Validated Stability-indicating HPTLC Method Development for Simultaneous Determination of Efonidipine hydrochloride and Metoprolol succinate as Bulk Drugs and in Combined Tablet Formulation

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***Abstract*—A new simple, accurate and precise stability-indicating high performance thin layer chromatographic (HPTLC) analytical method has been developed and validated for the simultaneous quantitative analysis of Efonidipine hydrochloride and Metoprolol as bulk drugs and in combined tablet formulation. The objective of this study was to explore degradation behaviour of both drugs under ICH-recommended stress conditions, employing a newly designed stability indicating HPTLC method and validating it. The chromatographic separation was carried out using precoated silica gel 60F254 (10 × 10 cm) plates as stationary phase and mixture of Toluene: Ethyl Acetate: Methanol: Formic acid (5.5: 2.5: 2: 0.5, v/v/v/v) as mobile phase. The retention factors for metoprolol and efonidipine were 0.26 ± 0.05 and 0.72 ± 0.03, separately. The method that was optimized has been inspected for linearity, accuracy, precision, limit of detection, limit of quantification, and robustness according to ICH guidelines. Linear results were observed for both drugs in the concentration range of 800-2800 ng band-1 for Metoprolol and 500-1750 ng band-1 for Efonidipine. The % drug content after application of the method to commercial tablet formulation was found to be (Mean ± S.D.) 99.29 ±1.11 and 98.26 ±1.29 for Metoprolol and Efonidipine hydrochloride, respectively. These drugs in combined tablet dosage form can be quantified simultaneously with the developed method, as well as for routine analysis in quality control laboratories.**

***Index Terms*—Efonidipine hydrochloride, Metoprolol, HPTLC, Stability Studies, Validation**

I. INTRODUCTION

Efonidipine hydrochloride, chemically, 2-(N-benzylanilino) ethyl 5-(5, 5-dimethyl-2-oxo-1, 3, 2λ5-dioxaphosphinan-2-yl)-2, 6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3-carboxylate; hydrochloride is a calcium channel blocker of the dihydropyridine class used in the treatment of acute or chronic vascular hypertension [1]. Metoprolol chemically, Bis [(2RS)-1-[4-(2-methoxyethyl) phenoxy] 3 [(1methylethyl) amino] propan-2-ol] butanedioate is used to treat angina and hypertension [2]. A thorough study of the literature showed that analytical methods such as Spectrophotometry [3, 4], High Performance Liquid

Chromatography (HPLC) [5-7] and High-Performance Thin Layer Chromatography (HPTLC) [8-10] has been reported for determination of Metoprolol in pharmaceutical dosage form either as single drug and/or in combination with other drugs. Analytical methods reported for determination of Efonidipine hydrochloride includes UV [11], HPLC [12], LC-MS [13-15] and HPTLC [16] either as single drug or in combination with other drugs. Analytical methods such as HPLC [17] were also found for simultaneous estimation of Metoprolol and Efonidipine hydrochloride in pharmaceutical formulations. To best of our information, no reports were found in the literature for simultaneous determination of Metoprolol and Efonidipine hydrochloride in combined tablet dosage form by stability-indicating HPTLC method. This paper describes the development and validation of a simple, precise, accurate and selective stability indicating HPTLC method for simultaneous determination of Metoprolol and Efonidipine hydrochloride in combined tablet dosage form in the presence of their

degradation products as per International Conference on Harmonization Guidelines.

II. MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade working standard Efonidipine hydrochloride ethanolate and Metoprolol was obtained as gift sample from Zuventus Healthcare Ltd. (Mumai, India). The pharmaceutical dosage form used in this study was Efnocar-MX tablets labelled to contain 25 mg of Efonidipine hydrochloride ethanolate and 40 mg of Metoprolol were procured from the local market. Ethyl acetate, Toluene, Methanol, Chloroform (All AR grade) were obtained from Thomas Baker Pvt Ltd (Mumbai, India). Ethyl acetate was obtained from Loba Chemie Pvt Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

The drugs were separated chromatographically on Merck TLC plates that were precoated with silica gel 60 F254 plates (10 cm×10 cm with 250 µm layer thickness) from E. MERCK, (Darmstadt, Germany) using a CAMAG Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland). Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using as mobile phase. The mobile phase was saturated in the chamber for 15 minutes. After development, TLC plates were dried in a current of air with the help of a hair drier. The CAMAG thin layer chromatography scanner III was used to scan for all developments using WinCATS software version 1.4.2 and was scanned at 264 nm and 212 nm. A deuterium lamp was the source of radiation that emitted UV spectrum between 200 and 400 nm.

Preparation of standard stock solutions

Standard stock solution of Metoprolol and Efonidipine were prepared by dissolving 10 mg of each drug in 10 mL of methanol to get concentration of 1000 µg mL-1 from which 4 mL of metoprolol and 2.5 ml of efonidipine were withdrawn and diluted separately with methanol to achieve final concentration of 400 ng µL-1 and 250 ng µL-1 for Metoprolol and Efonidipine, respectively.

Analysis of marketed formulation

Twenty tablets were weighed accurately and finely powdered. A quantity of tablet powder equivalent to 40 mg of Metoprolol (25 mg Efonidipine) was weighed and transferred to 10 mL volumetric flask containing 7 mL of methanol. The contents were sonicated for 20 min, filtered and volume was made with methanol. From this stock solution, 1 mL was further diluted to 10 mL with methanol. 3 µL volume of this solution was applied on TLC plate to obtain final concentration of 1200 ng band-1 for Metoprolol and 750 ng band-1 for Efonidipine. After chromatographic development peak areas of the bands were measured at 212 nm for Metoprolol and 264 nm for Efonidipine. The amount of each drug present in sample was estimated from the respective calibration curves. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies of bulk drugs

The stability studies were performed by subjecting the mixed standard solution of bulk drugs to the physical stress (hydrolysis, peroxide, heat and light) and stability was accessed. The stress degradation studies were carried out at initial drug concentration of 400 µg mL-1 and 250 µg mL-1 for Metoprolol and Efonidipine respectively. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 400 ng band-1 for Metoprolol and 250 ng band-1 for Efonidipine. The oxidative degradation was carried out in 30 % H_2O_2 and the sample was diluted with methanol to obtain solution having concentration 400 and 250 ng band-1 of both the drugs. Thermal stress degradation was performed by keeping the solid drugs individually in oven at 60ºC for a period of 4 h. Photolytic degradation studies were carried out by exposing both drugs individually to UV light up to 200-watt h square meter-1. Thermal and photolytic samples were diluted with methanol to get the concentration of 400 and 250 ng band-1 of both the drugs.

III. RESULTS AND DISCUSSION

Method optimization

The main aim in developing this stability indicating HPTLC method is to achieve the satisfactory resolution of drugs from each other and also from their

degradation products. To obtain better separation, a number of method trials were conducted initially using different mobile phases. Finally the mobile phase comprising Toluene: Ethyl Acetate: Methanol: Formic acid (5.5: 2.5: 2: 0.5, v/v/v/v) was selected as optimum to attain well-defined and resolved peaks for the drugs. The retention factors were found to be 0.26 ± 0.05 and 0.72 ± 0.03, for Metoprolol and Efonidipine hydrochloride, respectively.

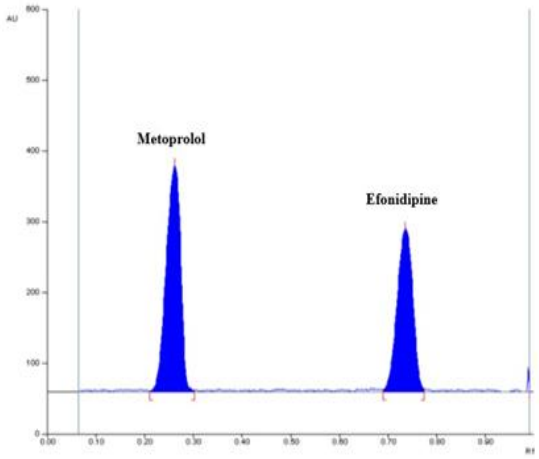

Fig 1: Densitogram of mixed standard solution of Metoprolol (1600 ng band-1, Rf = 0.26±0.05) and Efonidipine (1000 ng band-1, Rf = 0.72±0.03)

Forced degradation studies

The stress degradation results revealed the susceptibility of both the drugs to hydrolytic, oxidative, thermal stress conditions and stability under photolytic stress conditions. Marked degradation in the densitograms was observed with appearance of degradation products for Metoprolol and Efonidipine hydrochloride under acid and thermal stress conditions. Both the drugs were found to undergo degradation after treatment with peroxide and after effect of alkaliwith reduction in the peak area and without appearance of peak for degradation products. Fig 2 and 3 shows the densitograms of acid and thermal degradation. Peak purity results greater than 995 indicate that peaks for both drugs are homogeneous in all stress conditions confirmed the stability indicating power of the method.

The findings of degradation studies are represented in Table 1.

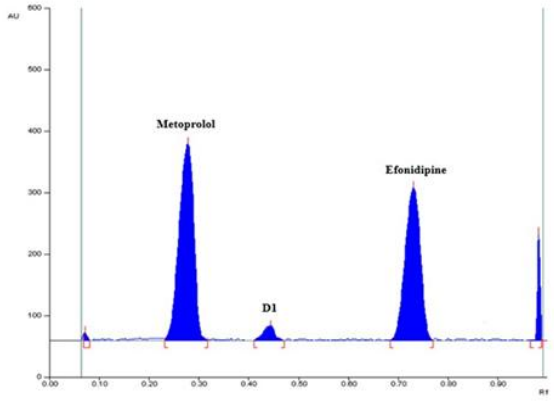

Fig 2: Densitogram of mixed standard solution of Metoprolol (D1, Rf = 0.45) and Efonidipine obtained after acid hydrolysis

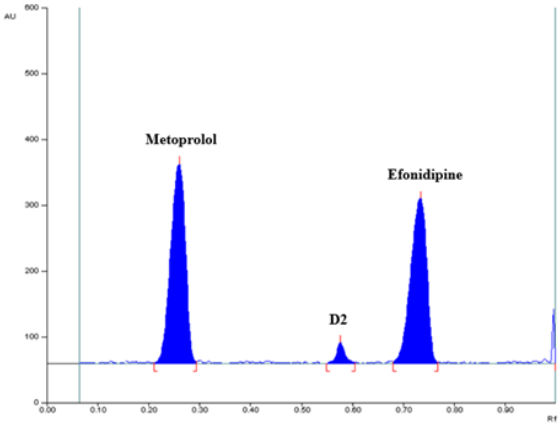

Fig 3: Densitogram of mixed standard solution of Metoprolol (D2, Rf = 0.58) and Efonidipine obtained after thermal stress

Table 1: Forced degradation studies

Stress conditions/ duration	Metoprolol	Efonidipine
	% degradation	% degradation
Acid / 0.1N HCl Kept at RT for 2 h	10.22	14.10
Alkali/0.05 N NaOH Kept at RT for 2 h	13.60	12.79
Oxidative /30 % H_2O_2 Kept at RT for 2 h	21.23	18.89
Dry heat/ 60ºC/ 4 h	11.24	10.88
Photolysis UV light	09.63	11.00

Method Validation

The optimized method was validated in accordance with ICH guidelines with respect to linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness.

Linearity

The standard stock solutions of Metoprolol and Efonidipine (400 and 250 ng µL-1 each) were applied by overspotting on TLC plate in range of 2, 3, 4, 5, 6 and 7 µL. Straight-line calibration graphs were obtained in the concentration range 800-2800 ng band-1 for Metoprolol and 500-1750 ng band-1 for Efonidipine. The linear regression equation and correlation coefficient were found to be y =2.582x + 399.4 and R2 =0.993 for Metoprolol and y = 3.9631x - 737.57and R2 = 0.995 for Efonidipine, respectively.

Precision

Set of three different concentrations in three replicates of mixed standard solutions of Efonidipine hydrochloride and Metoprolol were prepared. All the solutions were analyzed on the same day and on three consecutive days in order to record intra-day and inter-day variations in the results. The % R.S.D. values for intra-day and inter-day precision were found to be in the range of 1.03 to 1.64 and 0.93 to 1.53 for Efonidipine hydrochloride and 1.10 to 1.83 and 1.46 to 1.73 for Metoprolol, respectively. The smaller values of % R.S.D. obtained indicate that the developed method is precise.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. LOD and LOQ were found to be 103.10 ng band-1 and 312.48 ng band-1 for Metoprolol and 47.39 ng band-1 and 143.63 ng band-1 for Efonidipine hydrochloride, respectively.

Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to pre-analysed sample solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 1200 ng band-1 for Metoprolol and 750 ng band-1 for Efonidipine drugs from tablet solution.. The drug concentrations were calculated from respective linearity equation. The mean % recovery was found to be 99.56±1.23 and 100.5±1.46 for Metoprolol and Efonidipine, respectively which indicated the accurateness of developed method for estimation of drugs in combined tablet dosage form.

Robustness Robustness of the method was determined by making deliberate variations in method parameters. The parameters, such as the wavelength (± 1 nm) and composition of the mobile phase (± 1% methanol), were changed, and the impact on the drug area was observed. The areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

IV. CONCLUSION

The high-performance thin layer chromatography method developed for the simultaneous estimation of metoprolol succinate and efonidipine hydrochloride in the presence of their degradation products is selective, precise and accurate. Both the drugs (Metoprolol and Efonidipine hydrochloride) were prone to hydrolysis, oxidative and thermal stress conditions and found stable under photolysis. The degradation achieved for both drugs under tested stress conditions were within acceptable limit as proposed by ICH. As products of degradation and excipients do not show any interference with peaks of active drugs, the method was stability indicating. Validation results confirmed that the values achieved for all parameters are in the limit according to ICH guidelines. The proposed stability demonstrating method may be utilized by pharmaceutical industry for routine analysis and also for checking the stability of these drugs as bulk and in their combined formulations.

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