SIMULTANEOUS ESTIMATION OF IVABRADINE HYDROCHLORIDE AND BISOPROLOL FUMARATE IN BULK AND FAST DISSOLVING FILM FORMULATION

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ABSTRACT:
When treating ischemic heart disease, beta-blockers are the most often prescribed medication. Newer pharmacological therapies, however, offer the greatest alternative treatment when beta-blockers develop intolerance. Bisoprolol Fumarate is beta blocking agent. Ivabradine hydrochloride is an antianginal drug. It reduces dose-dependent heart rate without modification of blood pressure and may be used in combination with beta-blockers or as an alternative to them. But there's no way to figure out how to take both of these medications together. Thus, in the presence of bisoprolol fumarate, this work gives a novel simple way of simultaneous evaluation of Ivabradine hydrochloride both in bulk and fast dissolving film form through UV spectrophotometric measurement.
First, the λ_{max} of each medications calculated. bisoprolol fumarate shows λ_{max} at 223 nm and ivabradine hydrochloride shows λ_{max} 286 nm. Regression values (R^2) of 0.9998 & 0.9935 indicated that both Ivabradine hydrochloride and Bisoprolol fumarate obeyed linearity. The calibration curves plotted by measuring the absorbance at a particular concentration. For Ivabradine, the minimum Limit of detection was found to be 1.944767 µg /ml and Limit of quantification was found to be 5.893234 µg /ml. Similarly, the Limit of detection for Bisoprolol fumarate was found to be 5.698735862 µg /ml and Limit of quantification of 17.26889655 µg /ml. RSD of the precision study was assessed to be within appropriate limits (RSD < 2.0%). Accuracy was demonstrated by the recovery values of both Ivabradine & Bisoprolol, which fell between 97% and 101%. The suggested Ultraviolet spectroscopic method used for the Simultaneous determination of Ivabradine and Bisoprolol is innovative, yet it also seems straightforward, accurate, dependable, and affordable, according to the results obtained.

KEYWORDS:
Ivabradine hydrochloride, Bisoprolol fumarate, Simultaneous Determination, Ultraviolet spectroscopy

INTRODUCTION

As a result of an imbalance in heart's blood stream and need for Oxygen, the most common ailment is ischemia. A patient's quality of life (QoL) can be negatively impacted and mortality is a common outcome of ischemic heart disease, which is present in angina [1]. A single medication may not be useful for ischemia due to the many causes involved. Pharmacological treatment, lifestyle control, myocardial revascularization, and a multimodal, customised approach are all essential components of an effective treatment plan. It has been detected that in various case studies of angina, co-occuring conditions including Diabetes & COPD might increases the development of β-blocker intolerance. In such situations, innovative pharmacological treatments are best substitute [2].

IVA belongs to BCS class-I &molecular weight is 505g/mo. Its molecular formula is $C_{27}H_{37}N_2O_5Cl$ IVA is selected as the primary treatment used for restoring a normal heart rate because it selectively targets the cardiac pacemaker current flowing via the sinoatrial node and lowers heartbeat rate without altering myocardial contractility or any pre-existing (if any) vascular tone. Ivabradine is not like beta-blockers or calcium channel blockers [1] [3]. It can be used in place of or in addition to beta-blockers, as it lowers dose-dependent heart rate without changing blood pressure. In 2015, the US Food and Drug Administration (USFDA) authorized Ivabradine hydrochloride as a medication to reduce heart rate [4].

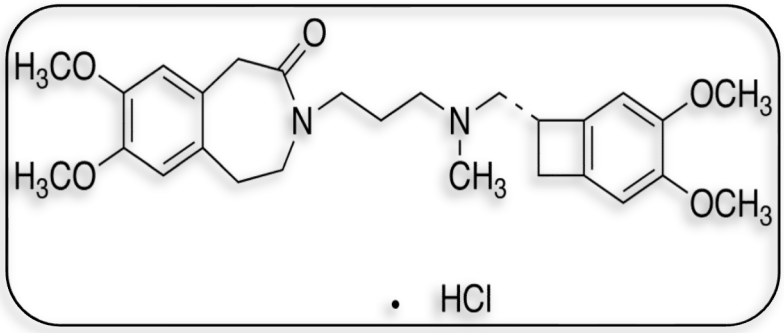

Figure 1: Structure of IVA [4]

Chemical compound BIS has the molecular formula $C_{40}H_{66}N_2O_{12}$ and the molecular weight 339.30 gm / mol. It is a 1-[(2,3,4-trimethoxyphnyl) methyl] piperazine dihydrochloride. BIS has anti-ischemic properties without changing hemodynamic parameters [5–9].

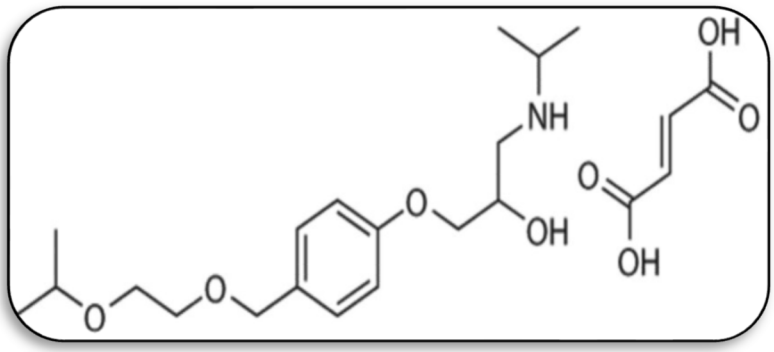

Figure 2: Structure of BIS

The evaluation said that, despite the lack of simultaneous estimations (SE) for this combination, basic Ultraviolet spectroscopy, High performance liquid chromatography, High performance thin layer chromatography, and Liquid chromatography-Mass spectroscopy methods were considered when evaluating these chemicals alone or in combination with other compounds.

Chemical mixtures containing two or more chemicals are often evaluated using the SE method within the same measuring equipment. This method has a few disadvantages when compared to other analytical methods. Thus, in order to facilitate assessment of given API in Pharmaceutical Formulations, attempts have been made to create exclusive and straightforward SE approach. The presented method has been effectively applied to develop SE method for IVA and BIS combined formulation. It has been validated. 10–14]
According to the related survey, there is currently no UV spectroscopic analysis that can be used to estimate the IVA and BIS combination simultaneously in a single unit-dosage form. Thus, the aim of this research work is to create and validate a new, straightforward UV analysis method for simultaneous IVA & BIS determination.

MATERIAL & METHOD:

Apparatus: UV-Vis Spectrophotometer (Shimadzu 1800 having a broad wavelength ranging 190 to 1100 nm used to measure the experimental study. Its features include two 10 mm quartz cells, an automated wavelength correction system, and a 1.5 nm spectral bandwidth. For sonication, a 2.5-liter Biomedica Sonicator was utilized. Glassware was used, including pipettes, beakers, and volumetric flasks.

Chemicals and Reagents: As gift samples, we received ivabradine hydrochloride from Lupin Limited in Sikkim, India, and bisoprolol fumarate from Sava Research Ltd in Maharashtra, India. The author created the 2.5 mg of BIS and IVA in the film.

Standard (stock) solutions of IVA: IVA stock solutions (100 μg/mL) were produced in simulated salivary fluid by dissolving 10 mg IVA in 50ml solvent & sonicated by sonicator for a sometime. After that make up the volume up to 100 mL using simulated salivary fluid.

Standard (stock) solutions of BIS: BIS stock solutions (100 μg/mL) were produced in simulated salivary fluid by dissolving 10 BIS in 50ml solvent & sonicated by sonicator for a sometime. After that make up the volume upto 100 mL using simulated salivary fluid.

Preparation of working standards of IVA:
To create working standards of 10–50μg/mL, 1–5 mL of the standard stock solution of IVA were taken into 10 ml volumetric flasks and make up the volume upto 10 ml by using simulated salivary fluid in a different volumetric flask.

Preparation of working standards of BIS:
To create working standards of 05–25 μg/ml, 0.5 – 2.5 mL of the standard stock solution of BIS were taken into 10 ml volumetric flasks and make up the volume upto 10 ml by using simulated salivary fluid in a different volumetric flask.

Plot of calibration curve for IVA & BIS:
λ_{max} of IVA found at value of 286 nm & λ_{max} of BIS found at value of 223 nm. These values were obtained from the standard (stock) solutions by preparing several working standards using Simulated Salivary fluid and examining them throughout the complete UV spectrum. In order to determine range of 10–50 μg/mL for ivabradine hydrochloride and 5–25 g/mL for bisoprolol fumarate, the linearity outline was investigated via progressively diluting the stock sample

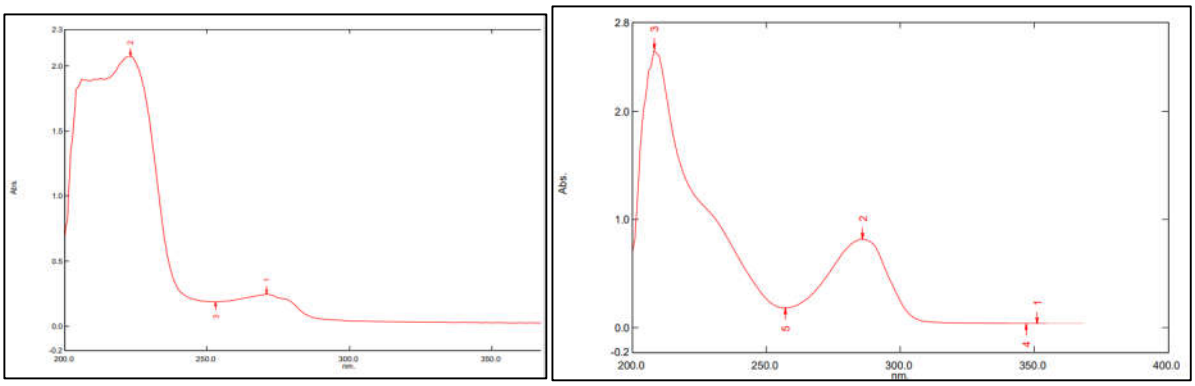

Figure 3: UV Spectra of BIS (223) and IVA (286)

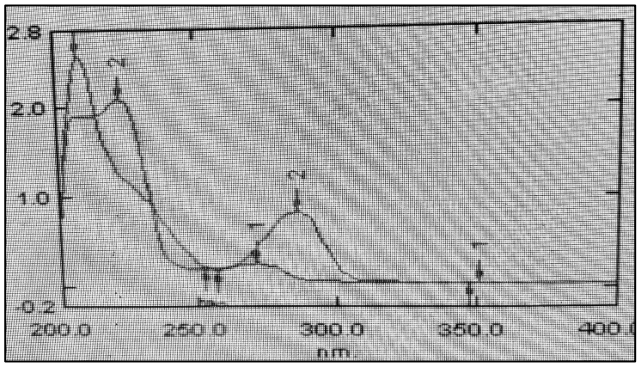

Figure 4: Overlay IVA and BIS spectra

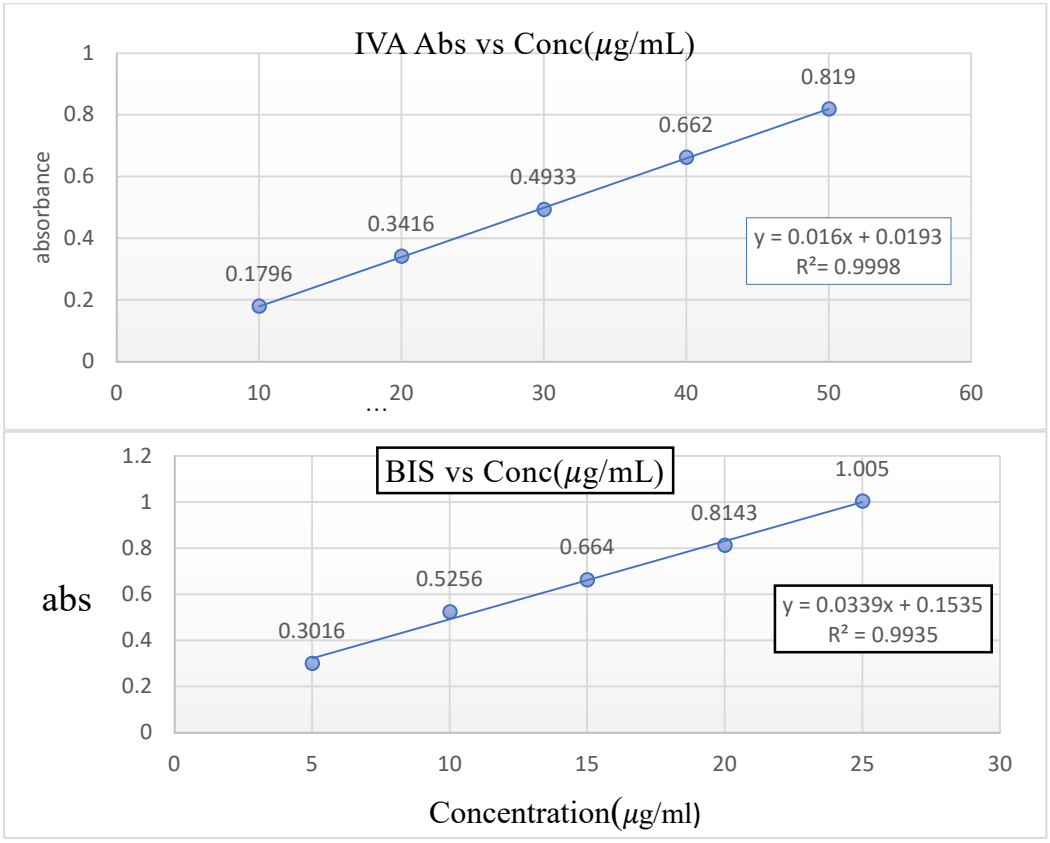

Figure 5: Calibration curve of IVA and BIS

Test number (IVA)	Absorbance at 286 nm	Conc.in (μg/ml)	Found Conc.	Recovery in %
01	0.1796	10	10.01	100.1
02	0.3416	20	20.14	100.7
03	0.4933	30	29.625	98.75
04	0.6620	40	40.16	100.4
05	0.8190	50	49.981	99.962
Mean				**99.9824**
SD				**0.745385**
LOD				**1.944767**
LOQ				**5.893234**
SLOPE(M)				**0.016**
INTERCEPT(C)				**0.0193**
Test number (BIS)	**Absorbance 223nm**	**Concentration (ppm)**	**Found Conc.**	**% Recovery**
01	0.3016	05	4.368	87.36
02	0.5256	10	10.976	109.76
03	0.664	15	15.05	100.33
04	0.8143	20	19.492	97.46
05	1.005	25	25.117	100.468
Mean				99.0756
SD				8.023935
LOD				**5.698735862**
LOQ				**17.26889655**
SLOPE(M)				**0.0339**
INTERCEPT(C)				**0.1535**

Table 1: Recovery result of IVA and BIS

Development of Simultaneous estimation of IVA and BIS:

Given procedure involved obtaining overlay spectra and scanning the prepared sample solutions of both medications in the ultraviolet range of 200–400 nm. The approach with 286 nm (IVA λmax) and 223 nm (BIS λmax) was used for overlap spectra for the study of given medications simultaneously by applying the simultaneous equation. In simulated salivary fluid, concentrations ranging from 10, 20, 30, 40, and 50 μg/mL for IVA and 5, 10, 15, 20, & 25 μg/mL for BIS were generated. The following formula was used to calculate the concentrations of medicines x (IVA) and y (BIS) in sample solutions using simultaneous estimation method:

$C_x = (A_2ay_1 - A_1ay_2) / (ax_2ay_1 - ax_1ay_2)$,

$C_y = (A_1ax_2 - A_2ax_1) / (ax_2ay_1 - ax_1ay_2)$

where IVA and BIS concentrations are represented by Cx and Cy, respectively; sample solution absorbances at 286 and 223 nm are represented by $A1$ and $A2$, respectively; IVA absorptivity

values are represented by $ax1$ and $ax2$, and BIS absorptivity values are represented by $ay1$ and $ay2$ at 286 and 223 nm, respectively [15–20].

Investigation of formulated fast dissolving film formulation and bulk API mixture:
IVA and BIS-containing film formulation was taken and cut into 2*2cm2 pieces, with 2.5 mg of IVA and BIS equivalent per square centimetre. Use a 100 ml volumetric flask to hold this film. After adding 50 mL of simulated saliva, the mixture was sonicated for a short while. Following sonication, Whatman's filter paper (0.45 microns) was used to filter the mixture and the volume was adjusted using the same solvent. To achieve a 5 µg/ml concentration, the filtered material that was left over was further diluted. In the end, this was assessed for IVA and BIS estimates at 286 nm and 223 nm.

Similarly, by weighing IVA and BIS in a 1:1 ratio, an API combination of the two was created. Five milligrams of powder were weighed out of this mixture. This mixture was transferred to a 100 ml volumetric flask, where it was mixed with simulated saliva and sonicated for a short while. Following that, the solution was diluted to a final conc. of 5 µg / ml. The final solution was measured for IVA and BIS at 286 nm and 223 nm, respectively. Table 3 displays the outcome.

VALIDATION OF METHOD:

1. **Linearity:** measuring absorbance on the Y-axis and conc. on the X-axis, the calibration curves for IVA and BIS were plotted independently. The working standard linearity ranges for IVA and BIS were determined to be 10–50 μg/mL and 5–25 μg/mL, respectively. These ranges were scanned at 286 nm and 223 nm, to obtain the absorbance at a certain concentration. Plotting the curve revealed that IVA and BIS had correlation coefficient values ($R2$) of 0.9998 and 0.9935, respectively, indicating a direct relationship between concentration and absorbance. Figures 3 and 4 display the IVA and BIS curves, respectively.

2. **LOD & LOQ (limit of detection & limit of quantification):** LOD & LOQ were calculated using the calibration curve and were derived using the formulas for LOD = 3.3*(σ/S) and LOQ = 10*(σ/S). Utilizing the y-intercept, where S is the plot's slope and sigma is the reaction's standard deviation (y-intercept). The LOD values for BIS and IVA were reported to be 5.698735862 µg/ml and 1.944767 µg/ml, respectively. The limit of quantification (LOQ) for BIS was 17.26889655 µg/mL, whereas IVA was 5.893234 µg/mL. IVA and BIS have tables with LOD and LOQ of 1 and 2, respectively.

3. **Precision:** By determining the interday and intraday independent assay for three distinct samples chosen from the calibration curve range—for IVA 20, 30, and 40 µg/mL and for BIS 10, 15, and 20 µg/mL—and estimating the percentage RSD, the precision and reproducibility of the method were assessed. Table 4 displays the precision results.

4. **Accuracy:** Three levels of accuracy were used in the accuracy study: 50%, 100%, and 150%. A recovery study that used the conventional addition approach was conducted on this. A recovery investigation was carried out to look for any excipient-related interferences. Results showing nearly 100% recovery showed that the suggested approach is accurate and that additional excipients did not cause any interference. Results of the accuracy investigation are displayed in Table 5.

Sr. No.	Solvent used	Name of sample	Amount present		Amount obtained		% Assay	
					Based on absorbance		(Based on amount found)	
			IVA	BIS	IVA	BIS	IVA	BIS
1	Simulated salivary fluid	PURE DRUG	2.5mg	2.5mg	2.397mg	2.192mg	95.88	87.68
2	Simulated salivary fluid	FILM	2.5mg	2.5mg	2.309mg	2.398mg	92.35	95.94

Table no. 2: Results of Analysis of prepared fast dissolving film and pure drug

Conc. (in ppm)	Intraday	%RSD	Inter-day			%RSD		
IVA								
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
20	0.3413	0.087822	0.3423	0.3427	0.3417	0.14707	0.147099	0.133915
	0.3416		0.3427	0.3421	0.3426			
	0.3419		0.3420	0.3417	0.3423			
30	0.4934	0.08107	0.4940	0.4934	0.4935	0.050917	0.04217	0.065155
	0.4930		0.4935	0.4938	0.4930			
	0.4938		0.4937	0.4937	0.4936			
40	0.6617	0.045317	0.6619	0.6623	0.6617	0.023072	0.23058	0.046151
	0.6623		0.6621	0.6626	0.6623			
	0.6620		0.6622	0.6625	0.6619			
10	0.5256	0.038052	0.5251	0.5244	0.5255	0.039368	0.066974	0.021971
	0.5254		0.5250	0.5247	0.5255			
	0.5258		0.5254	0.5240	0.5257			
15	0.6645	0.075301	0.6630	0.6633	0.6643	0.075855	0.075828	0.31328
	0.6635		0.6636	0.6643	0.6647			
	0.6640		0.6640	0.6637	0.6644			
20	0.8143	0.073683	0.8135	0.8129	0.8140	0.055401	0.055442	0.075001
	0.8137		0.8144	0.8133	0.8148			
	0.8149		0.8139	0.8138	0.8152			

Table 3: Precision Study

Level %	Sample ppm	Amt. Added	Total ppm	Abs. 286nm	Found Conc.	Recovery in %	Average	SD	RSD in %
IVA accuracy									
050	10	05	15	0.2593	15	100	0.259067	0.000208	0.080353
				0.2590	14.9815	99.875			
				0.2589	14.975	99.83333			
100	10	10	20	0.3390	19.98125	99.90625	0.339233	0.000252	0.074185
				0.3392	19.99375	99.96875			
				0.3395	20.0125	100.0625			
150	10	15	25	0.4194	25.00625	100.025	0.419133	0.000231	0.055099
				0.4190	24.98125	99.925			
				0.4190	24.98125	99.925			
BIS Accuracy									
50	10	5	15	0.6638	15.0531	100.354	0.664033	0.000252	0.037899
				0.6640	15.059	100.3933			
				0.6643	15.06785	100.4523			
100	10	10	20	0.8138	19.47788	97.38938	0.814033	0.000252	0.030915
				0.8140	19.48378	97.41888			
				0.8143	19.49263	97.46313			
150	10	15	25	1.004	25.0885	100.354	1.002333	0.003786	0.377713
				0.998	24.9115	99.6460			
				1.005	25.11799	100.472			

Table 4: Results of Recovery Studies

RESULTS AND DISCUSSION:

The suggested method was thought to be straightforward and basic in the focus range of 10–50 µg/ml for IVA and 5–25 µg/ml for BIS, respectively. The methods developed have been accepted in terms of examination, LOD, LOQ, specificity, accuracy, linearity, range, and precision. The reliability analysis suggested the validity of the methodology, and the RSD did not exceed 2. The established approach is hence efficient & transparent.

CONCLUSION:

For the SE of the IVA and BIS in the fast-dissolving film preparation, a quick, easy, and practical UV spectroscopic approach was tried. A broad range of concentrations produced the assay's linear response. The analytical method's efficiency was demonstrated by a very low

percentage of RSD. We may conclude that the established UV spectroscopy approach is accurate, exact, and precise based on all the examined validation factors. As such, the suggested approach may find daily application in the analysis and evaluation of IVA and BIS in conjunction with film formulation.

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CONFLICT OF INTEREST:

All Authors don't have any conflict of interest concerning the publication of this research paper.

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ABBREVIATION: Ivabradine hydrochloride (IVA); Bisoprolol fumarate (BIS); Active Pharmaceutical Ingredient (API); Relative Standard Deviation (RSD); UV (Ultraviolet); Standard Deviation (SD); Concentration (Conc). Amount (Amt).

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