

Stability indicating RP-HPLC method for the simultaneous estimation of ivabradine and metoprolol in bulk and tablet formulation

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ABSTRACT

A new, accurate, precise, and robust reverse phase-high-performance liquid chromatography method has been developed along with sensitive stability indicating attributes for the simultaneous estimation of ivabradine (IVA) and metoprolol (MET) in both bulk and tablet formulation. The estimation of the solutes was performed on a Denali C₁₈ column of dimension 150 mm × 4.6 mm, 5 μm. IVA and MET were eluted with orthophosphoric acid (0.1%) buffer: acetonitrile in the ratio of 60:40 V/V in a 6 minutes isocratic trial at a flow rate of 0.8 ml/minute having an ambient column temperature of 30°C and monitored at 260 nm wavelength. The retention time of IVA and MET was found to be 2.290 and 3.520 minutes, respectively. The Q2b validation of the analytical method revealed good linearity over the concentration range 5–30 μg/ml for IVA and 25–150 μg/ml for MET with r^2 of 0.999 in both the cases. The mean recovery % over the three tested ranges of 50%, 100%, and 150% were found to be 100.46%, 99.68%, and 99.68%, respectively. The acid, alkali, dry heat, and peroxide-induced stress studies presented the formation of a variety of degradation products. The developed analytical method was precise, accurate, and robust for the routine analysis of the drug combination in bulk and tablet formulations.

INTRODUCTION

Ivabradine (IVA), 3-[3-[(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 (Fig. 1a) is a direct and selective inhibitor of the hyperpolarization-activated cyclic nucleotide-gated (I_p) channel current in the pacemaker cells of the sinoatrial node of the heart (DiFrancesco and Camm, 2004). It is used for the symptomatic management of stable heart-related chest pain and heart failure not fully managed by beta blockers (Gokhroo *et al.*, 2016). It decreases the myocardial oxygen consumption while increasing diastolic time, without affecting myocardial contractility or coronary vasomotor tone

(Kaski *et al.*, 2018). Metoprolol (MET), 1-(isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol (Fig. 1b) is a selective β₁ receptor blocker used to treat high blood pressure, chest pain due to poor blood flow to the heart, and a number of conditions involving an abnormally fast heart rate (HR) (Zarifis *et al.*, 2016). It often remains the drugs of choice for reducing HR in CHF with reduced ejection fraction (Volterrani and Iellamo, 2016). IVA hydrochloride in combination with MET succinate or tartrate in use for the treatment of inappropriate sinus tachycardia and in acute inferior wall STEMI patients for lowering the HR with a significantly lesser risk of AV blocks (Ptaszynski *et al.*, 2013). This combination significantly improves the symptoms and quality of life in patients with stable angina pectoris (Werdan *et al.*, 2016). It is available in the market as IMPLICOR[®], IVAMET XL[®], etc., brand in the form of film coated tablet containing 5 mg IVA with 25 mg MET or 7.5 mg IVA with 50 mg MET.

So far, numerous reports employing validated analytical reverse phase-high-performance liquid chromatography (RP-HPLC) (Maheshwari *et al.*, 2010), ultra-performance liquid

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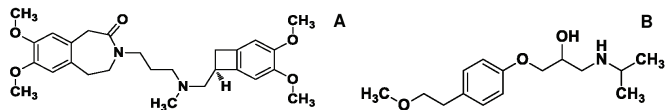


Figure 1. Structure of (a) ivabradine and (b) metoprolol.

chromatography tandem mass spectrometry (Sun *et al.*, 2015), fluorescence (Cui *et al.*, 2010), and spectrophotometric (Mostafa *et al.*, 2017) methods for individual estimation of IVA and its metabolic products (Li *et al.*, 2011) in plasma (rat, rabbit, and human) (Ranjha *et al.*, 2017; Zoerner *et al.*, 2013), bulk formulation, and pharmaceutical formulation (capsule, controlled release product, and tablet) (Patra and Panda, 2014; Rehman and Nagamallika, 2017; Seerapu and Srinivasan, 2010) by the global researchers have been into applications.

While going through the literature available in the standard global databases, not a single report have been found regarding any analytical RP-HPLC method for the routine simultaneous estimation of IVA and MET drug combination in bulk and pharmaceutical formulation along with validated stability indicating data. Understanding the fact, a simple, robust, precise, economical, and accurate method was developed to meet the challenge. The present work endeavors development of a validated stability indicating RP-HPLC method for simultaneous estimation of IVA and MET in bulk and tablet formulation.

MATERIALS AND METHODS

Materials

A generous gift sample of IVA and MET was obtained from SL Drugs and Pharmaceuticals Ltd., Hyderabad. The IVAMET XL[®] containing 5 mg of IVA and 25 mg of MET was purchased from Ajanta Pharma Ltd., Mumbai. Analytical grade chemicals (HiMedia Ltd., Mumbai) and HPLC grade solvents were employed for the study. The weighing was done on Shimadzu[®] AUW220D (Kyoto, Japan) balance. The pH was measured on VSI[®] VSI-1B digital pH meter (Mohali, India). Sonication was performed on Transonic Digital S sonicator (Mumbai, India). The method was developed on a reverse phase Denali C₁₈ column of dimension 150 mm × 4.6 mm having 5 μm particle size connected to a Waters[®] 2695 HPLC system equipped with PDA detector 2996 along with a manual rheodyne injector (20 μl loop), controlled by EMPOWERS v.2 software.

Selection of the mobile phase

The selection of the mobile phase is very critical for the elution of the solutes. The mobile phase was selected on the basis of theoretical plates, peak purity index, and peak symmetry. The trial commenced using the buffer systems with an eluant such as methanol, acetonitrile, etc. The elution with buffer KH₂PO₄: methanol in equal ratio produced low-intensity peaks with a high tailing. KH₂PO₄ buffer (pH 4.8) with acetonitrile led to the appearance of the broad peak with tailing, but an improvement was noticed in comparison to the previous trial. When the buffer was replaced with orthophosphoric acid (OPA) (0.1%), a significant excellence in the peak symmetry and reduced tailing

was observed when used along with methanol in equal ratio but was still not suitable to elute the solutes. For further perfection, the acetonitrile was employed along with OPA to get sharp peak with ideal Gaussian peak. The ratio of 60:40 v/v provided the highest peak purity index and the greatest number of theoretical plates. The mobile phase was degassed using the vacuum and filtered through 0.45 μm membrane filter. The mobile phase was allowed to equilibrate until a steady baseline was obtained.

Chromatographic conditions

IVA and MET were eluted with OPA (0.1%) buffer: acetonitrile in the ratio of 60:40 v/v in a 6 minutes isocratic trial at a flow rate of 0.8 ml/minute having an ambient column temperature of 30°C and monitored at 260 nm wavelength.

Preparation of analytical solutions

Preparation orthophosphoric acid (0.1%) buffer

An accurately weighed quantity of OPA (1 ml) was diluted with 1,000 ml of HPLC grade water and further degassed by sonication.

Preparation of mobile phase

The above-prepared buffer was thoroughly mixed with acetonitrile in the ratio of 60:40 v/v. The solution was then degassed by sonicating for the period of 5 minutes and filtered through 0.45 μm membrane filter under vacuum.

Diluent preparation

A 60:40 V/V ratio of water and acetonitrile was employed as the diluent for the preparation of the standard solution and the sample solution.

Standard preparation

An accurate amount of 5 mg IVA and 25 mg MET were added to 25 ml dry volumetric flask and 5 ml of diluent was added. The above content was sonicated for 10 minutes and the volume was made up to 10 ml to produce 500 ppm of IVA and 2,500 ppm of MET. Then, 1 ml of the produced content was pipetted out in a 10 ml volumetric flask and the volume was diluted to 10 ml to produce 50 and 250 ppm of the content.

Sample preparation

Five tablets were weighed accurately and the average weight was calculated accordingly. In a 100 ml of volumetric flask, weight equivalent to a tablet was transferred and half-filled with the diluent. The content was sonicated for the period of 20 minutes and filtered suitably to produce 500 ppm of IVA and 2,500 ppm of MET. Then, 1 ml of the solution was transferred to a volumetric flask of 10 ml volume and diluent was added to produce 50 and 250 ppm of the content.

Method validation

According to the Q2A and Q2B guidelines of the International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use and in compliance with the United States Food and Drug Administration (USFDA) guidance, the proposed method was validated.

Linearity and range

The linearity of the method was estimated by taking six concentrations of the solutes in the range of 5–30 µg/ml for IVA and 25–150 µg/ml for MET. The solutions were prepared with the diluent and equivalent volume was injected into the HPLC system to determine the peak area. A linearity graph was plotted between the concentration and average area for each solute. The regression coefficient value (r^2) was also determined (Sawale *et al.*, 2017).

Accuracy

The accuracy (recovery) was determined by spiking the standard drug solutions at concentrations of 50%, 100%, and 150% in the HPLC system. The study was performed in a triplicate way with data expressed in the form of % recovery \pm % relative error on the basis of definite concentrations (Deodhe *et al.*, 2017a).

Precision

The precision of the developed method was estimated in terms of inter-day and intra-day variability by spiking the concentrations of 50%, 100%, and 150% six times in a single day (intra-day) and also on three different days (inter-day). The data were expressed in precision \pm % relative error (Deodhe *et al.*, 2017b).

Robustness

The robustness of the method was judged by deliberately altering the mobile phase composition by \pm 5% v/v (i.e., 65:35% v/v and 55.45% v/v), flow rate by \pm 0.1 ml/minute (i.e., 0.9 and 1.1 ml/minute), and column temperature by \pm 5°C (i.e., 25°C and 35°C), keeping the other chromatographic parameters constant (Jha *et al.*, 2017).

Systems suitability parameters

The reproducibility profile of the analytical method was estimated by injecting five-times the standard solution and recording the parameters such as retention time, peak area, theoretical plates, and tailing factor (Prakash *et al.*, 2018).

Limit of detection and quantification

The limit of detection (LOD) may be defined as the lowest detectable concentration by any analytical method, but not necessary to measure the exact amount (Perumal *et al.*, 2014).

The LOD was determined by the formula:

$$\text{LOD} = 3.3 (\sigma/S)$$

Where σ = standard deviation of response; S = slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

The limit of quantification (LOQ) may be defined as the lowest detectable concentration by any analytical method with a particular level of accuracy and precision (Bauer *et al.*, 2014).

The LOQ is determined by the formula:

$$\text{LOQ} = 10 (\sigma/S)$$

Where σ = standard deviation of response; S = slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

Degradation studies

Acid degradation studies

0.5 mg of the equivalent amount of drug combination was taken in a 100 ml volumetric flask and 50 ml of the diluent was added. The content was sonicated for the duration of 15 minutes and the volume was made with the remaining amount of diluent. The content was stirred for half an hour and centrifuged for 5 minutes at 3,000 rpm. Five milliliters of the solution was taken carefully and mixed with equal volume of 2 N HCl. The content was allowed to boil for an hour and afterward neutralized with 2 N NaOH. The volume was made up to 100 ml and filtered cautiously with 0.45 µm membrane filter. Twenty microliters volume of sample was spiked into the system and the data were recorded.

Alkali degradation studies

0.5 mg of the equivalent amount of drug combination was taken in a 100 ml volumetric flask and 50 ml of the diluent was added. The content was sonicated for the duration of 15 minutes and the volume was made with the remaining amount of diluent. The content was stirred for half an hour and centrifuged for 5 minutes at 3,000 rpm. Five milliliters of the solution was taken carefully and mixed with equal volume of 2 N NaOH. The content was allowed to boil for an hour and afterward neutralized with 2 N HCl. The volume was made up to 100 ml and filtered cautiously with 0.45 µm membrane filter. Twenty microliters volume of sample was spiked into the system and the data were recorded.

Oxidation degradation studies

0.5 mg of the equivalent amount of drug combination was taken in a 100 ml volumetric flask and dissolved in 5 ml of H₂O₂ (30% w/w). The content was boiled for an hour to initiate the oxidative stress-induced drug degradation. The content was diluted with diluent and the volume was made up to the mark. The content was centrifuged, supernatant was collected, and 20 µl of volume was spiked into the system to record the data.

Dry heat degradation studies

0.5 mg of the equivalent amount of drug combination was taken in a 100 ml volumetric flask and exposed to heat at 90°C \pm 1°C for 1 hour. The content was diluted with the diluent and stirred for 30 minutes, further sonicated, and the volume was made up to 100 ml. The content was centrifuged for 5 minutes time at 3,000 rpm, filtered, and 20 µl of the volume was spiked into the HPLC system.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions

The novel method was based entirely on trials and errors as no previous similar methods are developed. However, some inspiration was drawn from the already available reports while selecting the stationary phase. The Denali reverse phase C₁₈ stationary phase of dimension 250 \times 4.6 mm i.d., particle size 5 µm was applied. The mobile phase OPA (0.1%): acetonitrile in the ratio of 60:40 v/v

was employed for the elution after several continuous trials run. A low pH was applied to the mobile phase as it ensured reduction in the peak tailing and significantly improves the robustness of the analytical method. Silica-based reverse phase columns are prone to dissolution due to high basic pH, therefore the use of acidic pH was justified to a greater extent. It was also ascertained that the pH of the mobile phase remains in close agreement with the pKa of the solute to make them remain in the unionized state. Therefore, the pH value in accordance with two units was chosen. The elution was performed using Denali C₁₈ column employing mobile phase OPA (0.1%): acetonitrile in the ratio of 60:40 v/v in isocratic mode run for 6 minutes duration. The flow rate was maintained at 0.8 ml/minute, keeping the column temperature at 30°C, and detected at 260 nm. The retention time of IVA and MET was found to be 2.290 and 3.520 minutes, respectively (Fig. 2a). The short-run method will conserve time and solvent and will provide peaks with better resolution. The tablet sample solution presented retention time of IVA at 2.289 minutes and for MET at 3.516 minutes (Fig. 2b). This undoubtedly revealed that the developed analytical method was precise, accurate, and robust for the routine analysis of the drug combination in bulk and tablet formulations.

Method validation

Linearity and range

Over the range of 10–30 µg/ml for IVA and 25–150 µg/ml for MET, an exceptionally high linearity was observed between the dose and peak area with linear regression equations found to be $y = 17451x + 775.43$ and $y = 24280x + 4295.11$, respectively (Table 1). The regression coefficient values were 0.999 in both the cases which significantly represent a high degree of linearity (Fig. 3).

Accuracy

By the application of the calibration curve, the Y-intercept and the slope of the graph played a pivotal role in the

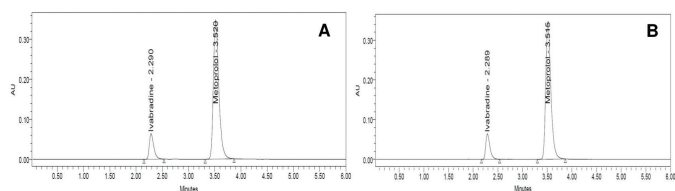


Figure 2. Chromatogram for ivabradine and metoprolol (a) after method optimization and (b) tablet sample solution.

Table 1. Linearity study of IVA and MET.

Linearity levels (%)	IVA		MET	
	Concentration (µg/ml)	Peak area (mV)	Concentration (µg/ml)	Peak area (mV)
25	5	91,815	25	633,581
50	10	175,590	50	1,219,283
75	15	260,273	75	1,808,568
100	20	349,645	100	2,430,079
125	25	428,697	125	3,003,949
150	30	531,783	150	3,681,392

determination of % recovery attribute of the proposed method for the simultaneous estimation. The established % RSD values were 0.36, 0.63, and 0.36 for IVA and 0.63, 0.78, and 0.31 for MET, respectively, which was within the USP pharmacopeia acceptance limit of ±2% (Table 2). Overall, the method signified good accuracy of the obtained recovery data.

Precision

In both the intra-day and inter-day variability studies for precision data, the method was found to be extremely precise over the tested range of 10–30 µg/ml for IVA and 25–150 µg/ml for MET. The peak area of the sample solution matched corresponding to the standard solution in both the cases, along with % RSD of <2%. The % RSDs were observed to be in the range of 0.34%–0.56% for IVA and 0.19%–0.39% for MET of the intra-day studies (Table 3), whereas the % RSDs were observed to be in the range of 0.21%–0.36% for IVA and 0.26%–0.53% for MET in the inter-day studies (Table 4) which reflected a high precision and minimum variation of the developed method.

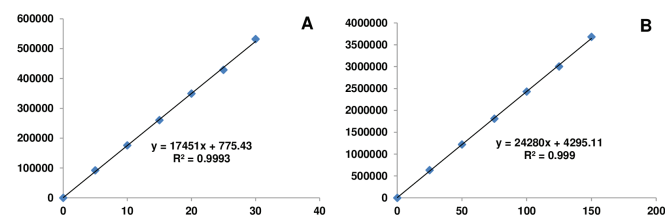


Figure 3. Linearity plot of (a) ivabradine and (b) metoprolol.

Table 2. Recovery for accuracy studies for the combination.

Spiked level (%)	Conc. of drug added (µg/ml)	Conc. of drug found (µg/ml)	Recovery (%)	Mean (%)	% RSD
Ivabradine					
	10	10.0871	100.87		
50	10	10.0349	100.35	100.46	0.36
	10	10.0185	100.18		
	20	19.9817	99.91		
100	20	20.0347	100.17	99.68	0.63
	20	19.7957	98.98		
	30	30.0311	100.10		
150	30	29.8422	99.47	99.68	0.36
	30	29.8422	99.47		
Metoprolol					
	50	49.8687	99.74		
50	50	49.7921	99.58	100.02	0.63
	50	50.3699	100.74		
	100	99.7799	99.78		
100	100	100.291	100.29	99.61	0.78
	100	98.7558	98.76		
	150	149.043	99.36		
150	150	148.13	98.75	99.04	0.31
	150	148.542	99.03		

Robustness

With the intentional variation in some key chromatographic parameters such as mobile phase composition, flow rate, and column temperature by $\pm 5\%$, ± 0.1 , and $\pm 5^\circ\text{C}$, a considerable change was observed in the chromatogram for both the drugs. When the mobile phase combination was varied to 65:35 v/v, the general retention peak of 2.290 minutes (IVA) and 3.520 minutes (MET) got shifted to 2.132 and 3.135 minutes, which was nearly 10% variation (Fig. 4a). In a similar manner, the alteration

Table 3. Precision data of intra-day variability.

Drug	Conc. ($\mu\text{g/ml}$)	Peak area of standard (mV)	Peak area of sample (mV)	% label claim	%RSD
IVA	10	353,332	353,390	100.01	0.56
	20	353,861	356,608	100.77	0.41
	30	356,766	356,573	99.94	0.34
	50	2,441,691	2,451,819	100.41	0.27
MET	100	2,455,467	2,456,204	100.03	0.39
	150	2,474,722	2,447,927	98.91	0.19

Conc. = concentration; SD = standard deviation; RSD = relative standard deviation.

Table 4. Precision data of inter-day variability.

Drug	Conc. ($\mu\text{g/ml}$)	Peak area of standard (mV)	Peak area of sample (mV)	% label claim	%RSD
IVA	10	338,983	338,106	99.74	0.36
	20	345,011	346,202	100.34	0.29
	30	346,218	345,215	99.71	0.21
	50	2,338,248	2,340,667	100.10	0.53
MET	100	2,382,253	2,387,851	100.23	0.34
	150	2,404,741	2,398,305	99.73	0.26

Conc. = concentration; SD = standard deviation; RSD = relative standard deviation.

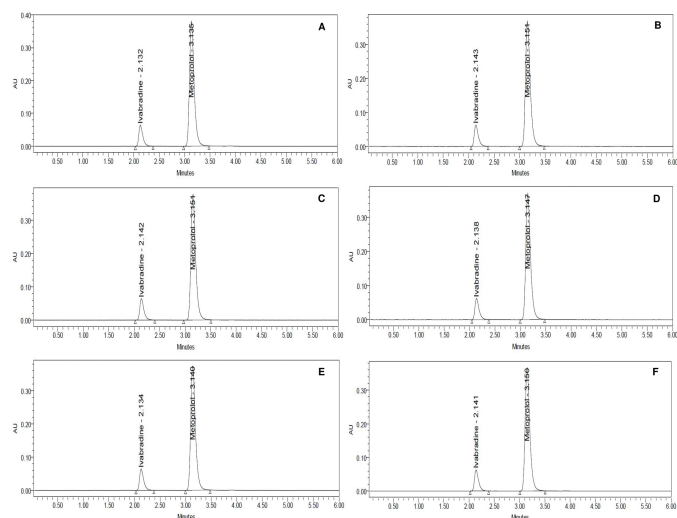


Figure 4. Robustness studies of ivabradine and metoprolol under (a) mobile phase composition 65:35 v/v; (b) mobile phase composition 55:45 v/v; (c) flow rate at 0.9 ml/minute; (d) flow rate at 0.7 ml/minute; (e) column temperature 35°C; and (f) column temperature 25°C.

in the composition by 55:45 v/v, the retention time was differed by nearly 8%–10% and the peaks were located primarily at 2.143 and 3.147 minutes (Fig. 4b). As the flow rate was enhanced by 0.1 ml/minute, the peaks emerged at 2.138 and 3.147 minutes (Fig. 4c). In contrast, the reduction in the flow rate by similar magnitude produced retention at 2.142 and 3.151 minutes (Fig. 4d). With an increase in the flow rate, the compound appeared earlier in the chromatogram whereas vice-versa phenomenon was observed when the flow rate was decreased. The disparity in the column temperature by 5°C produced retention peaks at 2.134 and 3.140 minutes at 35°C (Fig. 4e) whereas 2.141 and 3.150 minutes when the temperature was maintained at 25°C (Fig. 4f). The tailing factor was found to be $<2\%$ and the plate count was recognized to be $>2,000$, therefore matching the minimum requirements of the USP pharmacopeia (Table 5). In all the studies, the deliberate change in the parameters leads to an appearance of the peaks in a very close range of 2.132–2.143 minutes for IVA and 3.135–3.151 for MET which certainly indicated that the developed method possesses robust attributes.

System suitability parameters

The system suitability parameters of the developed method demonstrated a high degree of reproducibility and can be employed for the routine analysis of the drug combination. For IVA, the developed method expressed average retention time (R_t) of 2.287 minutes along with mean theoretical plates (TP) of 3,618. The R_t and TP were perceived to be 3.521 minutes and 5,760 in the case of MET (Table 6). The tailing factor of $<2\%$ signified no specific tailing in both cases. Good peak symmetry (asymmetric factor = 1) represented an ideal Gaussian peak where both the factors (symmetric and asymmetric) were of equal magnitude. A noteworthy resolution, significant separation, high column efficacy, and better reproducibility of the developed method can be judged from the fact that it met the minimum requirements of monographs of US Pharmacopoeia (minimum theoretical plates of 2,000 and tailing factor $<2\%$). The separation factor (α) and resolution factor (R_s) were found

Table 5. Robustness study of IVA and MET.

Parameter varied	Retention time	Peak area	USP plate count	USP tailing
Ivabradine				
Mobile phase 65:35 v/v	2.132	351,761	3,283	1.45
Mobile phase 55:45 v/v	2.143	350,038	3,665	1.47
Flow rate 0.9 ml/minute	2.138	350,566	3,345	1.48
Flow rate 0.7 ml/minute	2.142	353,438	3,786	1.51
Column temperature 35°C	2.134	353,220	3,397	1.46
Column temperature 25°C	2.141	354,250	3,892	1.46
Metoprolol				
Mobile phase 65:35 v/v	3.135	2,403,611	5,904	1.34
Mobile phase 55:45 v/v	3.151	2,415,903	5,702	1.35
Flow rate 0.9 ml/minute	3.147	2,398,045	5,346	1.36
Flow rate 0.7 ml/minute	3.151	2,401,986	5,557	1.37
Column temperature 35°C	3.140	2,410,497	5,796	1.34
Column temperature 25°C	3.150	2,417,631	5,526	1.35

Table 6. Systems suitability parameters.

IVA						MET					
Rt (min)	Area (mV)	Theoretical plates (TP)	Separation factor	Resolution factor	Tailing factor	Rt (minute)	Area (mV)	Theoretical plates (TP)	Separation factor	Resolution factor	Tailing factor
2.285	378,053	3,504	1.536	1.932	1.45	3.512	2,516,517	5,656	1.536	1.891	1.34
2.287	377,337	3,641	1.536	1.931	1.44	3.515	2,512,491	5,555	1.536	1.899	1.35
2.287	379,967	3,881	1.537	1.928	1.53	3.516	2,521,645	5,885	1.537	1.893	1.34
2.288	378,516	3,764	1.538	1.921	1.51	3.520	2,517,892	5,583	1.538	1.894	1.35
2.289	375,014	3,505	1.539	1.929	1.47	3.524	2,517,961	5,893	1.539	1.889	1.36
2.290	375,405	3,415	1.545	1.927	1.47	3.539	2,522,600	5,987	1.545	1.892	1.33
2.287 (Mean)	377,382 (Mean)	3,618 (Mean)	1.538 (Mean)	1.928 (Mean)	1.478 (Mean)	3.521 (Mean)	2,518,184 (Mean)	5,760 (Mean)	1.538 (Mean)	1.893 (Mean)	1.345 (Mean)
%RSD		0.08							0.28		

to be significantly high as mentioned in the minimum ICH limits and requirement guidelines of 1 and 1.5, respectively, which concluded that the proposed analytical method produces better separation of both the peaks with reduced tailing and better resolution. Therefore, the method can be employed for the routine analysis owing to high precision, reproducibility, and accuracy attributes.

Limit of detection and quantification

The LOD and LOQ of IVA were observed to be 0.38 and 0.69 µg/ml, whereas for MET it was found to be 0.27 and 0.71 µg/ml, respectively, which signified towards the tremendous detection ability of the method for the lowest possible concentration of the solute simultaneously from the combination or formulation.

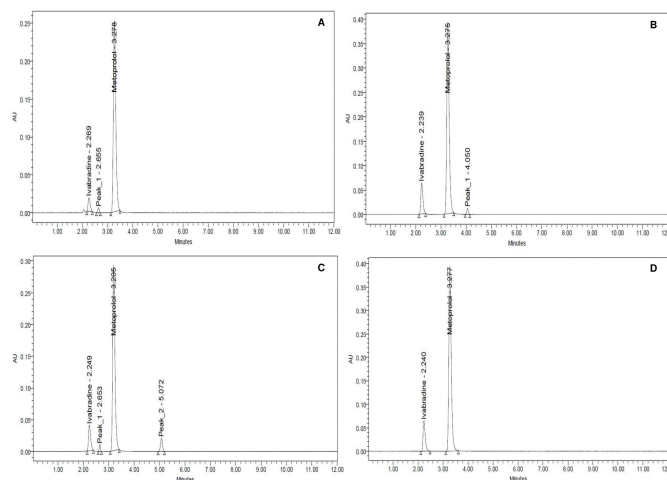
Forced degradation studies

The forced degradation study of both the molecules in combination revealed possible degradation under the influence of peroxide, thermal environment, and base treatment. However, the

compounds were resistant to the acidic treatment as no degraded products were observed in the chromatogram (Table 7). The treatment with hydrogen peroxide produced oxidative stress, which resulted in an acute shift of retention peaks of IVA and MET to 2.269 and 3.278 minutes along with the emergence of a degraded compound at 2.665 minutes (<0.5% of the total drug content) (Fig. 5a). When the combination was exposed to the thermal stress, a degraded material at 4.050 minutes (<0.005% of the total drug content) was observed in the chromatogram along with an alteration in the retention of IVA and MET at 2.239 and 3.275 minutes (Fig. 5b). The treatment under basic condition produced degradation of the compound to most severe form as suggested from the two prominent peaks at 2.653 minutes (<1.5% of the total drug content) and 5.072 minutes (<3.5% of the total drug content) along with drug peaks at 2.249 and 5.072 minutes (Fig. 5c). The degradation mechanism may be due to the mechanistic abstraction of the proton by the base (OH^{*}) radical from the compound by cleaving the weak bond between the alpha proton and the carbon atom. No degradant peaks were observed in the chromatogram under the acidic conditions, but a change in the retention time of the drugs was seen at 2.240 and 3.277 minutes,

Table 7. Forced degradation profile of IVA and MET.

Parameter varied	Retention time	Peak area	% peak area	USP plate count	USP tailing
Oxidative stress-induced degradation					
IVA	2.269	327,652	12.337	3,144	1.31
MET	3.278	2,316,102	87.208	5,577	1.32
PEAK-1	2.655	12,097	0.455	9,542	1.01
Thermal-induced degradation					
IVA	2.239	344,907	12.579	3,565	1.31
MET	3.275	2,395,316	86.835	5,946	1.32
PEAK-1	4.050	1,609	0.586	9,629	1.13
Base-induced degradation					
IVA	2.249	327,346	11.946	3,262	1.31
MET	3.205	2,281,899	83.274	1,806	1.32
PEAK-1	2.653	39,805	1.453	1,806	0.90
PEAK-2	5.072	91,190	3.327	51,871	1.02
Acid-induced degradation					
IVA	2.240	345,113	12.592	3,442	1.41
MET	3.277	2,395,482	87.408	5,911	1.33

**Figure 5.** Force degradation studies of ivabradine and metoprolol under (a) peroxide; (b) thermal condition; (c) basic treatment; and (d) acidic environment.

respectively (Fig. 5d). However, from the study, it was noticed that MET remained quite resistant as the retention time differed in a minimum amount. The future plans of the study will involve correct establishment of the chemical structures of the quantified degraded materials by using sophisticated techniques like LC-MS or GC-MS which will help in providing better quality control and quality assurance attributes for pharmaceutical industries.

CONCLUSION

The developed analytical method can be employed for the simultaneous estimation of IVA and MET in both bulk and tablet formulation. The ICH guidelines for validation indicated that the method has linearity over the range, accuracy, precision, and robustness. The % RSD, theoretical plates, and tailing values complied with the minimum requirements of monographs of US Pharmacopoeia. The validated stress degradation studies under thermal, oxidative, alkali, and acid presented the possible degraded components which will be of great importance to the chemists for quality control and assurance purpose. The method can be employed for the routine analysis owing to high precision, reproducibility, and accuracy attributes.

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CONFLICT OF INTEREST

No conflict of interest declared.

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