

RESEARCH ARTICLE

Validated Stability Indicating RP-HPLC Method for the Quantification of Process Related Impurities of Solifenacin and Mirabegron in Pharmaceutical Formulations

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ABSTRACT

To assess solifenacin (SOL) and mirabegron (MER) simultaneously, a verified reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed to indicate stability. The method was thoroughly evaluated and found to meet satisfactory criteria for precision, linearity, accuracy, limits on detection, and robustness, limits on quantitation. The quantitation wavelength of 231 nm was determined. Linearity was successfully demonstrated across concentration ranges of 5 to 25 µg/mL of solifenacin and 50 to 250 µg/mL of mirabegron. RPHPLC separations was conducted employing a Phenomenex L. C18 column measuring 250 x 4.6 mm and containing particles as small as 5 µm. The methanol and phosphate buffer (pH 7) were combined in a volumetric ratio of 25:75 to create the mobile phase. The separation is accomplished at a 0.7 mL per minute flow rate. Time spent in retention for mirabegron and solifenacin had been established at 5.521 and 9.161 minutes, respectively. Forced degradation studies validated the stability-indicating character of the approach, which included hydrolysis under acidic and basic conditions, exposure to H₂O₂, thermal degradation, and photodegradation. Mirabegron and solifenacin exhibited 10 to 20% degradation under the specified conditions. Importantly, the process evaluated the two prescription drugs in detail with all degradation products generated during the forced degradation experiments. This developed method is characterized as straightforward, specific, and cost-effective, making them suitable of the simultaneous estimate of mirabegron with solifenacin in tabs dose forms.

Keywords: Solifenacin, Mirabegron, RPHPLC technique, Validation, Forced degradation.

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INTRODUCTION

Mirabegron (MER), The compound with the chemical name 2-(pentamethylthiazol-4-yl) four-([(2R)-2-hydroxy-2-phenylethyl] aminoethyl) phenyl] A agonist that activates beta-3 adrenoceptors is acetamide. Large randomized placebo-controlled clinical trials have demonstrated its high safety and efficacy profile. This drug is used to treat urge urine incontinence, urgency, and increased frequency of urination, all of the symptoms of an overactive bladder. A muscarinic acetylcholine receptor antagonist, solifenacin (SOL), is utilized to manage an overactive bladder, where it competitively blocks these receptors to alleviate symptoms. The ingredient is 1-azabicyclo oct-8-yl(1S) chemically 1,4-dihydro-1-phenylhesoquinoline 1methoxylate -2.

A review of the literature reveals that numerous analytic techniques have been devised and published for the

estimation of MER by reverse-phase high-performance liquid chromatography (RP-HPLC), high-performance thin layer chromatography (HPTLC) and spectrofluorometry¹⁻⁵ and SOL individually or in combination with other drugs by chromatographic and spectroscopic techniques.^{6,10} Also, A literature review considers spectrophotometric, RP-HPLC, fluorometric and thin layer chromatography (TLC) methods for the combined dosage form.¹²⁻¹⁵ Even so, there isn't stability indicating the RP-HPLC technique for these two prescription drugs' simultaneous measurement. The "Stability testing of novel medicinal ingredients and products" guideline from stress testing is required by to ascertain the intrinsic durability qualities of the active ingredient by the International Harmonization Council (ICH). Achieving a perfect stability-indicating approach is crucial, providing excellent drug and degradation product resolution. Subsequently, it presents a

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challenge for pharmaceutical researchers to formulate an analytical methodology for the simultaneous measurement of SOL and MER in the presence of their breakdown products. In order to resolve this, the stability of MER and SOL was systematically studied under conditions involving acidic, alkaline, oxidative, UV, and photolytic. This research describes a well-established stability indicating, HPLC methodology towards estimating MER and SOL simultaneously when their degradation products are present. The proposed strategy is described in terms of simple, accurate, repeatable, and stability-indicating. It functions correctly for routinely determining SOL and MER in combination dose forms. The validation process adhered to the ICH guidelines, ensuring the method's reliability and robustness for pharmaceutical applications.¹⁷⁻¹⁹ The structure of mirabegron and solifenacin is shown in Figure 1.

MATERIALS AND METHODS

Materials

Pharmaceutical grade MER and SOL were generously provided as gift samples by Swapnaroop Drugs and Pharmaceuticals located in Sambhajinagar (431003), Maharashtra, India. Various chemicals and reagents were used in the study: Rankem in New Delhi provided sodium hydroxide, Fischer Scientific in Mumbai provided potassium dihydrogen phosphate, Himedia in Mumbai provided by the nylon 66 filter membrane of (0.45 μ), and Mumbai-based Loba Chemie Pvt. Ltd. supplied H₂O₂. For the analysis of the marketed formulation, Metrlex-M tablets including 5 mg for solifenacin and 25 mg for mirabegron. were acquired from a local pharmacy and used as the sample for the study.

Instrumentation

The HPLC system utilized in this study was an AGILENT (1100) equipped with a UV detector and a 20 μ L fixed loop for injections. Applying LC solution software, a chromatographic analysis was conducted on a 4.6 mm in the center and 250 mm in length in column. Additionally, a variety of analytical tools were used in the experimental procedures, including a hot air oven (Biotech) and a pH indicator (Frontline FS 4, Mumbai, India).

Method Development

Preparation of stock solutions

About 5 mg of SOL and MER were separately weighed and put into separate 50 mL volumetric flasks to make stock solutions. MER and SOL concentrations of 100 μ g/mL were attained by adding the mobile phase to the flasks once each one was full. For analysis with an HPLC, a technique of isocratic elution

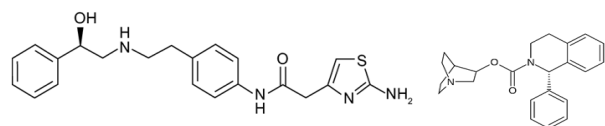


Figure 1: Structure of mirabegron and solifenacin

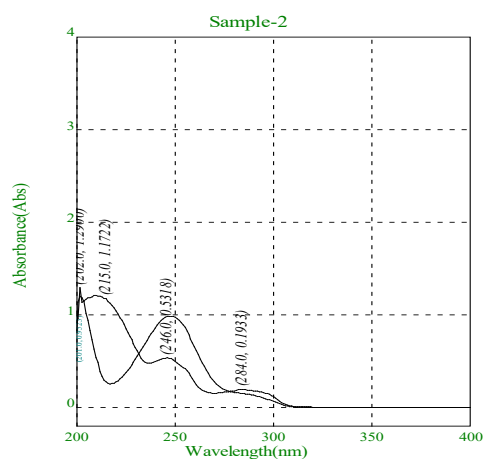


Figure 2: Overlain spectra of MER and SOL

in the reverse phase was implemented. Methanol and 0.1% orthophosphoric acid (OPA), and was the pH 4.2 adjusted with triethylamine (TEA), formed the mobile phase. The chemical separation happened on a column containing column 18 (250 x 4.6 mm, with particles with sizes of five microns) at 0.7 mL per minute. An ultraviolet detector was utilized to measure the wavelength at 231 nm. The capacity of this HPLC technique to extract and quantify solifenacin and mirabegron precisely led to its selection. The isocratic elution mode with the specified mobile phase composition on a C₁₈ column ensures accurate and reliable analysis of the substances. The UV detector at 231 nm provides the necessary sensitivity for detecting and measuring the concentration of the compounds in the samples. The overlain spectra of mirabegron and solifenacin are shown in Figure 2.

Calibration curves for MER and SOL

Tablets containing mirabegron (MER) and solifenacin (SOL) in a 5:1 ratio underwent analysis. Suitable aliquots of the individual MER and SOL stock solutions were measured and transferred into distinct volumetric flasks of 10 mL to achieve specific concentrations. The mobile phase was next added to each flask to the mark, yielding conclusive levels of 5 to 25 μ g/mL for SOL and 50 to 250 μ g/mL for MER. Chromatograms were produced when employing a preset loop of 20 μ L to introduce the prepared solutions into the chromatographic gadgets. The averages of the peak areas were plotted versus concentrations for generating calibration curves. Both mirabegron and solifenacin were computed regression equations that provided a quantitative relationship between each drug's concentration and corresponding peak area. The results are summarized in Table 1. This calibration process allows for accurate determination of the concentrations of MER and SOL in the tablet samples based on their respective chromatographic peak areas.

Analysis of marketed formulations

In the tablet analysis, weighed and powdered 20 pills. After that, the powder was placed in a 100 mL volumetric flask and the mobile phase was used to dissolve it, producing 200 mg of MER. Using 0.2 μ m nylon filter membrane paper, the finished

Table 1: Calibration curve data for linear regression

Parameter (units)	MER	SOL
Range of linearity (µg/mL)	50–250	5–25
r ²	0.999	0.998
Slope	35.12	162.1
Intercept	86.05	38.41

product was filtered. A 100 mL volumetric flask was filled with ten mL of the filtered solution; afterwards, the mobile phase was added to it to achieve the desired final concentration of 100 µg/mL. Ten milliliters from the filtered solution to a 100 mL volumetric flask, the mobile phase was added to dilute it to the desired final concentration of 100 µg/mL. Peak regions were evaluated under modified chromatographic conditions after the HPLC equipment was filled with 20 µL of the prepared sample solution. This analytical approach allows for the determination of the concentrations of both MER and SOL in the tablet samples based on their respective peak areas in the chromatogram.

Validation of Methods

The validation phase of the analysis method was conducted in accordance with the guidelines set forth by the International Council for Harmonization of Technical Standards for the Use of Pharmaceuticals in Humans (ICH). The validation approach encompassed several essential factors, including detection limitation, quantitation limitation, robustness, linearity, precision, and accuracy. The %recovery of MER and SOL was calculated to evaluate accuracy. The methods to drug samples were implemented with the standard addition method using established concentrations of MER and SOL for recovery tests. Six determinations were conducted at each level of the added amounts, and the obtained results were systematically compared. This validation step ensures that the method reliably and accurately quantifies MER and SOL even in the presence of added known amounts, demonstrating its suitability for analytical purposes across different concentration levels.

Intraday and interday precision

Precision studies, both intraday and interday, for SOL and MER were carried out by calculating their corresponding responses three times on three different days as well as within the same day. The intervals of concentration for these precision assessments were set at SOL: 15 to 25 µg/mL and MER: 150 to 250 µg/mL. This comprehensive evaluation provides insights into the method's repeatability and reproducibility, ensuring reliable results across varying time intervals and conditions.

Limitations of detection and quantitation

The following estimate techniques were used to calculate the limit for detection (LoD) along with the limit for quantitation (LoQ):

$$(\text{LoD} = 3.3 \times \text{SD}/S)$$

$$(\text{LoQ} = 10 \times \text{SD}/S)$$

Here, the calibration curve's average value slope can be represented by S, as SD refers for the response's standard

deviation (peak area). These computations quantify the method's sensitivity and ability to accurately detect and determine the samples' low quantities of MER and SOL.

Test for system suitability

System suitability testing is conducted to verify that the chromatographic system is repeatable, which is an essential part of chromatographic techniques. The system's efficacy was assessed by looking at certain system suitability characteristics. The repeatability of the system was tested by injecting the drug solution repeatedly at predefined concentration levels of 150–250 and 15–25 µg/mL of MER and SOL, in that order. The outcomes are displayed in Table 2.

Robustness

To assess the robustness of the HPLC process, several specific parameters were intentionally changed, including the amount of methanol in the mobile phase and its flow rate. At three different levels (-1, 0 +1) in relation to the optimal parameters, each factor was changed separately. The method's robustness was evaluated at concentrations for MER of 200 µg/mL and SOL of 20 µg/mL. This systematic approach provides insights into the method's ability to maintain performance and reliability under variations in selected parameters.

Studies on Forced Degradation

Studies on the induced degradation of both medications were carried out in a variety of environments, such as sunlight, oxidation, dry heat, and hydrolysis. About 20 tablets in all were weighed and pulverized. After that, 200 mg of MER were added to a 100 mL volumetric flask holding the powder that was produced. After the appropriate amount of powder dissolution using the mobile phase, 0.2 µm nylon membrane filter paper was used to filter the resulting solution. During filtration, 10 mL of the solution were mixed with the mobile phase in a second 100 mL volumetric flask to obtain a 100 µg/mL concentration. The sample solution was diluted to attain the ultimate concentrations of 150 µg/mL for MER and 15 µg/mL for SOL. This stock solution sample, which contained 100 µg/mL, was used for the research on forced degradation.

Table 2: SST Parameters and validation summary

Parameter- (units)	MER	SOL
Range of linearity (µg/mL)	150–250	15–25
Coefficient correlation	0.999	0.999
Limit of detection (µg/mL)	0.574	0.125
Limit of quantitation (µg/mL)	1.74	0.379
Percentage recovery	98.62–101.46	100.04–101.12
Precision (RSD)		
Inter-day	0.01–0.04	0.12–0.29
Intra-day	0.11–0.37	0.17–0.52
Robustness	Robust	Robust
Retention time ± %SD (min)	5.521 ± 0.015	9.161 ± 0.107
Theoretical plates	29091	7780
Tailing factor	1.073	0.923

This study aim to simulate and assess the stability and degradation patterns of the drugs under different stress conditions.

A total 0.3 mL of the MER sample base solution and SOL were obtained in separate round-bottom flasks to start the forced degradation process in alkaline conditions. Then, 5 mL of 0.1 N NaOH was added, and the mobile phase was injected to fill the remaining amount. The combination was left to rest at room temperature for an hour. 5.0 mL of 0.1 N HCl and 0.3 mL of the sample stock solution were brought into contact for forced degradation in an acidic environment. After the mobile phase was included to adjust the final amount, the mixture was let to stand at room temperature for a maximum of two hours. Five mL of 3% (w/v) hydrogen peroxide were mixed with 0.3 mL of the sample stock solution in the flask to initiate the hydrogen peroxide degradation process. This combination could only be used for an hour at most at room temperature. In order to assess photostability, 50 mg of the active pharmaceutical ingredients (API) for MER and SOL were subjected to direct sunlight for a whole day. All of the degraded sample solutions were diluted with the mobile phase in order to reach their final concentrations of 150 µg/mL for MER and 15 µg/mL for SOL in HPLC analysis. To evaluate the stability and drug degradation patterns, these investigations of drug degradation imitate various stressful environments.

RESULT AND DISCUSSION

The optimized mobile phase, which included 0.1% OPA (βH 4.2 with TEA) in a 25:75 (v/v) ratio with methanol at a flow rate of 0.7 mL/min, produced outstanding chromatographic performance. The arrangement produced two distinct, well-defined peaks for MER and SOL with low tailing factors (Figure 2). The design was discovered that the retention periods for SOL and MER were 9.161 and 5.521 minutes, respectively. Significant absorption at 231 nm was seen in the UV overlain spectra of both MER and SOL, leading to this wavelength's choice for detection. Calibration curves for MER and SOL correspondingly demonstrated linearity between the 50 to 250 µg/mL and 5 to 25 µg/mL concentration ranges. Table 1 summarizes the results of the calibration curve regression analysis. The amounts of MER and SOL in their combination tablet dosage form has been effectively determined through the recommended HPLC method. The combination's accomplished findings and the associated labeled amounts agreed well (Figure 3). This shows that the method employed to determine MER and SOL simultaneously in pharmaceutical formulations is accurate and applicable. Limit of quantitation (LoQ) of 1.74 and 0.379 µg/mL for MER and SOL, respectively, and limit of detection (LoD) of 0.574 and 0.125 µg/mL for SOL. Table 2 presents an overview of the outcomes of the validity and system compatibility test parameters. A comprehensive review of the robustness evaluation results for both medications is also included in Table 2, which shows a few minor variations in peak regions and retention durations. A discernible decrease in peak areas in the degradation research indicated drug degradation without the emergence of novel degradation peaks. To calculate

the percentage of deterioration, the areas of the peaks for both treatments under non-degradation conditions and the areas of the deteriorated peaks under each degradation condition were compared. The following protocols were applied: 3% v/v H₂O₂, 0.1 N NaOH for one hour, photodegradation for 30 minutes, and forced deterioration under 0.1 N HCl for two hours. Using the created HPLC method, it was discovered that under the stated conditions, the percent degradation for both SOL and MER in their tablet dosage form was between 10 and 20% (Figures 4-7). Table 3 presents a summary of the degradation study findings for both medications. For the simultaneous assessment of MER and SOL, a stability-indicating HPLC technique was created in this work and verified per ICH recommendations. Statistical analysis verified the procedure's accuracy, precision, and repeatability. Its defining features were the created method's simplicity, sensitivity, and selectivity for MER and SOL analysis in combination, free from excipient interference. Crucially, the approach precisely quantifies both medications when all degradants produced by forced degradation experiments are present. The combined dosage form's assay results, obtained through the suggested strategy, revealed 99.19 ± 0.454% of MER and 98.93 ± 0.757% of

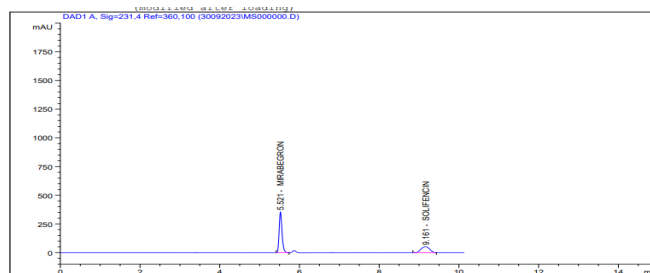


Figure 3: Chromatogram of MER and SOL combination

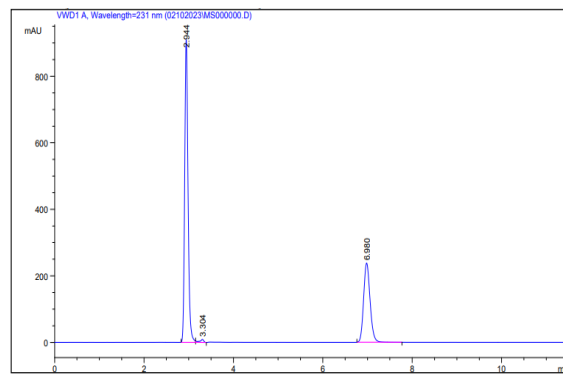


Figure 4: Force degradation acid

Table 3: Summary of research on tablet degradation for MER and SOL

State of forced degradation	Time (hours)	%Degradation	
		MER	SOL
Acid (0.1N HCL)	2	9.22	12.69
Alkali (0.1N NaOH)	1	5.31	7.19
Oxidation, (3% H ₂ O ₂)	1	14.34	14.30
Photolytic direct sunlight	24	0.2	0.1

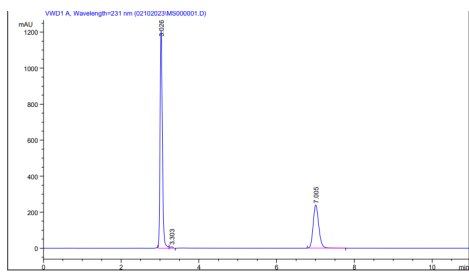


Figure 5: Force degradation alkali

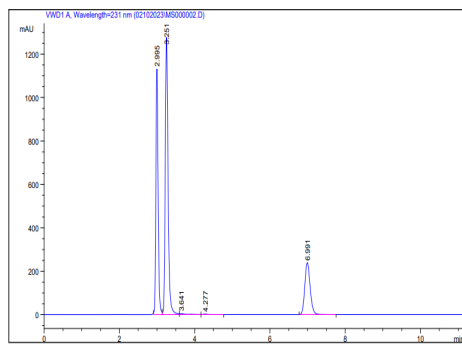


Figure 6: Force degradation by H₂O₂

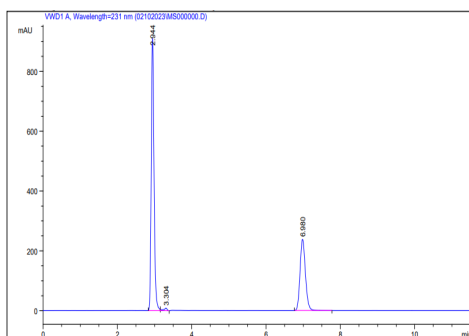


Figure 7: Photolytic force degradation by H₂O₂

SOL. The results demonstrate the technique's suitability for researching MER and SOL stability across a range of forced degradation scenarios, including oxidation, basic, acid, and photolytic degradation. Finally, the technique successfully extracts the medications from their degradation products, making it suitable for stability investigation of the drug's tablet dosage form. It is significant that this investigation did not involve the identification of degradation products.

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