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Robust RP-HPLC method with forced degradation studies for the simultaneous quantification of Efonidipine HCL Ethanolate and Metoprolol succinate

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Abstract

Background A robust and validated analytical method was developed for the simultaneous quantification of Efonidipine HCl Ethanolate and Metoprolol Succinate, which show a synergistic effect against thrombocytopenia. The analysis used a Shimpack C18 column (250×4.6 mm, 5 µm) with isocratic elution. The optimized mobile phase consisted of acetonitrile (ACN), Methanol, and phosphate buffer (pH 3.5) in a 65:20:15 (v/v/v) ratio, delivered at a flow rate of 1.0 mL/min. Detection was performed at 225 nm using a photodiode array (PDA) detector. The method was validated according to ICH Q2 (R2) guidelines, and forced degradation studies were conducted under acidic (1 N HCl), alkaline (1 N NaOH), oxidative (3% H_2O_2), thermal (110 °C for 3 h), and photolytic (UV light exposure) conditions.

Results Efonidipine and Metoprolol eluted at 7.17 and 2.77 min, respectively, showing good linearity for EFO (20–120 µg/mL, $r^2 = 0.9981$) and MET (12.5–75 µg/mL, $r^2 = 0.9961$), high recovery (98–102%), and acceptable precision. The method was accurate, robust, and sensitive. Both drugs were stable under thermal and photolytic conditions but degraded under acidic, alkaline, and oxidative stress.

Conclusion The stability-indicating method is reliable and ideal for routine analysis and stability studies in quality control lab.

Keywords Efonidipine HCl ethanolate, Metoprolol succinate, Forced degradation, Solution stability, Method validation, RP-HPLC

1 Introduction

EFO is a dihydropyridine derivative calcium channel blocker, providing efficacy in managing hypertension and angina [1 – 2] Efonidipine Hydrochloride Ethanolate (EFO) is a calcium channel blocker classified as a dihydropyridine derivative. Chemically, it is known as 2-[(3E)-3-(2,6-Dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-enyl]-6-methyl-4-(2-phenylethyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride ethanolate. Its molecular formula is $\mathbf{C_{22}H_{28}N_2O_5{\cdot}HCl{\cdot}C_2H_5OH}$ and has a molar mass of

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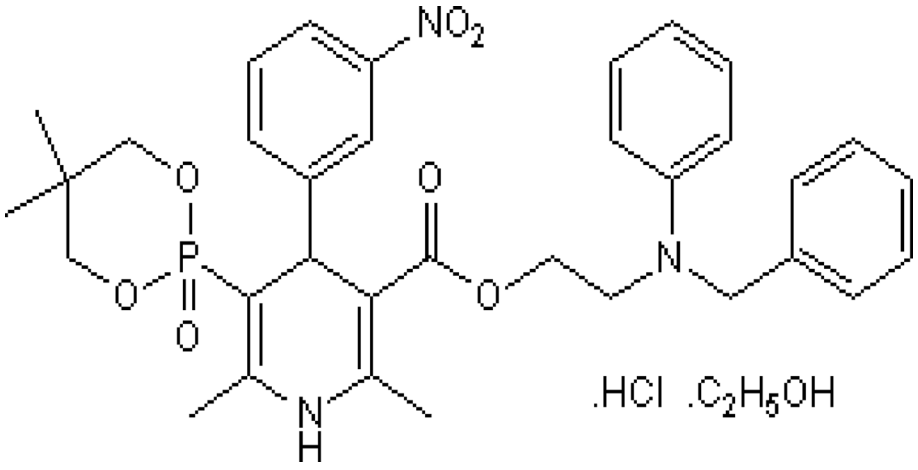

Fig. 1 Chemical structure of Efonidipine Hydrochloride Ethanolate

442.98 g/mol. The structure of EFO features a dihydropyridine ring, which is central to its calcium channel-blocking activity, with methoxy and ethoxy functional groups at specific positions on the aromatic rings. The presence of the ethanolate molecule in its formulation is significant as it stabilizes the compound and ensures prolonged release, contributing to its extended pharmacokinetic profile [3– 4] Fig. 1.

Metoprolol succinate, a β-adrenergic receptor antagonist, is therapeutically utilized in the management of angina pectoris, heart failure, myocardial infarction, atrial fibrillation, atrial flutter, and hypertension [3–5]chemically (RS)-1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol succinate with molecular mass & chemical formula of 652.8 g/mol & $C_{15}H_{25}NO_3 \cdot C_4H_6O_4$. Metoprolol Succinate is a **potent and selective β-blocker** that plays a vital role in managing several cardiovascular conditions Fig. 2.

The combination of *Metoprolol Succinate* and *Efonidipine HCl Ethanolate* provides a *synergistic* pharmacological approach to treating thrombocytopenia.

A literature review discloses that various analytical procedures have been established for the drugs, either on their own or in blend with other drugs. These include a range of various RP-HPLC [6–11], LC-MS [12–14], HPTLC [15] for EFO & RP-HPLC [16–26], UPLC [27], HPTLC [28–30], & UV Spectroscopy [31] for MET applied to pure drugs, pharmaceutical formulations, and biological fluids.

This study presents a rapid, reliable, and cost-effective RP-HPLC method for simultaneous quantification of Efonidipine and Metoprolol, addressing current analytical limitations. The method enables shorter analysis time, improved sensitivity and precision, and eliminates the need for separate assays. It is suitable for routine quality control and complies with regulatory requirements for linearity, accuracy, precision, and robustness.

Combine tablets of Efonidipine & Metoprolol in dosages of 40 & 25 mg with a brand name of EFNOCAR-MX 40/25 mg Tablet with the manufacturer's company name of Zuventus Healthcare Ltd.

In contrast with the previously developed RP-HPLC method for these combination [34].

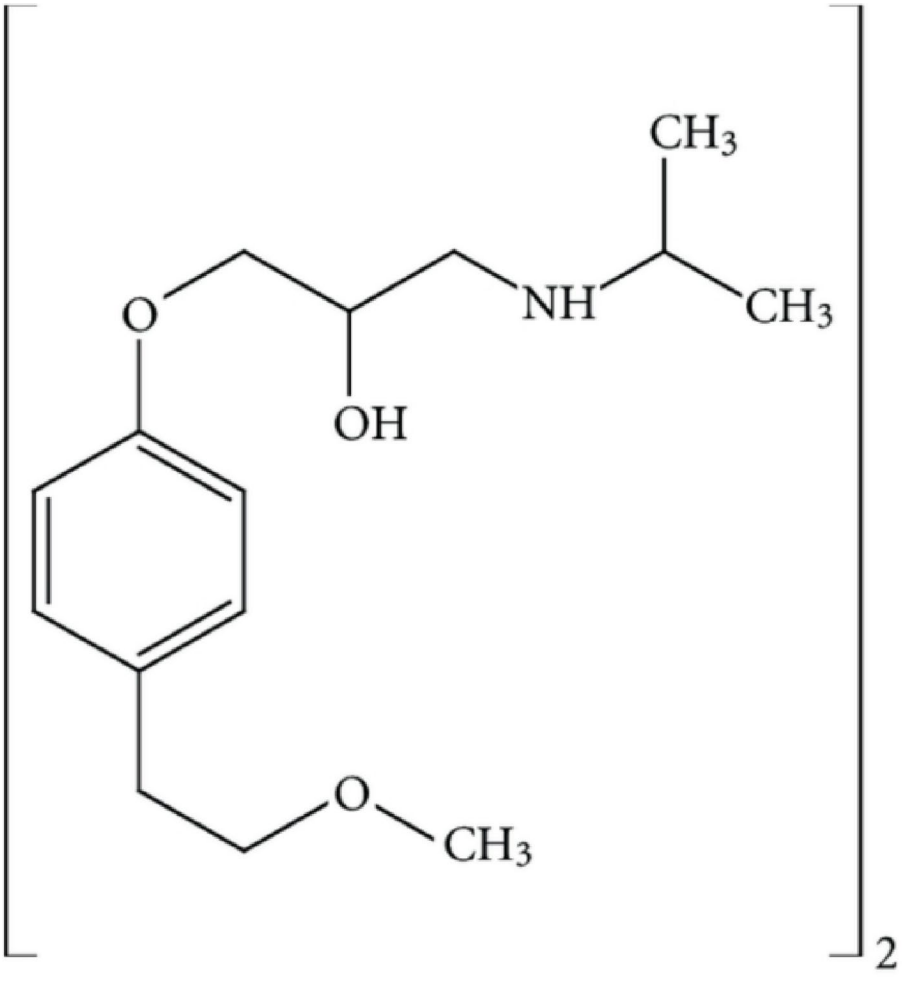

Fig. 2 Chemical structure of Metoprolol succinate

Feature	Published method	Our method
Objective	Develop a simple, precise, robust, and accurate stability-indicating RP-HPLC method	Develop a robust and validated method for simultaneous quantification with emphasis on synergistic action
Column	Zodiac CN (250×4.6 mm, 5 µm)	Shimpack C18 (250×4.6 mm, 5 µm)
Mobile Phase	20 mM Ammonium acetate: ACN (40:60)	ACN: Methanol: Phosphate buffer (pH 3.5) in 65:20:15 ratio
Flow Rate	1.0 mL/min	1.0 mL/min
Detection Wavelength	225 nm (PDA detector)	225 nm (PDA detector)
Linearity Range	EFO: 5–500 µg/mL; MET: 6.25–625 µg/mL	EFO: 20–120 µg/mL (r^2 = 0.9981); MET: 12.5–75 µg/mL (r^2 = 0.9961)
Degradation Conditions	Oxidation, hydrolysis, photolysis, thermal	Acidic, alkaline, oxidative, thermal (110 °C/3 h), photolytic (UV light)
Results	Good linearity (r^2 = 0.999), stability under stress not specified	High recovery (98–102%), stability under thermal and photolytic conditions; degradation in acidic/alkaline
Validation	According to ICH guidelines (implied)	Validated as per ICH Q2 (R2) guidelines
Conclusion	Suitable for simultaneous assay and stability-indicating purposes	Reliable, sensitive method ideal for routine quality control and stability studies

Although the published method addresses a wider concentration range, our chosen range is more closely aligned with actual pharmaceutical dosage levels. This makes our method more precise and practical for routine quality control. Additionally, our mobile phase—comprising ACN, methanol, and phosphate buffer at pH 3.5—is carefully optimized to deliver better separation and peak symmetry, which is especially valuable when analyzing complex formulations or compounds that elute closely together.

2 Methods

2.1 Materials and chemicals

Efonidipine HCl Ethanolate (EFO) (Batch No.: Z05CW24010, assay: 99:50%) and Metoprolol Succinate (MET) (Batch No.: C0CR2410, assay: 99.70%) standards were provided as gifts from Zuventus Pharma Ltd. & Globela Healthcare Ltd. The tablet EFOCAR-MX (Batch no.: Z05CW25201), containing 40 mg of EFO and 25 mg of MET, was purchased at a pharmacy shop. HPLC-grade Acetonitrile (Batch no.: 25251GRL25) and Methanol (Batch no.: B490012401), along with analytical-grade triethylamine and orthophosphoric acid (85% v/v), were sourced from Rankem India H_2O_2 (Batch no.: 5258) from Oxford Lab Fine Chem. Double-distilled water was prepared using a water distillation unit.

2.2 Equipment

The Materials were weighed using a Shimadzu analytical weighing balance (ATX224R). Sonication was done using an Athena SS316 ultrasonic bath. The chromatographic analysis was conducted using a Shimadzu P-SERIES-I LC-20AD HPLC system, featuring a PDA detector and an autosampler. pH meter (LT-49) manufactured by LLI was used to assess the pH of the solution. Lab Solution software was used to handle signal processing and monitoring. For photolytic degradation investigations, a UV chamber (SA Instruments) was used, and for thermal degradation tests, a hot air oven (Mercury).

2.3 Mobile phase Preparation

Preparation of Phosphate Buffer pH 3.5 Weigh accurately and transfer approximately 1.36 g of potassium dihydrogen orthophosphate and 2 mL of triethylamine into 800 mL of water. Adjust the pH to 3.5 with orthophosphoric acid and add sufficient water to produce 1000 mL.

A mobile phase was prepared by mixing Acetonitrile, Methanol, and Phosphate Buffer pH 3.5 solution in a 65:20:15 (v/v/v) ratio. The mixture was then filtered through a 0.45 µm membrane filter and transferred into a 500 mL glass reservoir under controlled conditions. To eliminate any dissolved gases, the solution was sonicated for 20 min. The resulting mobile phase was subsequently utilized as the diluent.

2.4 Chromatographic conditions

Column ODS C18 (250 mm x 4.6 mm, 5 µm).

Mobile Phase ACN: Methanol & phosphate buffer pH 3.5 (adjusted with o- phosphoric acid) in the volume (65:20:15 v/v/v).

Flow rate 1 ml/min.

Injection volume 20 µl.

Wavelength 225 nm.

Column temperature Ambient.

2.5 Preparation of standard

2.5.1 Preparation of working standard stock solution

60 ml of methanol was combined with 40 mg of EFO and 25 mg of MET, then dissolved in a 100 mL flask to prepare a mixture of 100 ml of methanol. The final concentrations of EFO and MET in the solution were 400 & and 250 µg/ml respectively.

2.6 Validation of RP-HPLC method

According to ICH Q2 (R2) guidelines, the settled RP-HPLC system was authenticated to ensure compliance with the required parameters, including specificity, linearity, precision (encompassing repeatability, intraday, and interday), accuracy, and robustness [32, 33].

Six replicates of a standard solution containing 40 µg/mL EFO and 25 µg/mL MET were injected to evaluate the system's relevance. To make sure there are no excipients that might be in the marketed product, specificity was investigated. To assess the linearity of EFO and MET concentrations in methanol, a stock solution was diluted to achieve concentrations ranging from 20 to 120 µg/mL for EFO & 12.5–75 µg/mL for MET ($n = 6$). A calibration curve of peak area versus concentration was plotted, and the correlation coefficient was calculated. The LOD and LOQ were determined using the calibration graph using standard deviation method. Intraday & interday precision were evaluated by conducting three independent analyses of each drug concentration—20, 60, and 120 µg/mL for EFO, and 12.5, 37.5, and 75 µg/mL for MET—on the same day and across three different days. Repeatability was assessed by analyzing solutions of EFO (60 µg/mL) and MET (37.5 µg/ mL) six times, recording the peak areas, and calculating the % RSD. Accuracy was evaluated by spiking a sample with a known drug concentration in triplicate. The concentrations of EFO and MET were assessed at three levels: 50%, 100%, and 150%. For routine qualitative assessment of EFO and MET in commercially available formulations, the validated RP-HPLC method is best suited. The LOD and LOQ were calculated by using this formula by standard deviation method.

$$LOD = 3.3 * \frac{\sigma}{Slope}$$

$$LOQ = 10 * \frac{\sigma}{Slope}$$

Where, σ = standard deviation of intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

The stability of both standard and sample solutions of EFO and MET was assessed under two storage conditions: room temperature (25 ± 2 °C). Aliquots were withdrawn at 0 and 48 h and analysed using the validated RP-HPLC method.

2.7 Analysis of marketed formulation

After calculating the average weight of twenty tablets, they were crushed into a fine powder. A 100 mL flask was filled with a portion of the powder equal to 40 mg of EFO (25 mg of MET). Twenty milliliters of diluent were added, and the mixture was sonicated for 30 min. The volume was then adjusted to 100 mL with the diluent. The resulting solution was filtered through a 0.45-micron syringe filter. From the filtered solution, 1 mL was taken and diluted to 10 mL with the mobile phase. The content of EFO and MET in the tablets was determined based on the chromatographic responses of their respective main peaks.

2.8 Forced degradation studies

Forced degradation studies were conducted under various conditions, including exposure to acid, alkali, 3% H_2O_2, UV light, and heat. Using standard solutions with concentrations of 40 µg/mL for EFO and 25 µg/mL for MET, these studies aimed to evaluate the inherent stability characteristics of the compounds.

2.9 Preparation of stock

(1) Acid degradation

The mixture was hydrolyzed by adding 5 mL of 1 N HCl, refluxed for an hour at 60 °C, cooled at room temperature, and neutralized with 1 N NaOH.

(2) Alkaline degradation

The mixture was hydrolyzed by adding 5 mL of 1 N NaOH, refluxed for an hour at 60 °C, cooled at room temperature, and neutralized with 1 N HCl.

(3) Oxidative Stress

Oxidative degradation was conducted by treating both drugs with 5 mL of 3% hydrogen peroxide and refluxing the solution at 60 °C for 60 min.

(4) Thermal degradation

The process was performed by subjecting both drugs to a hot air oven at 110 °C for 3 h.

(5) Photodegradation

The procedurse involved exposing both drug samples to UV light in a UV chamber for two days.

3 Results

Based on solubility and literature review, various solvent systems using acetonitrile, methanol, and water were evaluated. However, peak splitting was observed. Phosphate buffers at pH 2.5, 3.5, and 4.5 were tested, with optimal peak sharpness achieved at pH 3.5. Chromatographic parameters such as flow rate, column temperature, and detection wavelength were optimized. The final method employed a mobile phase of Acetonitrile: Methanol: Phosphate buffer pH 3.5 (65:20:15, v/v/v), yielding sharp peaks, good resolution, theoretical plates > 2000, and acceptable system suitability. A C18 column (4.6 × 250 mm, 5 µm) operated at 1.0 mL/min and detected at 225 nm (PDA) was found ideal .

3.1 System suitability

The %RSD was determined and reported within limits Table 1

3.2 Specificity

There was no interference detected when comparing the chromatograms of the marketed product with the standard solution of the medications Fig. 3.

Table 1 System suitability parameters MET (37.5 µg/ml) & EFO (60 µg/ml) by proposed HPLC method

	EFO		MET	
Parameters	Mean ± S.D. (*n* = 6)	% R.S.D.	Mean ± S.D. (*n* = 6)	% R.S.D.
Retention Time	7.173 ± 0.4	0.11	2.774 ± 0.2	0.22
Theoretical Plate	13,822 ± 215.7	1.31	1.016 ± 0.01	0.70
Tailing Factor	0.728 ± 0.022	1.40	8.72 ± 0.52	0.65

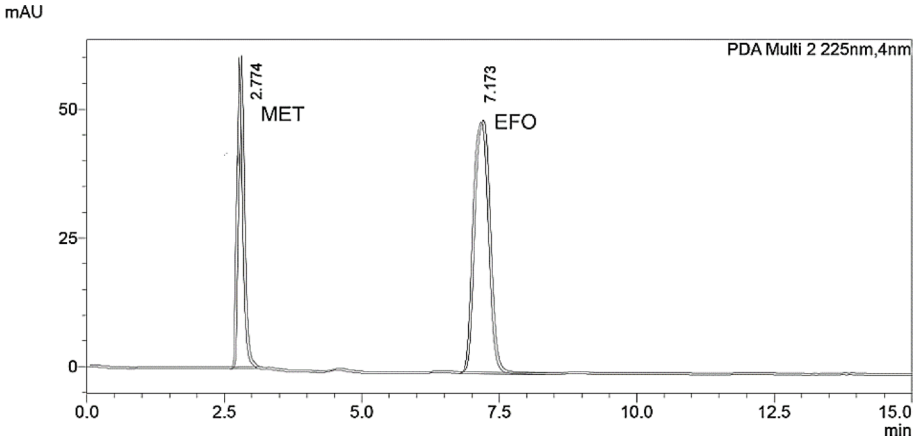

Fig. 3 Chromatogram of EFO (60 µg/mL) & MET (37.5 µg/mL) standard & Marketed formulation

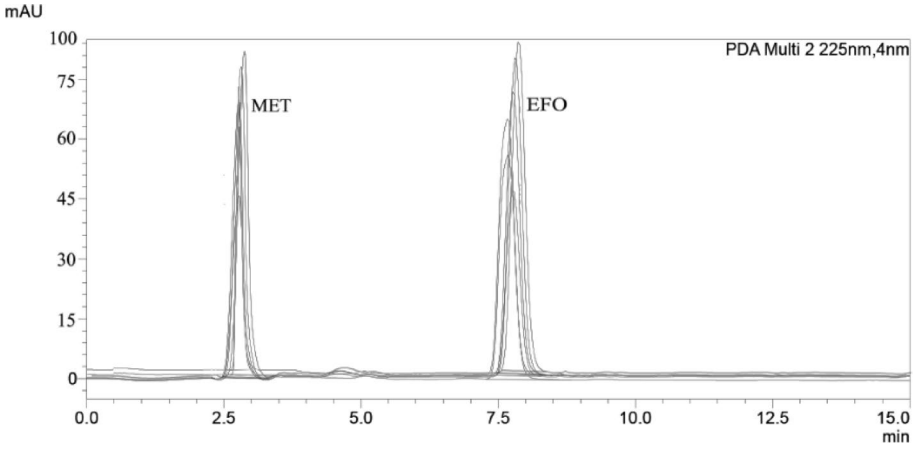

Fig. 4 Overlay Chromatogram of EFO (20–120 µg/ml) and MET (12.5–75 µg/ml),

3.3 Linearity and range

These parameters were assessed using a linear regression equation. Both drugs showed linearity within the concentration ranges of 20–120 µg/mL for EFO and 12.5–75 µg/mL for MET. A linear equation was determined to be 9691.4x + 138,011 for EFO and 14094x + 4306.6 for MET, with correlation coefficients (r^2) of 0.9981 and 0.9961, respectively. These results show within the ranges under study, peak area and analyte concentration have a significant linear relationship Fig. 4, 5 Tables 2, 3.

Table 2 Linearity data of EFO (20–120 µg/ml)

Conc (µg/ml)	Peak Area Mean ± SD (*n* = 6)	% RSD
20	330,181 ± 5067	1.53
40	521,647 ± 8229	1.58
60	714,656 ± 13,511	1.89
80	943,256 ± 4439	0.47
100	1,072,978 ± 16,352	1.52
120	1,288,704 ± 12,305	0.95

Table 3 Linearity data of MET (12.5–75 µg/ml)

Conc (µg/ml)	Peak Area Mean ± SD (*n* = 6)	% RSD
12.5	184,478 ± 3139	1.70
25	350,601 ± 3906	1.11
37.5	551,980 ± 5019	0.91
50	645,160 ± 7044	1.09
62.5	901,867 ± 14,894	1.65
75	1,045,684 ± 11,595	1.11

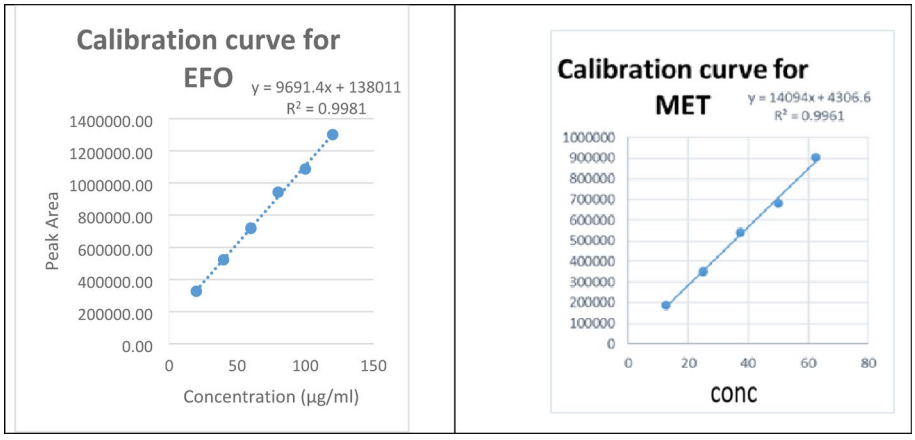

Fig. 5 Calibration curve for EFO & MET

3.4 Precision

The method's precision, both intraday and interday, as well as the system precision, demonstrated relative standard deviations (RSD) of less than 2%, indicating a high level of precision, as presented in Table 4.

3.5 Accuracy

The % recovery rates for EFO and MET were reported to range from 98.52 to 101.65% and 98.21–101.68%, respectively Table 5.

3.6 Robustness

The % RSD for small changes in the parameters was found to be below 2%, shown in Table:6 demonstrating the robustness of the developed method Table 6.

3.7 Limit of detection and quantitation

The lowest concentration level of the analysts that provide a quantifiable response in the current investigation outcome is displayed in Table 7.

Table 4 Result for precision by proposed HPLC method

Conc. (µg/ml)		Mean peak area ± SD EFO (*n* = 3)	% RSD	Mean peak area ± SD MET (*n* = 3)	% RSD
EFO	MET				
Intraday precision					
20	12.5	327,657 ± 1301	0.40	185,724 ± 544	0.29
60	37.5	716,217 ± 9224	1.29	554,125 ± 1456	0.26
120	75.0	1,283,075 ± 7179	0.56	1,044,648 ± 6598	0.63
Inter-day precision					
20	12.5	330,038 ± 5614	1.70	185,151 ± 1146	0.62
60	37.5	719,081 ± 10,696	1.49	549,834 ± 6859	1.25
120	75.0	1,310,915 ± 7179	0.55	1,044,648 ± 16,281	1.56
Repeatability (n = 7)					
EFO (60 µg/ml)		714,904 ± 13,432			1.87
MET (37.5 µg/ml)		552,174 ± 5162			0.93

Table 5 Percentage recovery results of both drugs

Level	Conc. of Synthetic Mixture (µg/ml)		Amount of Std. added		Total amount		Total amount Recovered Mean ± SD		% Recovery	
	EFO	MET	EFO	MET	EFO	MET	EFO	MET	EFO	MET
0	40	25	0	0	40	25	39.41 ± 0.60	24.80 ± 0.08	98.52%	99.19%
50	40	25	20	12.5	60	37.5	59.39 ± 1.39	37.66 ± 0.18	98.98%	100.44%
100	40	25	40	25	80	50	81.32 ± 0.25	49.10 ± 0.23	101.65%	98.21%
150	40	25	60	37.5	100	62.5	98.75 ± 0.71	63.54 ± 0.72	98.75%	101.66%

Table 6 Result of robustness for MET (37.5 µg/ml) & EFO (60 µg/ml) by proposed HPLC method

Parameters	Change in condition	MET		EFO	
		Peak Area	%RSD	Peak Area	%RSD
Detection wavelength	223 nm	548,037 ± 5518.65	1.00	711547.33 ± 4826.1	0.67
	227 nm	541440.33 ± 5743.6	1.06	720522.5 ± 3389.25	0.47
Flow rate	0.9	553713.33 ± 5479.8	0.98	723,810 ± 761.024	0.10
	1.1	545754.33 ± 2867.3	0.52	717919.67 ± 1627.4	0.22
Mobile phase	ACN: MeOH: Phosphate Buffer pH 3.5 in the volume ratio 80:10:10 v/v/v)	552852.6 ± 1527.5	0.30	722,098 ± 5390.8	0.75
	ACN: MeOH: Phosphate Buffer pH 3.5 in the volume ratio 60:30:10 v/v/v)	556989.66 ± 2076.3	0.37	725686.66 ± 5766.6	0.79

Table 7 Result for LOD & LOQ by proposed HPLC method

	EFO	MET
	µg/ml	
LOD	1.55	3.12
LOQ	4.72	9.47

3.8 Solution stability data

The results showed in Table 8 significant change in assay values (variation < ± 2%), retention time, or peak shape over the study period. No additional peaks were observed, indicating the absence of degradation products.

Table 8 Result for solution stability by proposed HPLC method

Sr. No	Drug	Conc. Taken	% Recovery	
			% Assay ± SD	
			0 h	48 h
1	EFO	40	99.64 ± 2.88	98.86 ± 1.88
2	MET	25	97.66 ± 1.14	96.66 ± 0.89

Table 9 Percent content of the marketed formulation

Sr. No	Drug	Conc. taken	Amount Found µg/ml	% Assay ± SD
1	EFO	40	39.73	99.64 ± 2.88
2	MET	25	24.42	97.66 ± 1.14

3.9 Percent content of marketed formulation

The result mentioned in Table 9 is in range.

3.10 Forced degradation studies

ICH degradation studies were directed under various stress conditions, including exposure to acid and alkali (1 N HCl and 1 N NaOH), oxidative hydrolysis (5% H_2O_2), thermal degradation (heating at 110 °C for 3 h), and photolytic degradation (UV light in a UV chamber for 48 h). The results of these stress studies are presented in Table 10; Figs. 6, 7, 8, 9 and 10.

4 Discussion

The forced degradation study conducted on Efonidipine HCl Ethanolate (EFO) and Metoprolol Succinate (MET) under various ICH-recommended stress conditions demonstrated that both compounds maintain excellent mass balance (~ 100%) across all degradation pathways, confirming the suitability of the analytical method for stability-indicating purposes. Efonidipine exhibited the highest susceptibility to alkaline hydrolysis, with 12.52% degradation, and consistent formation of a major degradation product at a retention time of 5.89 min, suggesting a common degradation pathway. In contrast, Metoprolol showed relatively low degradation under all tested conditions, with the highest degradation observed in alkaline media (8.10%). Multiple degradant peaks were identified primarily under acidic and alkaline conditions, with prominent retention times at 4.65 and 6.65 min. The complete mass balance across all conditions validates the reliability and accuracy of the analytical method in quantifying both the parent compound and degradation products. These findings confirm that the developed method is robust, stability-indicating, and suitable for routine quality control and stability testing of both EFO and MET.

This study successfully developed and validated a stability-indicating technique for EFO and MET, effectively separating the two drugs and their degradation products. The elution times for EFO and MET were recorded at 7.17 & 2.77 min, respectively, with a resolution of greater than 2. System suitability and repeatability tests evaluated parameters such as retention time, theoretical plate count, tailing factor, resolution, and percentage relative standard deviation (% RSD) across standard, sample, and mobile phase preparations. The method also included forced degradation studies and duplicate standard preparations to ensure the reproducibility of the chromatographic system. Validation results demonstrated that the proposed method is simpler, faster, and more precise, with improved linearity, accuracy, specificity, and robustness compared to previously reported methods.

Table 10 Result for forced degradation

Condition	Amt of drug taken (µg/ml)	Peak Area	Amt of drug found (µg/ml)	% Drug Assay	% Degradation	Mass balance (%) = %Drug Assay+% Degradation	Retention time of Degradant
Efonidipine HCl Ethanolate (EFO)							
Acidic hydrolysis	40	486,642	35.97	89.93	10.07	100	5.89 min and 9.87 min
Alkali hydrolysis	40	477,142	34.99	87.48	12.52	100	5.89 and 11.07 min
Oxidative Degradation	40	492,755	36.60	91.51	8.49	100	5.89 min
Thermal Degradation	40	504,225	37.78	94.47	5.53	100	-
Photo Degradation	40	512,125	38.60	96.51	3.49	100	-
Metoprolol Succinate (MET)							
Acidic hydrolysis	25	333,799	23.3782	93.51	6.49	100	4.12, 4.65, 5.11, 6.65 min
Alkali hydrolysis	25	328,126	22.97569	91.90	8.10	100	3.12, 4.65, 5.11 and 6.65 min
Oxidative Degradation	25	332,391	23.2783	93.11	6.89	100	3.12, 4.65, 6.65 min
Thermal Degradation	25	339,215	23.76248	95.05	4.95	100	-
Photo degradation	25	337,770	23.65995	94.64	5.36	100	-

5 Conclusion

The aim of investigate a simple, accurate, sensitive, & selective method of analysis of EFO and MET in pharmaceuticals using RP-HPLC technology. Currently, this is the sole technique available for quantifying EFO and MET in tablet dosage forms. The method has been validated according to ICH guidelines, confirming its selectivity, accuracy, linearity, precision (for both intra- and inter-day measurements), sensitivity, robustness, and ruggedness. Results from stress testing, which involved examining the degradation of both drugs and assessing their stability under stressful conditions, indicate the method's selectivity and stability, highlighting its capability to analyse EFO and MET even in the presence of their degradation products.

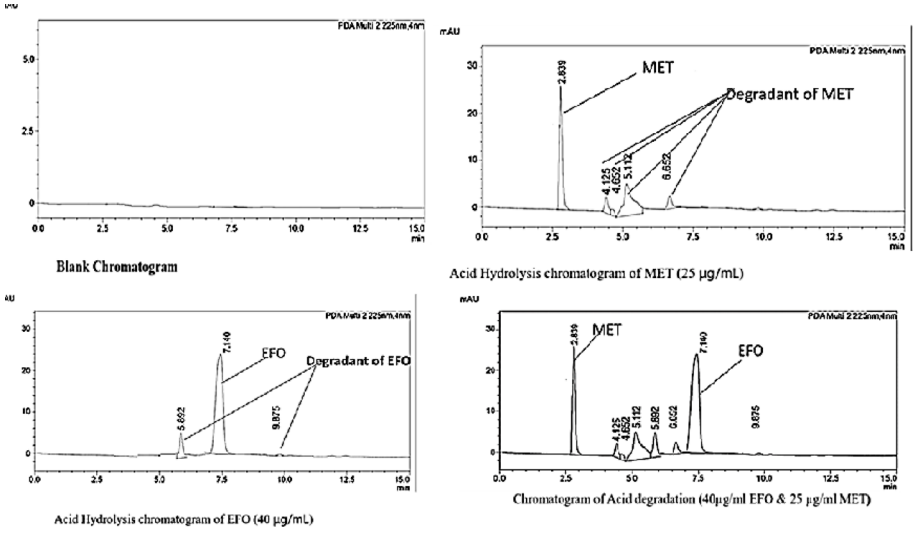

Fig. 6 Chromatogram of Acid Hydrolysis of EFO (40 µg/ml) and MET (25 µg/ml) in 1 N HCl reflux for 1 h at 60 °C

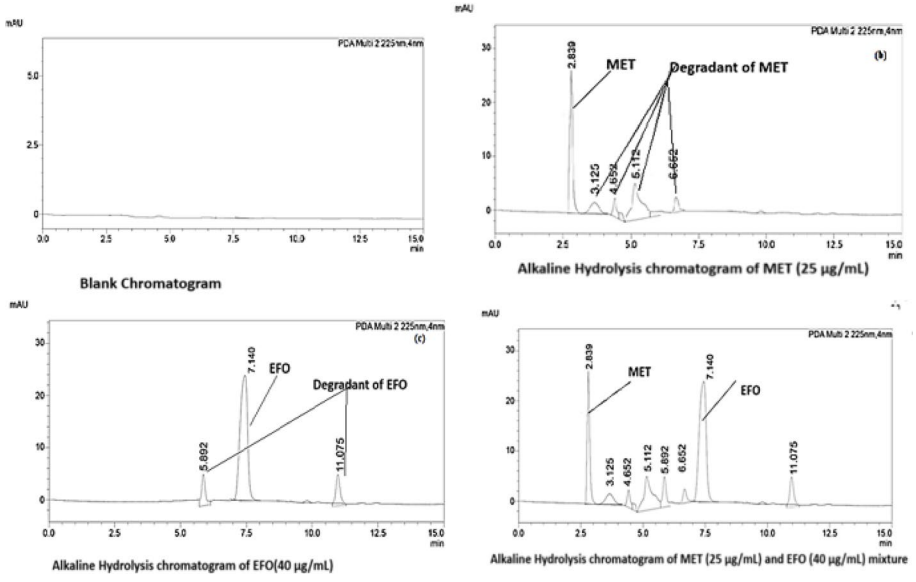

Fig. 7 Chromatogram of Alkaline Hydrolysis of EFO (40 µg/ml) and MET (25 µg/ml) in 1 N NaOH reflux for 1 h at 60 °C

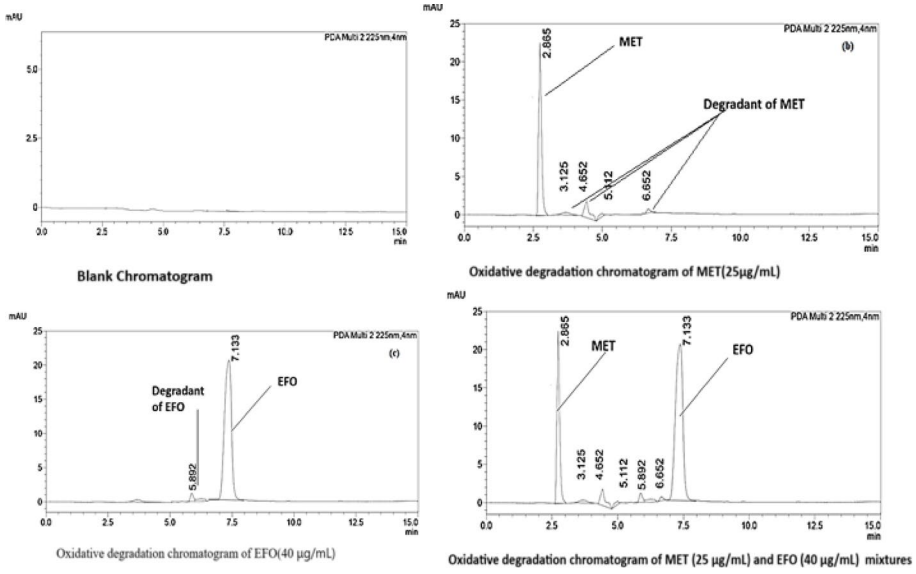

Fig. 8 Chromatogram of Oxidative degradation EFO (40 µg/ml) and MET (25 µg/ml) in 3% hydrogen peroxide reflux for 1 h at 60 °C

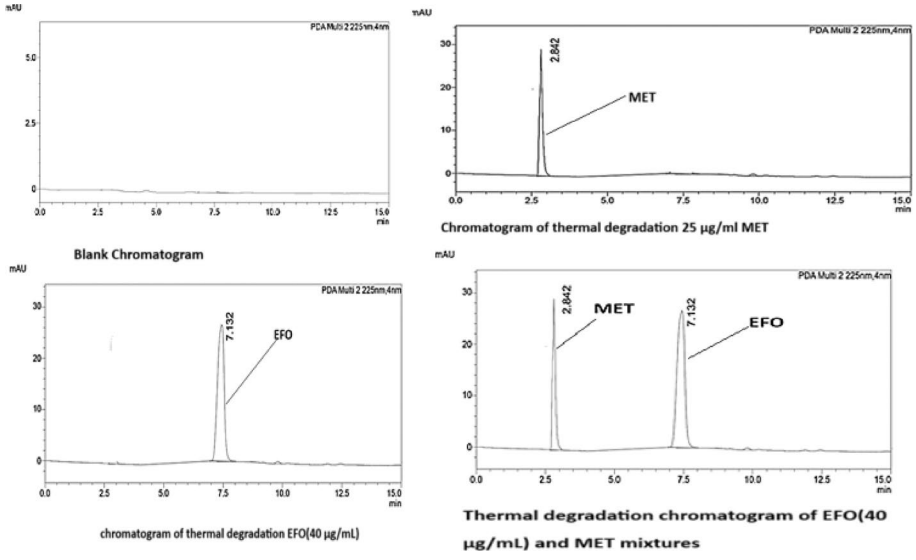

Fig. 9 Chromatogram of Thermal degradation of EFO (40 µg/ml) and MET (25 µg/ml) in dry heat for 110 °C for 3 h

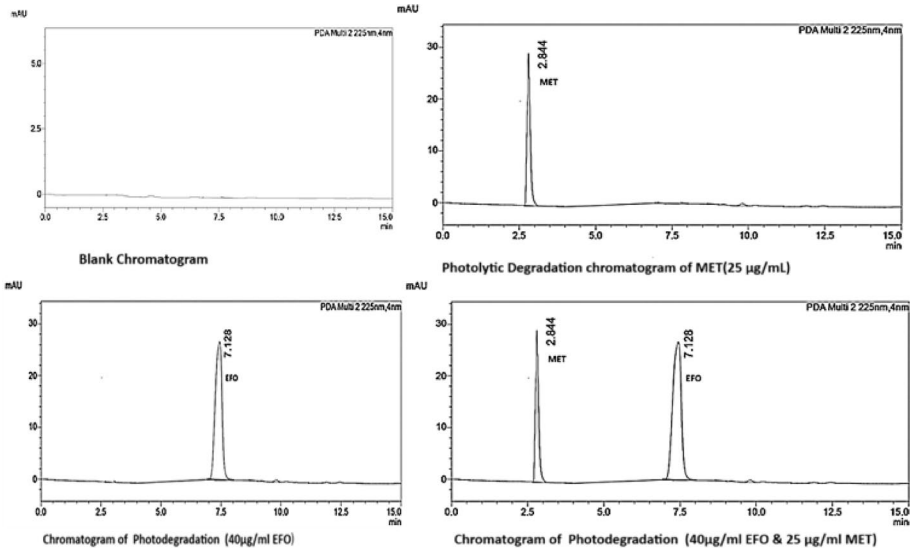

Fig. 10 Chromatogram of EFO (40 µg/ml) and MET (25 µg/ml) Photolytic degradation in UV exposure for 48 h

Abbreviations

EFO	Efonidipine HCl Ethanolate
MET	Metoprolol Succinate
CAN	Acetonitrile
PDA	Photodiode Array
ICH	International Council for Harmonisation
HPLC	High-Performance Liquid Chromatography
RP-HPLC	Reverse Phase High-Performance Liquid Chromatography
NaOH	Sodium Hydroxide
HCl	Hydrochloric Acid
H_2O_2	Hydrogen Peroxide
r^2	Coefficient of Determination

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Author contributions
Author ContributionsPriyanka Malani contributed to the conceptualization, methodology design, data collection, and manuscript writing.Vineet C. Jain provided supervision, validation of the analytical methods, and critical review of the manuscript.Zarna Dedania was involved in data analysis and manuscript editing.All authors have read and approved the final version of the manuscript.

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Data availability
The data supporting the finding of this study are available from corresponding author upon reasonable request.

Declarations

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