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Development and Validation of RP-HPLC Method for the Quantification of Efonidipine Hydrochloride Ethanoate and Metoprolol Tartrate

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ABSTRACT

The present work describes a Reversed-Phase High-Performance Liquid Chromatographic method for the simultaneous estimation of Efonidipine hydrochloride ethanolate and Metoprolol tartrate in tablet dosage form. The estimation was carried out by using a C8 column as a stationary phase with the mixture of Acetonitrile: Methanol: HPLC grade water pH 3 (1% O-Phosphoric Acid) in the ratio of 50:30:20 % v/v/v as mobile phase. The flow rate of the mobile phase was maintained at 1.0 ml/min. To achieve the highest precision in the analysis, Simvastatin was used as an internal standard. All analytes were detected by measuring the absorbance at 220 nm. Total run time was 10 min. Efonidipine hydrochloride ethanolate, Metoprolol tartrate and Simvastatin were eluted at the retention times of 5.742, 3.798 and 7.119 min respectively. The method was found linear over the concentration ranges of 10-320 µg/ml for Efonidipine hydrochloride ethanolate and 6.25-150 µg/ml for Metoprolol tartrate. The method was validated for accuracy, precision, linearity, specificity and sensitivity as per ICH norms. From the validation study it was found that the method is specific, rapid, accurate and precise.

Keywords: RP-HPLC**,** Efonidipine hydrochloride ethanolate, Metoprolol tartrate, Simvastatin, Validation, Internal Standard

INTRODUCTION

Hypertension, also known as high blood pressure, is a long-term chronic medical condition in which the blood pressure in the arteries is persistently elevated. A chronic medical illness commonly known as the “silent killer” is characterized by a persistent elevation of either the systolic or diastolic pressure above 140/90 mm of Hg [1]. High blood pressure typically does not cause symptoms. Long-term high blood pressure, however, is a major risk factor for coronary artery disease, stroke, heart failure, atrial fibrillation, and chronic kidney disease. To achieve the therapeutic objectives, the majority of hypertension patients will require a combination of antihypertensive medications[2]. To lower blood pressure below the prescribed level, over 70% of hypertension individuals need to take at least two antihypertensive medications together i.e., Diuretics, ACE (Angiotensin-Converting Enzyme) inhibitors, angiotensin II - type 1 receptor antagonists (angiotensin receptor blockers ARBs), adrenoceptor antagonists (blockers), renin inhibitors, calcium channel blockers, and central sympatholytic are some of the main drug classes used to treat hypertension therapeutically[3].

Efonidipine hydrochloride ethanoate is a calcium channel blocker, which inhibits both L-type and T-type channels in the dihydropyridine class, with a phosphonate nucleus at the 5th position. It has a negative chronotropic and vasodilator effect. It has a weak inotropic effect, which causes relaxation of afferent and efferent arterioles and reduces proteinuria. It has organ- protective effects on the heart and kidneys. Efonidipine hydrochloride ethanoate works by blocking calcium channels in blood vessels and the heart, leading to relaxation of blood vessels and reducing the pressure on them, making it easier for the heart to pump blood throughout the body[4].

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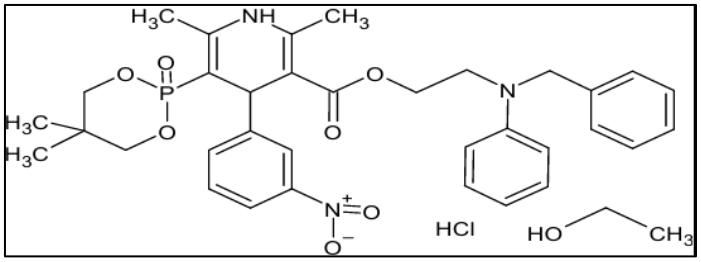

Figure 01: Structure of Efonidipine hydrochloride ethanoate

Metoprolol tartrate is a beta-1-adrenergic receptor inhibitor specific to cardiac cells with negligible effects on beta-2 receptors. Without exhibiting any action toward membrane stability or intrinsic sympathomimetics, this inhibition lowers cardiac output via having detrimental Chronotropic and Inotropic effects[5].

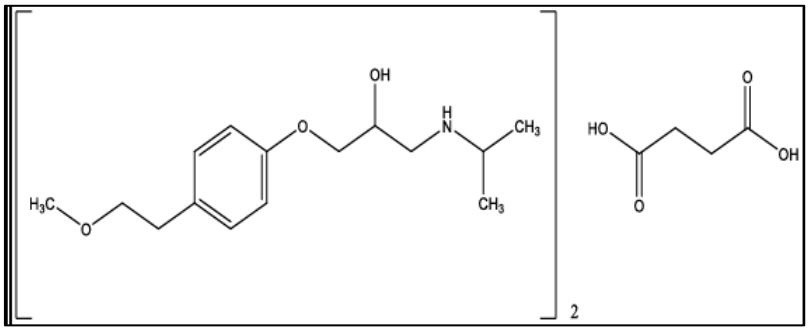

Figure 02: Structure of Metoprolol tartrate

The literature includes several analytical methods, such as UV-visible spectrophotometry[6-9], HPLC[10-15], HPTLC[16-17,] and LC-Q-TOF-MS[18], for the estimation of Efonidipine Hydrochloride Ethanoate and Metoprolol tartrate, either as individual drugs or in combination with other compounds. However, there is no HPLC method with internal standard reported for quantitative simultaneous estimation of EFO and MET in tablet dosage form. Hence in the present work attempts have been made for the development and validation of simple, rapid, sensitive and precise HPLC method, using internal standard (IS).

MATERIALS AND METHODS

Reagents and Chemicals

The reagents like O- phosphoric acid, acetonitrile (ACN), methanol (MeOH) and water used were HPLC grade. EFO and MET standard drugs were procured as a gift sample from Zuventus Healthcare Ltd, Hinjewadi, Pune, and CTX Life Sciences Ankleshwar, Gujarat, respectively. Marketed formulation EFNOCAR-MX is manufactured by Zuventus Healthcare Ltd.

Instrumentation

The HPLC system used was Shimadzu LC-20AT pump, Rheodyne injector (20 µl), SPD-20A UV detector and the system was controlled through Spinchrom CFR software (version 2.1.4.93). Analytical column used for this method was Shimadzu Shim Pack C8 (250 mm x 4.6 mm, 5µm), Abcare Export digital pH meter, GT Sonic (GT-1730QTS) sonicator and vacuum pump were used throughout the experiment.

Chromatographic Condition

- Analytical Column**:** Shimadzu Shim pack C8 (250 mm×4.6 mm, 5 µm)
- Mobile Phase: ACN: MeOH: HPLC grade water pH 3.0 (50:30:20 % v/v/v)
- Internal Standard**:** Simvastatin (50 µg/ml)

- Injection volume: 20 µl
- Flow rate: 1 ml/min
- Detection Wavelength: 220 nm
- AUFS: 0.1000
- Pressure ~ 7.6 MPa

Standards and Sample Solutions Preparation

The Standard stock solutions of EFO (1000 µg/ml), MET (2500 µg/ml) and SIM (1000 µg/ml) were prepared using HPLC grade Methanol. Serial mixed dilutions were prepared from concentration range of 10-320 µg/ml for EFO and 6.25 -150 µg/ml for MET using 50 µg/ml of SIM as an Internal Standard. These solutions were filtered through Nylon 25 mm, 0.2 µm filter and 20 µl sample of each solution were injected in the chromatographic system and the chromatograms were observed for their peak areas and other system suitability parameters.

Twenty Tablets EFNOCAR-MX (EFO-40 mg and MET-25 mg) were weighed and crushed to obtain fine powder. The volume was made up to the mark with the HPLC grade Methanol (EFO 400 µg/ml and MET 250 µg/ml). The solution was filtered using Whatman filter paper No. 41, labelled as 'Sample Stock A'.

From the above 'Sample Stock A' solution, 2 ml of the aliquot was pipetted out and transferred to a 10 ml volumetric flask along with 0.5 ml of 'Std Stock SIM' (1000 µg/ml) solution. The volume was made up to the mark with the mobile phase. (80 µg/ml of EFO, 50 µg/ml of MET, and 50 µg/ml of SIM).

Similarly, from the 'Std Stock EFO' (1000 µg/ml) solution 0.8 ml of aliquot was pipetted out in a 10 ml volumetric flask and from 'Std Stock MET-B' (250 µg/ml) solution 2 ml aliquot was pipetted out in the same 10 ml volumetric flask along with 0.5 ml of 'Std Stock SIM' (1000 µg/ml) was added. The volume was made up to the mark with mobile phase to obtain a solution with a final concentration of 80 µg/ml of EFO, 50 µg/ml of MET and 50 µg/ml of SIM.

Both solutions (Standard and Sample) were filtered through a Nylon 25 mm, 0.2 µm filter using a syringe and followed by injection into the Rheodyne injector (20 µl) of the HPLC system using a Hamilton Syringe. Both the sample and standard chromatograms were recorded under the finalised chromatographic conditions as described above, after getting a stable baseline. Peak areas were recorded for all the peaks.

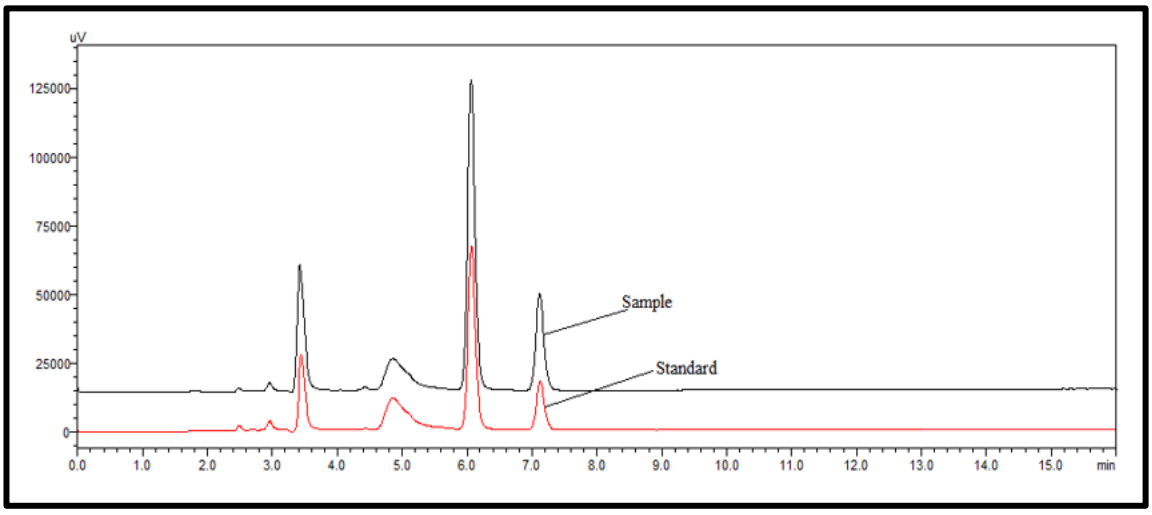

Figure 03: Overlain Chromatograms of Sample and Standard Solution of EFO (80 µg/ml), MET (50 µg/ml), and SIM (50 µg/ml)

Table 1: Results of Assay of EFNOCAR-MX tablet

Component	Label claim (mg)	Mean amount found (mg) n=6	Mean % Assay ± SD
EFO	40	40.10	101.64±0.6548
MET	25	25.31	100.16± 0.3744

Method Validation

Method validation was carried in accordance to the International Conference on Harmonization (ICH) guidelines for validation of analytical procedures[19]. The assay was validated with respect to linearity, precision, accuracy, sensitivity and robustness.

Accuracy/Recovery

Recovery studies were carried out by the standard addition method by adding the known amount of EFO and MET (Reference Standard) to the pre-analysed sample at three different concentration levels, i.e., 80%, 100%, and 120% of assay concentration, and per cent recoveries were calculated.

Precision

The precision of the method was determined by repeatability, intermediate precision (intra-day, inter-day) and was expressed as % Relative Standard Deviation (%R.S.D.). Intra- day precision was determined by performing analysis of triplicate injections of three different concentrations of combination on the same day at different time intervals and on three different days for inter- day precision.

Linearity and Range

The concentration ranges 10-320 µg/ml for EFO and 6.25-150 µg/ml for MET were prepared and analysed. Linearity of the method was decided by observing R^2 value.

Sensitivity

Sensitivity of the method was determined by means of the detection limit (LOD) and quantification limit (LOQ). Calculations for LOD and LOQ were based on the standard deviation of the Y-intercepts of the six calibration curves (σ) and the average slope of the six Calibration curve (S), using the equation LOD= 3.3×σ/S and the equation LOQ= 10×σ/S.

Robustness

Combined standard solutions of EFO (80 µg/ml), MET (50 µg/ml) with SIM (50 µg/ml) was prepared and analyzed at different flow rate (0.98, 1.00 and 1.02 ml/min) and different organic solvent content in mobile phase (49.29:22, 50:30:20 and 51:31:18 % v/v/v of Acetonitrile: Methanol: HPLC grade water pH 3), separately and variation of the results were observed.

System Suitability

Combined standard solutions of EFO (80 µg/ml) and MET (50 µg/ml) with SIM (50 µg/ml) were prepared and analyzed six times. Chromatograms were studies for different parameters such as tailing factor, resolution and theoretical plates to see that whether they comply with recommended limit or not.

RESULTS AND DISCUSSION

Accuracy

Method accuracy was checked by standard addition method and percentage recovery and percentage relative standard deviation were calculated. The results obtained (Table 2) indicate that recoveries were good, not less than 98% and percentage relative standard deviations were less than 2%.

Table 2: Results of Recovery Studies

Component	Concentration added (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

Precision

Three different concentrations of combination of EFO and MET were selected for intra-day and inter-day precision. The % RSD of the study was found to be less than 2% as shown in table 3.

Linearity

The linearity of this method was found to be in the concentration ranges 10-320 µg/ml for EFO and 6.25-150 µg/ml for MET. Y=0.0302x+ 0.2174 and Y = 0.0212x+0.0895 are linear regression equations with correlation coefficients of 0.9991 and 0.9998 for EFO and MET, respectively.

Table 3: Results of Precision Studies

Component	Concentration (µg/ml)	% RSD Intra-day (n=3)	% RSD Inter-day (n=3)
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

Limits of Detection and Quantification

The limits of detection (LOD) and quantification (LOQ) were established by evaluating the minimum level at which the analyte could be readily detected and quantified with accuracy, respectively. The LOD was found to be 1.841 µg/ml and 3.253 µg/ml for EFO and MET respectively, and the LOQ was found to be 5.580 µg/ml and 9.858 µg/ml for EFO and MET, respectively.

System Suitability

System suitability was performed to confirm that the equipment was adequate for the analysis to be performed. The test was carried out by making six replicate injections of a standard solution containing 80 µg/ml EFO, 50 µg/ml MET and 50 µg/ml Simvastatin (IS) and analyzing each solute for their peak area, theoretical plates (N), resolution (R) and tailing factor (T). The results of system suitability study in comparison with the required limits are shown in Table 4. The proposed method fulfils these requirements within the accepted limits.

Table 4: System Suitability Results of the Proposed Method (n=6)

Analyte	R	N	T	%RSD	
				Rt	Peak Area Ratio
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%	

R- Resolution factor, N-Number of theoretical plates, T-Tailing factor

CONCLUSION

In the present research work to achieve highest precision in quantitative estimation of Efonidipine hydrochloride ethanolate and Metoprolol tartrate from tablet dosage form, a reversed phase liquid chromatography method was developed and validated using Simvastatin as an IS. The method was validated in terms of linearity, precision, accuracy, detection limit, quantification limit and robustness.

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