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Development and Validation of UV-Vis Spectroscopy Method for the Determination of Ivabradine Hydrochloride and Metoprolol Succinate in Tablet Dosage Form

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Abstract: ***A simple, rapid and specific UV spectroscopic method with good sensitivity was developed and validated for the simultaneous determination of Ivabradine hydrochloride and Metoprolol Succinate in combined pharmaceutical dosage form. In distilled water the λ max of Ivabradine hydrochloride and Metoprolol Succinate were fixed as 286.4 and 224 nm respectively using a Shimadzu UV-Visible spectrophotometer. In this proposed method both drugs obeyed linearity within the concentration range of 1-5 µg/ml and 5-25 µg/ml for Ivabradine hydrochloride and Metoprolol Succinate respectively. The low RSD values indicate good precision and high recovery values indicate accuracy of the proposed method. The proposed method has been applied to the determination of drugs in commercial formulations. Assay results were in good agreement with label claim. The method was validated as per ICH guidelines. The developed method was simple, accurate, precise, specific, sensitive and reproducible which can be efficiently and easily applied to pharmaceutical dosage forms.***

Keywords: Ivabradine Hydrochloride, Metoprolol Succinate, simultaneous estimation, validation distilled water, UV spectroscopy

1. Introduction

Ivabradine Hydrochloride (IVA), chemically, 3-[3-[[(7S)-3,4-dimethoxy-7-bicyclo [4.2.0]octa-1,3,5-trienyl] methylmethylamino] propyl]-7,8-dimethoxy-2,5-dihydro-1H- 3-benzazepin-4-one; hydrochloride. Ivabradine is a heart-rate-lowering agent that acts by selectively and specifically inhibiting the cardiac pacemaker current (I_f), a mixed sodium-potassium inward current that controls the spontaneous diastolic depolarization in the sinoatrial (SA) node and hence regulates the heart rate. The molecular channel belongs to the HCN family.[10] Inhibition of this channel disrupts I_f ion current flow, thereby prolonging diastolic depolarization, slowing firing in the SA node, and ultimately reducing the heart rate.[14] The cardiac effects of ivabradine are specific to the SA node, and the drug has no effect on blood pressure, intracardiac conduction, myocardial contractility, or ventricular repolarization.[10,13]

Ivabradine also inhibits the retinal current (I_h), which has properties similar to that of cardiac I_f.[15] I_h participates in the temporal resolution of the visual system by curtailing retinal responses to bright light stimuli.[15] Under triggering circumstances, such as rapid changes in luminosity, partial inhibition of I_h may underlie the luminous phenomena (phosphenes) experienced by patients.[1-6]

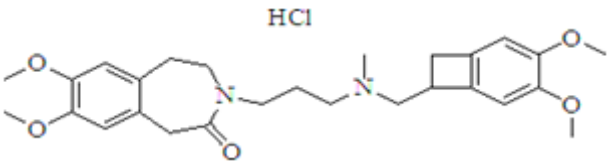

Figure 1: Structure of Ivabradine Hydrochloride

Metoprolol succinate (METO), chemically 1-[4-(-methoxyethyl)phenoxy]-3-(propan- ylamino)propan-2-ol. Whcich used as cardiovascular drug. METO is a beta1-selective (cardioselective) adrenergic receptor blocker. This preferential effect is not absolute, however, and at higher plasma concentrations, METO also inhibits beta2-adrenoreceptars, chiefly located in the bronchial and vascular musculature. Reduction in heart rate and cardiac output at rest and upon exercise. Reduction of systolic blood pressure upon exercise, inhibition of isoproterenol-induced tachycardia, and reduction of reflex orthostatic tachycardia[7].

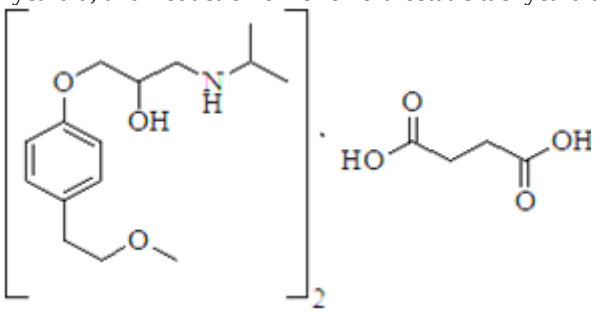

Figure 2: Structure of Metoprolol Succinate

It is soluble in water and methanol. For this combination literature survey revealed that there are a very few methods available for individual and simultaneous estimation of IVA and METO. The present work aims at developing a simple, sensitive, accurate and precise method for the effective quantitative estimation of IVA and METO as Active Pharmaceutical Ingredient (API) as well as in pharmaceutical preparations without the interference of other constituents in the preparation. In summary, the primary objective of the proposed work is to develop a simple, sensitive and accurate method for the determination of IVA and METO in combination dosage form by UV- Spectrophotometry

2. Materials and Methods

Instruments

UV Spectroscopy. (Model: UV-1800, Shimadzu), Digital ultrasonic cleaner (sonicator): CD-4820, HMG India, Digital PH meter: Systronic, HPLC grade water system: Millipore,

Analytical weighing balance: Anamed; Model AA-2200. [max.200gm, min. 0.01gm; e = 0.0001g], UV probe software version-1.4.2, Hamilton syringe of 100 ul capacity.

Chemicals

Manufactured pure Ivabradine hydrochloride and metoprolol succinte were obtained as gift sample from Lupin Research park , Aurangabad. The tablet dosage form Ivamet XL 5/25 (claim: Ivabradine 5mg and 25mg metoprolol) was procured from local market.

Preparation of standard stock solution

An accurately weighed quantity of (100 mg) of IVA and METO was dissolved in separate 100 ml volumetric flask with a part of distilled water and then volume was made up up to 100 ml with distilled water.

Preparation of working standard (10µg/ml)

The standard stock solution was further diluted with distilled water to get 10 µg/ml concentrations of each drug. It was scanned in UV range of 200-400 nm.

Selection of λ max

From the overlain spectra of IVA and METO at concentration 10µg/ml of each, the λ max 286.4 nm for IVA and 224 nm for METO were selected. The overlain spectra of both the drug is shown in figure 3.

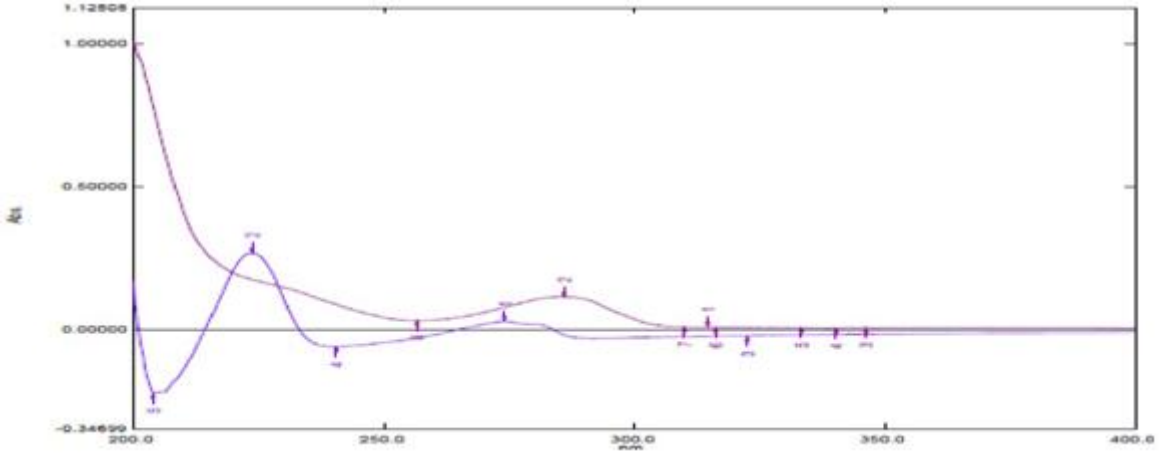

Figure 3: Overlain UV spectra of IVA Hcl and METO succinate in water

Equation for simultaneous estimation

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2} \ldots\ldots\ldots (1)$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2} \ldots\ldots\ldots (2)$$

Where,

Cx = Concentration of IVA in µg/ ml
Cy = Concentration of METO in µg/ml
A1 = Absorbance of mixture at λ1 (286.4 nm)
A2 = Absorbance of mixture at λ2 (224)
ax_1 = absorptivity of IVA at λ1 (286.4 nm)
ax_2 = absorptivity of IVA at λ2 (224)
ay_1 = absorptivity of METO at λ1 (286nm)
ay_2 = absorptivity of METO at λ2 (224)

3. Preparation of sample solution

Twenty tablets were accurately weighed and average weight was calculated, the tablets were triturated in mortar and pestle to form fine powder. The powder equivalent to 5 mg of IVA and 25 mg of METO was transferred in 100 ml of volumetric flask and volume was made up to 100 ml with distilled water. It was filter through Whatmann filter paper No. 41 and further diluted with water to get concentration of 5 µg/ml and 25 µg/ml of IVA and METO respectively and its absorbance was recorded at 286.4 and 224 nm. Its drug content was estimated by developed simultaneous equation at 286.4 and 224 nm respectively.

Analysis of marketed formulations

The effective use of developed method has been demonstrated by analysis of combined tablet formulation. Hence, the developed method was employed for quantitative estimation of IVA and METO . In combine tablet dosage form. Data of marketed formulation and analysis showed in table no.1 & 2

Table 1: Marketed formulation

Formulation	Content	Company
IVA Met XL 5/25	Ivabradine 5mg Metoprolol 25 Mg	Ajanta pharma limited, Mumbai (w) 400 067, India

Table 2: Analysis of marketed formulations data

	Label claim (mg/tab)		Amount found (mg/tab)		% label claim	
Sr. No.	IVA	METO	IVA	METO	IVA	METO
1	5	25	4.928	25.254	98.56	101.06
2	5	25	4.866	24.947	97.32	99.78
3	5	25	5.07825	25.215	101.56	100.93
4	5	25	4.925	25.147	98.5	100.88
5	5	25	5.110	25.223	102.2	100.58
6	5	25	4.944	25.352	98.88	101.46
				Average	99.5033	100.782
				SD	1.7585	0.5680
				% RSD	1.767277	0.564

Method Validation

The proposed spectrophotometric method was validated with respect to following parameters, as per ICH guidelines. Calibration curves were prepared in the concentration range of 1-5µg/ml for IVA and 5-25µg/ml for METO. Linearity was demonstrated by analysing five different concentrations of active compound. Absorbances were recorded for all the samples and calibration plot was constructed by plotting

absorbance Vs concentrations of IVA and METO. Accuracy was determined by recovery studies with Two standard solutions containing known concentration of drugs and the percentage recoveries of the added drugs were determined. Precision was evaluated in terms of intra-day and inter-day precision. The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding concentration six times on the same day and six times on the different days and the results were reported. LOD and LOQ values were calculated from the calibration curves.

1) **Linearity:**
   Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for IVA was found to be 1-5µg/ml and for METO was found to be 5-25µg/ml. Standard solutions of drugs containing IVA and METO was prepared and scanned for absorbance at 286.4 nm and 224 nm respectively. Linearity data showed in table 3,4 Calibration curve of IVA & METO showed in fig.4&5
2) **Repeatability**
   To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Six samples of the API were analyzed for the repeatability study. The standard deviation and relative standard deviation was calculated. Repeatability study is carried out for remove Instrumental error. The % RSD was found to be 1.189 and 0.7317 for IVA and METO. The % RSD is less than 2 indicate the given method can be repeatable. The results are shown in Table 5.
3) **Limit of detection and limit of quantitation**
   The LOD and LOQ were separately determined based on calibration curve. The residual standard deviation of a regression line or the standard deviation of y- intercepts of regression lines were used to calculate the LOD and LOQ. LOD and LOQ of IVA and METO showed in table no.6
   I. Formula for LOD (µg/ml);
   LOD = 3.3 x SD / S
   Where, SD = The standard deviation of the response
   S = The slope of the calibration curve (mean)

   II. Formula for LOQ (µg/ml);
   LOQ = 10 x SD / S
   Where, SD = The standard deviation of the response
   S = The slope of the calibration curve (mean).
4) **Accuracy:**
   The accuracy of the method was ascertained by carrying out recovery studiesusing standard addition method. The recovery studies are perfomed to determine if there was any positive or negative interference from excipients present in the formulation. The percentage recovery results revealed that the values were near to 100%, which indicates that the proposed method is accurate as the results are within the official limits. It also reveals that the commonly used excipients and additives in the formulation were not interfering with the proposed method. (Table no: 7, 8, 9)
5) **Precision:**
   Precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to the multiple samplings of a homogeneous sample. It is expressed as ±S.D or RSD of series of measurements. Precision was ascertained by replicate estimation of drugs by proposed method. Precision studies carried out for intraday and inter-day variations of the responses. The inter-day and intraday precision was carried out and the results were found to be within limits. Interday precision was performed using same procedure for the analysis of marketed preparations in different days. The percent label claim was calculated using formula as in analysis of marketed formulation. The results shown in Table10

Force Degradation Study

The ICH guidelines entitled stability testing of new drug substances and product that required stress testing to be carried out to elucidated the inherent stability characteristics of the active substances. The aim of stressed degradation study was to check the stability of the IVA and METO on different condition applied for degradation study involved acid, base, neutral hydrolysis, thermal, sunlight and photolytic degradation. Summary of the force degradation study of IVA and METO are given in table 10.

Acid hydrolysis : To perform acid hydrolysis, added 10ml of stock solution of both drugs separately in 100 ml conical flask, then added 10 ml of 0.1M Hcl, refluxed for half hrs and 6 hrs at 60 ^{0}C for IVA and METO respectively. These solution further diluted with distilled water to form 2 µg/ml and 10 µg/ml of IVA and METO; respectively. Finally absorbance of sample measured and compared with standard absorbance to evaluate percent assay. The degradation spectra are shown in figure 6 a and 6 b

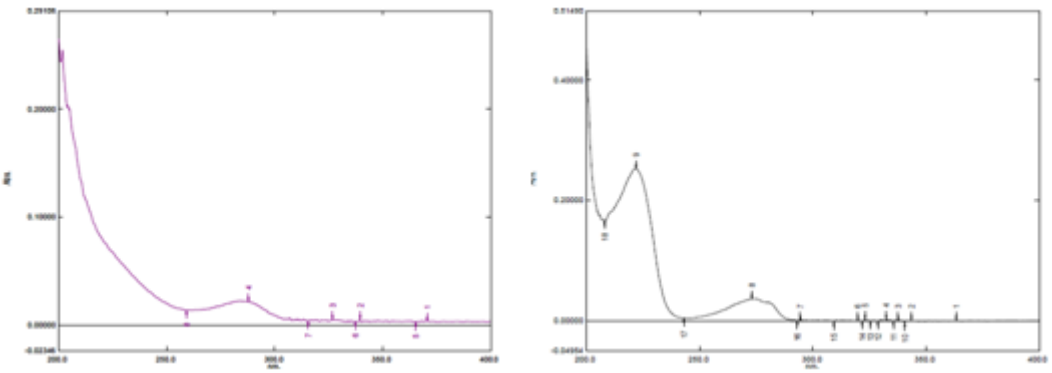

Figure 6 (a) and Figure 6 (b): Acid hydrolysis of IVA and METO respectively

Base hydrolysis: To perform base hydrolysis, added 10ml of stock solution of both drugs separately in 100 ml conical flask, then added 10 ml of 0.1 M NaoH, refluxed for 6 hrs and 8 hrs at 60 ^{0}C for IVA and METO; respectively. These solutions further diluted with distilled water to form 2µg/ml and 10 µg/ml of IVA and METO recepectively. Finally absorbance of sample measured and compared with standard absorbance to calculate percent assay. The degradation spectra are shown in figure 7a and 7b

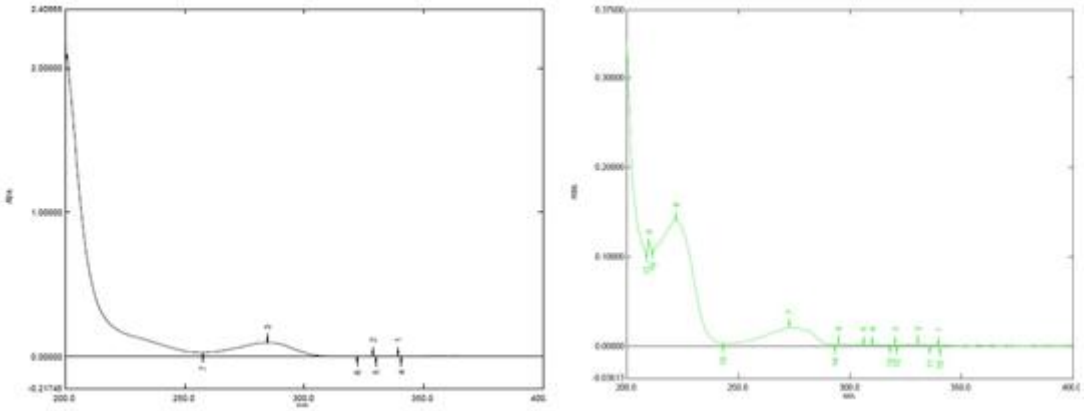

Figure 7 (a) and Figure 7 (b): Base hydrolysis of IVA and METO

Neutral hydrolysis: To perform neutral hydrolysis, added 10ml of stock solution of both drugs separately in 100 ml conical flask, then added 10 ml of distilled water,refluxed for 1.5 hrs and 8 hrs at 60 ^{0}C for IVA and METO respectively. These solutions further diluted with distilled water to form 2µg/ml and 10µg/ml of IVA and METO respectively. Finally absorbance of sample measured and compared with standard absorbance to calculate percent assay. The degradation spectra are shown in fig 8a and 8b.

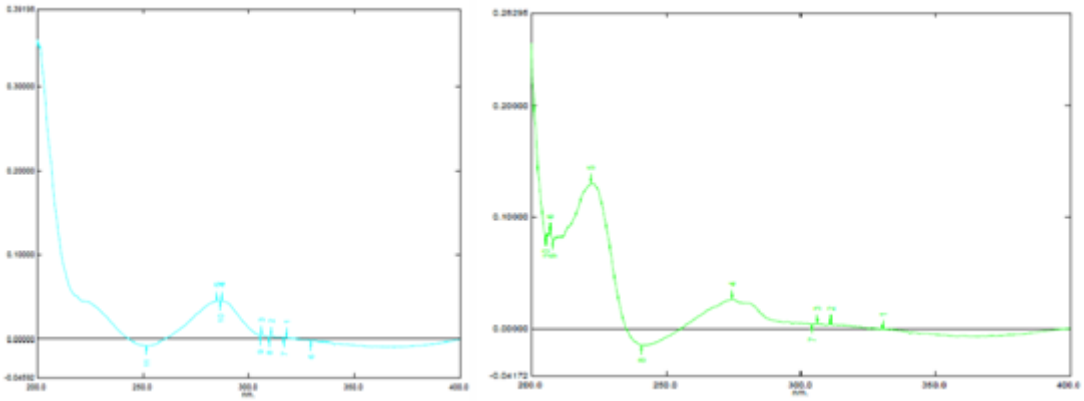

Figure 8 (a) and Figure 8 (b): neutral hydrolysis of IVA and METO

Oxidative hydrolysis: To perform oxidative hydrolysis , added 10ml of stock solution of both drugs separately in 100 ml conical flask, then added 10 ml of 3% H2O2, refluxed for 1 hrs and 4 hrs at 60 ^{0}C for IVA and METO respectively. These solutions further diluted with distilled water to form 2 µg/ml and 10 µg/ml of IVA and METO respectively. Finally absorbance of sample measured and compared with standard absorbance to calculate percent assay. The degradation spectra are shown in figure 9a and 9b.

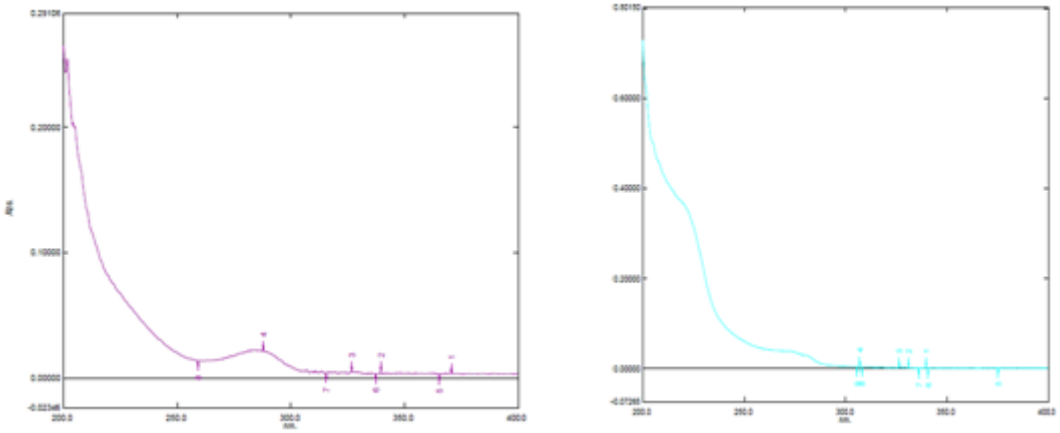

Figure 9 (a) and Figure 9 (b): Oxidative Hydrolysis of IVA and METO

Photolytic degradation: Photolytic degradation was carried out by exposing pure drug IVA and METO to UV radiation for 12 and 24 hrs respectively. The sample after exposure to light were diluted with distilled water to get 2 and 10 IVA and METO respectively. Finally absorbance of sample were measured and compared with standard absorbance to calculate percent degradation and percent assay. The degradation spectra are shown in figure 10a and 10b.

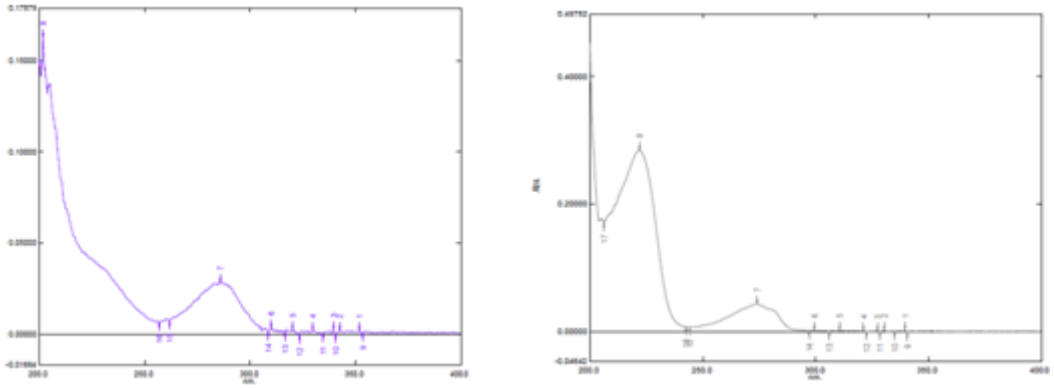

Figure 10 (a) and Figure 10 (b): Photolytic degradation of IVA and METO

Thermal degradation: Thermal degradation was carried out by exposing pure drug IVA and METO to dry heat at 80 ^{0}C for 24 hrs respectively. The sample after exposing to heat were diluted with distilled water to get 2 and 10 IVA and METO respectively. Finally absorbance of sample were measured and compared with standard absorbance to calculate percent degradation and percent assay. The degradation spectra are shown in figure 11a and11b

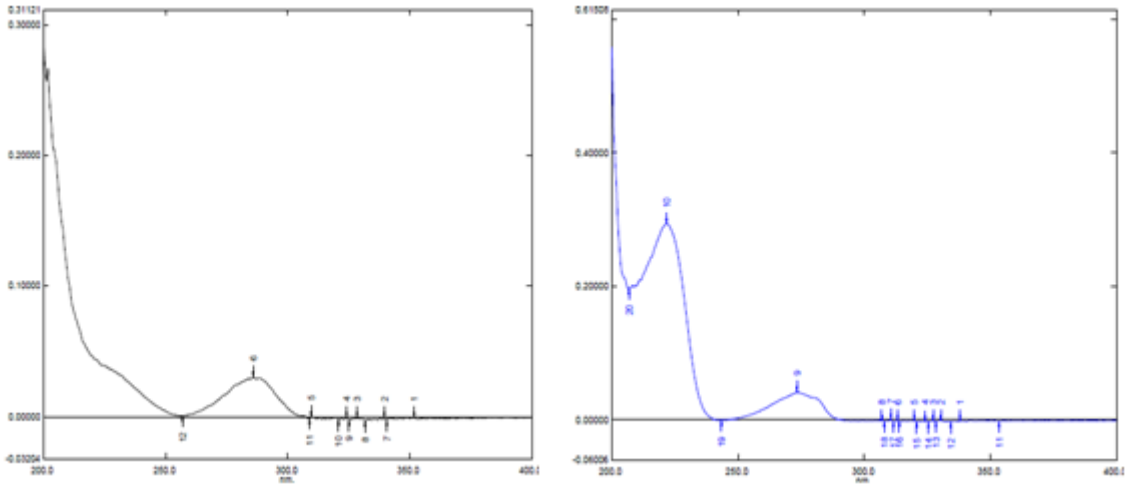

Figure 11 (a) and Figure 11 (b): thermal degradation of IVA and METO

4. Results and Discussion

Ivabradine Hydrochloride and Metoprolol Succinate showed maximum absorbance in Water at 286.4 and 224 nm. The proposed method for simultaneous estimation of both the drugs was validated as per the ICH guidelines The linearity was observed in the concentration range of 1-5 µg/ml for Ivabradine Hydrochloride and 5-30 µg/ml for Metoprolol succinate. The slope, and correlation coefficient values of Ivabradine Hydrochloride and Metoprolol Succinate are 0.0195, 0.9978 and 0.0296, 0.998. Amount of drugs estimated by the proposed method was in good agreement with the label claim. The accuracy of the method was assessed by recovery experiments .The precision of the method was studied as repeatability, intra-day and inter day variations; the %RSD less than 2, indicates proposed method is precise. Recovery was close to 100% for both the drugs.

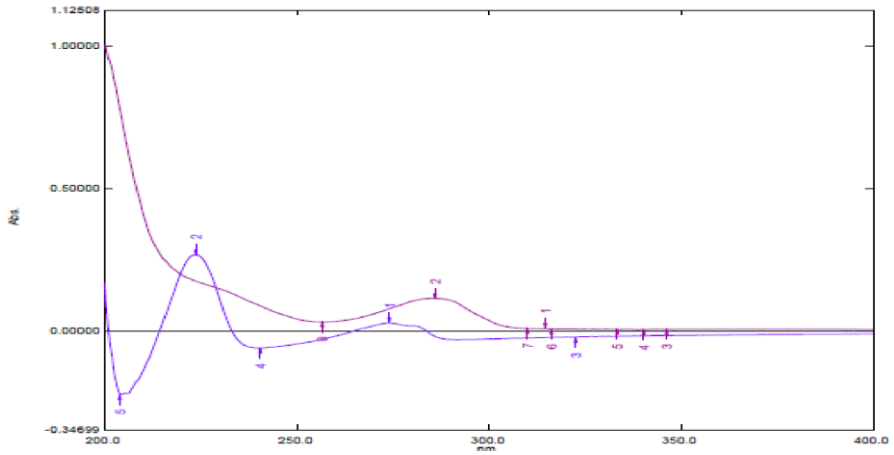

Figure 3: Overlain UV spectra of IVA Hcl and METO succinate in water Linearity of Calibration Curv

Table 3: Linearity data of IVA

Concentration (µg/ml)	Absorbance of IVA at 286.4nm
1	0.024
2	0.043
3	0.062
4	0.08
5	0.099

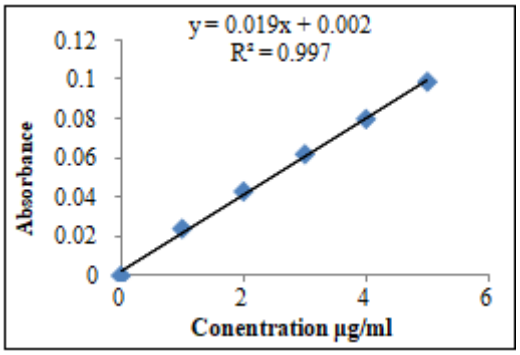

Figure 4: Calibration curve of IVA

Table 4: Liniarity of METO

Concentration (µg/ml)	Absorbance of METO at 224nm
5	0.116
10	0.265
15	0.434
20	0.556
25	0.711

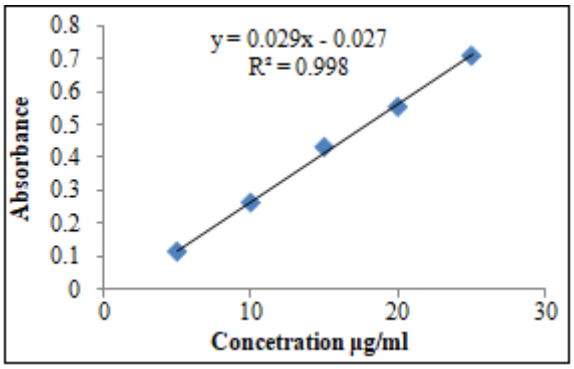

Figure 5: Calibration curve of METO

Table 5: Repeatability data

S no.	Concentration µg//ml		Absorbance		%Amount found	
	IVA	METO	IVA	METO	IVA	META
1	2	10	0.03010	0.345	98.56	99.18
2	2	10	0.0341	0.38699	99.78	100.27
3	2	10	0.03266	0.3696	99.34	98.95
4	2	10	0.03153	0.3568	99.89	98.72
5	2	10	0.03990	0.4515	102.21	100.75
6	2	10	0.03324	0.3761	99.47	99.81
				Average	99.7083	99.6133
				SD	1.1863	0.7288
				%RSD	1.189	0.7317

Table 6: LOD and LOQ

Drugs	LOD (µg/ml)	LOQ (µg/ml)
IVA	0.117815	0.357015
METO	3.068107	9.297295

Table 7: Recovery study data of Ivabradine hydrochloride

% drug added	Amount present in mg	Amount added in mg	Amount recovered in mg	% recovery
80	5	4	8.802	97.82
80	5	4	8.902	98.93
80	5	4	8.782	97.60
100	5	5	9.93	99.30
100	5	5	10.05	100.54
100	5	5	9.865	98.66
120	5	6	10.92	99.27
120	5	6	11.3	102.90
120	5	6	11.1	101.00
	5	4	8.802	97.82

Table 8: Recovery study data of Metoprolol succinate

% drug added	Amount present in mg	Amount added in mg	Amount recovered in mg	% recovery
80	25	20	45.25	100.57
80	25	20	45.05	100.11
80	25	20	45.95	102.14
100	25	25	51.425	100.22
100	25	25	48.775	97.55
100	25	25	50.3	100.61
120	25	30	53.72	97.71
120	25	30	54.37	98.89
120	25	30	54.9	99.84

Table 9: Statistical validation of accuracy data

Level of recovery	% mean recovery		SD		%RSD	
	IVA	METO	IVA	METO	IVA	METO
80%	98.11	100.94	0.580	0.320	0.591	0.310
100%	99.5	100.35	0.780	1.988	0.784	1.88
120%	101.05	98.81	1.482	0.871	1.466	0.86

Table 10: Results of precision study

Sr. No.	Interval of time	Concentration (µg/ml)		% Found	
		IVA	METO	IVA	METO
I	Intra-day	2	10	100.01	97.1
II		2	10	98.75	99.88
III		2	10	98.37	99.91
		Mean*		99.043	98.963
		SD		0.70092	1.317633
		% RSD		0.7076	1.331
I	Inter-day	2	10	101.56	99.86
II		2	10	98.3	100.018
III		2	10	97.32	103.52
		Mean*		99.06	101.13
		SD		1.812	1.689
		% RSD		1.829	1.67

Table 11: Summary of validation

Parameters	Results	
	IVA	**METO**
Absorbance Maximum	286.4nm	224 nm
Linearity range (µg/ml)	01-May	May-25
Regression equation (Y= mx+ C)	Y= 0.0195x	Y= 0.0296x
Correlation coefficient (R2)	0.9978	0.998
Precision(% RSD)		
Intraday (n=3)	0.707693	1.33144
Interday (n=3)	1.829	1.67
Limit of Detection (µg/ml)	0.117	3.068
Limit of Quantitation (µg/ml)	0.357015	9.297295
Accuracy 80%	0.591	0.3108
Accuracy 100%	0.784	1.887
Accuracy 120%	1.466	0.861

Table 12: Data of forced degradation study

Sr. no.	*Condition*	*% degradation*		*% assay*	
		IVA	*METO*	*IVA*	*METO*
1	Acid hydrolysis(0.1M HCl) 0.5 & 6 hr., 60^0	15.9277	29.56	84.0723	70.44
2	Base hydrolysis(0.1M NaOH) 6 & 8 hr. 60^0	4.77	33.33	95.23	66.67
3	Neutral hydrolysis (distilled water) 1.5&8 hr. 60^0	3.62	10.15	96.38	89.85
4	Oxidative degradation ($3\%H_2O_2$) 1&4hr. 60^0	27.76	4.6376	72.2495	95.36
5	Photolytic degradation (UV radiation) 12&14 hr.	7.061	17.98	92.939	82.02
6	Thermal degradation 24 hr. 80^0	4.762	1.29	95.238	98.71

5. Conclusion

The present study comprises a UV spectroscopic method of analysis for the simultaneous estimation of Ivabradine Hydrochloride and Metoprolol Succinate in tablet dosage form. From the study of validation parameters, it was observed that the method is specific, accurate, precise and reproducible .The proposed method could be applied to routine analysis in quality control laboratories.

6. Acknowledgement

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