple, precise, accurate RP-HPLC bioanalytical method for determination of valsartan drug in plasma. Apart from this that developed method should also be used for determination of valsartan in API and pharmaceutical formulations also. Simple, precise, accurate RP-HPLC bioanalytical method for determination of valsartan drug in plasma is so far not available which is applicable for all above mentioned reasons and moreover developed method can also be used for stability study of drugs also.[1-7] Chromatographic methods of ion separation have become particularly important in recent years. As is well known , chromatographic analysis was first introduced into science in 1903 by the eminent Russian botanist M. Tsvet.[8]



Fig,No.1: Schematic diagram of HPLC.

Instrumentation

Injection of the sample:

Septum injectors are available; using which sample solution is injected. Sample can be injected when the mobile phase is flowing or it is stopped. A new advanced rotary valve and loop injector can be used to produce reproducible results.

The detector:

There are several ways of detecting when a substance has passed through the column. Generally UV spectroscopy is attached, which detect the specific compounds. Many organic compounds absorb UV light of various wavelengths. The amount of light absorbed will depend on the amount of a particular compound that is passing through the beam at the time.

Interpreting the output from the detector:

The output is recorded as a series of peaks, each one representing a compound in the mixture passing through the detector and absorbing UV light. The area under the peak is proportional to the amount of substance, which is passed through detector, and this area can be calculated automatically by the computer linked to the display.

Experimental Work

MATERIALS AND METHODS

Chemicals and standards used

Table.No.1 List of chemicals and standards used.

S.No	Chemicals	Manufacturer Name	Grade
1.	Water	Merck	HPLC grade
2.	di-potassium hydrogen phosphate	Merck	HPLC grade
3.	Acetonitrile	Merck	HPLC grade
4.	Ortho phosphoric acid	Merck	G.R
5.	KH_2PO_4	Merck	G.R
6.	K_2HPO_4	Merck	G.R
7.	0. 22µ Nylon filter	Advanced lab	HPLC grade
8.	0.45µ filter paper	Millipore	HPLC grade
9.	Valsartan	In – House	In- House

Instruments used

Table. No.2. List of instruments used.

S.No	Instrument name	Model number	Soft ware	Manufacturers Name
1	HPLC-auto sampler –UV detector	Separation module 2695, PDA detector	Empower-software version-2	Waters
2	U.V double beam spectrometer	UV 3000+	U.V win soft ware	Lab India
3	Digital weighing balance(sensitivity 5mg)	ER 200A	-	Ascoset
4	pH meter	AD 102U	-	ADWA
5	Sonicator	SE60US	-	Enertech

Method development for the estimation of Valsartan by using RP-HPLC.

1. Selection of mobile phase
2. Selection of detection wavelength
3. Selection of column
4. Selection of solvent delivery system
5. Selection of flow rate
6. Selection of column temperature
7. Selection of diluent
8. Selection of test concentration and injection volume

Selection of mobile phase

ACE: di-potassium hydrogen phosphate (75:25)

Selection of wavelength:

10 mg of was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained .The overlay spectrum was used for selection of wavelength for Valsartan .

Selection of column :

Heart of HPLC made of 316 grade stainless steel packed with stationary phase.
Silica based columns with different cross linking's in the increasing order of polarity are as follows:

←------- Non-polar----------moderately polar--------Polar----------→

C_{18}< C_8< C_6< Phenyl < Amino <Cyano< Silica

- In reverse phase chromatography, hydrophobic interaction between drug molecule and the alkyl chains on the column packing material.
- Column is selected based on solubility, polarity and chemical differences among analytes and Column selected: i.e. Zodiac silC18 column 150×4.6 mm 5.0 µm.
- Reasons : Better separation,

Selection of solvent delivery system

- Always preferable solvent delivery system.
- More chance of getting reproducible result on retention time of analytes.
- More economic than gradient technique.

Selection of flow rate

Acceptable limit: - Not more than 2.5 ml/min

- Flow rate selected was 1 ml/min
- Flow rate is selected based on

1. Retention time
2. Column back pressure
3. Peak symmetry.
4. Separation of impurities.

Reasons:

❖For earlier elution of analyte and elution of all impurities within 6.0 min.
❖Information from the reference method in literature.

Selection of diluent

- Selection of diluent is based on the solubility of the analyte
- Diluent selected: ACE : di-potassium hydrogen phosphate (75:25)

Reason:

- Analyte is soluble in ACE.

Selection of column temperature

- Preferable temperature is ambient or room temperature.

Reasons:

❖To elute all impurities along with analyte with in 6.0 min of run time.
❖Less retention time
❖Good peak shape
❖Higher theoretical plates.
❖Good resolution.

Selection of test concentration and injection volume

Test concentration is finalized after it is proved that API is completely extractable at the selected test concentration.

- Test concentration is fixed based upon the response of API peak at selected detector wavelength.
- Label claimed 150 mg.
- And the test concentration selected is 90 ppm.
- Injection volume selected was 20µL.

Reason: good peak area, retention time, peak symmetry.

Chromatographic trials for estimation of Valsartan by RP- HPLC.
Trial-1
Chromatographic conditions

Column	:	Nucleosil C18 4.6x150mm 5µm
Mobile phase ratio	:	Me OH: H_2O (20:80%v/v)
Detection wavelength	:	247 nm
Flow rate	:	1ml/min
Injection volume	:	20µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	4min

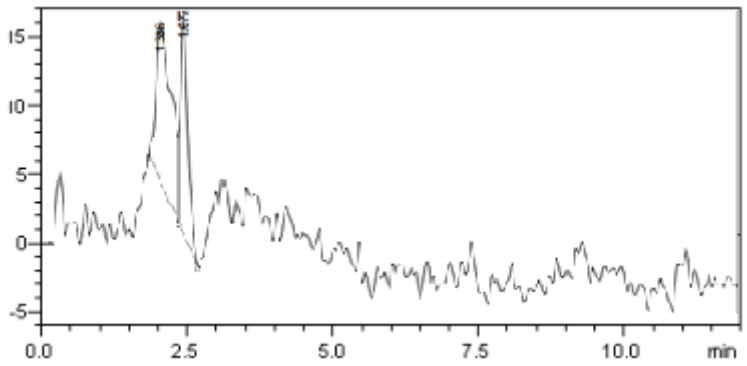


Fig.No.2. Chromatogram showing trial-1 injection.

Observation:

The trial shows no good peak separation, so more trials was required for obtaining peaks.

Trial - 2

Chromatographic conditions

Column	:	Zodiacsil C18 4.6x150mm 5µm
Mobile phase ratio	:	MEOH : H_2O (65:35%v/v)
Detection wavelength	:	247nm
Flow rate	:	1ml/min
Injection volume	:	20µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	7.0 min

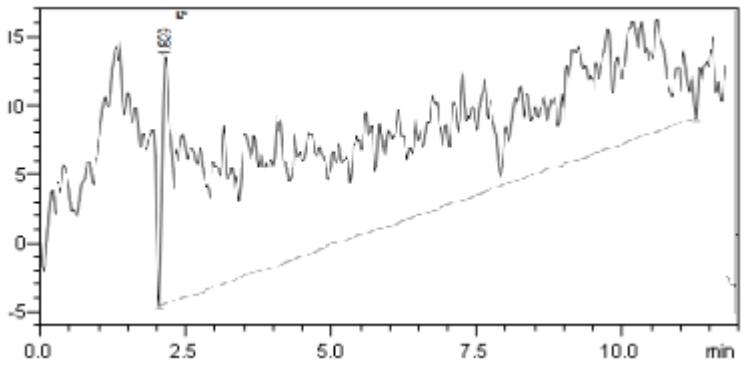


Fig.No.3. Chromatogram showing trial-2 injection.

Observation

In this trial no proper peak separation was observed, still more trials was required for good peaks.

Trial-3

Chromatographic conditions

Column	:	KROMOSIL RPC8 4.5×150mm 5.0 µm
Mobile phase ratio	:	MEOH: pH 4.5 buffer (70: 30% v/v)
Detection wavelength	:	247nm
Flow rate	:	1.0ml/min
Injection volume	:	20µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	9.0mins

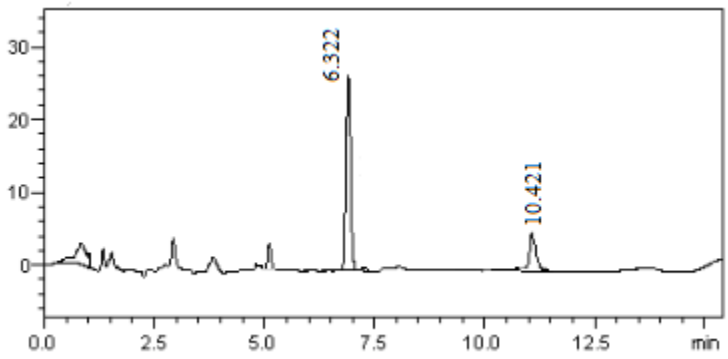


Fig.No.4. Chromatogram showing trial-3 injection.

Observation

In this trial Valsartan was eluted but there is no proper Tailing. Still more trials were required for better resolution in peaks

Trial-4 (optimized method)

Chromatographic conditions

Column	:	Kromosil RPC18 4.6×150mm 5µm
Mobile phase ratio	:	ACE: pH 4.5 di-potassium hydrogen phosphate (75:25% v/v)
Detection wavelength	:	247 nm
Flow rate	:	1.0ml/min
Injection volume	:	20µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	7 min

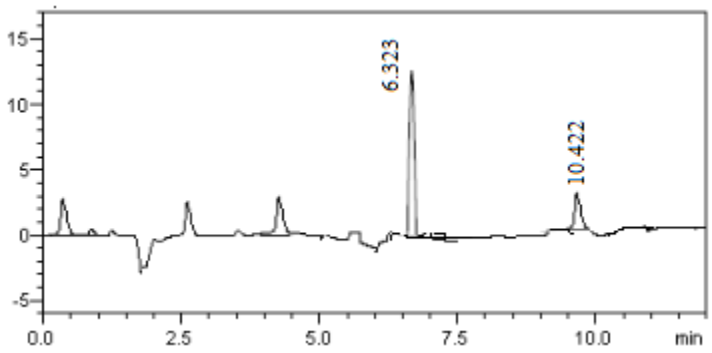


Fig.No.5. Chromatogram showing trial-4 injections.

Observation:

The separation was good; peak shape was good, still more trials were required to reduce the retention times of peaks final method

Procedure

Diluent preparation

Mobile phase was used as the diluent.

Preparation of mobile phase

A mixture of acetonitrile and 0.01M di-potassium hydrogen phosphate buffer in ratio of (75:25) and adjusted to pH 4.5 using o-phosphoric acid, filtered, degassed and used. 0.01M Di-potassium hydrogen phosphate buffer (pH 4.5) prepared in 100 ml volumetric flask, add 17.41 gm of dipotassium hydrogen phosphate and dissolve it in some of amount of HPLC grade water, and make up to volume with HPLC grade water. Adjust the pH 4.5 of resultant buffer by orthophosphoric acid, as required.

Preparation of the individual standard preparation

10 mg Amount of standard was mixed with 10 ml acetonitrile to +2ml of rat plasma(untreated) then vertically shaked for 30 min then centrifuged at 5000rpm for 1 hr .Then it was filterated using membrane filters to get clear organic solution .Then it was filled in to the sample vials of HPLC and loaded on to HPLC for Run.

Preparation of the Valsartan sample solution

Sample solution preparation

Blood samples are collected from the animals rats and then centrifuged at 5000rpm for 1 hr to separate the plasma from blood. Then the separated was mixed with acetonitrile then loaded on to the HPLC for Run.

System suitability

- Tailing factor for the peaks due to Valsartan in standard solution should not be more than 1.5.
- Theoretical plates for the Valsartan peaks in standard solution should not be less than 2000.

Assay calculation

$$Assay\ \% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where:

Avg.wt = average weight of tablets

P= Percentage purity of working standard

LC= Label Claim of Valsartan mg/ml

LC= Label Claim of Valsartan mg/ml

Analytical Method Validation [9]

Validation parameters

- ❖Specificity
- ❖Linearity
- ❖Range
- ❖Accuracy
- ❖Precision
- ➢ Repeatability
- ➢ Intermediate Precision
- ❖Detection Limit
- ❖Quantitation Limit

RESULTS AND DISCUSSIONS

The present investigation reported in the research work was aimed to develop a new method development and validation for the estimation of Valsartan by RP-HPLC method. Literature review reveals that there are no analytical methods reported for the estimation Valsartan by RP-HPLC method. Hence, it was felt that, there is a need of new analytical method development for the estimation of Valsartan in pharmaceutical dosage form.

METHOD DEVELOPMENT

The detection wavelength was selected by dissolving the drug in mobile phase(rat plasma) to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Valsartan was obtained and the point of Valsartan showed absorbance's maxima at 247 nm.

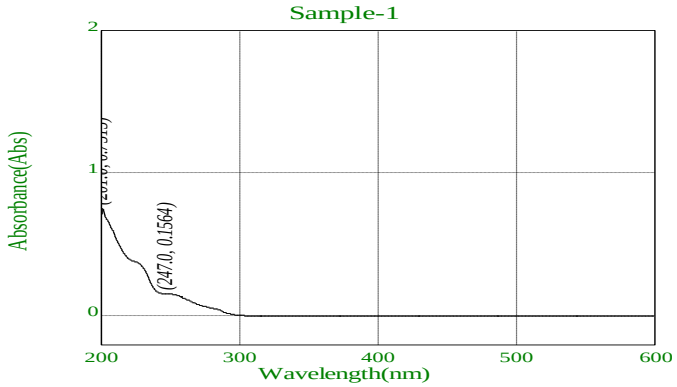
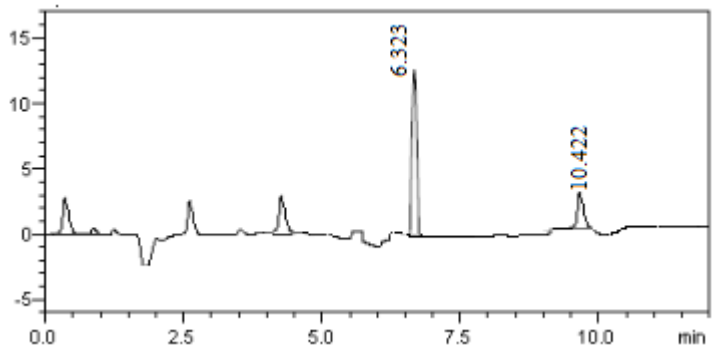


Fig.No.6. Spectrum showing wavelength Valsartan.

The chromatographic method development for the estimation of Valsartan was optimized by several trials for various parameters as different column, flow rate and mobile phase; finally the following chromatographic method was selected for the separation and quantification of Valsartan in API and pharmaceutical dosage form by RP-HPLC method.

Optimized chromatographic conditions for simultaneous estimations of Valsartan by RP-HPLC method



Column	:	Kromosil RPC18 4.6×150mm 5µm
Mobile phase ratio	:	ACE: pH 4.5 di-potassium hydrogen phosphate (75:25% v/v)
Detection wavelength	:	247 nm
Flow rate	:	1.0ml/min
Injection volume	:	20µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	7 min
Retention time	:	6.323 mints

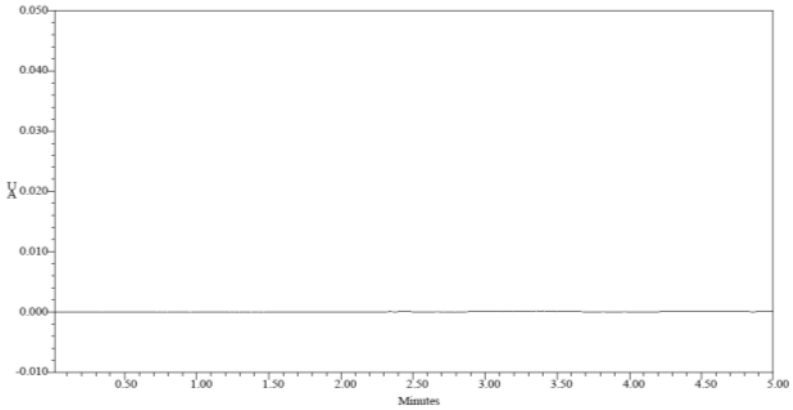
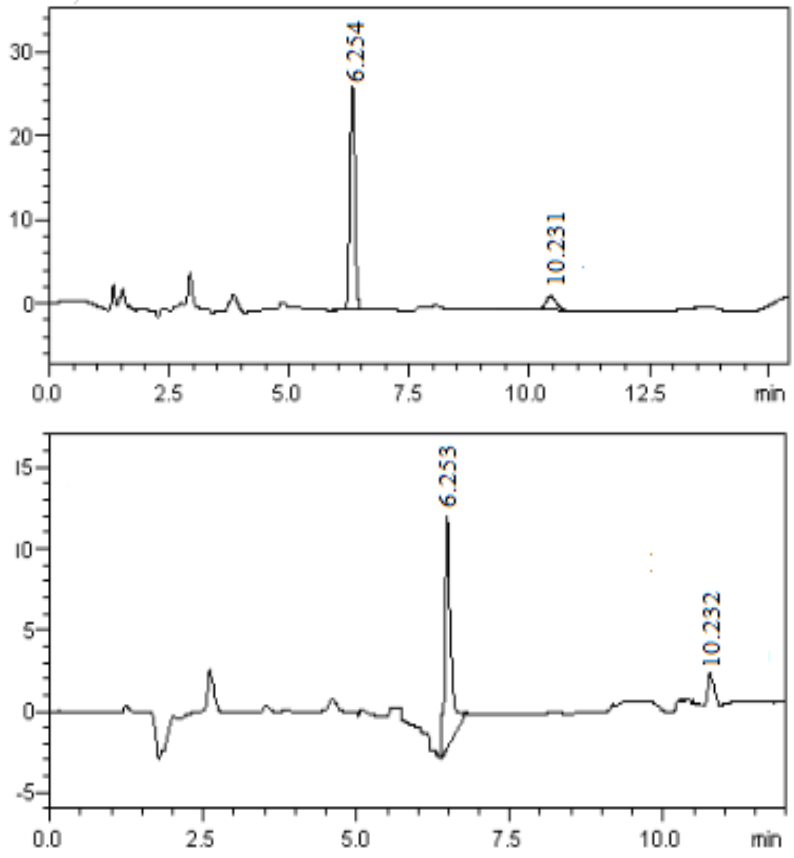


Fig.No.7 Chromatogram showing blank preparation (mobile phase).



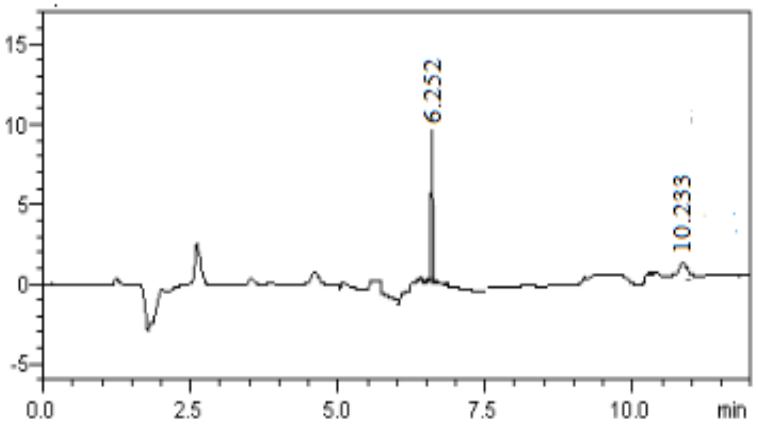


Fig.No.8 Chromatogram showing Valsartan standard.

Table.No.3 Retention time and area data for Valsartan by RP-HPLC method.

	NAME	RT	AREA
1	Valsartan	6.254	698557
2	Valsartan	6.253	699824
3	Valsartan	6.252	700069
Mean			699483
Std.Dev.			811.1
%RSD			0.12

Assay calculation for Valsartan:

The assay study was performed for the Valsartan. Each three injections of sample and standard were injected into chromatographic system. Results are tabulated in Table.

Table.No.4 Assay data for Valsartan by RP-HPLC method.

	NAME	RT	AREA	USP Plate Count	USP Taililng
1	Valsartan	6.262	695226	4159.0	1.5
2	Valsartan	6.263	694341	4059.3	1.4
3	Valsartan	6.264	694434	4059.3	1.5
Mean			694667	4092.6	
Std.Dev.			486.6		
%RSD			0.07		

Validation Report

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. The chromatograms are shown in Fig.

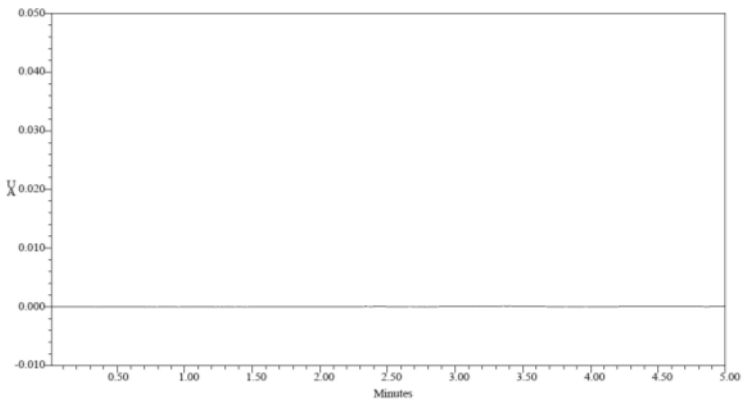


Fig. No.9 Chromatogram showing blank (mobile phase preparation).

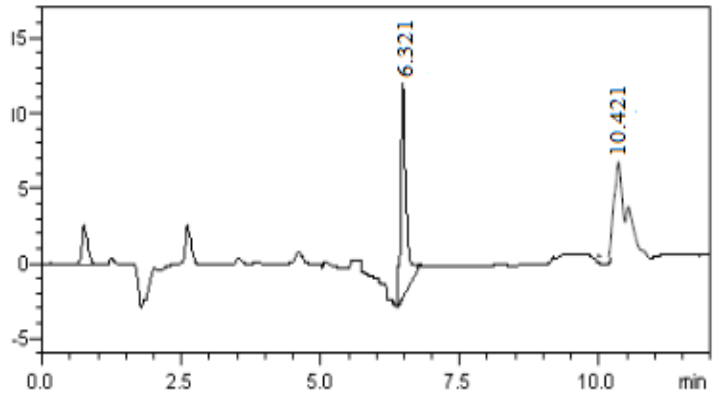


Fig. No.10 chromatogram showing standard injection.

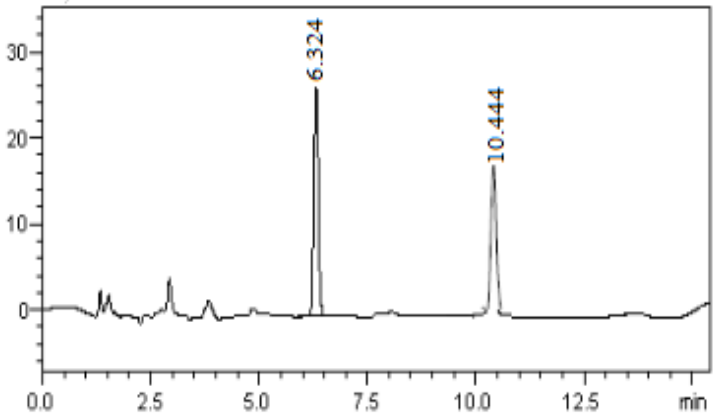


Fig. No.11 chromatogram showing Sample injection.

The specificity test was performed for Valsartan. It was found that there was no interference of impurities in retention time of analytical peak.

Linearity

The linearity study was performed for the concentration of 30 ppm to 150 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The chromatograms are shown in Fig. and results are tabulated in Table. Calibration graph for Valsartan shown in Fig.

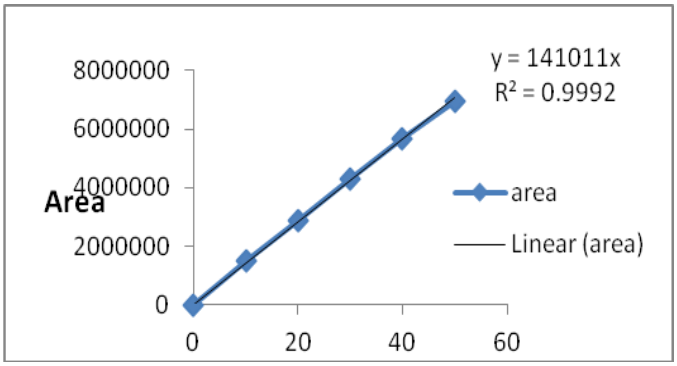


Fig. No.12 Plotting of calibration graph for Valsartan.

Table.No.5 showing the results for the linearity:

Name	Rt	Area
Valsartan	6.341	1508152
Valsartan	6.342	2892905
Valsartan	6.343	4278327
Valsartan	6.344	5670038
Valsartan	6.345	6949400
Co efficient of correlation (R^2)		0.999

Valsartan r2 = 0.999

The linearity study was performed for concentration range of 30µg/ml-150 µg/ml of Valsartan and the correlation coefficient was found to be 0.999 (NLT 0.99).

Accuracy

The accuracy study was performed for 50%, 100% and 150 % for Valsartan. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of recovery. Chromatograms are shown in Fig. and results are tabulated in Table.

Table.No.6 Table showing results for accuracy 50%:

	PEAK NAME	RT	AREA	HEIGHT(µV)
1	Valsartan	6.352	1046104	158415.9
2	Valsartan	6.353	1049450	157800.7
3	Valsartan	6.354	1049306	157865.4
Mean			1048287	
Std.Dev.			1891.6	
%RSD			0.18	

Accuracy -100%

Table.No.7showing results for accuracy 100%:

NAME: Valsartan

	NAME	RT	AREA
1	Valsartan	6.354	1376694
2	Valsartan	6.355	1377029
3	Valsartan	6.356	1380876
Mean			1378200
Std.Dev.			2324.1
%RSD			0.17

Accuracy 150%.

Table.No.8 showing results for accuracy 150%:

NAME: Valsartan

		RT	AREA	HEIGHT (µV)
1	Valsartan	6.467	1714604	189510.2
2	Valsartan	6.468	1714196	189758.4
3	Valsartan	6.469	1717641	189550.4
Mean			1715480	
Std.Dev.			1882.1	
%RSD			0.11	

Table.No.9 Showing accuracy results for Valsartan.

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1048287	5	5.14	100.2%	
100%	1378200	10	10.01	98.8%	100.4%
150%	1715480	15	15.2	96.5%	

The accuracy study was performed for % recovery of Valsartan. The % recovery was found to be 100.4% (NLT 98% and NMT 102%).

Precision

❖Repeatability
❖Intermediate Precision

Repeatability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision/Ruggedness

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Repeatability

The precision study was performed for five injections of Valsartan. Each standard injection was injected in to chromatographic system. The area of each Standard injection was used for calculation of % RSD. The chromatograms are shown in Fig. and results are tabulated in Table.

Table.No.10 Table showing the results for precision:

NAME: Valsartan

	NAME	RT	AREA
1	Valsartan	6.453	693078
2	Valsartan	6.454	693338
3	Valsartan	6.455	695080
4	Valsartan	6.456	694843
5	Valsartan	6.457	695336
Mean			694335
Std.Dev.			1047.5
%RSD			0.15

The Method precision study was performed for the %RSD of Valsartan was found to be 0.5 and (NMT 2).

Ruggedness (intermediate precision):

Table.No.11 showing the results for intermediate precision:

NAME: Valsartan

	NAME	RT	AREA
1	Valsartan	6.458	693877
2	Valsartan	6.459	696531
3	Valsartan	6.475	693977
4	Valsartan	6.476	695278
5	Valsartan	6.477	697676
Mean			695468
Std.Dev.			1642.7
%RSD			0.24

The Method precision study was performed for the %RSD of Valsartan was found to be 0.3 and (NMT 2)

Detection limit

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$\mathbf{LOD = 3.3\ X\ \frac{\sigma}{S}}$$

Where

σ - Standard deviation (SD)
S - Slope

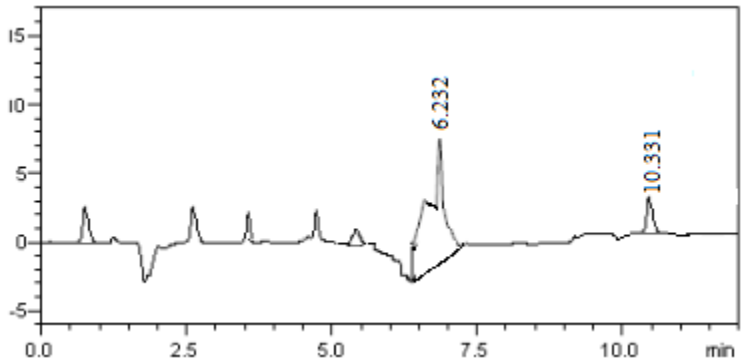


Fig. No.13. Chromatogram showing Limit of detection.

Table.No.12 Showing results for Limit of Detection.

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
VALSARTAN	371827.90	563365963	2.97

The LOD was performed for Valsartan was found to be 2.97.

Quantitation limit

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$LOQ = 10 \text{ X } \frac{\sigma}{S}$$

Where

σ - Standard deviation

S - Slope

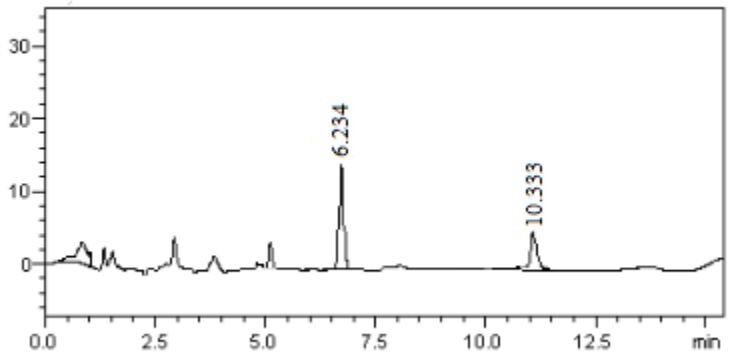


Fig. No.14 Chromatogram showing Limit of Quantitation.

Table.No.13 Showing results for Limit of Quantitation.

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Valsartan	371827.90	563365963	9.92

The LOQ was performed for Valsartan was found to be 9.92.

Robustness

The robustness was performed for the flow rate variations from 0.8ml/min to 1.2 ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Valsartan. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.The chromatograms are shown in Fig and results are tabulated in Table.

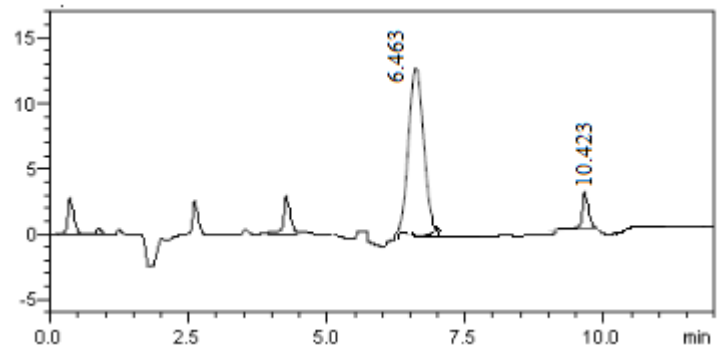


Fig. No.15 Chromatogram showing less flow rate 0.8 ml/min.

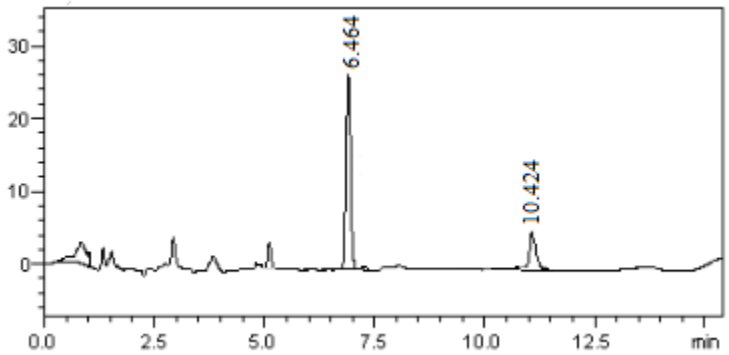


Fig. No.16 Chromatogram showing more flow rate 1.2 ml/min.

The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

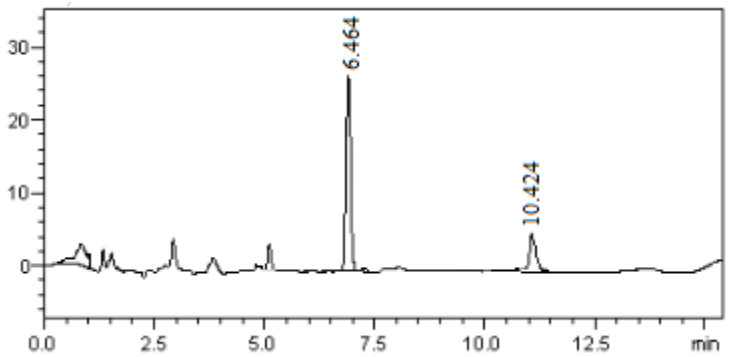


Fig. No.16 Chromatogram showing more flow rate 1.2 ml/min.

The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

Table.No.14 Showing system suitability results for Valsartan.

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	SP Tailing
1	0.8	4352	1.1
2	**1**	**4024**	**1.2**
3	1.2	3730	1.2

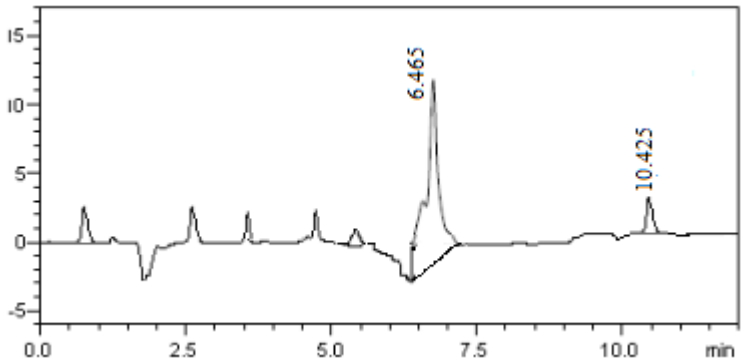


Fig. No.17 Chromatogram showing more organic phase ratio.

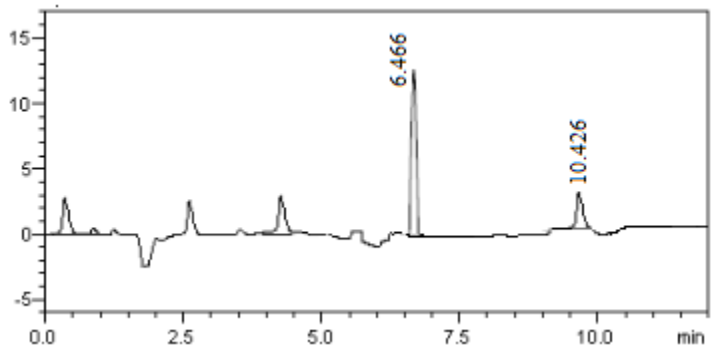


Fig. No.18 Chromatogram showing less organic phase ratio.

On evaluation of the above results, it can be concluded that the variation in±5%. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the mobile phase ±5%.

Table.No.15 Showing system suitability results for Valsartan.

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	10 % less	4331	1.20
2	***Actual**	**4024**	**0.87**
3	10% more	3693	1.26

Forced degradation studies

Forced degradation of Test sample was performed under acidic, alkaline, heat, photolytic and oxidative stress conditions.

Stock solution preparation:

Twenty Tablets were weighed and powdered. Tablet powder having weight equivalent to 20 mg of was weighed accurately and taken in a 10 mL volumetric flask. To it 5 mL of the mobile phase was added and sonicated for 15 minutes to dissolve the drug. The volume was made up to 10 mL with mobile phase. The resulting solution was then filtered through a 0.45 µm membrane filter to prepare a stock solution of the tablet sample. Further dilution was done by diluting 0.1 mL of stock solution to 10 mL mobile phase. The concentration of Valsartan in the solution was 10 µg/mL, respectively.

Acid hydrolysis

Forced degradation in acidic media was performed by adding 2 mL 0.1 M HCl to 10 mL of stock solution and the mixture is heated at 60°C for approximately 26hrs and the solution is neutralized by addition of 0.1 M NaOH. The prepared solution is injected and chromatograms were recorded.

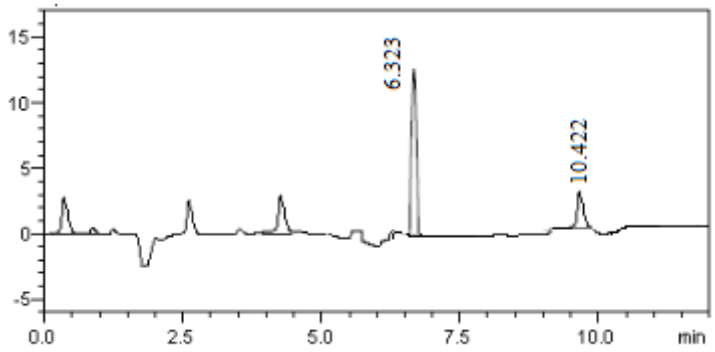


Fig. No.19 Chromatogram of Acid degradation sample.

Observation:

The study indicates that the drugs under study were degraded and assay results shows that mostly Valsartan (8.6%) was degraded following by the drug were well separated from the degradation products.

Alkaline hydrolysis

Forced degradation in basic media was performed by adding 2 mL 0.1 M NaOH to 10 mL of stock solution and the mixture is heated at 60°C for approximately 26hrs and the solution is neutralized by addition of 0.1 M HCl. The prepared solution is injected and chromatograms were recorded.

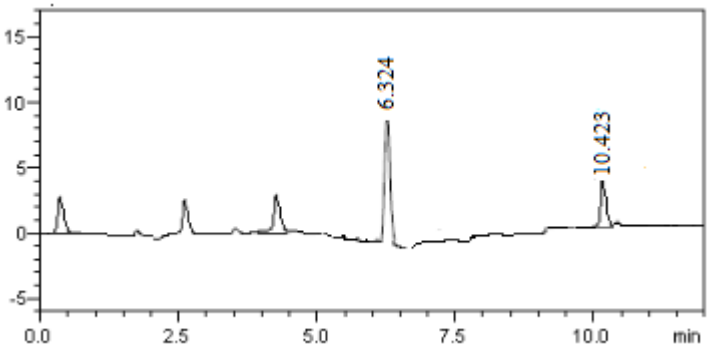


Fig. No.20 Chromatogram of Alkali degradation sample.

Observation:

The results of alkaline hydrolysis indicates that some degradation occurred and assay results shows that Valsartan was degraded by 13.7% was degraded and the drug were well separated from the degradation products.

Oxidative Degradation

To study the effect of oxidizing conditions, an aliquot of stock solution was added to 1 mL 30 % H_2O_2 solution. The prepared solution is injected and chromatograms were recorded.

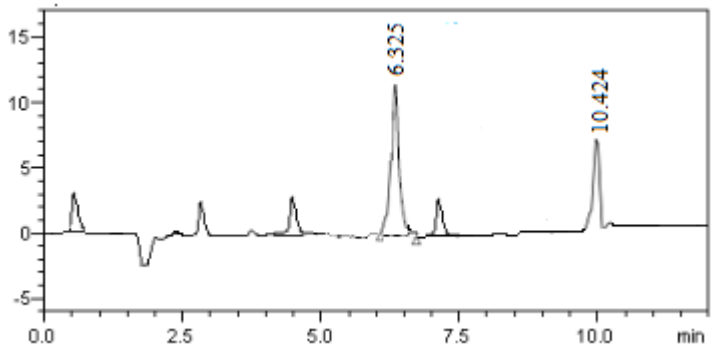


Fig. No.21 Chromatogram of Oxidative degradation sample.

Observation:

From the observation it was found that the Valsartan were found stable i.e, no significant peaks were found. So it is stable in the above condition.

Thermal Degradation

To study the effect of temperature an aliquot of stock solution was kept at 70^0C for 26 hrs. Resulting solution was injected into HPLC and chromatograms were recorded.

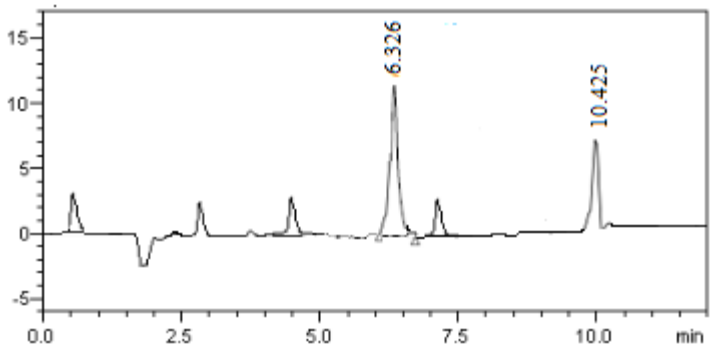


Fig. No.22 Chromatogram of Thermal degradation sample.

Observation:

From the observation it was found that both Valsartan were found to be degraded by 13.4% respectively.

Photolysis

To study the effect of photolysis, an aliquot of stock solution was exposed to UV light for 4hrs.
Resulting solution was injected into HPLC and chromatograms were recorded.

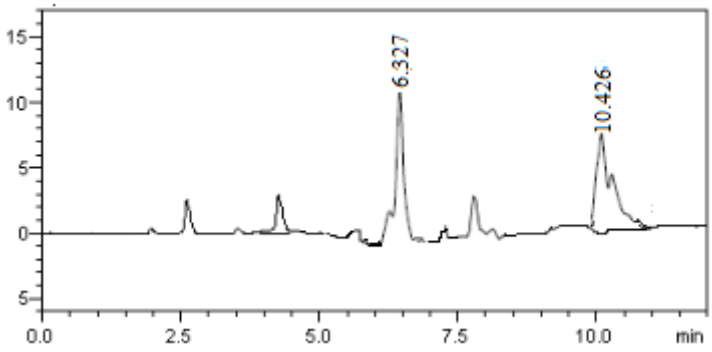


Fig. No.23 Chromatogram of Photolytic degradation of sample.

Observation:

From the observation it was found that the Valsartan were found stable i.e, no significant peaks were found. So it is stable in the above condition.

Summary on degradation studies

Table. No. 16 Results of Stress degradation studies.

Stress Condition	Sample-1 (Valsartan)		
	Area	%Assay	%Degradation
Acidic	110473	91.7	8.7
Alkaline	124364	92.0	12.8
Photolytic	113269	87.5	13.7
Thermal	101474	96.3	14.5
Oxidative	106734	94.3	11.2

SUMMARY AND CONCLUSION

For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of plasma samples in a short time period with good robustness, accuracy and precision without any prior separation step. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. A new method was established for estimation of Valsartan by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Valsartan by using Kromosil C_{18} 4.5×150 mm 5.0 µm, flow rate was 0.8ml/min, and mobile phase ratio was 75:25% v/v ACE: di-potassium hydrogen phosphate, detection wavelength was 247 nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 6.323 mins. The % purity of Valsartan was found to be 99.87%. The system suitability parameters for Valsartan such as theoretical plates and tailing factor were found to be 4146, 1.23. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Valsartan was found in concentration range of 30µg-150µg and correlation coefficient (r^2) was found to be 0.997, % recovery was found to be 100.4%, %RSD for repeatability was 0.5, % RSD for intermediate precision was 1.0. The precision study was precision, robustness and repeatabilty.LOD value was 2.97 and LOQ value was 9.92.Hence the suggested RP-HPLC method can be used for routine analysis of Valsartan in API and Pharmaceutical dosage form.

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