Simultaneous Estimation of Ivabradine Hydrochloride and Trimetazidine Dihydrochloride in Bulk and Tablet Formulation

Ajay Patange[1], Sagar Jadhav[1], Atul Phatak[1]*

[1]Department of Pharmaceutics, PES Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India.

ABSTRACT

Beta-blockers are the most commonly used in the treatment of ischemic heart disorder. However, when beta-blockers develop intolerance, newer drug therapy is the best alternative treatment. Ivabradine hydrochloride and Trimetazidine dihydrochloride are both antianginal drugs. However, there is no method for determining the combination of both of these drugs. Hence, this work provides a new and simple UV (Ultraviolet) spectrophotometric-based simultaneous estimation of Ivabradine hydrochloride (IBH) in the presence of Trimetazidine dihydrochloride (TMZ) in bulk as well as tablet form.

Firstly, the lambda max of both drugs was determined for Ivabradine hydrochloride (286 nm) and Trimetazidine dihydrochloride (231 nm). Calibration curves for Ivabradine hydrochloride and Trimetazidine dihydrochloride were plotted by measuring the absorbance at a specific concentration, and it was found that both drugs obeyed linearity with regression values (R^2) of 0.999. The minimum concentration required (limit of detection) for UV spectrophotometric detection of Ivabradine hydrochloride was determined to be 2.350 µg/mL with a limit of quantification (LOQ) of 7.122 µg/mL, similarly the limit of detection (LOD) for Trimetazidine dihydrochloride observed to be 3.790 µg/mL with limit of detection of 11.485 µg/mL. The precision study showed a percentage deviation (relative standard deviation (RSD)) within acceptable values (RSD < 2.0%). The recovery value of both drugs was in the range of 98% to 102%, demonstrating accuracy. Based on the results, it is concluded that the proposed UV spectroscopic technique is new but simple, precise, reliable, and affordable for the simultaneous determination of Ivabradine hydrochloride and Trimetazidine dihydrochloride.

Key Words: *Ivabradine hydrochloride, Trimetazidine dihydrochloride, Simultaneous estimation, UV Spectroscopy*

eIJPPR 2023; 13(6):29-36

HOW TO CITE THIS ARTICLE: Patange A, Jadhav S, Phatak A. Simultaneous Estimation of Ivabradine Hydrochloride and Trimetazidine Dihydrochloride in Bulk and Tablet Formulation. Int J Pharm Phytopharmacol Res. 2023;13(6):29-36. https://doi.org/10.51847/LLg6fFFYTc

INTRODUCTION

Ischemia is the most prevalent disease that occurs due to an imbalance of oxygen demand and supply to the heart. Angina is a manifestation of ischemic heart disease, which predominantly contributes to death and impairment of a patient's quality of life (QoL) [1].

Multiple pathogenesis is involved in ischemia; hence, a single drug may or may not be effective. Effective treatment mainly includes a multifaceted and tailored approach depending on the patient's disease profile, lifestyle management, pharmacological treatment, and myocardial revascularization. It is observed that in many angina cases, comorbidities like diabetes and chronic obstructive pulmonary illness accelerate possible intolerance to beta-blockers, in such conditions, newer drug therapy is a good alternative [2]. Ivabradine hydrochloride is considered to be the first choice for normalizing heart rate which acts specifically and particularly on the present cardiac pacemaker current of the sinoatrial node and reduces the heart rate with no alteration in myocardial contractility and present (if any) vascular tone, which makes Ivabradine unique from beta-blockers and calcium channel blockers [1, 3].

Ivabradine hydrochloride's chemistry nomenclature is 3-(3-{[((7S)-3,4-Dimethoxybcyclo [4,2,0] oct-1,2,3-trien-7-

Corresponding author: Atul Phatak
Address: Department of Pharmaceutics, PES Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India.
E-mail: ✉ atulphatak1305@gmail.com
Received: 03 September 2023; **Revised:** 04December 2023; **Accepted:** 10 December 2023

yl) methyl] methyl amino} propyl)- 1,3,4,5-tetrhydro-7,8-dimethoxy-2H-3-benzazpin-2-one, hydrochloride with molecular formula $C_7H_6NO_5$. HCL and molecular mass 505.05 g/mol. US Food and Drug Administration (USFDA) approved Ivabradine hydrochloride in 2015 as a heart rate-lowering agent [4].

Trimetazidine dihydrochloride is a chemically 1-[(2,3,4-trimethoxyphnyl) methyl] piperazine dihydrochloride with a molecular formula of C_{14} $H_{22}N_2O_3$.2HCl and a molecular mass 339.3 g/mol. it is a clinically effective agent that shows anti-ischemic effects without inducing hemodynamic changes, unlike other beta-blockers, calcium channel blockers, or long-acting nitrates [5-9].

The text summary indicated that basic UV, HPLC, HPTLC, and LC-MS methods were considered when evaluating these compounds separately or in combination with other compounds, but simultaneous estimates (SE) were not shown about this new hybrid. The simultaneous estimation (SE), is usually used to evaluate chemical mixtures containing two or more chemicals in the same measurement system. In comparison with other analytical methods, this method has a few unique drawbacks. Consequently, efforts have been made to develop a novel and simple SE method to support the estimation of these active substances in health and pharmaceutical products. The developed method has been validated and successfully used in the formulation development of TMZ and simultaneous estimation of IBH in the unmodified compounds [10-14].

The background survey has confirmed that, at present, no UV spectroscopic analysis is possible for simultaneous estimation of the IBH and TMZ combination in a single unit dosage form. Therefore, the objective of this research work is the development and validation of a new but simple UV spectroscopic analysis tool for simultaneous estimation of IBH and TMZ.

MATERIALS AND METHODS

Apparatus (Jasco V-630 UV-visible spectrophotometer)

For experimental work, a UV-visible spectrophotometer (model Jasco-V 630) with a wide wavelength range of 190 to 1100 nm was employed for measurement. Its facilities include a spectral bandwidth of 1.5 nm, automatic wavelength corrections, a pair of 10 mm quartz cells, etc.

A Sonicator of Biomedica of 2.5 liters was used for sonication purposes. Glassware such as volumetric flasks, pipettes, and beakers were used.

Chemicals and reagents

Ivabradine hydrochloride and Trimetazidine dihydrochloride were both received as gift samples from Lupin Limited, Sikkim, India, and Sharon Bio-Medicine Ltd, Maharashtra, India, respectively. The marketed tablets of IBH Coralan 5 mg and TMZ, Flavedon MR 35 mg were manufactured by Servier India Pvt. Ltd. and Les Laboratoires Servier Industry, respectively, and also purchased from an approved vendor of the institute.

Standard (stock) solutions of IBH

The stock solutions of Ivabradine hydrochloride (100 μg/mL) were prepared in methanol and sonicated for a few minutes which was adjusted further to 100 mL using methanol.

Standard (stock) solutions of TMZ

Trimetazidine dihydrochloride (100 μg/mL) was prepared by dissolving 10 mg of Trimetazidine dihydrochloride in 50 mL of methanol and was sonicated for a few minutes, and the volume was adjusted with methanol.

Preparation of working standards of IBH

From the standard stock solution of IBH, 0.5 mL to 2.5 mL were withdrawn using a pipette in a separate volumetric flask and mixed with methanol to prepare working standards of 5-25μg/mL.

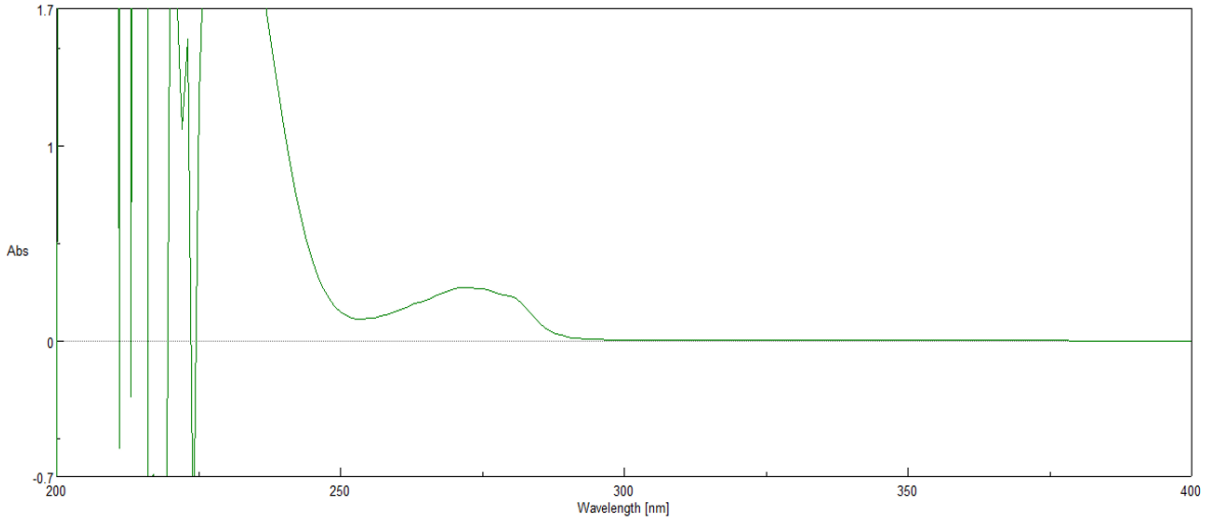
Preparation of working standards of TMZ

From TMZ standard stock solution, 1 mL to 5 mL were drawn using a pipette and mixed with methanol as a solvent to prepare working standards of 10-50μg/mL.

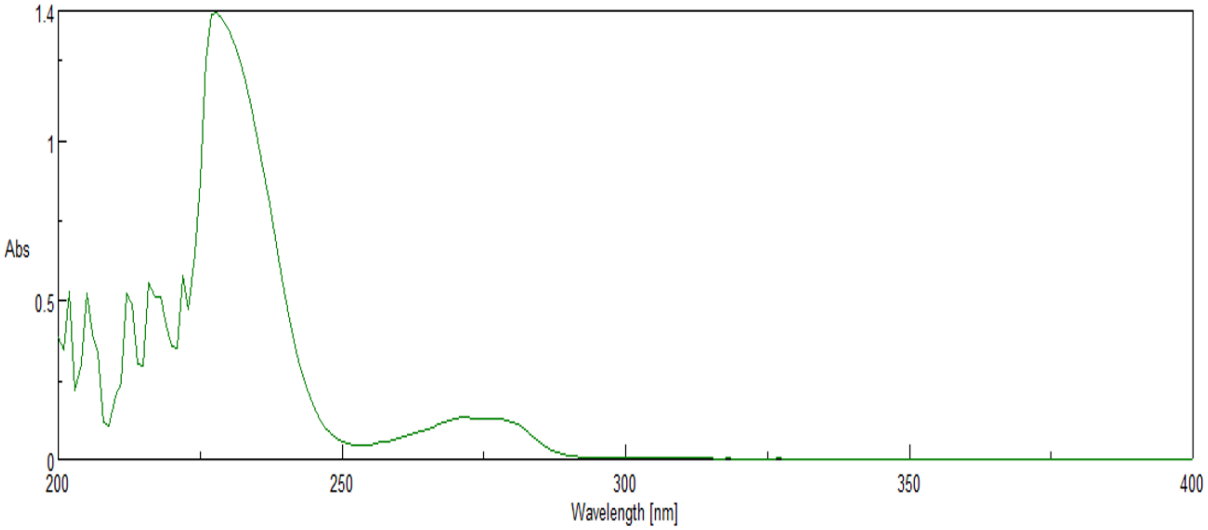
Calibration curve for IBH and TMZ

From these standard (stock) solutions, different working standards were prepared with methanol and examined in the whole UV range to determine that Ivabradine hydrochloride is at 286 nm and Trimetazidine dihydrochloride is at 231 nm λ max. The linearity framework was explored by sequentially diluting the stock solution to fix the range of 5–25 μg/mL for Ivabradine hydrochloride and 10–50 g/mL for Trimetazidine dihydrochloride **(Figure 3)**.

Table 1 represents recovery study of both drugs based on concentration range.



a)



b)

Figure 1. UV Spectra of IBH(286) & TMZ (231)

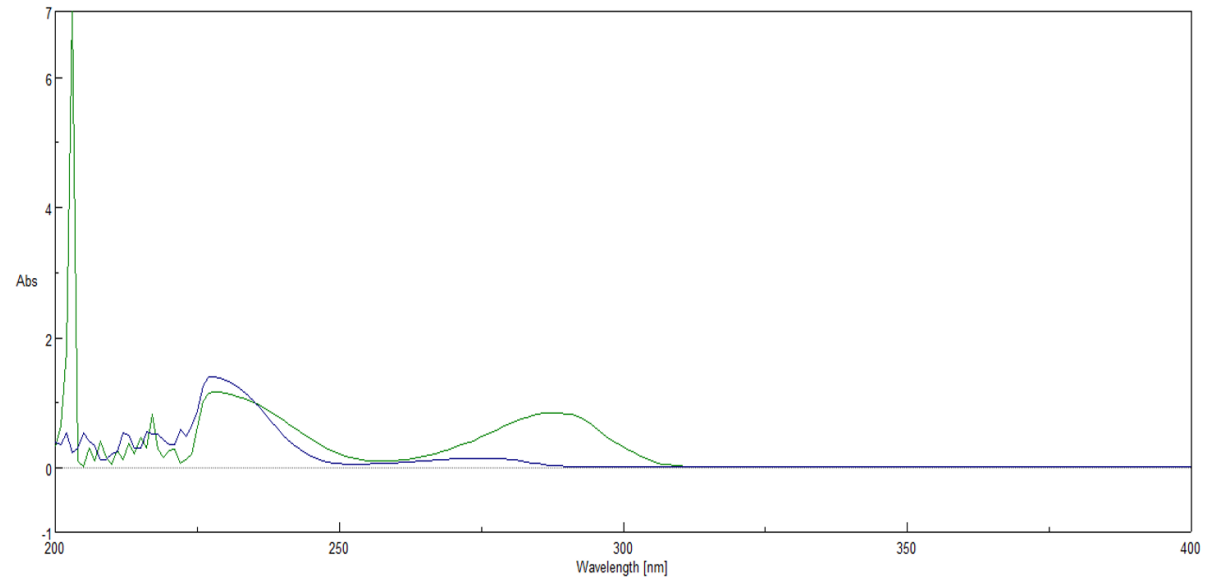
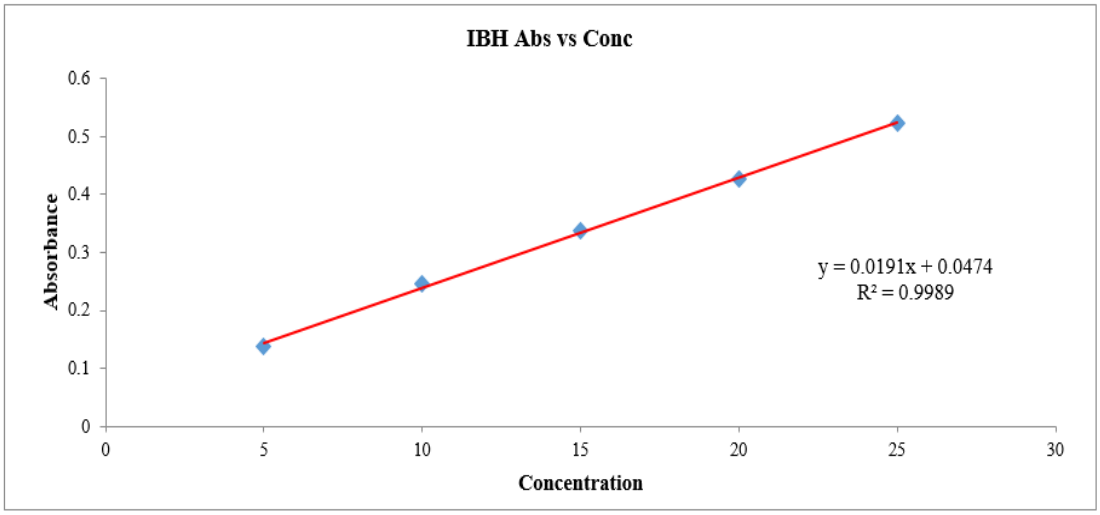
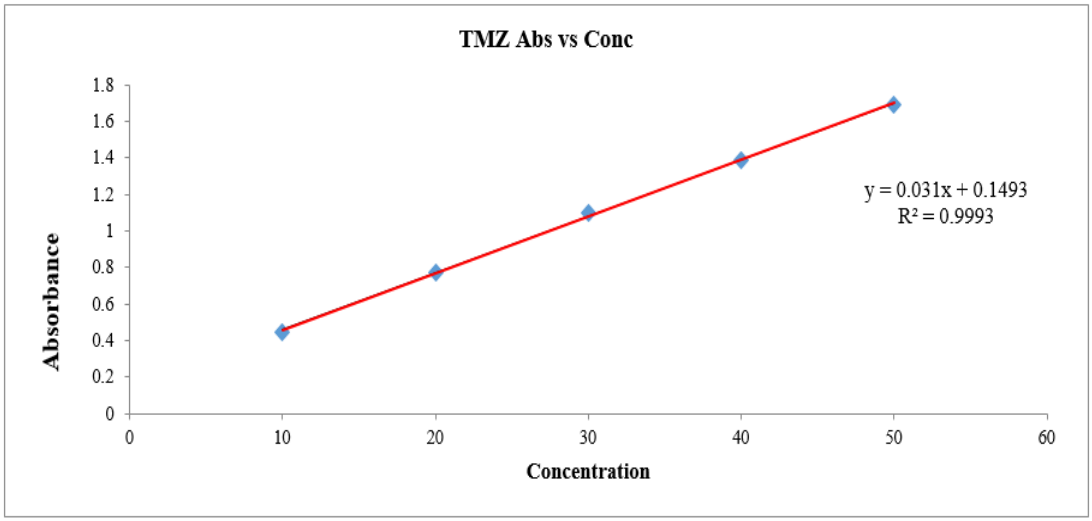


Figure 2. Overlay IBH and TMZ spectra



a)



b)

Figure 3. Calibration curve of IBH and TMZ

Table 1. Recovery result of IBH and TMZ

Test no [IBH]	Absorbance 286nm	Concentration (ppm)	Found Concentration	% Recovery
1	0.1369	5	4.685	93.717
2	0.2454	10	10.366	103.664
3	0.3375	15	15.188	101.256
4	0.4277	20	19.910	99.554
5	0.5239	25	24.947	99.790
Mean				**99.596**
SD				**3.671**
LOD				**2.350**
LOQ				**7.122**
SLOPE[M]				**0.0191**
C INTERCEPT				**0.0474**
Test No [TMZ]	**Absorbance 231nm**	**Concentration (ppm)**	**Found Concentration**	**% Recovery**
1	0.4482	10	9.641	96.419
2	0.7715	20	20.070	100.354
3	1.1017	30	30.722	102.408

4	1.387	40	39.925	99.814
5	1.6916	50	49.751	99.503
Mean				**99.700**
SD				**2.155**
LOD				**3.790**
LOQ				**11.484**
SLOPE[M]				**0.031**
C INTERCEPT				**0.1493**

Development of simultaneous estimation of IBH and TMZ

In this step, the prepared sample solutions of both drugs were scanned in the UV range of 200–400 nm, and overlay spectra were taken. By application of the simultaneous equation, the method with 286 nm (IBH λmax) and 231 nm (TMZ λmax) was selected for overlay spectra for analysis of both drugs together. Concentrations range of 5, 10, 15, 20, and 25 μg/mL for IBH and 10, 20, 30, 40, & 50 μg/mL for TMZ were prepared in methanol. The concentration of drugs x (IBH) and y (TMZ) in sample solutions was determined by the SE method using the following formula:

$$Cx = [A2ay1 - A1ay2/\ ax2ay1] - ax1ay2, \quad (1)$$

$$Cy = [A1ax2 - A2ax1/ax2ay1] - ax1ay2, \quad (2)$$

Where, Cx and Cy are the concentration of IBH and TMZ, $A1$ and $A2$ are the absorbance of sample solution at 286 nm and 231 nm, respectively, $ax1$ and $ax2$ are absorptivity of IBH at 286 nm and 231 nm, and $ay1$ and $ay2$ are absorptivity of TMZ at 286 nm and 231 nm, respectively [15-20].

Analysis of marketed tablet formulation and bulk API (Active Pharmaceutical Ingredient) mixture

Marketed tablet formulation, 10 tablets of IBH (Corlanor 5mg) and TMZ (Flavadon MR 35 mg) taken into a mortar and pestle, triturated into powder form. The powder mixture equivalent to 5 mg IBH and 35 mg TMZ was taken into a 100 mL flask. To this, 50 mL of methanol was mixed and sonicated for a few minutes. After sonication, the volume was adjusted with the same solvent and filtered through Whatman's filter paper (0.45 microns). The resultant filtered sample was further diluted to get a 20 µg/ml concentration. Finally, this was evaluated at 286 nm and 231 nm, for IBH and TMZ estimation.

Similarly, an API mixture of IBH and TMZ was prepared by weighing a 1:7 ratio of IBH and TMZ. From this mixture, 40 mg of powder was weighed, which equates to 5 mg of IBH and 35 mg of TMZ. This mixture was taken to a 100 mL flask, and methanol was mixed and sonicated for a few minutes. The resulting solution was then diluted to for final concentration of 20 µg/mL. The resulting solution was evaluated at 286 nm and 231 nm for IBH and TMZ respectively. The result is shown in **Table 2**.

Valıdatıon of method

1. *Linearity:* The calibration curves for IBH and TMZ were separately plotted using absorbance on the y-axis and concentration on the x-axis. For IBH, the working standard linearity range was found to be 5–25 μg/mL, and for TMZ 10–50 μg/mL, which were scanned using 286 nm and 23nm, respectively, to get the absorbance at a specific concentration. After plotting the graph, the correlation coefficient values ($R2$) of IBH and TMZ were 0.9989 and 0.9993, which indicates a direct relation between concentration and absorbance. The curves of IBH and TMZ are shown in **Figures 1 and 2**, respectively.

2. *LOD and LOQ (Limit of detection and limit of quantification):* The calibration curve was used to determine both LOD and LOQ, which were estimated based on the formula for LOD =3.3*(σ/S) and LOQ =10*(σ/S). Using y-intercept, where sigma is the standard deviation (SD) of the reaction (y-intercept) and S is the slope of the plot. The LOD of IBH and TMZ were reported at 2.350 µg/ml and 3.790 µg/ml, respectively. IBH had a limit of quantification (LOQ) at 7.122 µg/mL, while that for TMZ was 11.485 µg/mL. The table of LOD and LOQ of IBH and TMZ are 1 and 2, respectively.

3. *Precision:* Evaluation of precision or reproducibility of the method performed by determination of inter-day plus intra-day independent assays for 3 different samples selected from the calibration curve range, for IBH 10, 15, and 20 µg/mL, and for TMZ 20, 30, and 40 µg/mL, and percentage RSD was estimated. These precision results are shown in **Table 3**.

4. Accuracy: The accuracy study was performed at three levels using 50%, 100%, and 150%. This was studied by a recovery study using the standard addition method. A recovery study was performed to check for

any interferences due to excipients. Close to 100% recovery results revealed that the developed method is accurate and there was no interference due to other excipients. The accuracy study results is shown in **Table 4**.

Table 2. Results of Analysis of Marketed Formulation and Bulk API

Sample No.	Solvent	Sample	Label Claim		Amount Found (Based On Absorbance)		% Assay (Based On Amount Found)	
			IBH	TMZ	IBH	TMZ	IBH	TMZ
1	Methanol	API	5mg	35mg	4.497	34.591	89.94	98.83
2	Methanol	TABLET	5mg	35mg	4.902	34.795	98.04	99.41

Table 3. Precision Study

Concentration (in ppm)	Intraday	%RSD	Interday			%RSD		
IBH								
			1st Day	2nd day	3rd Day	1st Day	2nd day	3rd Day
10	0.122	1.5489	0.1987	0.204	0.1941	1.2751	0.8907	1.3499
	0.123		0.1951	0.2054	0.1971			
	0.1257		0.1939	0.2018	0.1994			
15	0.3314	1.6231	0.2909	0.2987	0.3048	0.5515	0.4809	1.1753
	0.3423		0.2887	0.2995	0.299			
	0.3377		0.2878	0.3015	0.2984			
20	0.4151	0.0482	0.3628	0.3823	0.3747	1.6566	0.8983	0.5439
	0.4149		0.3737	0.3834	0.3741			
	0.4147		0.3731	0.377	0.3779			
TMZ								
20	0.8011	1.6682	0.6631	0.6822	0.718	0.8719	0.5150	1.2738
	0.7788		0.6564	0.6849	0.7078			
	0.778		0.6679	0.6892	0.7			
30	1.0991	1.4972	0.9937	0.9948	1.0393	0.5841	1.8144	0.6938
	1.0747		1.0007	1.0212	1.0538			
	1.1058		1.0053	0.9864	1.0459			
40	1.425	0.8198	1.3319	1.3301	1.3799	1.3168	0.8661	1.3602
	1.4037		1.3092	1.3122	1.3827			
	1.4065		1.3436	1.3089	1.3491			

Table 4. Results of Recovery Studies

Level %	Sample ppm	Amount Added	Total ppm	Absorbance 286nm	Found Concentration	% Recovery	Mean	SD	%RSD
IBH Accuracy									
50	10	5	15	0.334	15.127	100.851	101.740	0.770	0.757
				0.3375	15.322	102.148			
				0.3377	15.333	102.222			

				0.4145	19.6	98			
100	10	10	20	0.4147	19.611	98.055	98.055	0.055	0.056
				0.4149	19.622	98.111			
				0.5135	25.1	100.4			
150	10	15	25	0.5155	25.2111	100.844	100.748	0.311	0.309
				0.5162	25.25	101			
TMZ Accuracy									
				1.108	30.251	100.838			
50	20	10	30	1.1058	30.184	100.613	100.548	0.326	0.325
				1.1017	30.058	100.194			
				1.4138	39.631	99.079			
100	20	20	40	1.4037	39.322	98.305	98.634	0.399	0.405
				1.4065	39.407	98.519			
				1.7609	50.279	100.558			
150	20	30	50	1.7544	50.079	100.159	100.265	0.256	0.255
				1.7531	50.039	100.079			

RESULTS AND DISCUSSION

The proposed technique was viewed as basic and direct in the focus scope of 5-25 μg/ml for Ivabradine hydrochloride and 10-50 μg/ml for trimetazidine dihydrochloride respectively. The created techniques have been approved concerning linearity, range, precision, specificity, accuracy, examination, LOD, and LOQ. The methodology seemed to be valid, as indicated by the reliability analysis, and the RSD was not more than 2. Thus, the developed methodology is clear and effective.

CONCLUSION

A rapid, simple, and convenient UV spectroscopic method was attempted for simultaneous estimation of the IBH and TMZ in tablet formulation. The linear response of the assay was obtained at a wide range of concentrations. A very low percentage of RSD revealed the effectiveness of the analytical method. Based on all the studied validation parameters we may summarize that the developed UV spectroscopic method is specific, up to the mark, and precise. Therefore, this developed method can be potentially used for daily evaluation and analysis of IBH and TMZ together in tablet formulation. Further deliberate degradation or stress testing can be performed to investigate the impact of light, moisture, temperature, and acid on the stability of IBH and TMZ.

Acknowledgments: The authors would like to acknowledge Lupin Limited, Sikkim, India, and Sharon Bio-Medicine Ltd, Maharashtra, India for API gift samples and PES's Modern College of Pharmacy, Nigdi, Pune-44, Maharashtra, India, for providing all the facilities required during conduct and execution of this research work.

Conflict of interest: None

Financial support: None

Ethics statement: None

REFERENCES

[1] Gowdak LHW. The role of ivabradine in managing symptomatic patients with chronic coronary syndromes: A clinically oriented approach. Cardiol Ther. 2022;11(1):163-74.
[2] Sagar J, Atul P. Stable Angina and its treatment with newer antianginals: A comprehensive review. The Indian Pharmaceutical Association. Pharma Times. Mumbai; Pharma Times. 2023;55(02).
[3] Sharma V, Dewangan HK, Maurya L, Vats K, Verma H. Rational design and in-vivo estimation of Ivabradine Hydrochloride loaded nanoparticles for management of stable angina. J Drug Deliv Sci Technol. 2019;54:101337.
[4] Thete PG, Saudagar RB. Quantitative determination and validation of a novel derivative spectrophotometric method for estimation of Ivabradine hydrochloride in bulk and marketed formulation. Asian J Pharm Pharmacol. 2018;4(5):697-701.
[5] Venugopalaiah PV, Prakash KPBP, Ramesh Y, Alagusundaram M. Formulation and evaluation of extended-release tablets of trimetazidine HCL. World J Adv Res Rev. 2023;18(3):993-1002.

[6] Shu H, Peng Y, Hang W, Zhou N, Wang DW. Trimetazidine in Heart Failure. Front Pharmacol. 2021;11:569132.
[7] The British Pharmacopoeia (BP). Ninth edition. H.M, The stationary office. London. 2019;2:1193-5.
[8] Agrawal S, Gurjar P, Katheriya B. Analytical method development and validation for simultaneous estimation of Trimetazidine hydrochloride and Metoprolol succinate using HPTLC. Curr Pharm Anal. 2019;15(3):243-50.
[9] Andal P, Kshatriya AG, Mhaske A, Naik MS. A new validated analytical method for simultaneous estimation of Trimetazidine dihydrochloride and Bisoprolol Fumarate from dosage forms using HPLC. J Pharm Negat Res. 2022;31:7398-407.
[10] Kuwar AV, Patil AP, Jadhav KD, Patil SR, Sonar KV. Development and Validation of UV Spectroscopic Method for Estimation of Ivabridine HCl in Tablet Dosage Form. Am J Pharm Tech Res. 2019;9(06):114-22.
[11] El-Alfy W, Ismaiel OA, El-Mammli MY, Shalaby A. Determination of atenolol and trimetazidine in pharmaceutical tablets and human urine using a high-performance liquid chromatography-photo diode array detection method. Int J Anal Chem. 2019;2019:1-8.
[12] Atia NN, Tawfeek HM, Rageh AH, El-Zahry MR, Abdelfattah A, Younis MA. Novel sublingual tablets of Atorvastatin calcium/Trimetazidine hydrochloride combination; HPTLC quantification, in vitro formulation and characterization. Saudi Pharm J. 2019;27(4):5409.
[13] Gandhi NK, Ezhava SB. Stability indicating analytical method development using quality by design (qbd) approach for simultaneous estimation of ivabradine and metoprolol. Res J Pharm Technol. 2021;14(11):5937-44.
[14] Ma D, Xu T, Cai G, Wu X, Lei Z, Liu X, et al. Effects of ivabradine hydrochloride combined with trimetazidine on myocardial fibrosis in rats with chronic heart failure. Exp Ther Med. 2019;18(3):1639-44.
[15] Yosrey E, Elmansi H, Sheribah ZA, Metwally ME-S. Micellar-emphasized simultaneous determination of ivabradine hydrochloride and felodipine using synchronous spectrofluorimetry. Luminescence. 2022; 37(4):569-76.
[16] Kanthale SB, Thonte SS, Mahapatra DK. Stability indicating RP-HPLC method for the simultaneous estimation of ivabradine and metoprolol in bulk and tablet formulation. J Appl Pharm Sci. 2019;9(4):137-44.
[17] Veeshma A, Priyanka S, Kumar KP, Sirisha K. Simultaneous estimation of ciprofloxacin and metronidazole in bulk and tablet formulation by UV spectrophotometry. Int J Pharm Sci Res. 2021;42:2247-56.
[18] Aayushi MS, Surati JS. UV-spectrophotometric method development and validation for simultaneous estimation of hydroxychloroquine sulfate and nitazoxanide in synthetic mixture. Int J Pharm Sci Res. 2022;13(3):1374-82.
[19] Polker A, Padmavathi Y, Kumar R, Babu N. Quantitative bioanalytical and analytical methods for estimation of ivabradine hydrochloride in pure and pharmaceutical dosage form. J Rep Pharm Sci. 2020;9(1):1-0.
[20] Gandi S, Manikandan A, Rao SV. Novel Stability indicating RP-UPLC Method for simultaneous Determination of Ivabradine and Metoprolol drug materials in bulk and their Pharmaceutical Dosage Forms. Res J Pharm Technol. 2020;13(1):250-4.