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High-Performance Thin Layer Chromatographic Method Development and Validation in Tablet Dosage Form

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Abstract
A sensitive, selective, accurate, precise and robust high performance thin layer chromatographic method has been developed and validated for the estimation of Mirabegron in pure and pharmaceutical tablet formulation form. Methanol was used as a solvent. In this method the stationary phase used is HPTLC aluminium plates pre-coated with silica gel 60F-254 and the mobile phase used is n-butanol: acetic acid: water (6:2:2 V/V). TLC plate then detected in absorbance mode at wavelength 249 nm, the Rf value was observed to be 0.64. The method was linear in the range of 2-8 µg per band. The method was validated as per the ICH Q2 (R1) guidelines.

Keywords:*HPTLC,Mirabegron, ICH Q2(R1).*

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I. INTRODUCTION

Mirabegron, commonly known as 2-[2-Amino-1, 3-thiazol-4-yl]-N-[4-[2-{[[2R]-2-hydroxy-2-phenylethyl] amino} ethyl] phenyl] acetamide.[1]The International Continence Society defines overactive bladder (OAB) as urgency with or without urine incontinence, typically accompanied by frequency and nocturia, is a widespread, complex health condition that has negative consequences on quality of life and is quite expensive.[2,3]A drug called mirabegron is used to treat an overactive bladder.[4] It is marketed under the trade names Myrbetriq and others. Its advantages are comparable to those of other antimuscarinic drugs like solifenacin or tolterodine.[5] It is less popular than antimuscarinic drugs like oxybutynin in the United Kingdom.[6] It is ingested orally. For the treatment of OAB, mirabegron, a selective 3-adrenoceptor (AR) agonist, is a first-in-class medication.[7] High blood pressure, headaches, and urinary tract infections are typical adverse effects.[8] Angioedema, abnormal heartbeat, and urine retention are other important adverse effects.[9] It functions by stimulating the bladder's 3 adrenergic receptor, which causes the bladder to relax.[10]

In the United States, mirabegron's medical use was authorized in 2012.[11] As of 2019, the NHS in the United Kingdom pays around £29 for a month's supply.[12] The cost of this amount at wholesale in the US is roughly 369 USD. With more over a million prescriptions written in 2016, it was the 263rd most often prescribed medicine in the US.[13] The primary goal of the specified research was to create and validate a straightforward, accurate, economical, and sensitive spectrophotometric technique for mirabegron in both its bulk and tablet formulations.[14]

2-[2-Amino-1,3-thiazol-4-yl]-N-[4-[2-{[[2R]-2-hydroxy-2-phenylethyl] amino} ethyl] phenyl]

Figure1: Chemical structure of Mirabegron

II. EXPERIMENTAL

2.1. High-Performance Thin-Layer ChromatographicInstrument

The HPTLC instrument consisted of a CAMAG (Muttenz) Linomat V sample applicator with a 100-µL applicator syringe (CAMAG). Chromatography was performed on 20 cm × 10 cm aluminum HPTLC plates pre-coated with silica gel F254 (E. Merck, Darmstadt,Germany). A CAMAG TLC scanner 4 was used for the scanning TLC plates and develop chromatogram. All the drugs and chemicals were weighed on Wensar electronic balance (MAB220).

2.2 Chemicals And Reagents

Analytically pure Mirabegron (99.99% *w/w*) were obtained as gift samples. HPLC-grade methanol, n-Butanol, Acetic Acid were used for this practical work.

2.3 Chromatographic System

2.3.1 Sample Application

Standards and Tablet formulation samples of Mirabegron wereapplied on the HPTLC plates in the form of 4-mm length narrow band, with an 8 mm space between bands. The bands were placed 5 mm from the plate's left and bottom edges. The samples were applied while being dried continuously by a nitrogen gas stream.

2.3.2 Mobile Phase and Development

The mobile phase consisting of n-butanol: Acetic acid:water (6:2:2, *V*/*V*) was used for developing TLC plates. In a twin-trough glass chamber filled with the mobile phase vapours for 30 min., linear ascending development was done. Each development employed 10 mL of the mobile phase, which was allowed to migrate 90 mm (5 mL in the trough containing the plate and 5 mL in the other trough). After development, the HPTLC plates were dried completely at 80^0 C.

2.3.3 Scanning and Analysis

CAMAG TLC scanner 4 was used for scanning. This was performed in the absorbance mode. The deuterium lamp was used as a source of radiation, and the bands were scanned at 249 nm. The slit dimensions were 5 mm length and 0.45 mm width, with a scanning rate of 20 mm/sec.Utilizing a linear regression equation, the chemical concentrations were calculated from the intensity of light that was diffusely reflected and analysed as peak areas versus concentrations.

2.3.4 Preparation of Standard Stock Solution

10 mgof Mirabegron were weighed accurately andtransferred to 100mL volumetric flasks and dissolved in a few mL of methanol. The volumes were made up to the mark with methanol to get a solution containing 100 µg/ml (stock solution).From this stock solution further take 5 ml in the 10 mlvolumetric flask and diluted with methanol to get a working standard of 50 µg/ml.

2.4 Validation

Validation of this developed HPTLC method was carried out according to the ICH guidelines Q2(R1) for specificity, accuracy, precision, Specificity, solution stability and robustness.

2.4.1 Linearity of Calibration Curves

Linearity of the method was evaluated by constructing calibrationcurves at 7 concentration levels over a range of 2–8 µg per band of Mirabegron. The calibration curves were developed by plotting peak area *versus* concentration.

2.4.2 Accuracy

The recoveries of Mirabegron using the method of standard additions were calculated in order to assess the method's accuracy.Mirabegron was spiked at known concentrations of 50, 100, and 150%. Using the TLC plate and the aforementioned chromatographic conditions, the solutions were analysed. Amount of Mirabegron was determined using a regression equation after measuring the peak area.

2.4.3 Precision

In the Precision study, intra-day and inter-day precisions carried out. Intra-day and inter-day precision was determined by analyzing sample solutions of Mirabegron in pure and tablet formulation. The % assay and %RSD values were calculated fromobtainedpeak areas.

2.4.4 Specificity

To assess the analyte in the presence of components, specificity was examined, which may include impurities, excipients, and matrix. The tablet was used to determine the specificity, and the interference of excipients was noted.

2.4.5 Sensitivity

The lowest concentration of analyte that may be detected was established to be the limit of detection (LOD), and The lowest amount of analyte that the method can quantify was identified as the limit of quantitation (LOQ). LODand LOQ were calculated using the following equations as perthe ICH guidelines.

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

Where,σ is the standard deviation of the *y*-intercepts of the regression lines and *S* is the slope of the calibration curve.

2.4.6. Robustness

To validate Robustness of the method, aSmall changes were made in the detecting wavelength and mobile phase ratio, and the effects on the results were examined. The *%RSD* was calculated from the peak areas of samples applied.

2.5 Solution Stability

The stock solutions of Mirabegron was stored at room temperature and another one at 2°Cfor 48 h and analyzed at an interval of 12, 24, 36 and 48 h.

III. RESULT AND DISCUSSION

To select the mobile phase for develop an HPTLC method forMirabegron, polarity of solvent play important role. For this development, compact and well-resolved spots were essential. Different solvents like methanol, ethyl acetate, butanol, hexane alone and in combination with other solvents were tried in differentratios, so that spot of Mirabegron well resolved. A mixture of n-butanol: Acetic acid: water (6:2:2, *V/V*).was selected as the mobile phase, which gave a well-defined, compact band of Mirabegron with Rf values of 0.64.30 minutes was the ideal mobile phase chamber saturation time, and solvent migration distance was 90 mm.

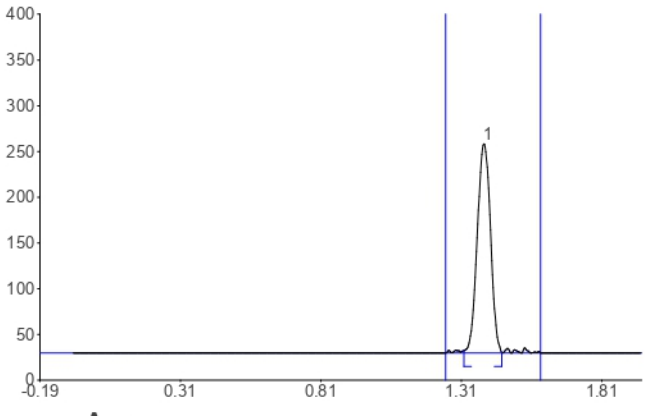

Fig.1. Mirabegron densitogram using mobile phase n-butanol: Acetic acid: water (6:2:2, V/V)

3.1 Validation

3.1.1 Linearity and Calibration Curves

Mirabegron's calibration curve was plotted between 2 and 8 g per band, which shows a linear correlation coefficient (r^2) of 0.9996.

The regression data shown in **Table 3**reveal a good linear

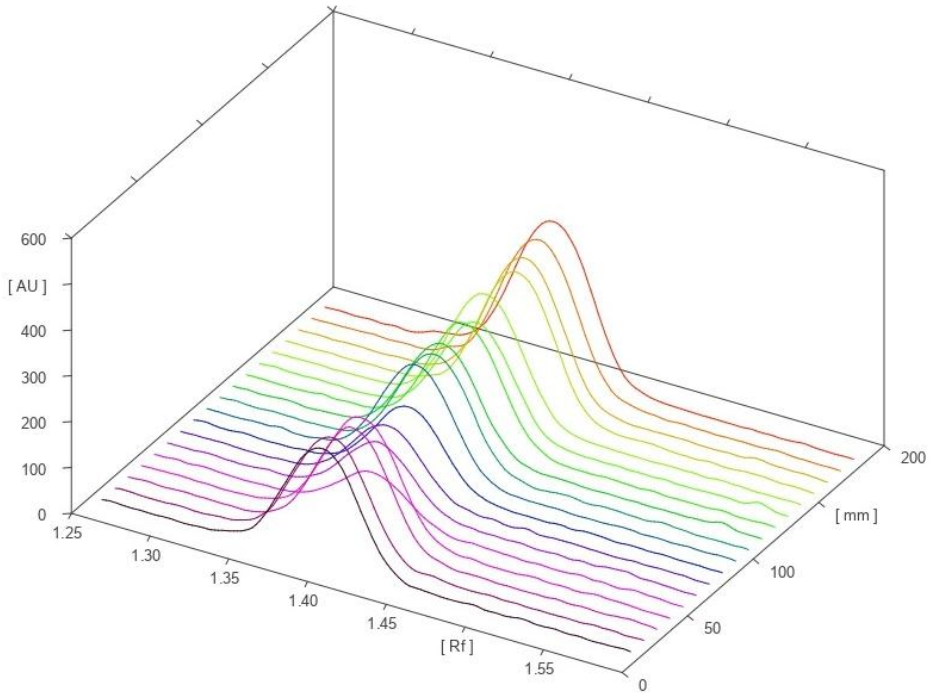

Fig.2. Overlay 3-D diagram of Mirabegron

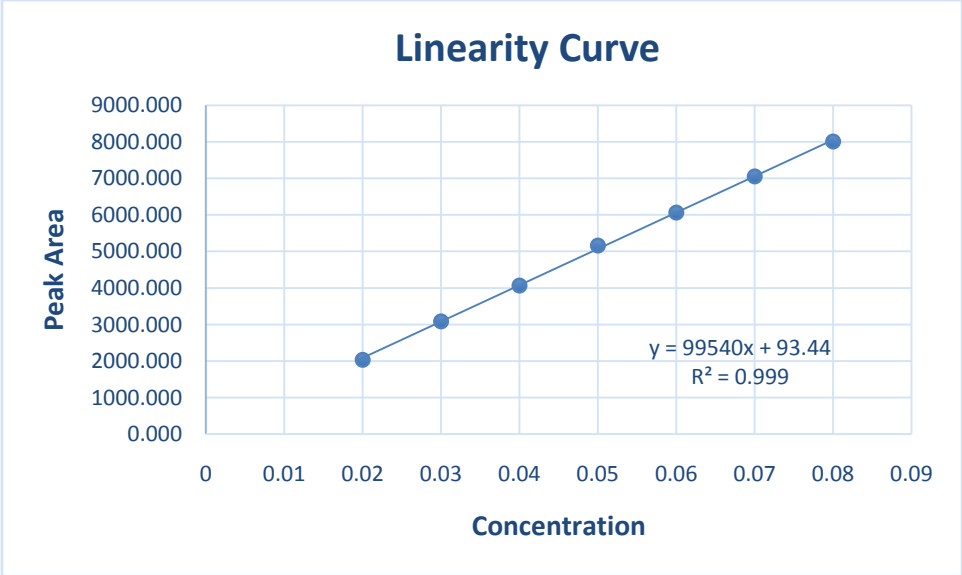

Fig.3. Linearity curve of Mirabegron

Relationship over the concentration range studied, demonstratingthe suitability of the method for analysis. The overlaid three-dimensionaldensitogram is reported in **Figure 2** and regressioncurves are shown in **Figure 3**.

Table 1 Regression analysis of the calibration curve.

Parameters	Mirabegron
Linearity [µg per band]	2–8 µg per band
Correlation coefficient(*r*2)	0.9996
Slope of regression equation	99540
Standard deviation of slope	52.98
Intercept of regression	93.441

3.1.2. Accuracy

Accuracy study reveals the amount of drug recovered at three levels of standard addition. Percentage recovery of Mirabegron was found in the range of 98.53–99.87% *w*/*w*, Results near to the true value (100%) indicate that the method is accurate **(Table 2).**

3.1.3 Intermediate Precision

Precision study was evaluated inIntra-day and Inter-day Precision.Intra-day precision was checked by measuring the responseof same concentrations from 6 different set measured 3 times a day. *%RSD* values for Mirabegronwere found to be 0.87% for intra-day precision,whereas in inter-day precision %RSD value was 1.42%.

3.1.4 Limit of Detection and Limit of Quantification

The LOD for Mirabegronwas found to be 0.047 µg perband The LOQs of Mirabegronwasfound to be 0.12 µg per band, whichindicate that the method is sensitive, and the Nano gram quantityof drug can be estimated accurately and precisely. The validationparameters are summarized in Table 2.

3.1.5 Robustness

Robustness studies were performed by introducing small changes in the method parameters like the mobile phase ratio and detection wavelength in UV-VIS spectroscopy. The mobile phase composition n-butanol: Acetic acid: water (6:2:2, *V*/*V*) was changed to n-butanol: Acetic acid: water (8:1:1, *V*/*V*)and then further changed to n-butanol: Acetic acid: water (4:3:3, *V*/*V*). None of these changes affected the performance of the developed HPTLC method. No significant change in the Rf value was observed, thus proving that the method is robust.

Table 2Summary of the validation parameter

Parameters	Value
Linearity [µg per band]	**2-8**
Detection limit [µg per band]	**0.047**
Quantitation limit [µg per band]	**0.12**
Accuracy [%]	**98.53-99.87%**
Intra-day precision *[%RSD]*	**0.87%**
Inter-day precision *[%RSD]*	**1.42%**

IV. CONCLUSION

For the measurement of Mirabegron, a precise and exact high performance thin-layer chromatographic method has been created.The HPTLC approach is both time and money efficient because just 10 mL of mobile phase are needed for a single analysis.The results are comparable in terms of accuracy and precision to the liquid chromatographic technique.The mobile phase used in the method's development was n-butanol:acetic acid:water (6:2:2, V/V).For Mirabegron, the Rf values were found to be 0.64.In the range of 2–8 g per band for mirabegron, the approach was discovered to be linear. Following ICH criteria, the procedure was validated Q2 (R1). When the procedure was used to estimate the medication Mirabegron, a percentage recovery of more than 98% was obtained. The approach can be utilised for routine quality control examinations of the in-process quality control of samples and dosage forms.

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