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IVABRADINE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION BULK AND PHARMACEUTICAL DOSAGE FORMS USING REVERSE PHASE HPLC

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ABSTRACT

The present work focusses on the analytical development and validation of ivabradine in bulk and pharmaceutical dosage form by RP-HPLC method. It is a simple, Accurate, precise method, using the C8 column with length of 250mm and 4.5mm diameter, 5µm, contain mobile phase potassium hydrogen phosphate: Acetonitrile 45:55 was pumped through column at a flow rate of 1.0ml/min. At temperature of 30°C. With wave length of 260.0nm. The Retention time of Ivabradine was found to be 2.517min, %RSD found to be 0.6. %Recovery was obtained as 99.85% for ivabradine, by the regression equation the LOD, LOQ, R were 0.33, 0.99 and 0.998, the linearity was found to be y = 17338x + 1317.6 R^2 = 0.9998. Based on the result the method can useful for routine quality control for analysis of ivabradine.

KEYWORDS

Ivabradine, Retention time, LOD, LOQ and RP-HPLC.

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INTRODUCTON

Ivabradineis benzazepines class of drug with IUPAC name **[**{[(7S)-3, 4-dimethoxybicyclo [4.2.0] octa-1, 3, 5-trien-7-yl] methyl} (methyl) amino] propyl}. Used to treat the angina pectoris with - patient having in tolerance with beta blockers. It having the cardiotonic activity[1].

MATERIAL AND METHODS[2-15]

Materials

Ivabradine (API), Ivabradine tablet (PROCORALAN with labeld claim 5mg), Distilled water, Acetonitrile, Methanol, water, phosphate buffer, Methanol, ortho phosphoric acid were AR Grade and purchased from the Merck and instruments like Electronics Balance from Shimazu,

pH meterelico India, Ultrasonic ate from Lab man India, HPLC LC SYSTEM, UV spectrometer from Shimazu.

Methods[15-35]

Optimized Method

Preparation of standard stock solution

Weigh exactly 5mg of ivabradine in a 25ml of volumetric flask to this add ¾th ml of diluent sonicate it for 15 min, make up remaining with diluent labeled as standard solution contain 200µg/ml concentration.

Preparation of standard working standard

Take 1ml of solution from stock solution and make up to 10ml in a 10ml of volumetric flask labeled as 20µg/ml concentration.

Preparation of sample stock solution

Take 10 tablets weighed and average weight was calculated then the equivalent 1 tablet was transfer into 50ml of volumetric flask add 25 ml of diluent sonicate it for 15 min and add the reamoing diluent and filter it through HPLC filters.

Preparation of sample solution[36-43]

Take 2ml of sample stock add 10 ml of diluent into a 10ml of volumetric flask and labeled as 20µg/ml concentration.

RESULTS AND DISCUSSION

Optimized Chromatographic Conditions

The column with Intersil C8 with length of 250mm with diameter of 4.6mm loaded stationary phase with 5µm, mobile phase contains 45:55 ration of phosphate buffer and acetonitrile with flow rate of 1.0ml/min with PDA detector wavelength 260 nm at ambient temperature. With injection volume 10µL runtime 6.0 min.

SUMMARY

The Linearity Range (µg/ml) for ivabradine was found to be 5-30µg/ml, Regression coefficient was 0.998 the Regression equation was to be y = 17338x + 1317.6. Assay (% mean assay) was found to be 100.332, Specificity No peak interference System precision %RSD was found to be 0.8886. Method precision %RSD was found to be 0.620 Accuracy % recovery was found to be 99.75%, LOD was found to be 0.33, LOQ was found to be 0.99, in robustness Flow rate changes 0.3 %RSD Flow rate changes was found to be 0.4, Mobile phase changes was found to be 0.6 Mobile phase changes was found to be 1.2, Temperature changes was to be 1.0, Temperature changes was to be 0.3.

System suitability

Table No.1: Ivabradine system suitability parameters

S.No	Ivabradine		
	Retention time	Plate count	Tailing factor
1	2.517	11256	1.18
2	2.564	11294	1.17
3	2.566	11035	1.16
4	2.566	11235	1.13
5	2.566	10978	1.16
6	2.566	11189	1.14

Linearity

Table No.2: Ivabradine linearity

S.No	Ivabradine	
	Concentration (µg/mL)	Peak Area
1	0	0
2	88024	88024
3	173030	173030
4	263152	263152
5	351256	351256
6	437530	437530
7	516672	516672

Precision

System precision

Table No.3: Ivabradine system precision

	System precision	Repeatability	Reproducibility
S.No	Area of Ivabradine	Area of Ivabradine	Area of Ivabradine
1	357442	360929	345312
2	357352	360297	350027
3	360548	358659	349558
4	359490	362290	347522
5	358401	345860	344554
6	352357	362229	347720
Mean	357630	357867	347876
S.D	3180.04	6879.14	2158.78
%RSD	0.8892	1.92226	0.62056

Accuracy

Table No.4: Ivabradine accuracy

S.No	% Level	Spiked volume µg/ml	Amount recovered in µg/ml	% of Recovery	% Mean Recovery
1	50%	10	9.99	100.01	99.75%
		10	10.05	100.35	
		10	9.92	99.03	
2	100%	20	20.14	100.54	
		20	19.93	99.71	
		20	19.97	99.74	
3	150%	30	29.71	99.14	
		30	29.99	99.85	
		30	29.79	99.65	

Sensitivity

Table No.5: Sensitivity for Ivabradine

S.No	Compound	LOD	LOQ
1	Ivabradine	0.33	0.99

Robustness

Table No.6: Robustness data for Ivabradine

S.No	Condition		%RSD of Ivabradine
1	Change in Flow rate	0.9ml/min	0.3
2		1.1ml/min	0.4
3	Change in mobile phase composition	55B:45A	0.6
4		45B:55A	1.2
5	Change in temperature	25°C	1.0
6		35°C	0.3

Table No.7: Ivabradine assay results

S.No	Standard area	Sample area	% Assay
1	357552	360729	100.7
2	360448	358699	99.84
3	359550	364490	100.77
4	358401	357760	99.97
5	352447	367719	100.94
6	355671	357705	99.77
Mean	357345	361184	100.332
SD	2913.46	4095	0.52648
%RSD	0.81531	1.13377	0.52474

Table No.8: Ivabradine degradation studies data

S.No	Degradation type	Ivabradine		
		Area	%Recovered	% Degraded
1	Acid	332482	92.67	7.33
2	Base	340629	94.95	5.05
3	Peroxide	335706	93.57	6.43
4	Thermal	349197	97.33	2.67
5	UV	354448	98.80	1.20
6	Water	356146	98.80	1.20

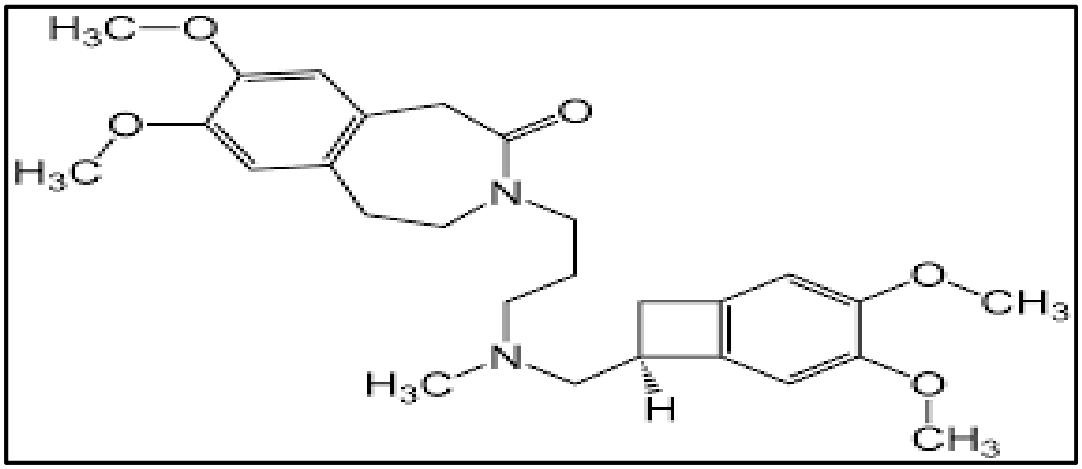

Figure No.1: Structure of Ivabradine

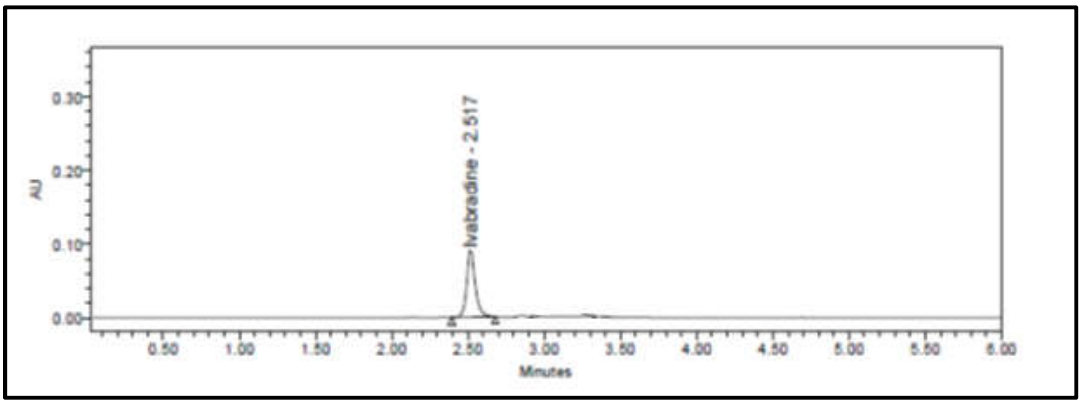

Figure No.2: System suitability chromatogram

Validation, Specificity

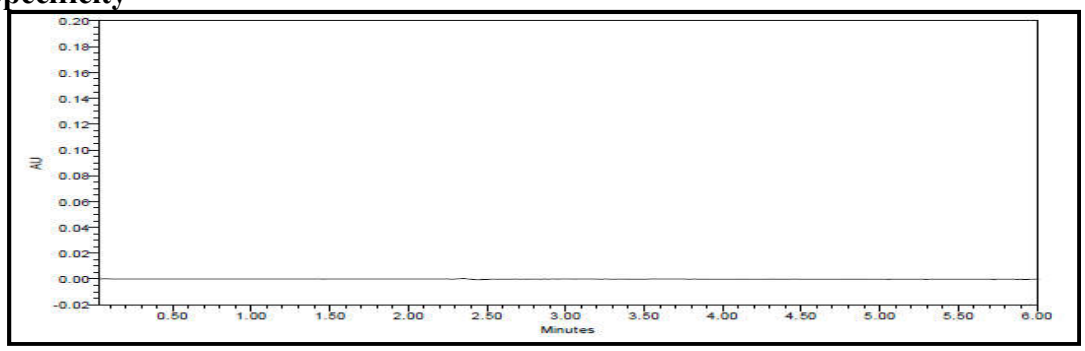

Figure No.3: Black chromatogram

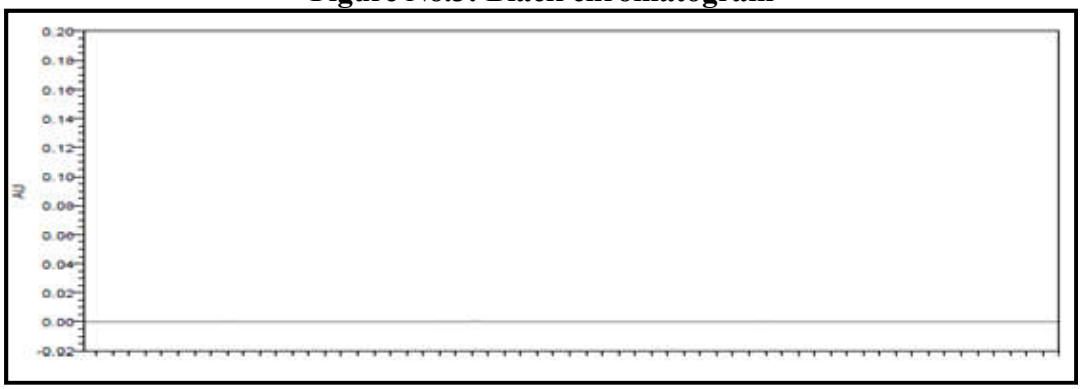

Figure No.4: Placebo chromatogram

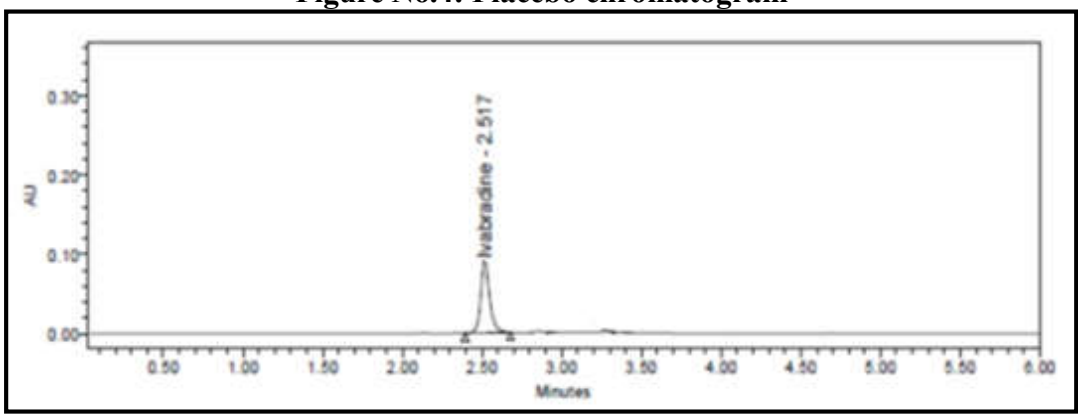

Figure No.5: Standard chromatogram

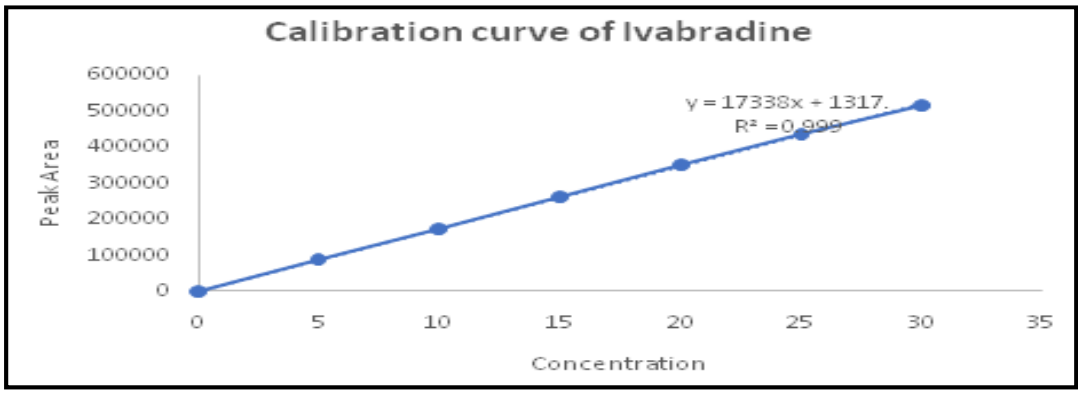

Figure No.6: Ivabradine calibration curve

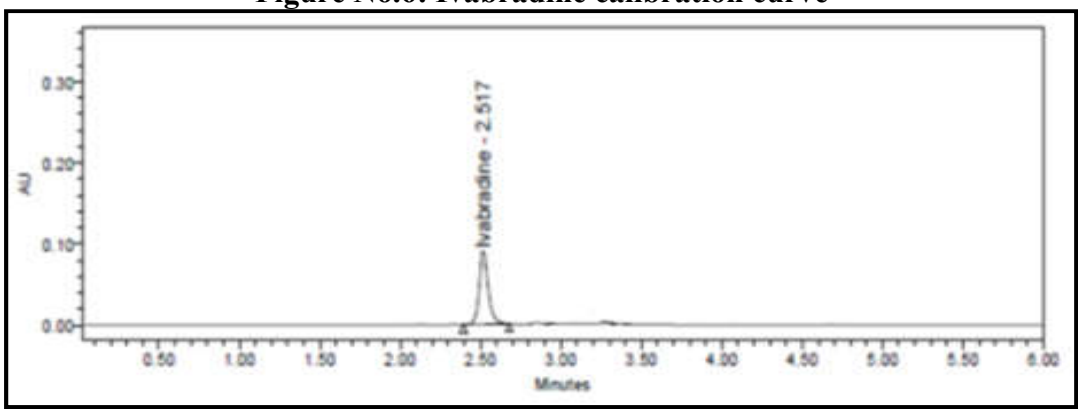

Figure No.7: Standard chromatogram

CONCLUSION

For estimation of Ivabradine the developed method was simple, precise, accurate and robust in tablet formulations. In the method the Rt was found to be 2.517min %RSD of the Ivabradine was found to be 0.620 respectively. %Recovery was obtained as 99.75 % for Ivabradine. LOD, LOQ values obtained from regression equations of Ivabradine were 0.33, 0.99. Regression equation of Ivabradine is y = 17338x + 1317.6.the passes the regression coefficient therefore the method was simple, precise, economical for quality control department pharmaceutical industry.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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