VOLUME - 11, ISSUE - 04, APRIL - 2022 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper | Pharmaceutical Science

SIMULTANEOUS ESTIMATION OF IVABRADINE HCl AND BISOPROLOL FUMARATE BY UV-SPECTROSCOPIC METHOD

Pushpak Pandit	Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, Gujarat, India.
Vraj Sheth	Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, Gujarat, India.
Savan Viradiya	Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, Gujarat, India.
Mansi Patel	Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, Gujarat, India.
Dimal A. Shah*	Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, Gujarat, India. *Corresponding Author
Usmangani K. Chhalotiya	Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, Gujarat, India.

ABSTRACT A simple UV spectrophotometric analytical method has been developed for simultaneous estimation of Ivabradine HCl and Bisoprolol used in the treatment of angina pectoris. Modified simultaneous equation method was developed with double beam UV-Visible spectrophotometer using water as a solvent. The method was found to be linear in the range of 4-16 µg/ ml of Bisoprolol and 08-32 µg/ ml of Ivabradine. Percentage recovery for bisoprolol and Ivabradine by this method was found in the range of 96.43-100.60% and 95.80-100.10 %, respectively. The method was validated with respect to accuracy, precision, specificity and robustness as per ICH guideline. The method was applied for the estimation of laboratory prepared mixture and was found to be accurate and precise.

KEYWORDS : Bisoprolol Fumarate, Ivabradine HCl, UV-Spectrophotometry, Validation, ICH (International conference on Harmonization)

INTRODUCTION

Ivabradine HCl (IVA) is used to treat the symptoms of stable angina pectoris. Ivabradine lowers heart rate and act on the ion current, which is located in the sinoatrial node. It is a mixed Na^+-K^+ inward current that's triggered by hyperpolarization and controlled by the autonomic nervous system [1]. Heart failure is a chronic condition in which the heart doesn't pump blood in the required quantity causing shortness of breath. Heart failure can happen if the heart cannot pump required quantity of blood. Symptoms are shortness of breath, fatigue, swollen legs and rapid heartbeat.

Ivabradine is hyperpolarization activated cyclic nucleotide-gated (HCN) channel blockers. Chemically IVA is 3-[3-[[(7S)-3,4-dimethoxy-7-bicyclo[4.2.0]octa-1,3,5-trienyl]methyl-methylamino]propyl]-7, 8-dimethoxy-2,5-dihydro-1H-3-benzazepin-4-one[2-3]. It acts by slowing the heart rate so that the heart can pump more blood through the body each time it beats. Ivabradine is used in chronic heart failure with systolic dysfunction in combination with standard therapy including beta-blocker therapy or when beta-blocker therapy is contraindicated or not tolerated[4].

Bisoprolol (BIS) is a competitive, cardio selective β1-adrenergic antagonist. Chemically it is 1-(propan-2-ylamino)-3-[4-(2-propan-2-yloxyethoxymethyl) phenoxy]propan-2-ol[5-6]. When β1-receptors (located mainly in the heart) are activated by adrenergic neurotransmitters such as epinephrine, both the blood pressure and heart rate increase, leading to greater cardiovascular work, increasing the demand for oxygen. Bisoprolol reduces cardiac workload by decreasing contractility and the need for oxygen through competitive inhibition of β1-adrenergic receptors[7].

Combining ivabradine with low dose of bisoprolol in angina patients produces additional antianginal and anti-ischemic benefits. This combination is under Clinical phase III trial.

Literature survey indicates that there was no analytical method published for simultaneous estimation of Ivabradine and Bisoprolol in combination. Liquid chromatography (LC) techniques for BIS or IVA alone and in combination with other drugs have been reported [8-38]. The present study involves development and validation of UV spectrosphotometric simultaneous equation method for the estimation of IVA and BIS in combination. Compared to liquid chromatographic methods, UV method offers advantage of less analysis time and cheaper. UV methods are comparable with liquid chromatographic method with respect to accuracy and precision. So, the present study describes accurate and precise UV spectroscopic method for simultaneous quantification of BIS and IVA in synthetic mixture.

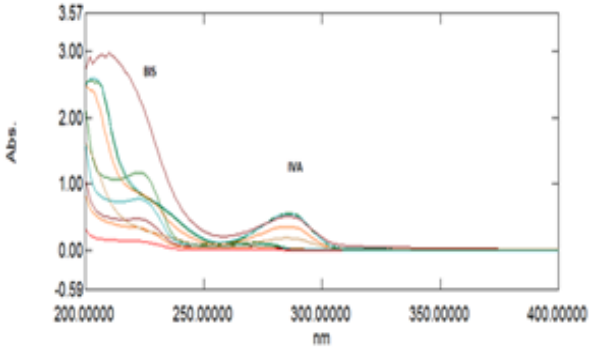

Figure 1: Overlaid UV spectra of BIS (4-16 µg/ml) and IVA (8-32 µg/ml) in water

EXPERIMENTAL

Materials and Instruments

IVA and BISO were obtained from reputed pharmaceutical industry, Gujarat, India. Methanol (AR grade) was purchased from S. D. Fine Chemicals Ltd., India. Double distilled water used in the study was prepared in the laboratory.

Double Beam UV-Visible spectrophotometer (UV-1900 I, Shimadzu, Japan) with 1 cm matched quart cell was used in the study. All materials were weighed on calibrated METTLER TOLEDO (Columbus, OH, USA) analytical balance.

Preparation of stock solution

BIS and IVA were weighed (10 mg each) and transferred to two separate 100 ml volumetric flask containing few ml of water. Drugs were dissolved in water and volume was made up to the mark with water, which gave 100 μg/ ml of both the drugs.

Selection of wavelength

From the stock solution of BIS and IVA, 2.5 ml was pipetted out and diluted up to 25 ml using Distilled water which gave 10 μg/ ml solution of both the drugs. Spectra of both the solutions were scanned between 400-200 nm. 223 nm was selected as wavelength maxima of BIS and 286 nm as wavelength maxima of IVA. BIS do not absorb at the wavelength maxima of IVA so 286 nm was selected for the estimation of IVA.

Calibration Curves for BIS and IVA

Appropriate aliquots of BIS and IVA from the stock solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with water to obtain final concentrations in the range of 4-16 μg/ ml of BIS and 08-32 μg/ ml of IVA, respectively. Spectra of solutions were scanned between 400-200 nm. Calibration curves were constructed relating to absorbance at 223 nm and 286 nm. Regression equations were computed for BIS and IVA. Absorptivities of both the drugs were evaluated at both the wavelength and simultaneous equation was computed. All the spectrophotometric estimations were carried out at controlled room temperature.

Method Validation

The method was validated as per ICH guidelines [39] for accuracy, precision, specificity, linearity, LOD, LOQ and robustness by following procedure.

Linearity and range

Linearity of the method was evaluated by constructing calibration curves at five concentration levels over a range of 4-16 μg/ ml of BIS and 08-32 μg/ ml of IVA at 223 nm and 286 nm, respectively. Calibration curves were constructed by plotting average absorbance versus concentrations and regression equations were computed for both the drugs (n=5).

Precision

The intra-day and inter-day precision studies were carried out by estimating corresponding responses 3 times on the same day and on 3 different days (first, second, third day) for 3 different concentrations of BIS (4,10,16μg/ ml) and IVA (8,20,32 μg/ ml) at 223nm and 286 nm, respectively and the results are reported in terms of relative standard deviation (RSD).

The repeatability studies were carried out by analysing of BIS (10 μg/ml) and IVA (20 μg/ml) six times and results are reported in terms of relative standard deviation.

Accuracy

The accuracy of the method was determined by calculating recoveries of BIS and IVA by method of standard additions. Known amount of BIS (3,6,9 μg/ ml) and IVA (6,12,18 μg/ ml) were added to a pre quantified (6 µg/ml and 12 µg/ml of BIS and IVA, respectively) sample solution. The amount of BIS and IVA were estimated by measuring response at the appropriate wavelengths. The recovery was verified by estimation of drugs in triplicate preparations at each specified concentration levels.

LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using following equation as per ICH guidelines.

$LOD = 3.3 \times \sigma / S$;
$LOQ = 10 \times \sigma / S$; Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Specificity

The specificity of the method was ascertained by analysing BIS and IVA in presence of excipients like Micro crystalline cellulose, Hydroxypropyl methylcellulose and Magnesium stearate were used for preparation of tablet formulations. Interference due to excipients was noted and amount of drug recovered were calculated.

Laboratory prepared mixtures

Appropriate aliquots of BIS working standard solutions were taken in different 10 ml volumetric flasks. To the same flask appropriate aliquots of IVA working standard solutions were added and the volume was diluted to the mark with distilled water to achieve final concentration of 4, 8, 10,12,16 μg/ ml of BIS and 8,16,20,24,32 μg/ ml of IVA. Spectra of solutions were scanned and absorbance was measured at 223 nm and 286 nm.

RESULTS AND DISCUSSION

Selection of solvent and wavelength

Both the drugs, BIS and IVA are freely soluble in Water. So, Water was selected as a solvent and further dilutions were carried using distilled Water. Stock solution of 10 g/ml of BIS and IVA were prepared and scanned between the ranges of 400-200 nm. From the overlaid spectra BIS gave wavelength maxima at 223 nm and IVA gave wavelength maxima at 286 nm. BIS do not absorb at the wavelength maxima of IVA, so IVA was estimated at 286 nm, while for estimation of BIS, simultaneous equation was formed (Figure 1)

Method Validation

Linearity and Range

The calibration curve was plotted for BIS in the concentration range of 4-16 µg/ml which showed linear absorbance at 223 nm with correlation coefficient (r^2) of 0.9970 and for IVA in the concentration range of 08-32 µg/ml which showed linear absorbance at 286 nm with correlation coefficient (r^2) of 0.9942. The absorptivities of both the drugs were determined at 223 nm and 286 nm and simultaneous equation was computed for further calculations.

At 286 nm, Concentration of IVA is

$A = \alpha_{286} b c_{IVA}$
$= 133 \times b \times c$
Where α_{286} = absorptivity of IVA at 286 nm
C = concentration of IVA

At 223 nm (Total absorbance of IVA and BIS)

$A = \alpha_{BIS} b C_{BIS} + \alpha_{IVA} b C_{IVA}$
A = Total absorbance of mixture at 223 nm
α_{BIS} = absorptivity of BIS at 223 nm
C_{BIS} = Concentration of BIS at 223 nm
α_{IVA} = absorptivity of IVA at 223 nm
C_{IVA} = Concentration of IVA at 223 nm

Precision

Intraday precision

The intraday studies were carried out by measuring response for 3 concentrations for 3 times a day. The % RSD was found to be 0.733-1.88% for BIS at 223 nm and 0.229-1.34% for IVA at

286 nm. These %RSD value was found to be less than ± 2.0 indicated that the method is precise.

Inter day precision

The interday studies were carried out by measuring response for 3 concentrations for 3 times at 3 different days. The % RSD was found to be 0.82-1.71% for BIS at 223 nm and 0.679-1.81% for IVA at 286nm. These %RSD value was found to be less than ± 2.0 indicated that the method is precise.

Repeatability study

The repeatability studies were carried out by measuring response for a single concentration for 6 times a day. The % RSD was found to be less than 1% at both the wavelength.

Accuracy

Accuracy of the method was determined by recovery study from marketed formulation at three level of standard addition. Percentage recovery for BIS and IVA by this method was found in the range of 96.43-100.60% and 95.80-100.10 %, respectively (Table 1). The value of %RSD within the limit indicated that the method is accurate and percentage recovery shows that there is no interference from the excipients.

Table 1: Accuracy data of BIS and IVA

Amt of std drug taken (µg/ml)		Amount of standard drug added (µg/ml)		% recovery ±SD		% RSD	
BIS	IVA	BIS	IVA	BIS	IVA	BIS	IVA
6	12	0	0	96.9±0.81	95.8±0.70	0.82	0.73
6	12	3	6	96.43±0.15	97.6±0.50	0.15	0.51
6	12	6	12	98.53±0.40	99.03±0.32	0.40	0.3
6	12	9	18	100.6±0.30	100.1±0.45	0.29	0.45

Limit of detection and limit of quantification

LOD is the lowest amount of the analyte that can be detected. From the visual observation of UV spectra, the LOD for BIS was found to be 1 µg/ml and for IVA was found to be 2.5 µg/ml. LOQ is the lowest amount of the analyte that can be detected and quantified. LOQ of the BIS was found to be 4 µg/ml and LOQ of the IVA was found to be 8 µg/ml.

Specificity

The specificity study was carried out to check the interference from the excipients. The spectra showed absorbance for both the drugs without any interference and the recoveries of both the drugs were above 96%.

The validation parameters are summarized in Table 2.

Table 2: Summary of Validation Parameters

PARAMETERS	Simultaneous Estimation		
	BIS	IVA	
	223 nm	286 nm	223 nm
Concentration range(µg/ml)	4-16	8-32	8-32
Regression equation			
Correlation Coefficient(r^2)	0.9942	0.9939	0.9967
Intra-day Precision (%RSD) (n=3)	0.733-1.88	0.22-1.34	0.22-1.39
Inter-day precision (%RSD) (n=3)	0.82-1.71	0.67-1.81	0.26-1.38
Repeatability (%RSD) (n=6)	0.94	0.97	0.92
Accuracy(%Recovery) (n=3)	96.43-100.60%	95.80-100.1%	
LOD(µg/ml)	1	2.5	
LOQ(µg/ml)	4	8	
Robustness	Robust	Robust	
Specificity	Specific	Specific	

Laboratory prepared mixtures

To check the validity of developed equation, laboratory prepared mixtures were analyzed. The percentage recovery of the BIS and IVA was found to be 97.56-99.58% and 97.5-99.7%, respectively.

CONCLUSION

A simple analytical method has been developed for simultaneous estimation of Ivabradine HCl and Bisoprolol in synthetic mixture. Modified simultaneous equation method was developed using water as a solvent. The method was found to be linear in the range of 4-16 µg/ ml of BIS and 08-32 µg/ ml of IVA. The method was validated with respect to accuracy, precision, specificity and robustness. The method was applied for the estimation of laboratory prepared mixture and was found to be accurate and precise.

REFERENCES:

[1] Drug profile, Ivabradine, https://go.drugbank.com/drugs/DB09083, accessed on 14th December 2021.

[2]. Drug profile Ivabradine, https://pubchem.ncbi.nlm.nih. gov/compound/Ivabradine, accessed on 14th December 2021.

[3] Drug profile and mechanism of action of Ivabradine, https://en. wikipedia. org/wiki/Ivabradine, accessed on 14th December 2021.

[4] Drug profile and mechanism of action of Bisoprolol, https://go. drugbank. com/drugs/DB00612, accessed on 16th December, 2021.

[5] Drug profile Bisoprolol, https:// pubchem. ncbi.nlm.nih. gov/compound/Bisoprolol, accessed on 16th December 2021.

[6] Drug profile and mechanism of action of Bisoprolol, https://en. wikipedia. org/wiki/Bisoprolol, accessed on 16th December 2021.

[7] Amosova E, Andrejev E, Zaderey I, Rudenko U, Ceconi C, Ferrari R (2011). Efficacy of Ivabradine in Combination with Beta-Blocker Versus Up titration of Beta-Blocker in Patients with Stable Angina. Cardiovascular Drugs and Therapy, 25(6):531-537.

[8] Motisariya M. H., Patel K. G., Shah P. A. (2013), Validated stability-indicating high performance thin layer chromatographic method for determination of Ivabradine hydrochloride in bulk and marketed formulation: An application to kinetic study. Bulletin of Faculty of Pharmacy, 51(2): 233-241.

[9] Maheshwari S., Khandhar A., Jain A (2010), Quantitative Determination and Validation of Ivabradine HCL by Stability Indicating RP-HPLC Method and Spectrophotometric Method in Solid Dosage Form. Eurasian Journal of Analytical Chemistry, 5 (1):53-62.

[10] Seerapu S., Srinivasan B. P. (2010), Development and Validation of RP-HPLC Method for the Estimation of Ivabradine Hydrochloride in Tablets. Indian Journal Pharmaceutical Science, 72(5):667-671.

[11] Thete P. G., Saudagar R. B. (2019), Analytical method development and validation for the determination of ivabradine HCL by RP-HPLC in bulk and pharmaceutical dosage form. AJP Tech, 9(2):89-92.

[12] Damle M. C., Bagwe R. A. (2015), Development and Validation of Stability-Indicating HPTLC Method For Ivabradine HCl. International journal of Pharmaceutical Science, 6(1): 141-152.

[13] Rahman M. R., Asaduzzaman Md., Ashraful Islam S. M. (2012), Development and validation of RP-HPLC method for analysis of Ivabradine Hydrochloride in tablet dosage forms. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2012, 3(3):1032-1043.

[14] Piotr P, Nowakowska J., Ciura K. (2013), Chromatographic analysis of ivabradine on polar, nonpolar and chemically modified adsorbents by HPTLC. Journal of Food and drug analysis, 21(2): 165-168.

[15] Xiaoli Y., Hui L., Cui L. (2011), Determination of the Related Substances in Ivabradine Hydrochloride and Its Tablets by HPLC. Chinese Pharmaceutical Affairs, 5: 022.

[16] Patel H., Jivani N. (2015), Development of Validated RP-HPLC Method For Simultaneous Estimation of Carvedilol and Ivabradine. World Journal of Pharmacy and Pharmaceutical Sciences, 4(5):630-639.

[17] Lu-ye C., Xiu-mei J., Yuan-wu B. (2014), Determination of Ivabradine hydrochloride in the human plasma and the bioequivalence study by LC-MS/MS. West China Journal of Pharmaceutical Sciences, (2):183-188.

[18] Mostafa N., Fayez Y., Farid J. F. (2016), Validated stability indicating chromatographic methods for determination of Ivabradine hydrochloride in the presence of its acidic degradation product. International Journal of Research and Reviews in Pharmacy and Applied sciences, 6(1):1370-1380.

[19] Patel P. N., Borkar R. M., Kalariya P. D., Gangwal R. P, Sangamwar A. T., Samanthula G., Ragampeta S (2015), Characterization of degradation products of Ivabradine by LC-HR-MS/MS: a typical case of exhibition of different degradation behavior in HCl and H2SO4 acid hydrolysis. Journal of Mass Spectrometry, 50(2):344-353.

[20] Bouchard M. F., Gilles S., JeanneBossant M., Neyret C. B. (2000), Simultaneous determination of ivabradine and its metabolites in human plasma by liquid chromatography–tandem mass spectrometry. Journal of Chromatography B: Biomedical Sciences and Applications, 745(2): 261-269.

[21] Rehman M., Naggamalika G. (2017), Validated RP-HPLC method for the determination of Ivabradine Hydrochloride in pharmaceutical formulation. International Journal of pharmaceutical sciences and drug research, 9(5): 228-233.

[22] Logoyda L., Kovalenko S., Abdel-megied A., Zhulkevych I., Drapak I., Demchuk I., Ndetsyuk O. (2019), HPLC method development for the analysis of bisoprolol in combined dosage form containing bisoprolol and enalapril and in vitro dissolution studied. International Journal of Applied Pharmaceutics, 11(3): 186-194.

[23] Arjun G., Sathus K. D., Bindu M. B., Naga M. M., Ramalingam R., Ravindernath A. (2009), A simple HPLC method for quantitation of bisoprolol fumarate in tablet dosage form. Indian Drugs, 46(7): 39–42.

[24] Piponski M, Balkanov T, Logoyda L (2021), Development and validation of a fast and simple HPLC method for the simultaneous determination of

bisoprolol and enalapril in dosage form. Pharmacia, 68(1): 69–77.
[25] Bhavyasri K., Soujanya J., Sewthasri R., Mogili S. (2020), Development and Validation of Stability Indicating RP-HPLC Method for the Estimation of Bisoprolol Fumarate in Bulk and Pharmaceutical Dosage Form. International Journal of Pharmaceutical and Phytopharmacological Research, 10(4): 49-70.
[26] Yadav S. S., Rao J. R. (2013), Simultaneous HPTLC analysis of bisoprolol fumarate and hydrochlorthiazide in pharmaceutical dosage form. International Journal of Pharmacy and Pharmaceutical Science, 5(2): 286-290.
[27] Bhoya P. N., Patelia E. M., Gautam (2013), Development and Validation of TLC-Densitometry Method for Simultaneous Estimation of Bisoprolol Fumarate and Hydrochlorothiazide in Bulk and Tablets. Chromatographic Separation Techniques, 4(1): 1-4.
[28] Vora D. N., Kadav A. A. (2008), Development and Validation of a Simultaneous HPLC Method for Estimation of Bisoprolol Fumarate and Amlodipine Besylate from Tablets. Indian Journal of Pharmaceutical Science, 70(4): 542–546.
[29] Joshi S. J., Karbhari P. A., Bhoir S. I., Bind K. S., Das C. (2010), RPHPLC method for simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in tablet formulation. Journal of Pharmaceutical and Biomedical analysis, 52(3): 362–371.
[30] Baokar S. S., Erande S. R., Shaikh G. S. (2011), Analytical method development and validation for simultaneous determination of bisoprolol fumarate and amlodipine besylate. Indo American Journal of Pharmaceutical Research, 2(1): 100–110.
[31] Mostafa A., Gindy A. E., Emara S. (2017), Simultaneous Spectrophotometric Estimation of Bisoprolol Fumarate and Hydrochlorothiazide in Tablet Formulation using Partial Least-Squares, Principal Component Regression Multivariate Calibrations and RP-HPLC Methods. Journal of Analytical & Pharmaceutical Research, 4(6):1-9.
[32] Rao G. S., Rao P. K., Ramachandran D. (2015), Development and Validation of RP-HPLC Method for the Assay of Bisoprolol in Pure and Formulations. Indian Journal of Pharmacy and Pharmaceutical Research, 3 (1): 15–24.
[33] Hefnawy M. M., Sultan M. A., Al-Shehri M. M. (2006), Development of an HPLC method for the quantitation of bisoprolol enantiomer in pharmaceutical products using a teicoplanin chiral stationary phase and fluorescence detection. Journal of Liquid Chromatography & Related Technologies, 29(20): 2901-2914.
[34] Panainte A. D., Vieriu M., Tantaru G., Apostu M., Bibire N. (2015), A HPLC Method for the Determination of Bisoprolol in Tablets and its Application to a Bioequivalence Study. Revista De Chimie, 6(11):1791-1795.
[35] Athota R. V., Jagarlapudi S. K., Singampalli M. R. (2016), Stability Indicating RP-HPLC Method for Simultaneous Assay of Bisoprolol and hydrochlo rothiazide in combined tablet dosage form. International journal of Pharmtech research, 9(7):329–339.
[36] Patel D. R., Mashru R. C., Patel M. M. (2011), Enantio-separation of bisoprolol fumarate by TLC and HPTLC using (+)-10-camphorsulphonic acid as a chiral selector. International Journal of Pharmacy & Technology, 3(1): 1593–1602.
[37] Tatar Ulu S., Aydogmuş Z. (2012), An HPLC method for the determination of bisoprolol in human plasma and its application to a pharmacokinetic study. Journal of Chromatographic Science, 50(7): 615–619.
[38] Indian pharmacopeia; Indian Pharmaceutical Commission, Ghaziabad; Volume I, 2018, p.p. 428.
[39] ICH Guidelines Q2(R1), Validation of Analytical Procedure: Text and Methodology, Geneva, Switzerland, November 2005.