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Stability Indicating RP-HPLC Method for the Estimation of Ivabradine in Tablet Dosage Form

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Pushpani Parve[1*], Ramesh Jadhav[2], Vijay Kumar M.[2], Sayali Warde[2], R. Mohan Singh[2], Smita Nayak[1], Vaidhun Bhaskar[1]

[1]Department of Quality Assurance, Gahlot Institute of Pharmacy, Plot no. 59, Sector-14, Koparkhairne, Navi Mumbai – 400709, Maharashtra, India.

[2]Analytical Research and Development, Central Drug Testing Laboratory, Zonal FDA Bhavan, GMSD Compound, Belasis Road, Mumbai Central, Mumbai – 400008, Maharashtra, India.

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Keywords: Ivabradine, RP-HPLC, Method Development, Validation, Stress Degradation Studies.

ABSTRACT

Objective: - The principal objective of this study was to develop and validate a new, simple, accurate, fast, precise, and reproducible Reverse Phase High- Performance Liquid Chromatography (RP-HPLC) for the estimation of Ivabradine in the tablet dosage form. **Method: -** The chromatographic separation was performed on HemochromIntsil C_{18} Column (250 x 4.6 mm; 5µm) via isocratic elution with a mobile phase comprising of 25mM potassium phosphate buffer containing 0.2% TEA(pH adjusted to 3.0 with orthophosphoric acid) and acetonitrile in the ratio of (30:70%v/v). The flow rate was kept at 0.6ml/min. The mobile phase was used as a diluent and detection was carried out at 287nm. **Results: -** The retention time of ivabradine was found to be about 5.2 min. Validation parameters such as specificity, linearity, precision, accuracy, recovery, and robustness were done according to ICH Q2 (R1) guidelines. The method was linear over the concentration range of 5-30 µg/ml with a regression coefficient of 0.99995. The percentage RSD of every parameter was found to be within the limit. The stress degradation studies were executed to give degradation products by exposing the drugs to hydrolytic, photolytic, oxidative, acid, alkali, and thermal degradation conditions. The acquired data showed that the degradation product successfully separated without any intrusion, which establishes the stability-indicating nature of a developed method. **Conclusion: -** Accurate, simple, precise, economic, reliable, and robust RP-HPLC method has been successfully developed and validated. The developed method can be applied for routine quality control analysis of Ivabradine in the tablet dosage form.

INTRODUCTION:

Ivabradine is a pure heart rate-controlling agent, acting by selective and specific inhibition of the cardiac pacemaker funny channel inhibitor (I_f) current that controls the spontaneous diastolic depolarization of the sinus node and regulates heart rate.[1] I_f the current is responsible for the automatic depolarization of the sinus node cell and is the result of an inward movement of sodium and potassium. By inhibiting the I_f current, ivabradine allows heart rate control without the deleterious consequences on the force of contraction, peripheral circulation, bronchial tone, bowel transit, and glucose and triglyceride metabolism associated with other anti-anginal agents.[2] Four HCN channels are known, labeled from 1 to 4. HCN4 is the most widespread isoform in the SA node which is responsible for diastolic depolarization.[6-8]

Ivabradine (CAS 148849-67-6) is an orally bioavailable, hyperpolarization-activated, cyclic nucleotide-gated channel inhibitor.[3]

On April 15, 2015 -- The U.S. Food and Drug Administration, approved Corlanor (ivabradine) to reduce hospitalization from worsening heart failure. Corlanor is approved for use in certain people who have long-lasting (chronic) heart failure caused by the lower-left part of their heart not contracting well.[4] The chemical name of Ivabradine is 3-[3-({[7S)-3,4-dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7yl]methyl}methylamino)propyl]-1,3,4,5-tetrahydro-7,8-dimethoxy-2H-3-benzazepine-2-one hydrochloride with its empirical formula is $C_{27}H_{37}ClN_2O_5$ and molecular weight is 505.0.[5]

A thorough literature survey revealed that Ivabradine is not official in any pharmacopeia. Few articles were reported but restricted to Assay only while other articles have been carried out in forced degradation studies.[5] Three articles have reported the stability-indicating methods and in one article the System suitability parameter have not mentioned[12] and in another article, the RT was found to be at 3.0 min, and at this RT the degradation products may get merged with the main peak.[13] Hence, trials were made to develop new quantitative stability-indicating the RP-HPLC method for the detection of Ivabradine in the pharmaceutical tablet dosage form.

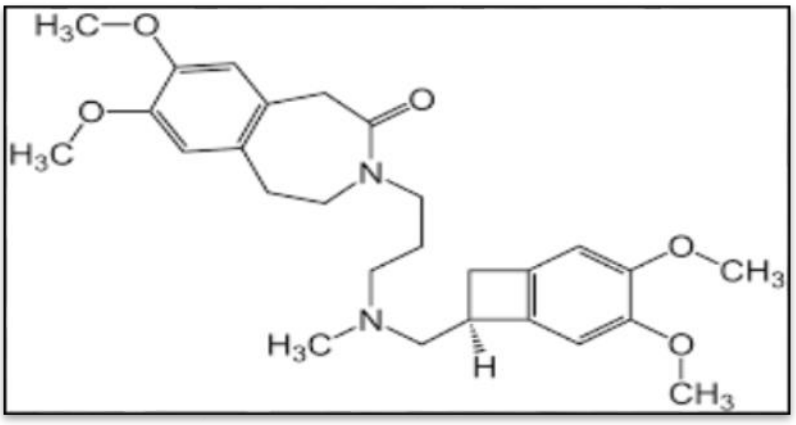

Figure No.1: Chemical structure of Ivabradine

MATERIALS AND METHODS:

Chemicals and Reagents:

An analytically pure Ivabradine reference standard was procured from Central Drugs Testing Laboratory, Mumbai with defined potency [99.52% as its basis]. IVAMAC®5 (5mg) Ivabradine Tablets were procured from the local market. Triethylamine (AR grade) from Rankem, Acetonitrile (HPLC grade) from Rankem, and Orthophosphoric acid from Molychem were used. Ultra-purified HPLC grade distilled water obtained from the Milli-Q® system (Millipore, Milford, MA, USA) water purification unit was used to prepare all the required solutions.

Instrumentation:

Perkin Elmer UV/VIS spectrophotometer lambda 45 equipped with software Perkin Elmer UV win lab was used for all the spectrophotometric measurements. The chromatography was performed on Thermo Scientific Ultimate 3000 HPLC system using chromeleon 7.2.6 software and other instruments like analytical weighing balance, pH meter, and ultrasonicator were used during method development. HemochromIntsil C18 (250 × 4.6mm, 5µm) column was used for the separation.

Chromatographic Conditions:

The chromatographic separation was achieved on HemochromIntsil C18 (250 × 4.6mm, 5µm) column at ambient temperature using a mobile phase comprising of 25mM potassium phosphate buffer containing 0.2% TEA(pH adjusted to 3.0 with orthophosphoric acid) and

acetonitrile in the ratio of (30:70% v/v) at flow rate 0.6ml/min. The run time was 10 min. The detection of the analyte was carried out at 287nm and the injection volume was 20µl. The retention time is about 5.1 min.

Determination of wavelength:

The reference standard solution (10 ppm) of Ivabradine was prepared and scanned in the range of 400 to 200 nm against the mobile phase as a blank. Ivabradine showed maximum absorbance at 287 nm as shown in **Fig. 2**and the same wavelength was selected for the analysis of Ivabradine.

Preparation of Mobile Phase:

The mobile phase comprising of 25mM potassium phosphate buffer containing 0.2% TEA(pH adjusted to 3.0 with orthophosphoric acid) and acetonitrile in the ratio of (30:70% v/v) was prepared and filtered through a 0.45µm membrane filter.

Preparation of Standard solution:

Accurately weighed about 10 mg of Ivabradine reference standard was transferred to a 100ml volumetric flask, dissolved in the mobile phase and volume adjusted up to the mark and mixed well and further dilutions were made to get the concentration of (10 µg/ml).

Analysis of Marketed Formulation:

20 tablets of IVAMAC®(5mg) were accurately weighed and the average weight was determined. Tablets were crushed to a fine powder. The powder equivalent to 10mg of Ivabradine was taken in a 100ml volumetric flask and added to about 50ml of mobile phase and kept in a sonicator for 5min and shake well, diluted up to the mark with mobile phase, and mixed well. Filtered using a syringe filter (0.45µm). Further dilutions were made to get the concentration of (10 µg/ml).

Method Optimization:

Chemical structure and solubility data showed that Ivabradine is weakly basic with a pKa value of 9.37 andis a non-polar drug. Considering the chemical nature of the molecule, initial trials were started with phosphate buffer and Acetonitrile of different proportions with different makes C18 columns. A HemochromIntsil column was found to be suitable when

compared to other C18 columns for the retention of the drug. Good peak shape and better SST parameters were found with a mobile phase comprising of25mM KH_2PO_4buffer containing 0.2% TEA (pH adjusted 3.0 with orthophosphoric acid) and acetonitrile in the ratio of (30:70% v/v). The flow rate was kept at 0.6 ml/min and UV detection wavelength of 287 nm and column oven temperature was maintained at 30˚C. The data are summarized in **Table. 1** and chromatograms are shown in **Fig. 3 and 4.**

Method Validation:

The method was validated for parameters such as specificity, linearity, precision, accuracy and recovery, and robustness as per ICH Q2 (R1) guidelines.[11]

Specificity:

The specificity of the method was carried out by forced degradation studies under different conditions like acidic, alkaline, hydrolytic, thermal, oxidative, and photolytic conditions.

Linearity:

The linearity of the method was determined by appropriate aliquots from reference standard ivabradine stock solutions prepared to obtain concentrations of 5-30 µg/ml. The linear calibration plot was constructed by analyzing the concentrations over the selected range versus the peak area. The peak areas were noted by injecting each level into the chromatographic system. The response to the drug was linear in the concentration range between 5-30 µg/ml. The linearity was observed in the expected concentration range, demonstrating its suitability for analysis shown in **Table. 2** and **Fig. 6** [10].

System Precision:

This was performed by injecting six replicate injections of a reference standard solution (10 µg/ml). The average, SD, and %RSD of peak areas of six replicate injections were calculated and reported. The results are shown in **Table 3**.

Method Precision (Assay Repeatability):

This was performed by injecting six replicate injections of standard solution (10 µg/ml) and six sample preparations of ivabradine (10 µg/ml) in triplicates into the HPLC system. The

%assay, average, SD, and %RSD were calculated and reported. The results are summarized in **Table 4**.

Intermediate Precision:

This was performed on two different days and different analysts. Three replicates of standard solution (10µg/ml) and three sample preparation (10µg/ml) in triplicates were injected into the HPLC system. The % Assay, average, SD, and %RSD were calculated and reported. The results are shown in **Table 5.**

Accuracy:

Accuracy is the closeness of experimental values to the true value and was done by the standard addition method. A known amount of ivabradine standard solution was added to the pre-analyzed formulation at 110,120, 130 % levels, and chromatograms were recorded. Good recovery of the spiked drug was obtained at each added concentration, to show that the method was accurate. The results at various levels of concentration are summarized in **Table 6**.

Robustness:

It is defined as a small or deliberate change in the parameter that should not affect any method. This was performed by a change in flow rate (± 0.2 ml/min), change in the column in temperature (±2 °C), change in wavelength (±2 nm), Change in buffer(± 0.2 nm). The results are summarized in **Table 7**.

Forced Degradation studies:

Forced degradation studies also known as stress testing happen to be an intrinsic part of pharmaceutical product development. The stability of Active Pharmaceutical Ingredients or formulation products can be predicted by carrying out stress testing and also impurities developed while the storage of drug products in different environmental conditions can be studied under forced degradation studies. Forced degradation of Ivabradine was carried out under acidic, alkaline, oxidative, thermal, hydrolytic, and photolytic conditions. PDA detector was used to determine the peak purity of Ivabradine and to confirm the stability-indicating nature of the developed method.

RESULTS:

Table No.1: Optimized Chromatographic Condition

Sr.No.	Parameters	Condition
1	Mobile phase	Phosphate buffer:Acetonitrile(30:70%v/v)
2	Flow rate	0.6ml/min
3	Runtime	10min
4	Volume of injection	20
5	Detection of Wavelength	287nm
6	Diluent	Mobile phase

Table No. 2: Linearity data of Ivabradine.

Concentration (µg/ml)	Area
5	2996
10	6029
15	9216
20	12508
25	15592
30	18723

Table No.3: System suitability and system precision study of Ivabradine.

Injection no	Area	Retention Time
1	1282.03	5.17
2	1291.53	5.16
3	1281.35	5.16
4	1287.85	5.16
5	1287.07	5.16
6	1282.42	5.16
AVERAGE	1286.04	5.32
SD	4.1707	0.0047
% RSD	0.32	0.09
LIMIT	NMT 2.0%	NMT 1.0%

Table No. 4: Method Precision (Assay Repeatability) data of Ivabradine

Sample No.	% Assay
1	101.89
2	101.65
3	101.70
4	101.70
5	101.33
6	100.92
AVERAGE	101.53
SD	0.320
%RSD	0.32
Limit	NMT 2%

Table No. 5: Intermediate precision data of Ivabradine

Sample No.	% Assay Day-1 HPLC-1	% Assay Day-2 Analyst -1	%Assay HPLC-1 Analyst-2
1	101.89	102.18	102.48
2	101.65	101.0	102.49
3	101.70	100.55	102.89
AVERAGE	101.75	101.25	102.62
SD	0.13	0.84	0.193
%RSD	0.124	0.830	0.19
LIMIT	NMT 2%	NMT 2%	NMT 2%

Table No. 6: Accuracy data of Ivabradine.

% level	STD spiked (µg/ml)	Amount recovered (mg)	% amountrecoverd	% recovery	Mean % recovery
110	1	5.59	111.8	101.6	101.83
120	2	6.11	122.3	101.9	
130	3	6.61	132.2	101.7	

Table No. 7: Robustness data of Ivabradine

Parameter	Change in parameter (±)	% Assay Estimation	AVERAGE	SD	% RSD	LIMIT
Flow rate (±0.2 ml/min)	0.58	99.28	100	0.965	0.964	NMT 2%
	0.6	101.15				
	0.62	99.80				
Column temperature (±2°C)	28	100.29	101	0.571	0.566	
	30	101.15				
	32	101.37				
Wavelength (±2 nm)	285	100.92	101	0.362	0.357	
	287	101.15				
	289	101.63				
Buffer (±2 ml/min)	32:68	100.74	100.74	0.409	0.406	
	30:70	101.15				
	28:72	100.33				

Table No. 8: Data of Forced Degradation Studies

Sr.no.	Stress Condition	Duration	Retention time	%Residual Drug	Peak Purity
1.	Acidic 0.2M HCL	3 h	5.267	85.05	100
2.	Basic 0.2M NaOH	3 h	5.268	94.71	99.99
3.	Oxidative 1%H2O2	3 h	5.237	95.58	100
4.	Photolytic	3 h	5.235	96.88	100
5.	Thermal	3 h	5.243	99.29	99.99
6.	Water	3 h	5.243	97.95	99.99

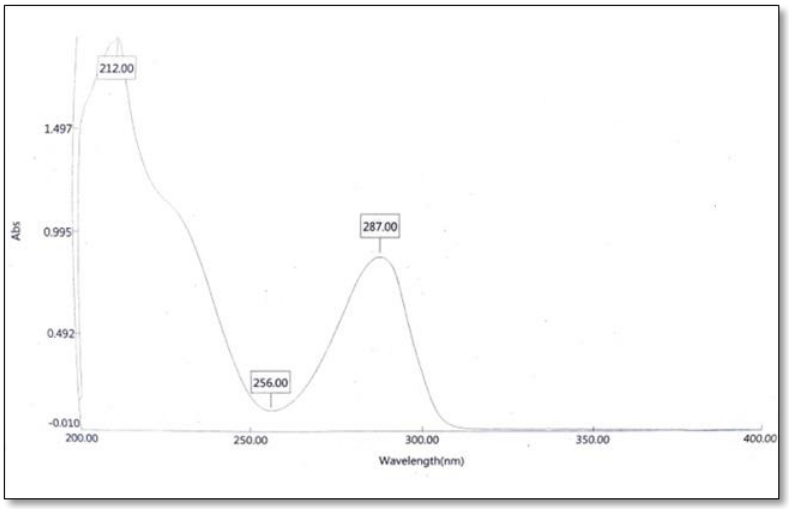

Figure No.2: UV spectra of Ivabradine

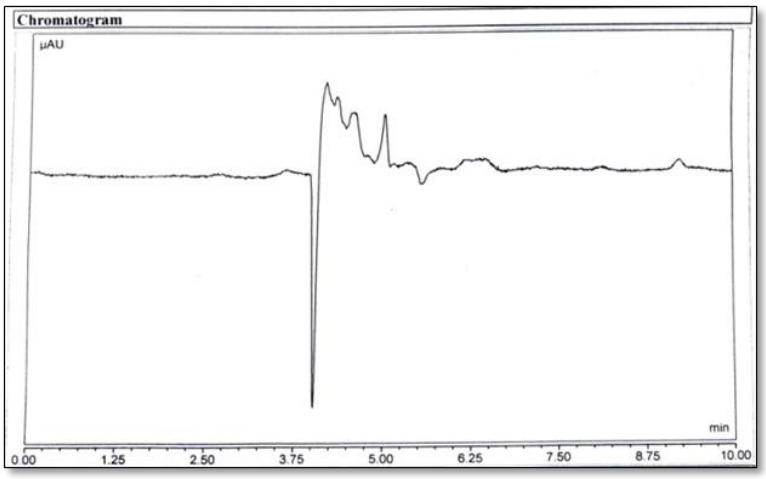

Figure No. 3: Chromatogram of Blank solution

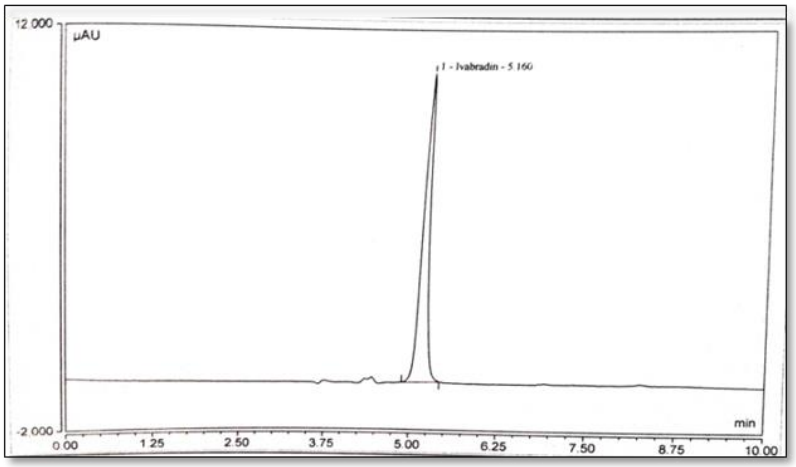

Figure No. 4: Chromatogram of Standard solution

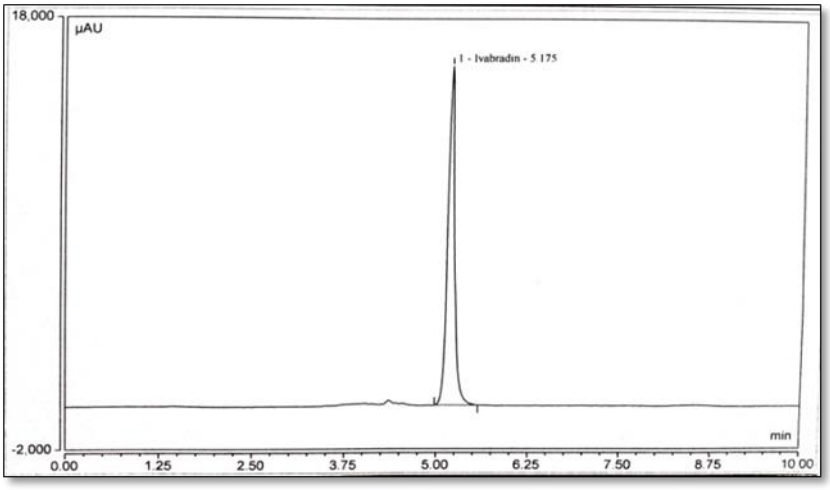

Figure No. 5: Chromatogram of Test solution

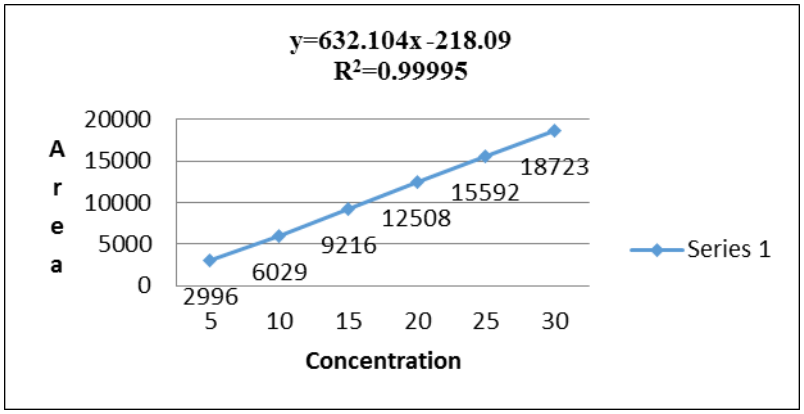

Figure No. 6: Linearity graph of Ivabradine

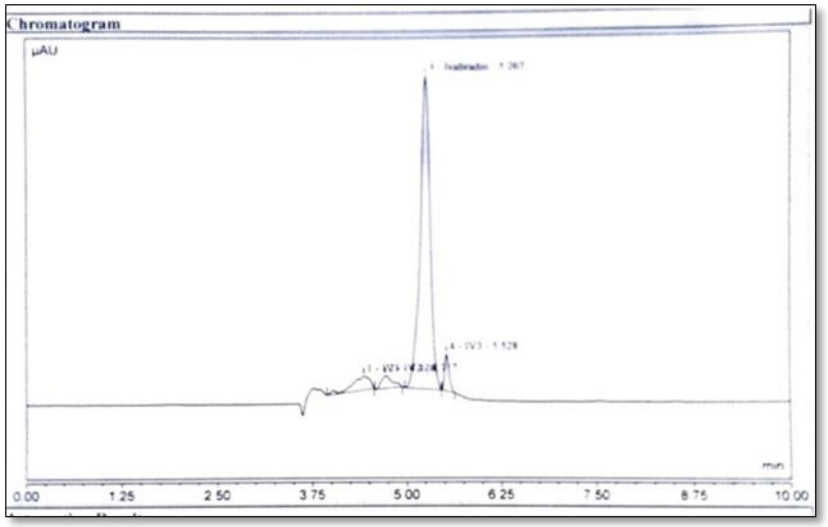

Figure No. 7: Chromatogram of Acid degradation of Ivabradine

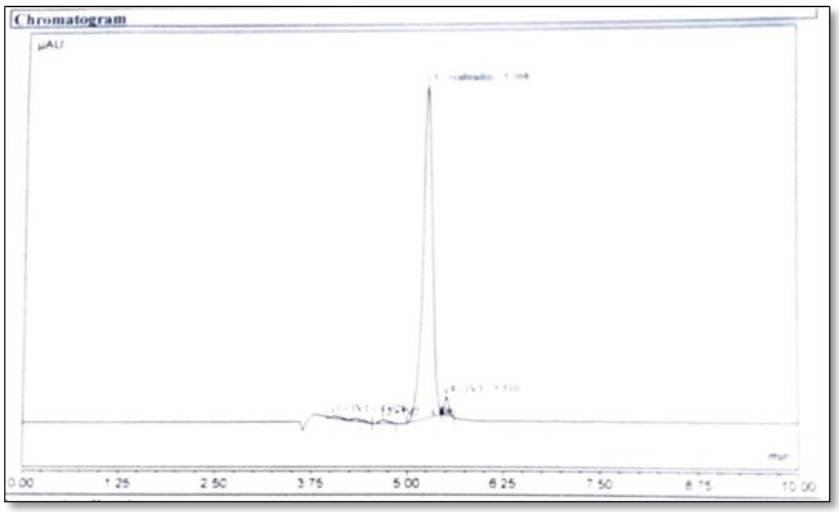

Figure No. 8: Chromatogram of Alkali degradation of Ivabradine

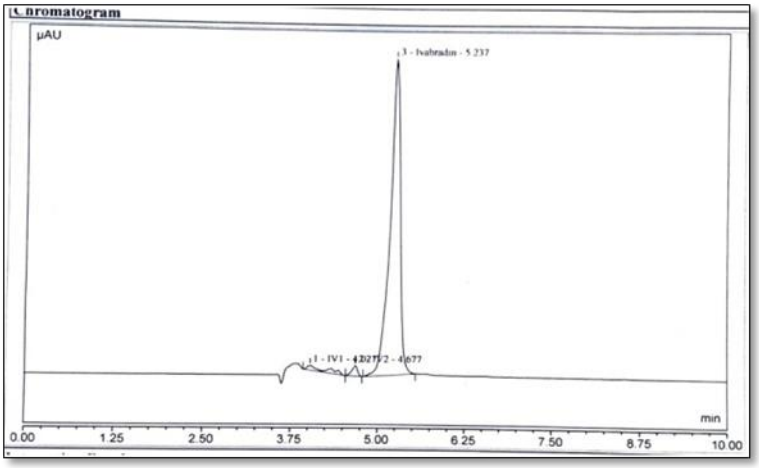

Figure No. 9: Chromatogram of Oxidative degradation of Ivabradine

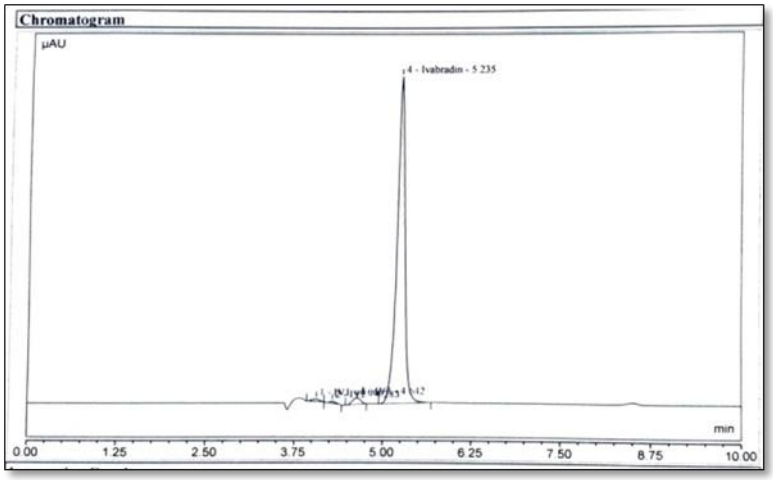

Figure No. 10: Chromatogram of Photolytic degradation of Ivabradine

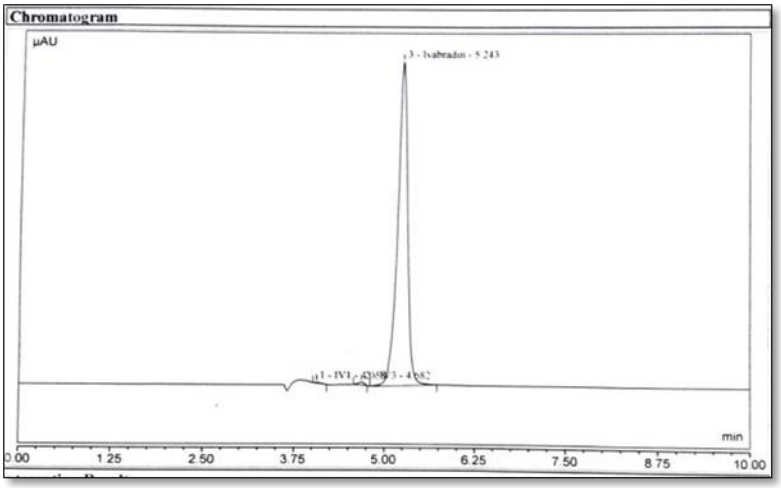

Figure No. 11: Chromatogram of Thermal degradation of Ivabradine

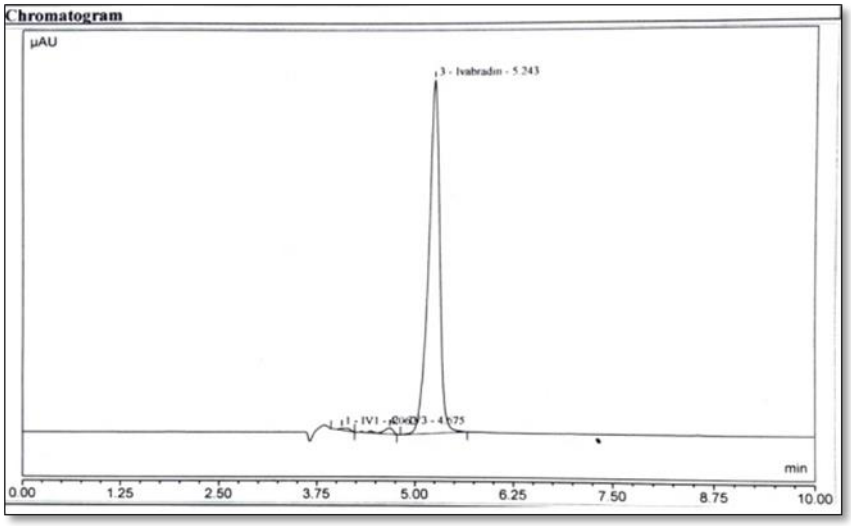

Figure No. 12: Chromatogram of Water hydrolysis of Ivabradine

DISCUSSION:

Forced Degradation Studies

Chromatograms of photolytic, thermal, and hydrolytic degradation showed extra peaks indicating mild degradation. e. 3.12 % degradation in photolytic condition, 0.71 % degradation in thermal condition, and 2.05 % degradation in hydrolytic condition. The most significant degradation was observed under acidic conditions i.e.14.95 %. Moderate degradation was observed under basic and oxidative conditions i.e. 5.29 % and 4.42 %.

Degradation products formed were well resolved under the proposed condition. Under each condition, peak purity was found to be 100% as the peaks of degradation were successfully separated and resolved from the main peak of Ivabradine. The results are summarised in **Table. 8** and the chromatograms recorded and shown in **Fig. 7 to 12**.

System Suitability Studies

This study was done to check the system performance by injecting a standard solution of Ivabradine (six replicates) of a concentration (10µg/ml) and a blank preparation (single injection) into the HPLC. The chromatograms were recorded and % RSD of parameters such as area, retention time, tailing factor and theoretical plates were evaluated. The % RSD of peak areas was found to be 0.32. Theoretical plates were found to be about 13848, tailing factor was found to be about 0.97. The chromatograms of blank and standard (10µg/ml) solution were recorded and shown in Fig. 3 and 4 and the data were summarized in **Table. 3** and chromatogram are shown in **Fig. 5.**

Method Validation

The developed method for estimation of Ivabradine in tablets is specific which was established by Forced Degradation Studies. The method was found to have good linearity over the concentration range of 5- 30 µg/ml with a correlation coefficient of 0.99995.

For system precision, the % RSD for peak areas of Ivabradine standard solution was found to be 0.32 and the mean assay percentage results of Ivabradine sample solutions were found to be within the limits, and the % RSD was found to be 0.32, hence the method was found to be precise. The % RSD of intermediate precision and assay was found to be within the limits.

Accuracy results at different levels of concentration are recorded in Table. 6. The method was found to be accurate as the mean percent recovery of Ivabradine sample solutions was found to be 101.83 % which is within the limit.

Reproducible results were obtained which proves the developed method to be robust. % RSD during changes in method parameters was less than 2.0 %i.e. the change in flow rate was found to be 0.96, the change in column temperature was found to be 0.56, the change in wavelength was found to be 0.35, the change in buffer was found to be 0.40 and the results were not adversely affected by these changes. High percent recovery values and very low SD

and % RSD values confirm that the currently developed method is suitable for routine analysis of Ivabradine in its pharmaceutical tablet dosage form.

CONCLUSION:

The RP-HPLC developed method was found to be specific, simple, selective, rapid, and can generate accurate and precise results. Moreover, the shorter duration of analysis time and lesser mobile phase consumption confirmed that the method is rapid and economical. As per ICH Q2 (R1) guidelines, all the parameters such as specificity, linearity, accuracy and recovery, precision, and robustness were successfully validated. The successful separation of the forced degradation products from the active pharmaceutical ingredients without any interference confirmed the stability-indicating nature of the developed method. The parameters were found to be within the acceptable limits according to ICH guidelines. Hence the proposed RP-HPLC method can be used for routine analysis and quality control of Ivabradine in the tablet dosage form.

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