Validated HPTLC method for simultaneous estimation of metoprolol succinate and ramipril in bulk drug and marketed formulation

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Abstract

This paper describes a new, simple, precise, and accurate HPTLC method for simultaneous estimation of Metoprolol succinate and Ramipril as the bulk drug and in tablet dosage forms. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F254 as the stationary phase and the solvent system consisted of Methanol: Toluene: Ethyl Acetate: Ammonia (2.5:3:5:0.7v/v/v/v). Densitometric evaluation of the separated zones was performed at 209 nm. The two drugs were satisfactorily resolved with RF values 0.67, and 0.37 for Metoprolol Succinate and Ramipril, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (2000-12000 ng/spot for Metoprolol succinate and 200–1200 ng/spot for Ramipril), precision (intra-day RSD 0.471–1.036% and inter-day RSD 1.085–1.580% for Metoprolol Succinate and intra-day RSD 1.057–1.63% and inter-day RSD 1.024–1.746% for Ramipril), accuracy (98.95 ± 0.16 % for Metoprolol and 98.98 ± 0.41 % for Ramipril), and specificity, in accordance with ICH guidelines.

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and Ramipril in a combined dosage form were purchased from the local market, brand name Starpress R XL-25 (Lupin). All chemicals and reagents (methanol, toluene, ethyl acetate, ammonia) used were of analytical grade and were purchased from Merck Chemicals, India.

Instrumentation: The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on precoated silica gel aluminium plate 60 F – 254, (20 × 10 cm) with 250 μm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai) using a Camag Linomat IV applicator (Switzerland). The plates were prewashed with methanol and activated at 110°C for 5 min prior to chromatography. Then the chromatoplate was saturated with ammonia vapours for 30 min. A constant application rate of 0.1 μl/s was employed and space between two bands was kept at 6 mm. The slit dimension was kept at 5 × 0.45 mm and 10 mm/s scanning speed was employed. The monochromator bandwidth was set at 20 nm with K 320 cut off filter, each track was scanned thrice, and baseline correction was used. The mobile phase consisted of methanol: toluene: ethyl acetate: ammonia (2.5: 3: 5.0, 0.7 v/v/v/v). 11.2 ml of mobile phase was used per chromatography. Linear ascending development was carried out in 20 × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). Dimensions: length × width × height = 12 × 4.7 × 12.5 cm. It was saturated (lined on the two bigger sides with filter paper that had been soaked thoroughly with the mobile phase) and the chromatoplate development was carried out in dark with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25°C ± 2) at relative humidity of 60 % ± 5. The length of chromatogram run was 8 cm and approximately the mobile phase. The optimized chamber saturation time for mobile phase was 20 min. Subsequent to the development, TLC plates were dried in a current of air with the help of an air dryer in wooden chamber with adequate ventilation. The flow of air in the laboratory was maintained unidirectional (laminar flow, towards exhaust). Densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorbance mode at 209 nm for all measurements and operated by CATS software (V4.06, Camag). The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was via peak areas with linear regression.

Preparation of Standard Stock Solutions: 20 mg of Metoprolol and 2 mg of Ramipril were accurately weighed and transferred to 10ml volumetric flask. Metoprolol and Ramipril were dissolved in 10ml of methanol to get standard solutions of a concentration of 2 mg/ml of Metoprolol and 0.2 mg/mL of Ramipril. The standard solution was stored at 2-8°C, protected from light.

Optimization of the HPTLC method: The TLC procedure was optimized with a view to develop a simultaneous assay method for Metoprolol and Ramipril respectively. Various solvent systems like toluene: ethyl acetate: methanol, chloroform: methanol: ethyl acetate, toluene: ethyl acetate: methanol: ammonia was tried in different concentrations to separate and resolve spots of Metoprolol and Ramipril from their impurities and other excipients of formulations. Methanol: toluene: ethyl acetate: ammonia (2.5: 3: 5.0: 0.7 v/v/v/v) was found to result in the compact spot and best peak shape of Metoprolol and Ramipril. Metoprolol and Ramipril were satisfactorily resolved with Rf 0.67±0.05 and 0.37±0.02 respectively with acceptable resolution and peak shape at wavelength of 209 nm (Figure 3). In order to reduce the needless effect TLC chamber was saturated for 20 min using saturation pads. The mobile phase was run up to a distance of 8 cm; which takes approximately 20 min for complete development of the TLC plate.

Validation of the method: Validation of the optimized TLC method was carried out with respect to the following parameters.

Linearity and range: From the mixed standard stock solution 2 mg/mL of Metoprolol and 0.2 mg/mL of Ramipril, 1 to 6 µL solution spotted on TLC plate to obtain final concentration 2000-12000 ng/spot for Metoprolol and 200-1200 ng/spot for Ramipril. Linearity of the method was studied by injecting six concentrations of the drug each concentration was applied three times to the TLC plates. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

Precision: The precision of the method was verified by repeatability and intermediate precision studies.

Repeatability studies were performed by analysis of three different concentrations (2000, 6000, 10000 ng/spot for Metoprolol and 200, 600, 1000 ng/spot for Ramipril) of the drug six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Limit of detection and limit of quantitation: Limit of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background by spotting a blank and calculating the signal-to-noise ratio for Metoprolol and Ramipril by spotting a series of solutions until the S/N ratio 3 for LOD and 10 for LOQ. To determine the LOD and LOQ, serial dilutions of mixed standard solution of Metoprolol and Ramipril were made from the standard stock solution in the range of 10–200 ng/spot. The samples were applied to TLC plate and the chromatograms were run and measured signal from the samples was compared with those of blank samples.

Robustness of the method: Following the introduction of small changes in the mobile phase composition (± 0.1 ml for each component), the effects on the results were examined. Mobile phases having different compositions, e.g. methanol: toluene: ethyl acetate: ammonia (2.6: 3: 5: 0.7 v/v/v/v), (2.5: 3: 5: 0.7 v/v/v/v), (2.5: 3: 5: 0.8 v/v/v/v), (2.5: 3: 5: 0.7 v/v/v/v), (2.5: 3: 5: 0.8 v/v/v/v), (2.5: 3: 5: 0.7 v/v/v/v), were tried and chromatograms were run. The amount of mobile phase was varied over the range of ± 5 %. The plates were prewashed with methanol and activated at 60°C for 2, 5, and 7 min respectively prior to chromatography. The time from spotting to chromatography and from chromatography to scanning was...
varied from +10 min. The robustness of the method was determined at three different concentration levels 4000, 8000, 12000 ng/spot for Metoprolol and 400, 800, 1200 ng/spot for Ramipril.

**Specificity:** The specificity of the method was determined by analyzing standard drug and test samples. The spot for Metoprolol and Ramipril in the samples was confirmed by comparing the R<sub>r</sub> and spectrum of the spot with that of a standard. The peak purity of Metoprolol and Ramipril was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

**Accuracy:** Accuracy of the method was carried out by applying the method to drug sample (Metoprolol and Ramipril combination tablet) to which know amount of Metoprolol and Ramipril standard powder corresponding to 80, 100 and 120% of label claim had been added (standard addition), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

**Analysis of a marketed formulation:** To determine the content of Metoprolol and Ramipril in conventional tablet (Brand name: Starpress R XL25 Label claim: 25 mg Metoprolol and 2.5 mg Ramipril per tablet), ten tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 25 mg Metoprolol and 2.5 mg Ramipril was transferred into a 25 mL volumetric flask containing 10-15 mL methanol, sonicated for 30 min and diluted to 25 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (1000 and 100 µg/mL for Metoprolol and Ramipril respectively). 2μL of this solution (2000 and 200ng/spot for Metoprolol and Ramipril respectively) was applied to a TLC plate which was developed in optimized mobile phase. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined.

**Results and discussion**

The results of validation studies on simultaneous estimation method developed for Metoprolol and Ramipril in the current study involving Methanol: toluene: ethyl acetate: ammonia (2.5: 3.0: 5.0: 0.7 v/v/v/v) as the mobile phase for TLC are given below.

**Linearity:** The drug response was linear (r<sup>2</sup> = 0.997 for Metoprolol and 0.999 for Ramipril) over the concentration range between 2000-12000 ng/spot for Metoprolol and 200-1200 ng/spot for Ramipril. The slope and intercept for Metoprolol and Ramipril (mg/tablet) were 2.974 (± 0.862) and 658 (± 1.06), respectively.

**Precision:** The results of the repeatability and intermediate precision experiments are shown in (Table 1). The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guidelines.

**LOD and LOQ:** Signal-to-noise ratios of 3: 1 and 10: 1 were obtained for the LOD and LOQ respectively. The LOD and LOQ were found to be 50 ng/spot and 100 ng/spot for Metoprolol and 50 ng/spot and 150 ng/spot for Ramipril, respectively.

The standard deviation of peak areas was calculated for each parameter and the % RSD was found to be less than 2 %. The low values of the % RSD, as shown in indicated robustness of the method (Table 2).

**Specificity:** The peak purity of Metoprolol and Ramipril was assessed by comparing their respective spectra at the peak start, apex, and peak end positions of the spot i.e. r (S, M)=0.998 and r (M, E)=0.999. A good correlation(r=0.9997) was also obtained between the standard and sample spectra of Metoprolol and Ramipril, respectively. Also, excipients from formulation were not interfering with the assay.

**Recovery Studies:** Analysis of a formulation was executed in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients, which are normally present in tablets. The average drug content was found to be 99.52 % and 99.6 % for Metoprolol and Ramipril, respectively, using the proposed procedures and the results are summarized in (Table 4).

**Conclusion**

The developed TLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of Metoprolol and Ramipril as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Metoprolol and Ramipril also for its estimation in plasma and other biological fluids. The developed TLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of Metoprolol and Ramipril as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Metoprolol and Ramipril also for its estimation in plasma and other biological fluids. The

**Table 1.** Precision study for metoprolol and ramipril

| Drug       | Concentration ng per band | Intra-day (n=3) | Inter-day (n=3) |
|------------|---------------------------|----------------|----------------|
|            |                           | SD RSD%        | SD RSD%        |
| Metoprolol |                           |                |                |
| 60         | 14.28                     | 1.040          | 18.15          | 1.326 |
| 120        | 6.96                      | 0.317          | 5.91           | 0.269 |
| 180        | 24.83                     | 0.865          | 32.73          | 1.141 |
| Ramipril   |                           |                |                |
| 60         | 56.65                     | 1.904          | 49.86          | 1.708 |
| 120        | 33.97                     | 0.671          | 31.33          | 0.618 |
| 180        | 40.51                     | 0.627          | 41.03          | 0.635 |

**Table 2.** Robustness testing of metoprolol and ramipril

| Parameters | Metoprolol | Ramipril |
|------------|------------|----------|
|            | SD %RSD*   | SD %RSD* |
| Mobile phase composition (± 0.1 ml) | 10.42 | 1.235 | 10.42 | 1.235 |
| Amount of mobile phase (± 0.5 %) | 20.14 | 1.018 | 20.14 | 1.018 |
| Time from spotting to chromatography (± 20 min) | 15.36 | 0.942 | 15.36 | 0.942 |
| Time from chromatography to scanning (± 20 min) | 20.10 | 1.085 | 20.10 | 1.085 |

**Table 3.** Recovery studies of metoprolol and ramipril

| Label claim (mg/tablet) | Amount Added (%) | Total amount (mg) | Amount recovered (mg ± % RSD) | Recovery (%) |
|-------------------------|------------------|-----------------|-------------------------------|--------------|
| Metoprolol 25           | 80 (20mg)        | 45              | 44.65 ± 0.222                 | 99.22        |
|                         | 100 (25mg)       | 50              | 49.16 ± 0.154                 | 98.32        |
|                         | 120 (30mg)       | 55              | 54.63 ± 0.130                 | 99.32        |
| Ramipril 2.5            | 80 (2mg)         | 5.5             | 4.46 ± 0.526                  | 99.11        |
|                         | 100 (2.5mg)      | 5.0             | 4.92 ± 0.360                  | 98.4         |
|                         | 120 (3mg)        | 5.5             | 5.47 ± 0.344                  | 99.45        |

**Table 4.** Results from assay of Metoprolol, Ramipril in Starpress R XL 25 tablet

| Component | Label claim(mg) | Amount found Mg ± SD, n=6 | Percentage of label claim (±SD) |
|-----------|-----------------|---------------------------|---------------------------------|
| Metoprolol| 25              | 24.88±35.84               | 99.52%±15.15                    |
| Ramipril  | 2.5             | 2.49±18.87                | 99.6%±10.21                     |

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proposed TLC method is less expensive, simpler, rapid, and more flexible than HPLC.

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