

A VALIDATED HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE AND ISOSORBIDE MONONITRATE IN COMBINED CAPSULE DOSAGE FORM

YOGESHWARI S. BHANGALE¹, SANTOSH V. GANDHI^{1*}, PADMANABH B. DESHPANDE¹ AND SACHIN E. POTAWALE²

¹Department of Pharmaceutical Analysis, A.I.S.S.M.S. College of Pharmacy, Pune, MH, India, ²Dept. of Pharmaceutical Chemistry, Bharati Vidyapeeth Deemed University (BVDU), Centre for Advanced Research in Pharmaceutical Sciences (CARPS), Poona College of Pharmacy, Pune, MH, India. Email: santoshvgandhi@rediffmail.com

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ABSTRACT

A simple, accurate and precise densitometric method for the simultaneous estimation of Metoprolol Succinate and Isosorbide Mononitrate in combined capsule dosage form has been developed and validated. Separation of drugs was carried out using Methanol: Ethyl acetate: Triethylamine (6: 4: 0.1 v/v/v) as mobile phase on precoated Silica Gel 60 F₂₅₄ plates. The densitometric evaluation of bands was carried out at 215 nm. The retention factor for Metoprolol Succinate and Isosorbide Mononitrate were found to be 0.58 ± 0.012 and 0.80 ± 0.011 , respectively. The method was validated with respect to linearity, accuracy, precision and robustness as per the International Conference on Harmonisation (ICH) guidelines. Results were found to be linear in the concentration range of 1000-7000 ng/band for Metoprolol Succinate and 3000-9000 ng/band for Isosorbide Mononitrate respectively. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. The % assay (Mean \pm S.D.) was found to be 99.42 ± 0.446 for Metoprolol Succinate and 99.53 ± 0.607 for Isosorbide Mononitrate. The method can be used for routine analysis of these drugs in combined capsule dosage forms in quality-control laboratories.

Keywords: Metoprolol Succinate, Isosorbide Mononitrate, Densitometry, Capsule dosage form.

INTRODUCTION

Metoprolol Succinate (METO), chemically, 2-Propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-Methylethyl) amino]-, (\pm)-, butanedioate is Beta 1 selective adrenoceptor blocking agent¹. Isosorbide Mononitrate (ISMIN), 1, 4, 3, 6-Dianhydro-D-glucitol 5-nitrate is used for Angina Pectoris².

Extensive Literature survey reveals Spectrophotometric^{3, 4}, High Performance Liquid Chromatographic (HPLC) ⁵⁻¹⁶ methods for determination of METO either as single or in combination with other drugs. Analytical methods have been reported for the determination of ISMIN includes HPLC¹⁷⁻²¹ as single component or in combination with other drugs.

To the best of our knowledge no HPTLC method of analysis has yet been reported for simultaneous analysis of METO and ISMIN in combination. This paper describes simple, accurate and precise HPTLC method for simultaneous determination of METO and ISMIN in combined capsule dosage form. The method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines²².

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade working standards METO and ISMIN were obtained from Ajanta Pharmaceuticals Ltd. (Mumbai, India), Twilight Litaka Pharma Ltd. (Mumbai, India) respectively used as such without further purification. The pharmaceutical dosage form used in this study was STARPRESS MN-XLCAPSULES (Lupin Pharmaceuticals Ltd., India) labelled to contain 50 mg METO and 60 mg ISMIN were procured from the local market. Methanol (AR grade), Ethyl Acetate (AR grade), Triethylamine (AR grade) purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Silica gel 60 F₂₅₄TLC Plate (E. MERCK, Darmstadt, Germany) were used as stationary phase. A camag HPTLC system containing Camag Linomat V semiautomatic sample applicator, Hamilton syringe (100 μ L), Camag Scanner-3 with winCATS software version 1.4.2 and Camag twin-trough chamber (10 x 10 cm) were used for the present study. The slit dimensions 5 mm x 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 10 cm x 10 cm twin trough glass chamber (CAMAG, Muttensz, Switzerland) using

Methanol: Ethyl acetate: Triethylamine (6: 4: 0.1, v/v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 20 min. The length of densitogram run was 9 cm and development time was approximately 20 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner 3 at 215 nm for all developments operated by winCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of standard stock solutions

Standard stock solutions of METO and ISMIN was prepared by dissolving 10 mg of each drug in 10 mL of methanol separately to get concentration of 1000 ng μ L⁻¹ of each drug.

Selection of Detection wavelength

After chromatographic development bands were scanned over the range of 200-400 nm and the spectra were overlain. It was observed that both drugs showed considerable absorbance at 215 nm. So, 215 nm was selected as the wavelength for detection (Figure 1).

Analysis of Capsule Formulation

Twenty capsules were weighed accurately and finely powdered. A quantity of powder equivalent to 10 mg of METO (12 mg ISMIN) was weighed and transferred to volumetric flask containing 5 mL of methanol, it is sonicated for 5 min and volume was made with methanol up to 10 mL. The solution was filtered using Whatman paper No. 41. After chromatographic development peak areas of the bands were measured at 215 nm. Procedure was repeated six times for the analysis of homogenous sample.

Method Validation

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines²².

Linearity

Aliquots of 1, 2, 3, 4, 5, 6, 7 μ L for METO and Aliquots of 3,4,5,6,7,8,9 μ L for ISMIN (1000 ng μ L⁻¹ each) were applied by over spotting on TLC plate with the help of CAMAG 100 μ L sample syringe, using Linomat 5 sample applicator. The plate was developed and scanned under above established chromatographic conditions. Each standard in five replicates was analysed and peak areas were recorded. Calibration curves of METO and ISMIN were plotted separately of peak area vs respective concentration of METO and ISMIN.

Precision

Set of three different concentrations in three replicates of mixed standard solutions of METO and ISMIN were prepared. All the solutions were analysed on the same day in order to record any intra- day variations in the results. For inter-day variation study, three different concentrations of the mixed standard solutions in linearity range were analysed on three consecutive days.

Recovery Studies

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80, 100 and 120 %. Densitogram was developed and the peak areas were noted. At each level of the amount, three determinations were carried out. The results of recovery studies were expressed as % recovery and are shown in Table 1.

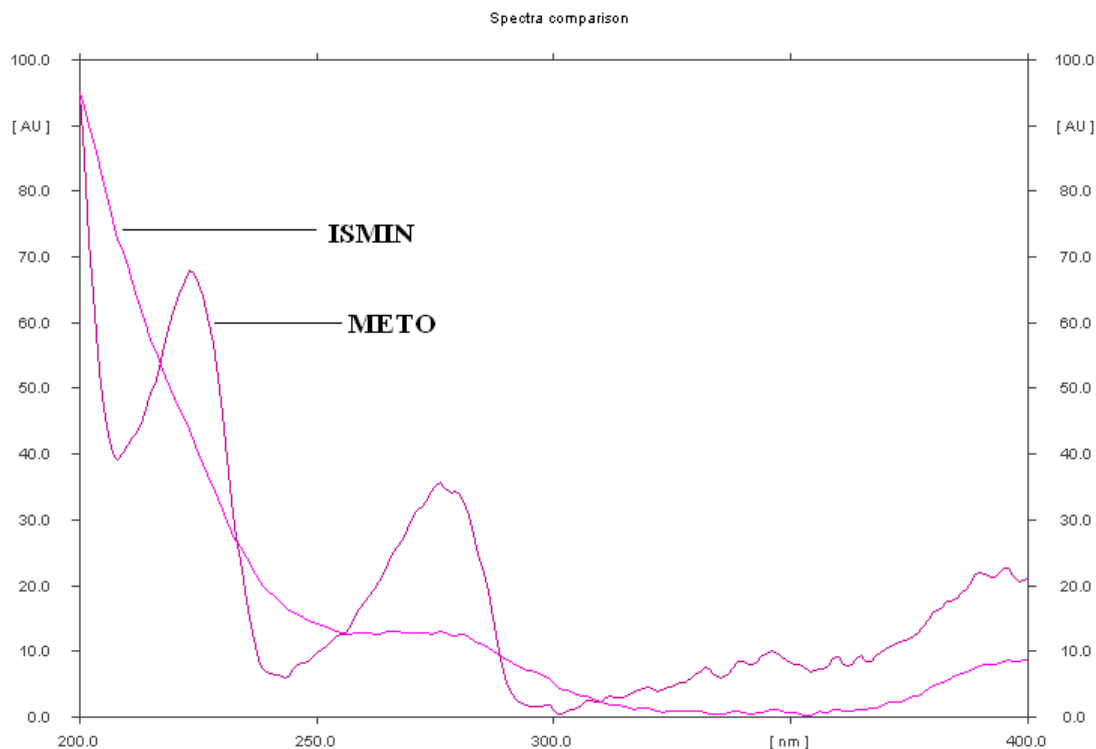


Fig. 1: In situ overlain spectrum of METO and ISMIN measured from 200 to 400 nm

Table 1: Recovery Studies of METO and ISMIN

| Drug | Amount taken (ng/band) | Amount added (ng/band) | Amount found (ng/band) | % Recovery | Mean \pm S.D. | % RSD* |
|-------|------------------------|------------------------|------------------------|------------------|------------------|--------|
| METO | 2000 | 1600 | 3565.06 | 99.02 \pm 0.04 | 99.28 \pm 0.56 | 0.55 |
| | 2000 | 2000 | 3955.74 | 98.89 \pm 0.13 | | |
| | 2000 | 2400 | 4397.31 | 99.93 \pm 0.49 | | |
| ISMIN | 4000 | 3500 | 7432.00 | 99.09 \pm 0.49 | 98.91 \pm 0.47 | 0.55 |
| | 4000 | 4000 | 7870.11 | 98.37 \pm 0.54 | | |
| | 4000 | 4800 | 8735.26 | 99.26 \pm 0.44 | | |

*Average of nine determinations

Robustness Studies

In the robustness study, the influence of small, deliberate variations of the analytical parameters on peak area of the drugs was examined. Factors varied were mobile phase composition ($\pm 1\%$), time from application to development (0, 10, 20, and 30 min) and from development to scanning (0, 30, 60, and 90 min). One factor at a time was changed to estimate the effect. Robustness of the method was checked at a concentration level of 3000 ng/ band for METO and 5000 ng/band for ISMIN. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% RSD < 2).

RESULTS AND DISCUSSION

Different mobile phases containing various ratios of n-Hexane, Ethyl acetate, Methanol, Chloroform, Toluene and Glacial acetic acid were examined (data not shown). Finally the mobile phase containing

Methanol: Ethyl Acetate: Triethylamine (6: 4: 0.1, v/v/v) was selected as optimal for obtaining well defined and resolved peaks. The optimum wavelength for detection and quantitation used was 215 nm. The retention factors for METO and ISMIN were found to be 0.58 ± 0.012 and 0.80 ± 0.011 respectively. Representative densitogram of mixed standard solution of METO and ISMIN is shown in **Figure 2**.

Straight-line calibration graphs were obtained for METO and ISMIN in the concentration range 1000-6000 ng/band and 3000-9000 ng/band with correlation coefficient 0.994 for METO and 0.995 for ISMIN. For METO, the recovery study results ranged from 99.02 to 99.93 % with % RSD values ranging from 0.050 to 0.315. For ISMIN, the recovery results ranged from 98.37 to 99.26 % with % RSD values ranging from 0.449 to 0.557. The proposed method was also evaluated by the assay of commercially available capsule containing METO and ISMIN. The % assay (Mean \pm S.D.) was found to be 99.42 ± 0.44 for METO and 99.53 ± 0.06 for ISMIN. The summary of validation parameters of proposed method are given in Table 2.

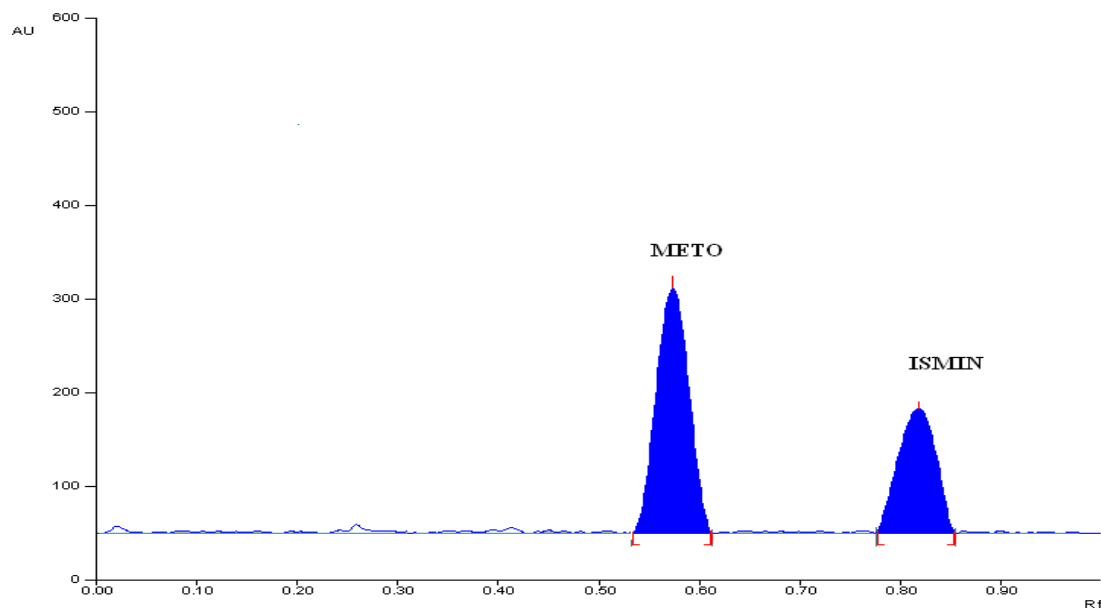


Fig. 2: Representative densitogram of mixed standard solution of METO (2000 ng/band, Rf= 0.58 ± 0.012) and ISMIN (4000 ng/band, Rf = 0.81 ± 0.011)

Table 2: Summary of validation parameters of proposed method

| Parameter | METO | ISMIN |
|---|---------------|-------------|
| Linearity Range (ng / band) | 1000-7000 | 3000-9000 |
| Correlation Coefficient (r ²) | 0.994 | 0.995 |
| LOD ^a (ng / band) | 110.41 | 352.124 |
| LOQ ^b (ng / band) | 334.58 | 1067.04 |
| Accuracy (% Recovery) | 99.02 - 99.93 | 98.37-99.26 |
| Precision (% RSD) ^c | | |
| Intra day (n ^d =3) | 0.50 - 0.80 | 0.33 - 0.68 |
| Inter day (n=3) | 0.43 - 0.86 | 0.40 - 0.62 |

LOD^a = Limit of Detection, LOQ^b= Limit of Quantitation, % RSD^c = Relative Standard Deviation, ^dn = Number of determination

CONCLUSION

The validated HPTLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of METO and ISMIN in combined capsule dosage form.

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