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DEVELOPMENT OF VALIDATED ANALYTICAL METHOD FOR ESTIMATION OF IVABRADINE IN PURE AND PHARMACEUTICAL DOSAGE FORM USING RP-HPLC

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Ivabradine tablet dosage form. Chromatogram was run through Discovery C8 250mm x 4.6 mm, 5µm. Mobile phase containing KH2PO4: Acetonitrile taken in the ratio 50:50 was pumped through column at a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 260.0nm. Retention time of Ivabradine was found to be 2.517min %RSD of the Ivabradine was found to be 0.6. % Recovery was obtained as 99.65% for Ivabradine, LOD, LOQ values obtained from regression equations of Ivabradine were 0.31, 0.95 and 0.68,Regression equation of Ivabradine is y = 17301x + 1713. Retention time, runtime was reduced, so the technique created has been easy and economical that can be used in periodic quality control tests in industries**.**

KEYWORDS: Ivabradine, retention time, LOD, LOQ RP-HPLC.

INTRODUCTION

Quality confirmation and control of pharmaceutical and compound plans is fundamental for guaranteeing the accessibility of protected and viable medication definitions to shoppers. Henceforth Analysis of unadulterated medication substances and their pharmaceutical measurements frames involves a crucial job in surveying the reasonableness to use in patients Ivabradine is a novel heart rate lowering medicine for the symptomatic management of stable angina pectoralis and symptomatic chronic heart failure. Ivabradine, brand name Corlanor, was approved by the FDA in April 2015 for the treatment of chronic heart failure in patients with an ejection fraction of ≤35%, in sinus rhythm with resting heart rate ≥70 beats per minute, who are not on beta-blockers due to contraindications or already receiving maximum beta-blocker dose. Recently a new indication was added to treat symptomatic heart failure from dilated cardiomyopathy for patients 6 months or more in age .Ivabradine acts by selectively inhibiting the "funny" channel pacemaker current (If) in the sinoatrial node in a dose-dependent fashion, resulting in a lower heart rate and thus more blood to flow to the myocardium. Although non-dihydropyridine calcium channel blockers and beta blockers also effectively lower heart rate, they exhibit adverse events due to their negative ionotropic effects. Therefore, as ivabradine is designed as a "pure" heart rate-lowering drug by selectively acting on the If channels, it may offer a more favorable side effect profile due to its lower likelihood of causing serious adverse effects.

Structure

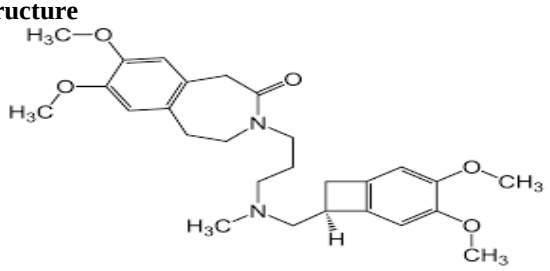

Fig. 1: Structure of Ivabradine.

MATERIALS AND METHODS

Materials

Ivabradine pure drug (API), Combination Ivabradine tablet (**Corlanor**), Distilled water, Acetonitrile, Methanol. All the above chemicals and solvents are from Rankem The chemicals acetonitrile, water, phosphate buffer, Methanol, ortho phosphoric acid were AR Grade and purchased from the Merck and instruments like Electronics Balance from shimadzu, pH meter elico India, Ultrasonicator Lab man India, HPLC LC

SYSTEM UV-VIS spectrophotometer PG Instruments T60 were used with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Ivabradine and Metoprolol solutions.

Methods

Optimized Method

Buffer: 0.01N Potassium dihyrogen ortho phosphate (3.0pH)

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.0 with dil. Orthophosphoric acid solution.

Label Claim: 5mgTablet

Brand name: Corlanor

Preparation of Standard stock solutions: Accurately weighed 5 mg of Ivabradine, transferred to 25 ml volumetric flask. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution. (200µg/ml of Ivabradine).

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml Ivabradine).

Preparation of Sample stock solutions: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 50 ml volumetric flask, 25ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (100µg/ml of Ivabradine and 500µg/ml of Metoprolol).

Preparation of Sample working solutions (100% solution): 2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of Ivabradine).

RESULTS AND DISCUSSION

Method development: Method development was done by changing various, mobile phase ratios, buffers and columns etc.

Optimized method

Optimized Chromatographic Conditions

Column	:	Intersil C8 250mm x 4.6 mm, 5µm.
Mobile phase	:	KH2PO4: Acetonitrile (50:50)
Flow rate	:	1.0 ml/min
Detector	:	PDA 260nm
Temperature	:	30^0C
Injection Volume	:	10µL
Run time	:	5.0 min

System suitability: All the system suitability parameters were with in the range and satisfactory as per ICH guidelines.

Table 1: System suitability parameters for Ivabradine and Metoprolol.

S. no.	Ivabradine		
Inj	RT(min)	USP Plate Count	Tailing
1	2.517	11545	1.19
2	2.564	11286	1.19
3	2.566	11029	1.18
4	2.566	11228	1.15
5	2.566	10969	1.17
6	2.566	11198	1.15

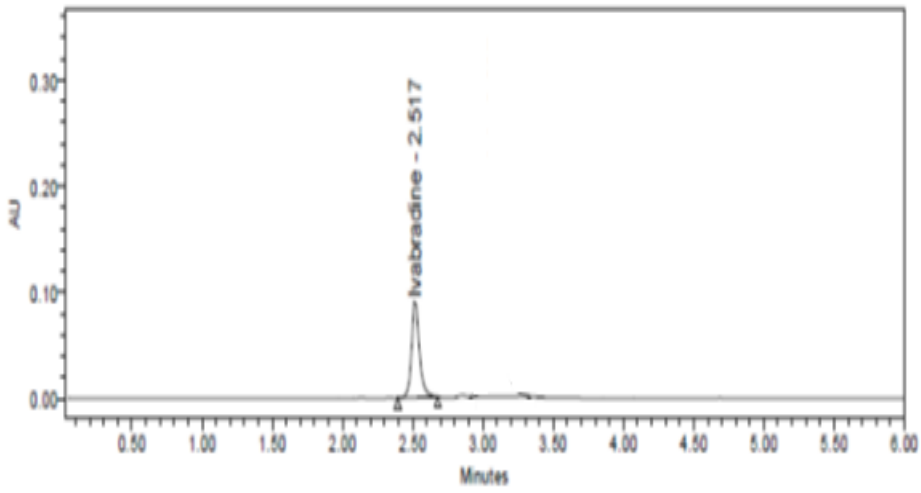

Fig. 2: System suitability Chromatogram.

Validation
Specificity

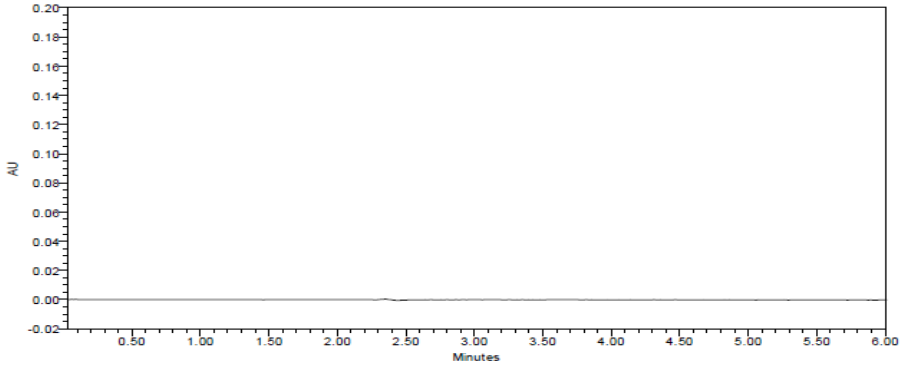

Figure 3: Chromatogram of blank.

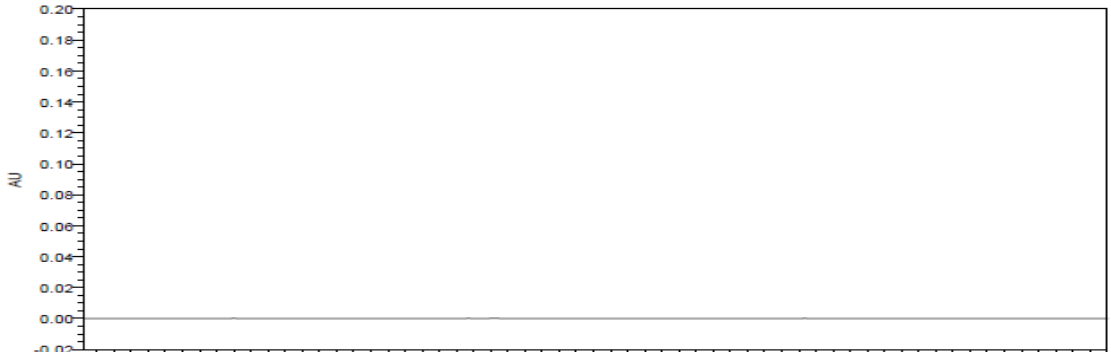

Figure 4: Chromatogram of placebo.

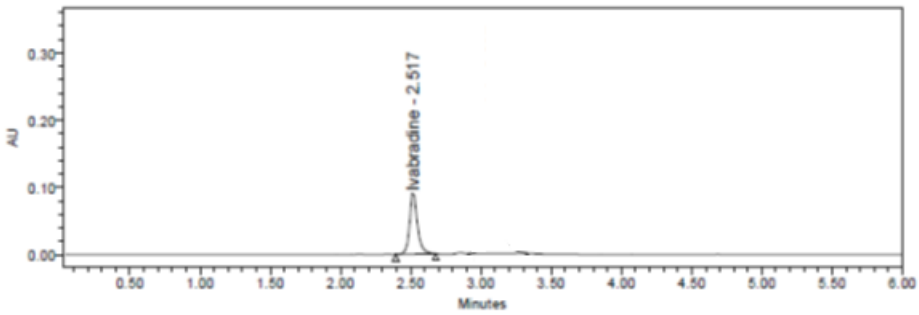

Figure 5: Chromatogram of Standard drugs.

Linearity
Table 2: Linearity table for Ivabradine and Metoprolol.

Ivabradine	
Conc (µg/mL)	**Peak area**
0	0
5	89022
10	174020
15	262132
20	350206
25	435600
30	517582

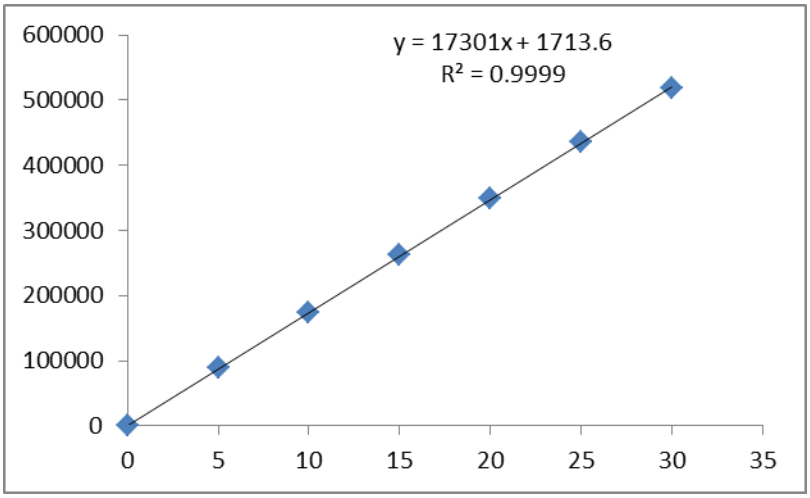

Figure 3: Calibration curve of Ivabradine.

Precision

System Precision

Table 6: System precision table of Ivabradine and Metoprolol.

S. No	Area of Ivabradine
1.	357442
2.	360848
3.	359290
4.	358201
5.	352527
6.	355661
Mean	357328
S.D	2927.2
%RSD	0.8

Repeatability

Table 7: Repeatability table of Ivabradine and Metoprolol.

S. No.	Area of Ivabradine
1.	360929
2.	358499
3.	362290
4.	358260
5.	362119
6.	356605
Mean	359784
S.D	2329.1
%RSD	0.6

Intermediate precision

Table 8: Intermediate precision table of Ivabradine and Metoprolol.

S. No.	Area of Ivabradine
1.	345312
2.	350027
3.	349898
4.	348422
5.	347554
6.	348020
Mean	348206
S.D	1735.5
%RSD	0.5

Accuracy

Table 9: Accuracy table of Ivabradine.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	10	9.9999422	100.00	99.65%
	10	10.044506	100.45	
	10	9.9031848	99.03	
100%	20	20.115485	100.58	
	20	19.92879	99.64	
	20	19.959193	99.80	
150%	30	29.733946	99.11	
	30	29.718109	99.06	
	30	29.745044	99.15	

Sensitivity

Table 10: Sensitivity table of Ivabradine.

Molecule	LOD	LOQ
Ivabradine	0.30	0.90

Robustness

Table 11: Robustness data for Ivabradine.

S. no.	Condition	%RSD of Ivabradine
1	Flow rate (-) 0.9ml/min	0.3
2	Flow rate (+) 1.1ml/min	0.4
3	Mobile phase (-) 55B:45A	0.6
4	Mobile phase (+) 45B:55A	1.2
5	Temperature (-) 25°C	1.0
6	Temperature (+) 35°C	0.3

Assay: Intas Pharmaceuticals Ltd (**IVA Met**), bearing the label claim Ivabradine 5mg, Metoprolol 25mg. Assay was performed with the above formulation. Average % Assay for Ivabradine and Metoprolol obtained was 100.28% and 100.03% respectively.

Table 12: Assay Data of Ivabradine.

S. no.	Standard Area	Sample area	% Assay
1	357442	360929	100.60
2	360848	358499	99.93
3	359290	362290	100.98
4	358201	358260	99.86
5	352527	362119	100.94
6	355661	356605	99.40
Avg	357328	359784	100.28
Stdev	2927.2	2329.1	0.65
%RSD	0.8	0.6	0.6

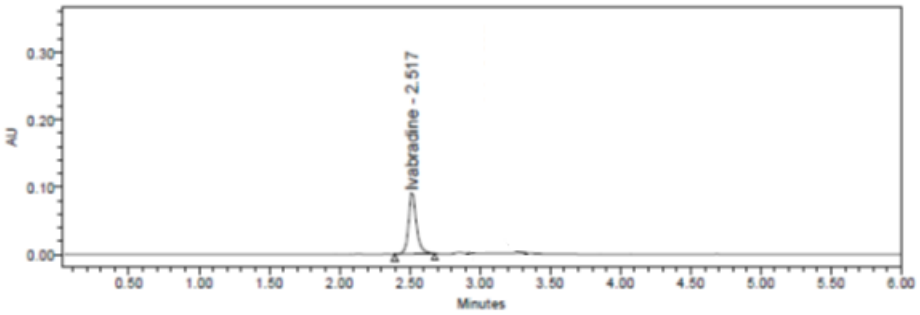

Fig. 4: Chromatogram of working standard solution.

Table 13: Degradation data for Ivabradine.

Type of degradation	Ivabradine		
	Area	%recovered	% degraded
Acid	332482	92.67	7.33
Base	340629	94.95	5.05
Peroxide	335706	93.57	6.43
Thermal	349197	97.33	2.67
Uv	354448	98.80	1.20
Water	356146	98.80	1.20

SUMMARY AND CONCLUSION

Parameters		Ivabradine	LIMIT
Linearity Range (µg/ml)		5-30µg/ml	R< 1
Regression coefficient		0.999	
Slope(m)		17301	
Intercept(c)		1713	
Regression equation (Y=mx+c)		y = 17301x + 1713.	
Assay (% mean assay)		100.28%	90-110%
Specificity		Specific	No interference of any peak
System precision %RSD		0.8	NMT 2.0%
Method precision %RSD		0.6	NMT 2.0%
Accuracy %recovery		99.65%	98-102%
LOD		0.31	NMT 3
LOQ		0.95	NMT 10
Robustness	**FM**	0.3	% RSD NMT 2.0
	FP	0.4	
	MM	0.6	
	MP	1.2	
	TM	1.0	
	TP	0.3	

CONCLUSION

A simple, Accurate, precise method was developed for the estimation of the Ivabradine in Tablet dosage form. Retention time of Ivabradine was found to be 2.517min %RSD of the Ivabradine was found to be 0.6 respectively. %Recovery was obtained as 99.65% for Ivabradine. LOD, LOQ values obtained from regression equations of Ivabradine and Metoprolol were 0.30, 0.90. Regression equation of Ivabradine is y = 17301x + 1713., the passes the regression coefficient therefore the developed method was simple and economical that can be adopted in regular Quality control test in Industries.

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