Original Article

Quantitative Bioanalytical and Analytical Methods for Estimation of Ivabradine Hydrochloride in Pure and Pharmaceutical Dosage Form

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Abstract

Introduction: New analytical and bioanalytical methods were developed for the estimation of Ivabradine hydrochloride in bulk and pharmaceutical dosage form by UV spectrophotometry and high-performance liquid chromatography (HPLC) technique. **Objective:** The primary objective of the study is to develop a new RP-HPLC method for estimation of Ivabradine Hydrochloride in pure and formulation and to develop a bioanalytical method for analysis of Ivabradine Hydrochloride in biological samples. The methods were validated as per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use and USFDA guidelines respectively. **Methods:** In Reversed-phase (RP)-HPLC method developed telmisartan is the internal standard used. After liquid–liquid extraction, the analyte and the internal standard were chromatographed on Waters 125A (10 µ, 300 × 3.9 mm) C18 column using 20-µL injection volume with a run time of 15 min. An isocratic mobile phase consisting of acetonitrile and ammonium acetate buffer pH 7.8 (60:40% vol/vol) is used to separate drug and internal standard. In Spectrophotometric bioanalytical method developed for the estimation of ivabradine hydrochloride in pure and pharmaceutical dosage form. The solvent system used is absolute methanol and detected at the wavelength of 287 nm. The solvent system used is absolute methanol and detected at the wavelength of 287 nm. **Results:** The Analytical method is validated according to ICH guidelines over the range of 2–16 µg/ mL, showing accuracy, precision, selectivity, and robustness. For Bioanalytical method the linearity is established in the range of 500–3500 ng/mL with the regression coefficient of r2 = 0.9994. The validated spectrophotometric method is used successfully to study ivabradine hydrochloride in rat plasma and also quantitative determination in marketed tablets. **Conclusion:** The proposed methods were successfully applied for the quantitation of ivabradine hydrochloride in pharmaceutical dosage form with good recovery and reproducibility.

Keywords: *Bioanalytical method, ivabradine hydrochloride, reversed-phase high-performance liquid chromatography, UV-spectrophotometric method*

Introduction

Introduction to drug

Ivabradine hydrochloride[1] is chemically 3-[3-({[(7S)-3,4-dimethoxybicyclo [4.2.0] octa-1,3,5-trien-7-yl]methyl}(methyl)amino) propyl]7,8-dimethoxy-2,3,4,5-tetrahydro-1H-3-benzazepin-2-one hydrochloride [Figure 1]. Its empirical formula is $C_{27}H_{37}ClN_2O_5$. Ivabradine hydrochloride is a first-in-class drug that is selective bradycardic agent with direct effect on the pacemaker I_f current[2] of the sinoatrial node. It is the only representative of the group of drugs that has been introduced for the treatment of chronic coronary artery disease and chronic heart failure.[3,4] It is white to off-white solid, soluble in organic solvents such as ethanol (<1 mg/mL at 25°C), DMSO (<20 mg/mL at 25°C), and dimethyl formamide (<25 mg/mL at 25°C), and its melting point is >190°C.[5]

Telmisartan (internal standard)

Chemically, telmisartan[6] is 2-(4-{[4-methyl-6-(1-methyl-1*H*-1,3-benzodiazol-2-yl)-2-propyl-1*H*-1,3-benzodiazol-1-yl]methyl} phenyl)benzoic acid [Figure 2]. The empirical formula is $C_{33}H_{30}N_4O_2$, which corresponds to a molecular weight of 514.617 g/mol. Telmisartan is white to off-white, odorless powder, slightly soluble in methanol, sparingly soluble in methylene chloride, dissolves in one molar NaOH, but practically insoluble in water. It is used for the treatment of hypertension.

Literature review[7-19] reveals that few UV spectrophotometric, high-performance liquid chromatography (HPLC), and liquid chromatography (LC)-mass spectrometry (MS)/MS-ESI methods have been reported

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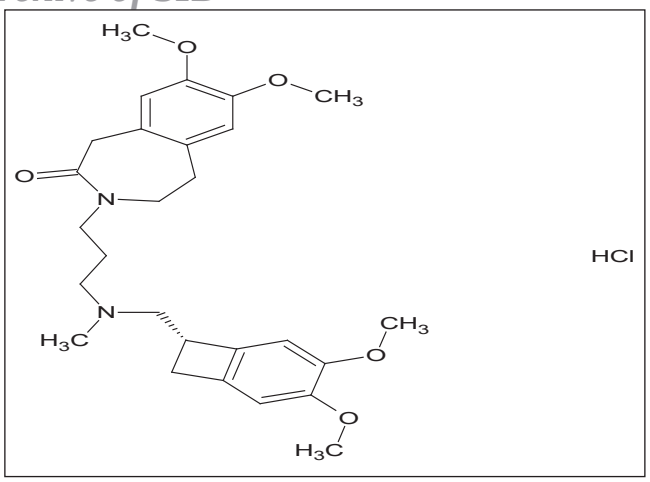

Figure 1: Structure of Ivabradine Hydrochloride

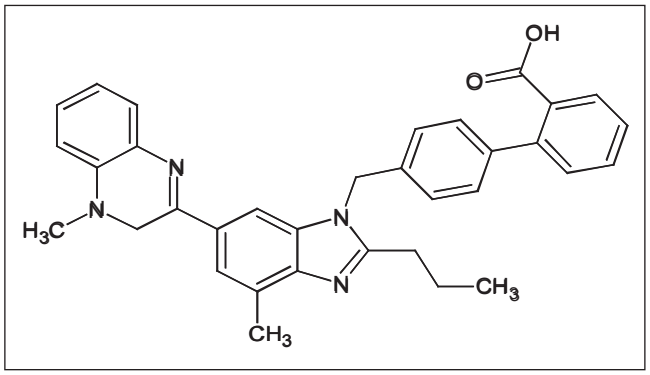

Figure 2: Structure of Telmisartan

so far for the determination of ivabradine hydrochloride alone and its combination with other drugs. There are few methods available for the estimation of ivabradine hydrochloride in biological samples. This study was planned for the development of bioanalytical and analytical methods for the analysis of ivabradine hydrochloride in pure and pharmaceutical dosage form. RP-HPLC method is extremely quick and efficient. Quantitation of drug with the use of internal standard makes the work unique.

Materials and Methods

Chemicals

Ivabradine hydrochloride was kindly provided by MSN Laboratories (Hyderabad, India), telmisartan (internal standard) by Sanofi (Banglore, India), Ivabrad 5 tablets (Lupin, Uttar Pradesh, India). The tested chemicals were supplied from the following sources: acetonitrile (HPLC Grade) from Merck (Mumbai, India), methanol (HPLC grade) from SDFCL (Thane India), water (HPLC grade) from Merck, ammonium acetate buffer (HPLC grade) from SDFCL, and disodium EDTA (LR grade) from SDFCL.

Instrumentation

HPLC analysis of ivabradine hydrochloride is carried out using an Agilent, 1100 series, RP-HPLC system equipped with automatic injector, Chemstation software, and variable wavelength detector. Chromatographic separation is achieved on C18 column (300 × 3.9 mm, 10 µm). Bioanalytical estimation is carried out using UV-Visible spectrophotometer (Shimadzu, 1800 series), a semi microbalance (Shimadzu, BL220H, Japan), pH meter (Elico, LI 127, India), ultrasonic bath sonicator (PCI Analytics, India 6.5 L 200 H), refrigerated centrifuge (Eltek, India), and hot air oven (Tempo Equipment, India).[20-31]

Methods

Analytical method

Preparation of standard stock solution: A stock solution of ivabradine hydrochloride 1000 µg/mL is prepared in acetonitrile, and the working standard solutions were prepared daily by appropriate dilution of the stock solution with diluent.

Mobile phases (MPs) of varying compositions of solvents such as acetonitrile, water, and ammonium acetate buffer (pH, 7.8) were used for the method development.

Preparation of internal standard stock solution: A stock solution of standard telmisartan 1000 µg/mL is prepared in methanol, and the working standard solutions were prepared daily by appropriate dilution of the stock solution with diluent.

Preparation of mobile phase: MP consists of acetonitrile and 0.01 M ammonium acetate buffer (60:40). The pH of ammonium acetate buffer is maintained to 7.8. The MP is filtered through 0.2-µm cellulose acetate filters and degassed in sonicator before use.

Preparation of 0.01M Ammonium acetate buffer pH 7.8: A total of 0.28 g of ammonium acetate is dissolved in 300 mL of HPLC water to produce 0.01 M solution. The pH of this solution is adjusted to 7.8 with sodium hydrochloride solution.

Bioanalytical method

Preparation of stock solutions: A stock solution of ivabradine hydrochloride 1000 µg/mL is prepared in methanol, and the working standard solutions were prepared daily by appropriate dilution of the stock solution with methanol.

The absorbance of resulting solution is measured against respective blank solution in visible region, that is, 200–400 nm, which shows a maximum absorbance at 287 nm.

Plasma sample preparation: The blood samples were collected from retro-orbital puncture into disodium ethylenediaminetetraacetic acid (EDTA) vials (20 mg disodium EDTA in 1 mL water, 1 mL of blood requires 50 µL of disodium EDTA). Plasma is separated from blood samples by centrifugation at 10,000 revolutions per minute for 10 min. After centrifugation, plasma layer gets separated, and it is collected and stored at -20°C for further use.[32,33]

Extraction procedure:

- A pool of blank rat plasma is obtained.
- A total of 100 µL of plasma sample is spiked with appropriate volume of the stock solution, and 400 µL of acetonitrile is added.

- The mixture is vortexed for 1 min.
- Centrifuged at 10,000 rpm for 10 min at 4°C.
- The organic layer is separated and filtered through a 0.2-µm cellulose acetate filter.
- The organic layer is evaporated on a Savant vacuum evaporator at about 60°C. The residue is reconstituted in 10 mL of solvent and is used for the measurement of the absorbance at the absorption maxima of ivabradine hydrochloride.

Blank plasma and standard preparation: The separated organic layer is taken from processed blank plasma, and after filtration, its absorbance is measured directly without spiking it with either sample or solvent. Standard solution of ivabradine hydrochloride 500 ng/mL spiked in plasma is prepared, and absorbance is measured. Blank plasma absorbance is also recorded.

Preparation of quality control (QC) standards in plasma: Calibration samples are prepared by spiking with appropriate amounts of sample into 90 µL of control plasma. From 1 mg/mL stock solution of drug, 5, 10, 15, 20, 25, 30, and 35 µL samples are taken and volume is made up to 10 mL with methanol. From each concentration, 10 µL of sample is taken and spiked into 90 µL control plasma to get 500, 1000, 1500, 2000, 2500, 3000, and 3500 ng/mL, and the absorbance of these concentrations is measured spectrophotometrically at 287 nm against blank plasma.

Matrix effect: Calibration sample is prepared by spiking with appropriate amounts of sample. From 1 mg/mL stock solution of drug, 5, 10, 15, 20, 25, 30, and 35 µL samples are taken and volume is made up to 10 mL with methanol. From each concentration, 10 µL of sample is taken and the final volume is made with methanol to get 500, 1000, 1500, 2000, 2500, 3000, and 3500 ng/mL, and the absorbance of these concentrations is measured spectrophotometrically at 287 nm against methanol as blank, and the matrix effect is calculated and tabulated in Table 1.

Results and Discussion

For the validation of the developed methods in the determination of ivabradine hydrochloride under experimental conditions, the analytical and bioanalytical characteristics were calculated.

Validation of analytical method

The optimized RP-HPLC method is validated according to ICH Q2 (R1) guidelines.[34]

Linearity

Linear regression analysis is performed for checking the linearity of the data obtained. The response of the drug is found to be linear in the concentration range 2–16 µg/mL. The linear regression equation for ivabradine hydrochloride is $y = 0.1954x + 0.0828$ with $r^2 = 0.9997$.

The calibration curve data are shown in Table 1, and the calibration plot is shown in Figure 3 and chromatogram in Figure 4.

Acceptance value for coefficient of determination should not be less than 0.999 and the found value is 0.999 which falls in the acceptance criteria.

System suitability tests

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established as shown in Table 2.

Specificity

The specificity study is performed by injecting the blank. The chromatograms of blank and standard solutions were compared, and no interference due to solvent from MP at retention time of ivabradine hydrochloride peak was found. No interfering peaks were noticed in the chromatogram, suggesting that solvents do not interfere in the estimation of drug.

Analyte solution stability

Sample solution of ivabradine hydrochloride is prepared and analyzed initially and at different time interval by keeping at room temperature. The results are given in Table 3.

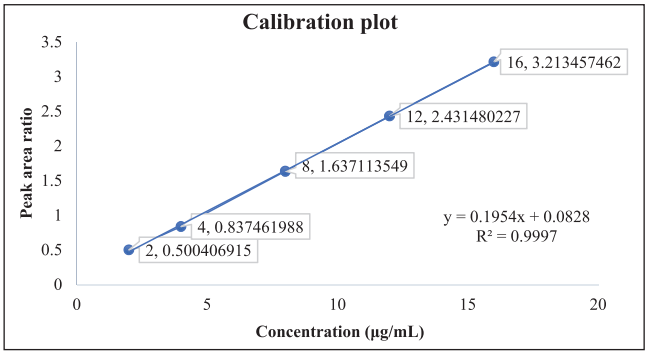

Figure 3: Calibration curve of Ivabradine Hydrochloride

Table 1: Calibration curve data of reversed-phase high-performance liquid chromatography method

Concentration (µg/mL)	Ivabradine hydrochloride	Telmisartan	Peak area ratio[a]
2	35.97657	71.89463	0.500406915
4	75.35962	89.98572	0.837461988
8	141.01862	86.13857	1.637113549
12	215.46297	88.61391	2.431480227
16	300.50204	93.51362	3.213457462

[a]Average of three determinations

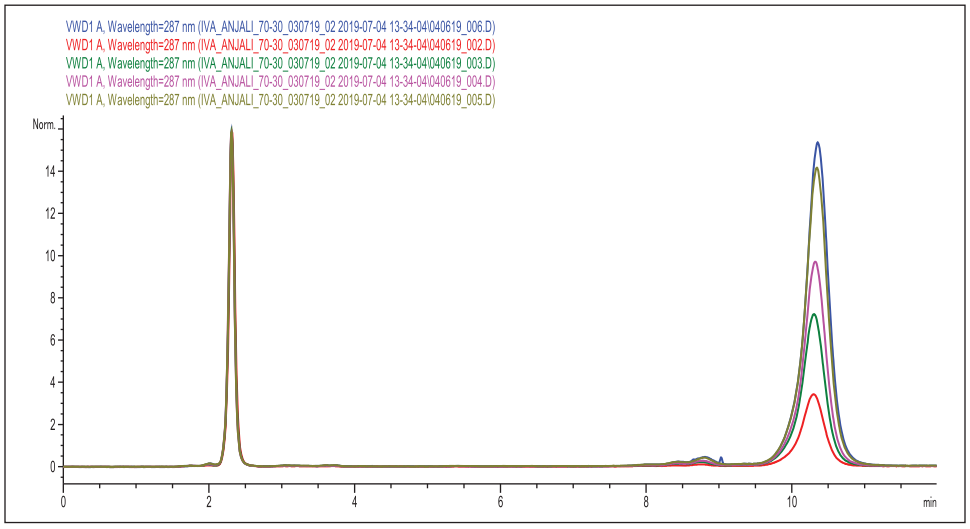

Figure 4: Overlay chromatogram of Ivabradine Hydrochloride (2–16 µg/mL)

Table 2: Results of system suitability parameter

S. no	Parameter	Ivabradine
1.	Retention time	10.71 min
2.	Theoretical plates (N)	5801
3.	Tailing factor	1.07
4.	Peak area	146.65694 mAU
5.	Resolution (R)	20

Table 3: Analyte solution stability data of reversed-phase high-performance liquid chromatography method

Concentration (µg/mL)	Time (h)	Peak area ratio	Mean peak area ratio	Standard deviation	%CV
8	0	3.0647	3.06105	0.0052	0.1698
	6	3.0574			

Acceptance criteria—Less than or equal to 2

Table 4: Sensitivity data of reversed-phase high-performance liquid chromatography method

S. no	Drug	LOD	LOQ
1	Ivabradine hydrochloride	0.7402 µg/mL	2.2433 µg/mL

Stability for analytical solution showed the %CV- % coefficient of variation in the acceptance range, proving the stability of the analytical solution.

Sensitivity (limit of quantification)

LOQ is the least concentration in the calibration curve, which is 2.2 µg/mL [Table 4].

The analyte response at the LOQ is more than five times the blank response. Analyte peak (response) is identifiable, discrete, and reproducible. It agreed the acceptance criteria.

Precision

Repeatability: The repeatability data are shown in Table 5.

Interday precision: The interday precision data are shown in Table 6.

Intraday precision: The intraday precision data are shown in Table 7.

The calculated % relative standard deviation values are very low, indicating the precision of the method. The %RSD values less than or equal to 2 is the acceptance criteria.

Table 5: Repeatability data of reversed-phase high-performance liquid chromatography method

Ivabradine hydrochloride	Telmisartan	Peak area ratio	Mean[a] ± Standard deviation	% RSD
149.7861	64.5735	2.3196	2.3218 ± 0.0067	0.2923
149.6413	64.4732	2.3209		
149.2154	64.5485	2.3116		
149.8920	64.3620	2.3288		
149.9896	64.3723	2.3300		
149.3293	64.3745	2.3196		

[a]Average of six determinations

Accuracy (drug substance)

Accuracy of the method is determined at three different concentration levels. Mean and %RSD values were calculated and are shown in Table 8.

The calculated %RSD values indicate that the method is accurate.

Recovery studies (drug product)

The recovery values indicate that extraction efficiency for the analyte is consistent and reproducible. The results are shown in Table 9.

The % recovery should be between 90% and 110%, and the found results were between the acceptance range.

Assay (drug product)

The assay of Ivabrad 5 tablets containing ivabradine hydrochloride is found to be 96.04%. The assay values are shown in Table 10.

Validation of bioanalytical method

After completion of several trials with different solvents and varying solubility-enhancing techniques, spectrum with good absorbance and stability, that is, trial 6 was selected [Table 11]. The absorption spectra are recorded in wavelength region of 200–400 nm (UV region). The λ_{max} of the drug was found to be at 287 nm.

Matrix effect

It is necessary to develop an extraction method, which gives consistent and reproducible recovery of the analyte from

Table 6: Interday precision data of reversed-phase high-performance liquid chromatography method

Precision level	Time points	Ivabradine hydrochloride	Telmisartan	Peak area ratio	Mean[a] ± standard deviation	%RSD
80%	Day 1	117.905	47.417	2.486	2.456 ± 0.027	1.111
	Day 2	115.378	47.424	2.432		
	Day 3	116.101	47.373	2.450		
100%	Day 1	147.915	47.687	3.101	3.065 ± 0.034	1.122
	Day 2	147.306	48.559	3.033		
	Day 3	146.529	47.881	3.060		
120%	Day 1	168.498	46.511	3.622	3.572 ± 0.044	1.248
	Day 2	168.903	47.755	3.536		
	Day 3	169.547	47.640	3.558		

[a]Average of three determinations

Table 7: Intraday precision data of reversed-phase high-performance liquid chromatography method

Precision level	Time points	Ivabradine hydrochloride	Telmisartan	Peak area ratio	Mean[a] ± standard deviation	%RSD
80%	Morning	116.889	48.104	2.429	2.412 ± 0.016	0.698
	Afternoon	116.096	48.116	2.412		
	Evening	115.816	48.333	2.396		
100%	Morning	158.143	51.009	3.100	3.082 ± 0.015	0.501
	Afternoon	157.761	51.362	3.071		
	Evening	157.650	51.251	3.076		
120%	Morning	168.815	47.915	3.523	3.518 ± 0.005	0.146
	Afternoon	167.892	47.792	3.512		
	Evening	167.781	47.681	3.518		

[a]Average of three determinations

Table 8: Accuracy data of reversed-phase high-performance liquid chromatography method

Accuracy level	Ivabradine hydrochloride	Telmisartan	Peak area ratio	Mean[a] ± standard deviation	%RSD
80%	118.84202	62.2459	1.9092	1.9088 ± 0.0108	0.56644
	119.4466	62.22942	1.9194		
	119.35729	62.89108	1.8978		
100%	150.66374	64.50385	2.3357	2.3372 ± 0.0013	0.0569
	150.47194	64.35564	2.3381		
	150.45505	64.35404	2.3379		
120%	186.25127	63.85784	2.9166	2.9106 ± 0.0068	0.2353
	185.57573	63.92154	2.9031		
	186.1443	63.92217	2.9120		

[a]Average of three determinations

Table 9: Recovery studies data of ivabradine formulation

Accuracy level	Ivabradine hydrochloride	Telmisartan	Peak area ratio	Mean[a]	Conc. of sample practically	% Recovery
80%	250.59729	84.88966	2.9520	2.9442	14.38772	99.9147
	250.99866	86.23467	2.9106			
	251.01246	84.51043	2.9701			
100%	274.69354	85.25449	3.2220	3.2541	15.9021	99.3881
	275.75879	84.34561	3.2693			
	275.84968	84.32802	3.2711			
120%	304.53903	83.9169	3.6290	3.6095	17.6388	100.2205
	305.99866	84.84388	3.6066			
	305.61725	85.05636	3.5931			

[a]Average of three determinations

Table 10: Assay of ivabradine hydrochloride tablets

Formulation	Ivabradine hydrochloride	Telmisartan	Peak area ratio	Mean[a]	Amount of drug	% Purity
Ivabrad 5 (tablet)	189.23371	35.43927	5.33966	5.3504	4.8021	96.04
	188.49611	35.53148	5.30504			
	191.54312	35.42816	5.40652			

[a]Average of three determinations
90%–110% is the acceptance range, and the results found (96.04%) fits in the range

Table 11: Trials for method development and method optimization

Trials	Solvent used	Wave length	Absorbance	Observation	Result
Trial 1	Ammonium acetate buffer (pH 7.8)	287 nm	0.156	Negligible absorbance	Method rejected
Trial 2	Phosphate buffer (pH 3.0)	287 nm	0.603	Unstable	Method rejected
Trial 3	NaCl as hydrotropic agent	287 nm	0.613	Unstable	Method rejected
Trial 4	Urea	287 nm	0.917	Unstable	Method rejected
Trial 5	Tridistilled water (pH 7.6)	287 nm	0.839	Unstable	Method rejected
Trial 6	Methanol	287 nm	0.439	Stable with good absorbance	Method accepted

Table 12: Matrix effect data

Concentration (ng/mL)	Extracted plasma	Standard solution	Matrix factor	Matrix effect
500	0.005	0.006	0.833333333	0.934505073
1000	0.015	0.016	0.9375	
1500	0.025	0.027	0.925925926	
2000	0.037	0.039	0.948717949	
2500	0.048	0.051	0.941176471	
3000	0.058	0.059	0.983050847	
3500	0.069	0.071	0.971830986	

plasma. Drug is extracted from plasma by solvent extraction technique using acetonitrile. It is shown in Table 12], and the spectra are shown in Figure 5.

The quantitative measure of matrix effects[35] is the matrix factor (MF). The value of MF less than one signifies suppression, MF value greater than one signifies enhancement, and MF value equal to one implies that the analytical method is free from matrix effect. The obtained value of MF is 0.9994. It can be considered as free from matrix effect. The values are noted in Table 12.

As such in untreated plasma, disturbance was noted; therefore, it made the plasma treatment necessary step. The plasma is treated with different volumes of acetonitrile to enhance the

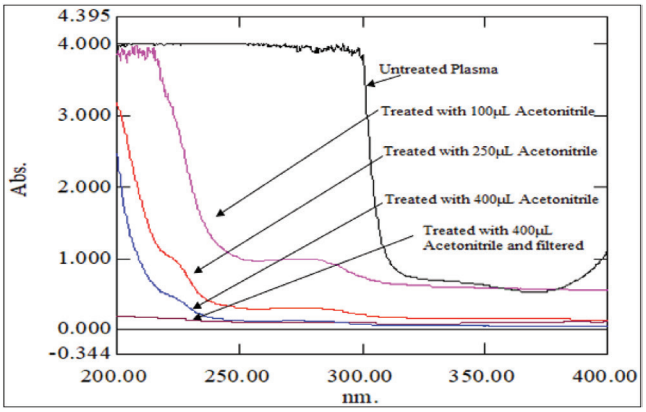

Figure 5: Spectra of treated plasma

Table 13: Linearity data of ivabradine hydrochloride in plasma

Concentration (ng/mL)	Absorbance[a] (nm)
500	0.005
1000	0.015
1500	0.025
2000	0.037
2500	0.048
3000	0.058
3500	0.069

[a]Average of five determinations

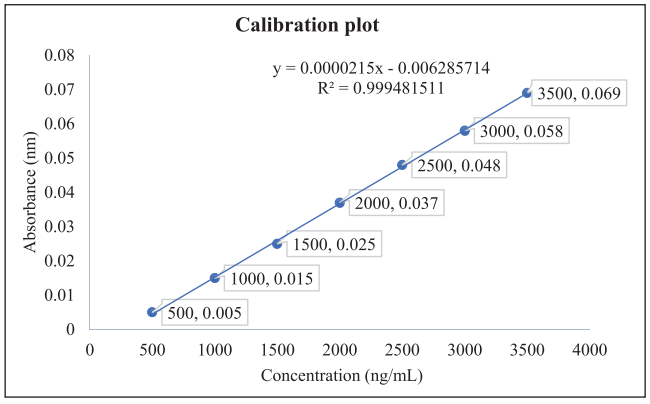

Figure 6: Calibration graph of ivabradine hydrochloride spiked in plasma

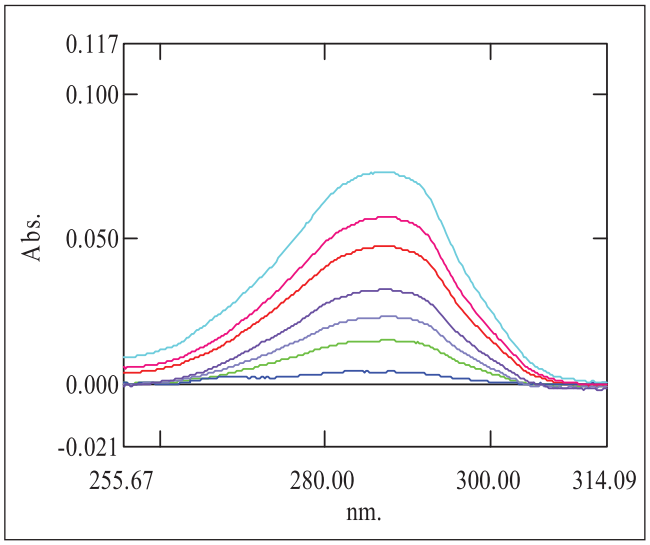

Figure 7: Overlay spectrum of ivabradine hydrochloride spiked in plasma (500–3500 ng/mL)

results. 400 µL of acetonitrile showed good results. The treated plasma is then filtered and this process is implemented for the preparation of samples throughout the study. The spectrum is shown in Figure 5.

Method validation

The optimized UV-spectrophotometric method is validated according to the United States Food and Drug Administration (USFDA) guidelines[36] "Bioanalytical Method Validation."

Linearity

Linear regression analysis is performed for checking the linearity of the data obtained. The response of the drug is found to be linear in the concentration range 500–3500 ng.

The linear regression equation for ivabradine hydrochloride is $y = 0.0000215x - 0.00628571$ with $r^2 = 0.9994$.

The calibration curve data are shown in Table 13, and calibration plot is shown in Figure 6, and calibration spectra in Figure 7.

Selectivity

There is no interference observed in blank plasma.

Sensitivity (lower limit of quantification)

LLOQ is the least concentration in the calibration curve, which is 500 ng/mL.

The analyte response at the LLOQ is more than five times the blank response. It can be accepted.

Optical conditions and statistical data of regression equation

The optimum conditions such as Beer's Law limits, sensitivity, and other regression characteristics such as slope (m), intercept (C), and correlation coefficient were calculated and presented in Table 14.

Accuracy (DS)

Accuracy of the method is determined at four different concentration levels. Mean and %CV values were calculated, and are shown in Table 15. The results indicate that the method is accurate.

% CV for analyte concentration in five replicate standard samples should not be more than 15.0 in the acceptance criteria.

Recovery studies

They represent the extraction efficiency of a method. They are performed at lower quality control (LQC), middle quality control (MQC), and higher quality control (HQC). Six replicates at LQC, MQC and HQC level were prepared for recording determination. Mean relative recovery was found to be 101.62. The data for relative recovery are given in Table 16. The results indicate that extraction efficiency for the analyte is consistent and reproducible.

Recovery data of ivabradine hydrochloride tablets is $\%C: (B/A) \times 100$.

% Recovery between 85% and 115% is the acceptance criteria.

Precision

Precision of the data is reported in terms of repeatability [Table 17], intraday [Table 18], and interday precision [Table 19].

The calculated %CV values are very low, indicating that the method is precise. The %CV for acceptance of sample should not be more than 15%.

Stability of analyte solutions

After completion of the storage times for different types of stabilities, sample is tested by comparing the instrument response with that of freshly prepared solution.

Freeze and thaw, short-term temperature, and stock solution stability studies were performed for the plasma samples spiked with the drug. They are noted in Tables 20–22.

Table 14: Optimized UV-spectroscopic conditions

Conditions	Ivabradine hydrochloride
Method	Bioanalytical method
Solvent	Methanol
λ_{max}	287 nm
Beer's Law range	500–3500 ng/mL
Lower limit of quantitation (ng/mL) (LLOQ)	500 ng/mL
Regression equation	$y = 0.0000215x - 0.006285714$
Slope	0.00002
Intercept	0.00628
Correlation coefficient (r^2)	0.9994

There should not exist much difference between test and freshly prepared solutions in terms of absorbance in the set acceptance criteria.

Assay of ivabradine hydrochloride tablets

The data obtained after analyzing five replicates of formulation samples spiked in plasma is given in Table 23.

The % recovery found is 98%, which falls in the acceptance range, which is 90%–110%.

Conclusion

Analytical and bioanalytical methods were developed for the estimation of ivabradine hydrochloride in pure and pharmaceutical dosage form.

In analytical method, ivabradine hydrochloride is estimated using telmisartan as internal standard on Agilent RP-HPLC. The developed analytical method is validated according to ICH guidelines. MP consisted of acetonitrile and ammonium acetate buffer pH 7.8 (60:40% vol/vol), and Waters 125A (10 µ,

Table 15: Accuracy data of ivabradine hydrochloride in plasma

Accuracy level	Concentration	Mean absorbance ratio[a] ± standard deviation	% CV
LLOQ	500 ng/mL	0.0104 ± 0.00055	5.26656
LQC	750 ng/mL	0.0206 ± 0.00055	2.65885
MQC	2000 ng/mL	0.0402 ± 0.00045	1.11247
HQC	3250 ng/mL	0.0726 ± 0.00055	0.75444

[a]Average of five determinations

Table 16: Recovery data of ivabradine hydrochloride tablets

Formulation	Amount of sample added (ng/mL)	Amount of standard added (ng/mL)	*Mean absorbance	Total amount added (A)	Total amount recovered (B)	%Recovery (%C)
Ivabrad 5	750	2000	0.052	2750	2650	96.36
	2000	2000	0.081	4000	3968	99.2
	3250	2000	0.132	5750	6286	109.3

*Average of five determinations

Table 17: Repeatability data of ivabradine hydrochloride in plasma

S. no.	Concentration	Absorbance	Mean[a] ± standard deviation	%CV
1	2000 ng/mL	0.038	0.03816±0.00041	1.06965
2	2000 ng/mL	0.038		
3	2000 ng/mL	0.038		
4	2000 ng/mL	0.038		
5	2000 ng/mL	0.039		
6	2000 ng/mL	0.038		

[a]Average of six determinations

Table 18: Intraday precision data of ivabradine hydrochloride in plasma

S. no	Concentration (ng/mL)	Mean absorbance ratio[a]		Mean[b] ± standard deviation	%CV
		Morning	Afternoon		
1	LLOQ (500)	0.0222	0.0202	0.0212 ± 0.00114	5.3552
2	LQC (750)	0.0294	0.0292	0.0293 ± 0.00048	1.6486
3	MQC (2000)	0.0522	0.0482	0.0502 ± 0.00215	4.2827
4	HQC (3250)	0.0922	0.0918	0.092 ± 0.00047	0.5124

[a,b]Average of five determinations

300 × 3.9 mm) C18 column is used. The method is linear in the range of 2–16 µg/mL.

A new sensitive bioanalytical method is developed on Shimadzu, UV-1800 spectrophotometer for the estimation of ivabradine hydrochloride in rats. Analysis is carried out at λ_{max}, 287 nm. The developed method is optimized and validated according to USFDA guidelines. The sensitivity of the method LLOQ is determined at 500 ng/mL. The method is linear in the range of 500–3500 ng/mL. All the validation parameters were found to be within the acceptable limits. As the method is sensitive, it is successfully applied to the analysis of ivabradine hydrochloride in spiked rat plasma.

Developed RP-HPLC method is sensitive and reproducible, which can be used for routine QC of ivabradine hydrochloride dosage forms. Bioanalytical method developed can be used for pharmacokinetic studies of new formulations developed for ivabradine hydrochloride.

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Nil.

Table 19: Interday precision data of ivabradine hydrochloride in plasma

S. no	Concentration (ng/mL)	Mean absorbance ratio[a]		Mean[b] ± standard deviation	%CV
		Day 1	Day 2		
1	LLOQ (500)	0.0156	0.016	0.0158 ± 0.0019	12.22
2	LQC (750)	0.0204	0.0172	0.0188 ± 0.0018	9.65
3	MQC (2000)	0.0498	0.0498	0.0498 ± 0.0027	5.50
4	HQC (3250)	0.0844	0.0916	0.088 ± 0.0047	5.38

[a,b]Average of five determinations

Table 20: Freeze and thaw stability of plasma samples

S. no	Concentration (ng/mL)	Time (h)	Mean absorbance ratio[a]	Mean assay[b] ± standard deviation	%CV
1	LLOQ (500)	0	0.016	0.0175 ± 0.0064	12.12183053
		72	0.019		
2	HQC (3250)	0	0.089	0.0825 ± 0.0092	11.14228867
		72	0.076		

[a,b]Average of five determinations

Table 21: Short-term stability of plasma samples

S. no	Concentration (ng/mL)	Time (h)	Mean absorbance ratio[a]	Mean assay[b] ± standard deviation	%CV
1	LLOQ (500)	0	0.02	0.0165 ± 0.0021	12.85648693
		24	0.015		
2	HQC (3250)	0	0.106	0.097 ± 0.0127	13.12156914
		24	0.088		

[a,b]Average of five determinations

Table 22: Stock solution stability of plasma samples

S. no	Concentration (ng/mL)	Time (h)	Mean absorbance ratio[a]	Mean assay[b] ± standard deviation	%CV
1	LLOQ (500)	0	0.022	0.0175 ± 0.0021	12.12
		6	0.016		
2	HQC (3250)	0	0.095	0.095 ± 0.0056	5.59
		6	0.091		

[a,b]Average of five determinations

Table 23: Assay data of ivabradine hydrochloride tablets

Formulation	Labeled amount (mg)	Absorbance	*Amount obtained (mg)	% Recovered
Ivabrad 5 (tablet)	5	0.104	4.9	98
		0.104		
		0.105		
		0.104		
		0.107		

*Average of five replicates

Conflicts of interest

There are no conflicts of interest.

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