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Original Article

Chromatographic Simultaneous Estimation of Efonidipine Hydrochloride Ethanoate and Metoprolol Tartrate

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ABSTRACT

Background: Hypertension is a widely prevalent cardiovascular disorder associated with significant morbidity and mortality, and its effective management often necessitates combination drug therapy. **Objectives:** The present study was undertaken to develop and validate a Reversed-Phase High-Performance Liquid Chromatographic (RP-HPLC) method for the simultaneous estimation of Efonidipine hydrochloride ethanolate and Metoprolol tartrate in a fixed-dose combination using Simvastatin as an internal standard. **Materials and Methods:** Separation of the analytes was achieved on a C8 column using a mobile phase comprising (Acetonitrile: Methanol: Water) with the pH 3.0 using 1% orthophosphoric acid, in the proportion of 50:30:20 (v/v/v). The mobile phase was pumped at a constant flow rate of (1.0 mL/min), and detection was performed at 220 nm with a UV detector. **Results:** The retention times of Metoprolol, Efonidipine and Simvastatin were found to be 3.79 min, 5.74 min and 7.11 min, respectively. The method exhibited linearity over the concentration range of (10-320 µg/mL) for Efonidipine and (6.25-150 µg/mL) for Metoprolol, with correlation coefficients greater than 0.999. **Conclusion:** The developed method was validated in accordance with ICH guidelines for parameters such as accuracy, precision, sensitivity and robustness. The results demonstrated that the method is reliable and suitable for routine analysis of these drugs in pharmaceutical formulations.

Keywords: Efonidipine hydrochloride ethanolate, Internal Standard, Metoprolol tartrate, Simvastatin, Validation.

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INTRODUCTION

Chronic elevation of arterial blood pressure is a defining feature of hypertension and contributes significantly to cardiovascular risk. It is frequently progresses without any measurable symptoms while significantly increasing the risk of cardiovascular complications. Sustained elevation of blood pressure above 140/90 mmHg is generally considered hypertensive. Long-term uncontrolled hypertension can lead to serious complications, including arrhythmia, coronary artery disease, heart failure, renal impairment, and stroke.[1]

In clinical practice, effective control of hypertension frequently necessitates the use of combination therapy. Clinical evidence suggests that a large proportion of patients require combination therapy to achieve the desired therapeutic outcome. Several pharmacological classes are commonly used for this purpose, like diuretics, inhibitors of angiotensin-converting enzyme, blockers of angiotensin receptors, blockers of beta-adrenergic receptors, renin inhibitors, blockers of calcium channels, and centrally acting sympatholytic agents.[2,3]

Efonidipine hydrochloride ethanolate exerts its antihypertensive effect by inhibiting both L-type and T-type calcium channels. The drug produces vasodilation and reduces heart rate, thereby lowering blood pressure. It also demonstrates renoprotective and cardioprotective properties by improving renal hemodynamics and reducing proteinuria. The therapeutic effect of EFO arises from preventing calcium from reaching smooth muscle cells and cardiac tissue, which leads to relaxation of blood vessels and improved blood circulation (Figure 1).[4]

Metoprolol Tartrate (MET) acts as a selective β1-adrenergic receptor blocker that primarily acts on cardiac tissue. It reduces myocardial contraction and pulse rate, thereby decreasing output from the heart and controlling the hypertension (Figure 2). The drug exhibits minimal activity on β2 receptors and lacks intrinsic sympathomimetic activity.[5]

Various analytical method found in the literature for the estimation of EFO and MET as a single drug or in presence of other components. These methods including UV spectrophotometry.[6-10] RP-HPLC[11-14] Stability Indicating HPLC method[15-17] planar chromatography (HPTLC)[18,19] and LC-Q-TOF-MS methods.[20]

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However, no analytical procedures are available for their simultaneous chromatographic determination using an internal standard. Therefore, the present investigation aimed to develop and validate a simple, rapid and reliable RP-HPLC method employing an internal standard for the quantification of EFO and MET in combination from the fixed dosage.

MATERIALS AND METHODS

Reagents and Chemicals

Acetonitrile, methanol, orthophosphoric acid, and purified water used throughout the study were HPLC-grade. Zuventus Healthcare Ltd., had provided the reference standards of Efonidipine and Metoprolol, Pune and CTX Life Sciences, Ankleshwar, respectively. The marketed formulation, EFNOCAR-MX tablets, was used for analysis

Instrumentation

In the present work, the LC-20AT HPLC system of Shimadzu was used, equipped with Spinchrom CFR software (version 2.1.4.93). The samples were injected by a Rheodyne loop, and analytes were detected using a SPD-20A UV detector. An Abcare Export digital pH meter, GT Sonic (GT-1730QTS) ultrasonic bath, and vacuum pump were used during analysis.

Chromatographic Condition

For the separation of analytes, a Shimadzu Shim-pack C8 column (25 cm X 0.46 cm id, 5 µm particle size) was used as a stationary phase. The mixture in the ratio 50:30:20 %v/v/v of solvents containing of acetonitrile, methanol, and water, whose pH 3 was adjusted with 1% OPA, was used as the mobile phase. The flow rate was maintained at 1.0 mL/min, and a 20 µL volume of each solution was injected for analysis. Detection was carried out at 220 nm using a UV detector. The accuracy of the method was enhanced by addition of internal standard. In the present study 50 µg/mL Simvastatin was used for this purpose.

Standards Preparation

Stock solutions of EFO (1000 µg/mL), MET (2500 µg/mL), and SIM (1000 µg/mL) were prepared in methanol. Appropriate dilutions were prepared from these stock solutions to obtain working concentrations of 10-320 µg/mL and 6.25-150 µg/mL for EFO and MET respectively. Internal standard i.e. 50 µg/mL of Simvastatin was added in each solution. To avoid any blockage of the components of the chromatographic system a 0.2 µm nylon membrane filter was used for filtering each solution.

Sample Preparation

Twenty tablets of the combined dosage form containing EFO (40 mg) and MET (25 mg) (EFNOCAR-MX, Zuventus Healthcare Ltd.,) were accurately weighed and finely powdered. An amount of the powder equivalent to 10 mg of EFO (corresponding to 6.25 mg of MET) was transferred into a 25 mL volumetric flask. The sample was dissolved in approximately 15 mL of HPLC-grade methanol and sonicated for 10 min to achieve complete extraction. The resulting solution was made up to volume with methanol to obtain a stock solution containing 400 µg/mL of Efo and 250 µg/mL of Met, and was subsequently filtered through Whatman filter paper No. 41. An aliquot of 2 mL of this stock solution was further diluted to 10 mL with the mobile phase, along with the addition of 0.5 mL of Sim internal standard solution (1000 µg/mL). This procedure yielded a final solution containing 80 µg/mL of Efo, 50 µg/mL of Met, and 50 µg/mL of Sim. A corresponding standard solution of identical concentration was prepared. Both standard and sample solutions were analysed under optimised chromatographic conditions after achieving baseline stability. Peak areas were recorded, and the drug content was quantified using a single-point calibration method based on the ratio of analyte response to internal standard, applying the equation:

$$C_1 = \frac{(R_1 \times C_2)}{R_2}$$

Where C_1 and C_2 represent concentrations of sample and standard solutions, respectively, and R_1 and R_2 denote their corresponding peak area ratios. The obtained results are presented in the respective (Table 1and Figure 3).

RESULTS AND DISCUSSION

Selection of Chromatographic Conditions

Various stationary phases, including C18 and C8 columns, were evaluated during method development. However, the C8 column provided better peak symmetry and shorter retention time for both analytes compared to C18.Different combinations of methanol, acetonitrile and water were investigated to achieve optimum resolution. Initial trials with binary mobile phases resulted in poor separation and peak tailing. Finally, A ternary mobile phase composed of acetonitrile, methanol, and water in the ratio of 50:30:20 (v/v/v) was ultimately chosen. The pH was adjusted to 3.0 using 1% orthophosphoric acid. This composition produced well-defined, symmetrical peaks and ensured satisfactory resolution between the analytes and the internal standard. A detection wavelength of 220 nm was selected based on overlain UV spectra of both drugs, where adequate sensitivity was observed.

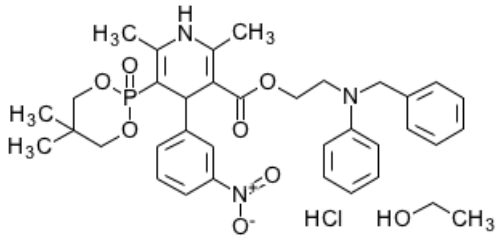

Figure 1: Efonidipine hydrochloride ethanoate Chemical Structure.

Validation of an Analytical Method

ICH Q2 guidelines were followed for the validation of the developed chromatographic method.[21]

Specificity

Specificity of the method was established by analyzing blank, standard, and sample solutions. The chromatograms showed no interfering peaks at the retention times corresponding to Efonidipine, Metoprolol, and the internal standard, demonstrating that the method is specific.

Accuracy/Recovery

To check whether the developed method is accurate or not, the addition of standard API was done at 80%, 100%, and 120% concentration levels. The percentage recovery results were within acceptable limits, indicating the reliability of the developed method (Table 2).

Precision

Precision of the method was determined by performing a repeatability study and an intermediate precision study. The analysis was performed at various drug concentrations. The percentage relative standard deviation values obtained were not more than 2%, demonstrating good precision of the method (Table 3).

Range and Linearity

The method showed Linearity in the concentration of 10-320 µg/mL and 6.25-150 µg/mL for EFO and MET, respectively. Calibration curves were plotted, and regression analysis showed correlation coefficients close to unity, indicating excellent linearity (Figure 4).

LOD and LOQ

The (LOD) and (LOQ) were determined in accordance with ICH guidelines using the standard deviation of the response (σ) and the slope of the calibration curve (S). The calculations were performed using the following relationships: LOD = 3.3σ/S and LOQ = 10σ/S. The LOD values were found to be 1.841 µg/mL for EFO and 3.253 µg/mL for MET, while the LOQ values were 5.580 µg/mL and 9.858 µg/mL for EFO and MET, respectively.

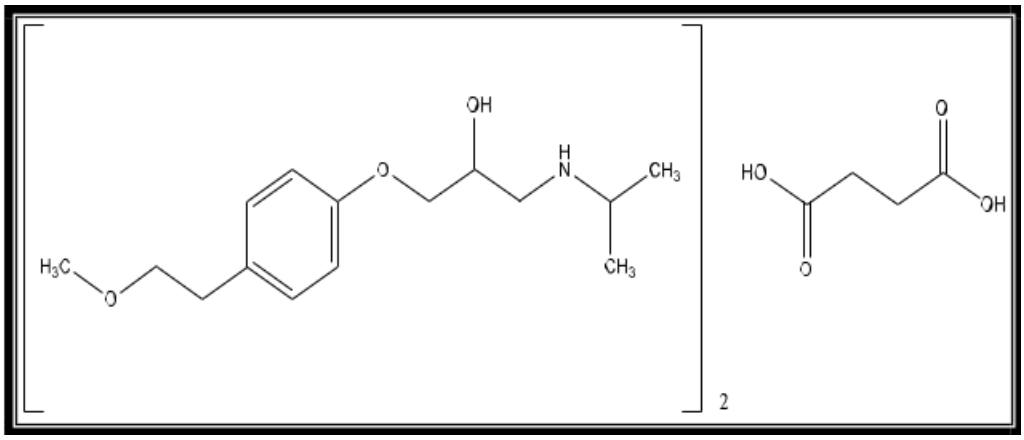

Figure 2: Structure of Metoprolol tartrate.

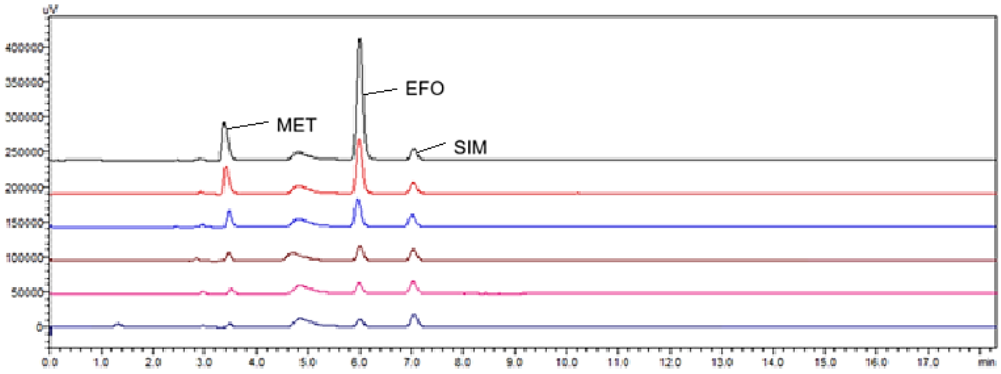

Figure 3: Overlain Chromatograms of Serial Dilutions of MET (6.25-150 µg/mL), EFO (10-320 µg/mL), SIM (50 µg/mL).

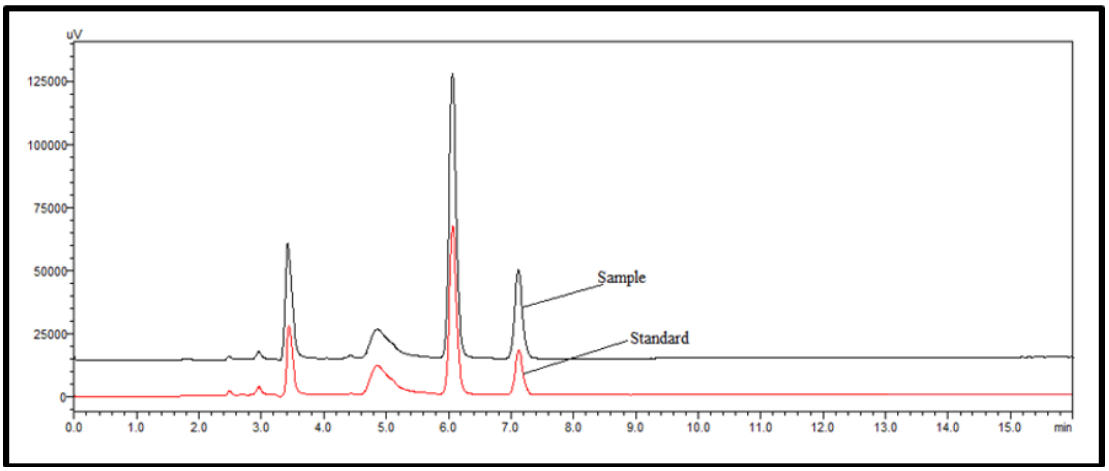

Figure 4: Overlain Chromatograms of mixed API and tablet formulation.

Table 1: Data for EFNOCAR-MX Tablet Assay.

Analyte Name	Label Claim (mg)	Calculated Value (mg) *n*=6	% Assay ±SD
EFO	40	40.10	101.64±0.6548
MET	25	25.31	100.16± 0.3744

Table 2: Results of Recovery Studies.

Component	Concentration spiked (µg/mL)	Concentration recovered (µg/mL)	%Recovery	% RSD (*n*=3)
EFO	64	63.74	99.95	0.6057
	80	81.55	101.05	1.1222
	96	96.62	100.10	0.5255
MET	40	40.08	101.04	0.8248
	50	50.14	100.54	0.8922
	60	60.21	101.09	1.5199

Table 3: Data for Precision Studies.

Component	Concentration (µg/mL)	Intra-day (*n*=3) % RSD	Inter-day (*n*=3) % RSD
EFO	40	0.5082	0.7755
	80	0.5504	1.3227
	160	1.2242	1.9899
MET	25	0.5533	1.6837
	50	0.4726	1.3075
	100	1.3512	1.5761

Table 4: Data for System Suitability Study (*n*=6).

Drug Component	Resolution	No of Theoretical Plates/Meter	Tailing Factor	%RSD	
				Retention Time	Area Under the Curve
MET	-	26113	1.146	1.5921	0.5254
EFO	2.222	64183	1.152	0.5870	0.7996
SIM	3.536	93528	1.136	0.5435	-
Required limits	R>2	N>2000	T<2	RSD < 2%	

Robustness

Robustness was assessed by introducing deliberate, minor variations in chromatographic conditions, specifically in the flow rate (±0.1 mL/min) and mobile phase composition (±2%). The impact of these changes on key parameters such as retention time, peak area, and resolution was evaluated. The findings demonstrated that these slight modifications did not significantly influence the analytical performance, thereby confirming the robustness of the method.

System Suitability

Parameters like the number of theoretical plates per meter, resolution between the drug peaks, the precision in retention time and area under curve, and tailing factor of each peak were evaluated to check the proper functioning of the chromatographic system (Table 4).

CONCLUSION

A chromatographic method was developed for the simultaneous determination of Efonidipine and Metoprolol in tablet dosage form. The developed method demonstrated better retention behaviour and shorter analysis time compared to previously reported HPLC methods for Efonidipine and Metoprolol. The use of an internal standard improved precision and minimised injection variability. The resolution between peaks was greater than 2, indicating adequate separation. Validation results were within acceptable limits as per ICH guidelines, confirming the reliability of the method for routine analysis.

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ABBREVIATIONS

HPLC: High-Performance Liquid Chromatography; **ICH:** International Council for Harmonisation; **EFO:** Efonidipine; **MET:** Metoprolol; **SIM:** Simvastatin; OPA**:** Orthophosphoric Acid; **RSD:** Relative Standard Deviation; **LOD:** Loss on Drying; **LOQ:** Limit of Quantification.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Tripathi KD. Essentials of Medical Pharmacology. 8th ed. New Delhi: Jaypee Brothers Medical Publisher Ltd., 2019; 604-15.
2. Sharma HL, Sharma KK. Principles of Pharmacology. 3rd ed. New Delhi: Paras Medical Publisher; 2017; 262-65.
3. Harrison, David G *et al.* "Pathophysiology of Hypertension: The Mosaic Theory and Beyond." Circulation Research, 2021; 128, 7:847-63. DOI: 10.1161/CIRCRESAHA.
4. Oh, I. Y., Seo, M. K., Lee, H. Y., Kim, S. G., Kim, K. S., Kim, W. H., *et al.* Beneficial Effect of Efonidipine, an L- and T-Type Dual Calcium Channel Blocker, on Heart Rate and Blood Pressure in Patients with Mild-to-Moderate Essential Hypertension.*Korean circulation journal*, 2010;*40*(10):514-9. DOI: https://doi.org/10.4070/kcj.2010.40.10.514
5. Atmakuri LR, Peeriga R, Begum S, Gaddamedi N, Vallamkonda B, Baratam A. Novel bioanalytical LC-MS/MS method for determination of metoprolol in human plasma. Journal of Applied Pharmaceutical Science;2024;14(12)131-8. DOI:10.7324/JAPS.2024.657641
6. Darji H, Dedania Z, Dedania R, Jain V. Eco-friendly based HPLC and UV-spectrophotometric methods for simultaneous estimation of Efonidipine hydrochloride ethanolate and Chlorthalidone in their dosage form. Green Analytical Chemistry. 2024;8:1-11. DOI: https://doi.org/10.1016/j.greeac.2024.100092
7. Jalkote NR, Kaulage MS, Kshirsagar PN, Khan BW, Mane PS, Kuber GV. Analytical method development and validation of Efonidipine hydrochloride ethanoate in bulk and dosage form by UV-visible spectrophotometry. International Journal of Innovative Research in Technology. 2022;9(1):838-43.
8. Shelar P, Nazareth C, Khorjuwenkar R, Pinto O, Kamat G, Naik B. A novel UV spectrophotometric method for simultaneous estimation of Metoprolol tartrate and Atorvastatin calcium based on absorbance correction principle. Rasayan Journal Chemistry. 2022;15(4):2822-7.
9. Dudhrejiya A, Patel A, Chavda J, Gol D, Koli P. Spectrophotometric simultaneous determination of Efonidipine hydrochloride ethanolate and Telmisartan in synthetic mixture by first order derivative method. J Med Pharma Allied Sci. 2021;11(2):4547-51.
10. Gopika V C, Remi S L. Validation UV spectrophotometric method for simultaneous estimation of Metoprolol succinate and Benidipine hydrochloride in their combined tablet dosage form. Journal of Pharmaceutical Health Sciences. 2018;8(3):1968-77.
11. Nathe V, Bharsawde J, Polshdttiwar P, More A, Choudhari G. Bio-analytical study of Efonidipine hydrochloride ethanolate and Telmisartan in human plasma by RP-HPLC. Journal Medical Pharmaceutical and Allied Sciences. 2025;13(3):16-23.
12. Patel D B, Vekaria H. Central composite design expert-supported RP-HPLC Optimization and quantitative evaluation of Efonidipine hydrochloride ethanolate and Chlorthalidone in tablet. Journal of Chromatographic Science. 2024;62(6):585-92.
13. Mujawar T, Ahmad S, Gadilohar H, Deshmukh N, Gaikwad A, Sable V. Analytical method development and validation for the simultaneous estimation of Metoprolol and Benidipine by RP-HPLC in bulk and tablet dosage forms. Journal of Health Sciences. 2022;6(6):8188-202.
14. Hussain S, Munjewar R R, Farooqui M. Development and validation of a simultaneous HPLC method for quantification of Amlodipine Besylate and Metoprolol Tartrate in tablets. J Pharma Sci Tech. 2012;1(2):1-5.
15. Malani P, Jain VC, Dedania Z. Robust RP-HPLC method with forced degradation studies for the simultaneous quantification of Efonidipine HCl Ethanolate and Metoprolol Succinate. Discover Chemistry. 2025;2(197):1-16.
16. Patel AB, Pithadiya SB, Zala RV, Parmar DU, Dudhrejiya AV, Vyas AJ. Stability Indicating RP-HPLC method for simultaneous estimation of Efonidipine Hydrochloride Ethanolate and Metoprolol Succinate. 2024;17(5):7605-13.
17. Shah P, Dr Gajjar A. Development and validation of stability indicating RP-HPLC method for Efonidipine hydrochloride ethanolate. International Journal of Innovative Research in Technology. 2021;8(6):340-5.
18. Prajapati P, Patel M, Shah S. A robust High-Performance Thin-Layer Chromatography method for the simultaneous estimation of Chlorthalidone and Metoprolol succinate using quality risk assessment and design of experiments-based enhanced analytical quality by design approach. J Planar Chromatography-Modern TLC. 2021;34:229-42.
19. Deshmukh TB and Deo SS. Development and validation of novel HPTLC method for the simultaneous estimation of Atorvastatin calcium and Metoprolol succinate in tablet dosage form. International Journal of Pharmaceutical Chemical and Biological Sciences. 2018;8(1):1-82.
20. Pandya CP, Rajput SJ. Forced degradation study of Efonidipine HCl ethanolate characterization of degradation products by LC-Q-TOF-MS and NMR. Journal of Applied Pharmaceutical Science. 2020;10(4):75-99.
21. International Conference for Harmonization. Q2(R2) Harmonized tripartite guidelines, validation of analytical procedures: text and methodology. Geneva; 2023; 1-14.

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