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Analytical Method Development and Validation for The Simultaneous Estimation Of Mirabegron and Solifenacin In Bulk and Pharmaceutical Dosage Form by RP-HPLC

K. Keerthana, K.Vinutha*, P. Sridevi

*Department of Pharmaceutical Analysis , Sri Venkateshwara College of Pharmacy, Madhapur,
India*

ABSTRACT

A rapid stability-indicating RP-HPLC was developed and validated for the estimation of Mirabegron and Solifenacin combination in bulk and tablet dosage form using Thermo C18 column (250 x 4.6 mm, 5m) as a stationary phase and a mixture solution of 0.1 percent Diazanium sulphate buffer: Acetonitrile (60:40 v/v) as the mobile phase at a flow rate of 1 ml/min. A photodiode array detector was used for detection at 246 nm. The linearity, sensitivity, selectivity, robustness, specificity, precision, and accuracy were all determined. The peak area response-concentration curve was rectilinear over the concentration ranges of 25-75 g/ml (Mirabegron) and 2.5-7.5 g/ml (Solifenacin), with quantitation limits of 0.793 g/ml (Mirabegron) and 0.307 g/ml (Solifenacin). The proposed method was validated for the simultaneous determination of mifepristone and misoprostol in combined tablet dosage form. In comparison to previously reported RP-HPLC methods, the performance of the proposed method was found to be rapid and cost-effective. The developed and validated stability-indicating RP-HPLC method was suitable for quality control and drug analysis.

Keywords: RP-HPLC, stability-indicating, tablet dosage form.

*Corresponding Author Email: vinutha08.ch@gmail.com

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INTRODUCTION

Overactive bladder syndrome (OAB) is a chronic medical condition that significantly impairs quality of life. Mirabegron, a novel orally active beta-3 agonist, has received clinical approval for the treatment of overactive bladder. It is also utilized in the management of neurogenic detrusor over activity (NDO), a bladder disorder resulting from neurological disability¹. Activation of the beta-3 receptor induces relaxation of the detrusor smooth muscle during the storage phase of the urinary bladder fill-void cycle, thereby increasing bladder storage capacity and reducing sensations of urgency and frequency². Solifenacin is a novel once-daily competitive M3 receptor antagonist that demonstrates both efficacy and tolerability in patients with overactive bladder^{3, 4}. The combination of a Beta-3 adreno receptor agonist and muscarinic receptor antagonist exhibits a higher likelihood of successful treatment of OAB symptoms compared to monotherapy^{5,6}. Multiple clinical trials have demonstrated that fixed dose combinations of solifenacin and mirabegron are highly efficacious in treating patients with overactive bladder disorder (OAB)⁷. Mirago S®, a novel combination therapy comprising Solifenacin and Mirabegron, received approval for the treatment of OAB in 2018.

Several HPLC, HPTLC, and UV methods have been reported in the literature for the determination of Solifenacin¹⁰⁻¹⁷ and Mirabegron¹⁸⁻²⁴ alone or in combination with other drugs in bulk, as well as in pharmaceutical formulations. To date, no HPLC method for determining Solifenacin and Mirabegron in pharmaceutical dosage form has been reported. The current study describes a simple, selective, and sensitive method for simultaneous quantitation of Solifenacin and Mirabegron using greener solvents and photo diode array detection.

MATERIALS AND METHOD

Materials:

Acetonitrile, HPLC grade (Lichroso IR, Merck Life sciences Pvt. Ltd., Mumbai, India), HPLC grade water (Thermo Fischer Scientific Pvt Ltd., Mumbai, India), and triethylamine (S D Fine – Chem. Ltd., Mumbai, India) were utilized in the study. Glenmark and Cipla Pharmaceuticals Ltd., India, provided the working standards for mirabegron and solifenacin succinate. Mirago S® tablet containing 50mg of mirabegron and 5mg of solifenacin succinate was obtained from the local market.

Instrumentation:

Chromatographic separation was accomplished utilizing a Waters Alliance HPLC system equipped with an auto sampler and PDA detector. Empower 2 software was employed for processing the eluted components. For thermal degradation, a hot air oven was utilized, and for photolytic

degradation, a UV crosslinker with a series of 23400 model UV chambers equipped with a UV fluorescence lamp with a wavelength range of 200 – 300nm was selected. The study employed an ultrasonic bath (Unichrome), a digital pH meter (Eutech), and a UV/VIS spectrophotometer (Lab India UV 3000). Operating conditions of HPLC

Analytes were separated using a Thermo C18 column (250 x 4.6 mm, 5m) at ambient temperature. With a flow rate of 1 mL/min and injection volume of 10 µL, the samples were eluted using phosphate buffer: acetonitrile (60:40 v/v) as the mobile phase. The mobile phase and samples underwent ultrasonic degassing for 20 minutes prior to filtration through 0.45 µm Nylon (N66) 47mm membrane filter. The eluted compounds were detected at 246 nm, and all determinations were conducted at ambient temperature. column temperature (25°C). The chromatograms of mirabegron and solifenacin standard stock solutions were recorded under optimized chromatographic conditions.

Solutions Preparation:

Preparation of 0.1% Diammonium sulfate buffer: 13.214g (NH₄)₂SO₄ was weighed into a 1000ml beaker and dissolved in water that had been filtered through a 0.45-micron membrane filter and sonicated for 10 minutes.

Preparation of mobile phase: 600 ml (60%) of Diammonium sulfate buffer and 400 ml of Acetonitrile (40%) were combined and degassed in an ultrasonic water bath for 15 minutes, then filtered through a 0.45 µ filter under vacuum filtration.

Preparation of Standard Solutions

A primary stock solution was prepared by dissolving 50mg of mirabegron reference standard and 5mg of solifenacin reference standard in diluent to achieve concentrations of 50 µg/mL and 5 µg/mL of mirabegron and solifenacin, respectively.

Preparation of sample solution

Ten tablets were finely pulverized using a mortar and pestle. A quantity equivalent to 50mg of mirabegron and 5mg of solifenacin was weighed and transferred to a 100mL volumetric flask. 140mL of diluent was added, and the mixture was sonicated for 30 minutes with intermittent agitation to disperse the content before diluting to volume with diluent to yield a solution containing 50 µg/mL mirabegron and 5 µg/mL solifenacin. This solution was filtered through a PVDF syringe filter with a pore size of 0.45 µm.

Validation of Method Developed:

In accordance with ICH guidelines, the proposed method was validated for system suitability, specificity, recovery, precision, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ).

System suitability test

The chromatographic conditions were utilized to optimize the HPLC system. In the chromatographic system, 10 μ l of drug standard solutions were injected in triplicate. The parameters retention time, theoretical plates, and tailing factor were calculated to determine the system's suitability for the proposed method.

Specificity

The method's specificity was evaluated to assess potential interference from impurities in the retention time of analyte peaks. The specificity was examined by injecting blank, placebo, and standard drug solutions.

Linearity

Mirabegron and solifenacin standard stock solutions were diluted with mobile phase to yield a series of solutions containing 25, 37.50, 50, 62.5, and 75 μ g/mL of mirabegron and 2.5, 3.75, 5, 6.25, and 7.5 μ g/mL of solifenacin, respectively. The linearity was determined by calculating a regression line from a plot of the drug's peak area ratio and IS versus concentration. The method was evaluated using the ICH guidelines for determining the correlation coefficient and intercept values.

Precision

Precision is defined as the degree of agreement among a series of measurements obtained from multiple samplings of the same homogeneous sample. Six replicate injections of mirabegron (50 μ g/mL) and solifenacin (5 μ g/mL) were analyzed on the same day by injecting them into an HPLC column. The intermediate precision was determined by injecting samples prepared at the same concentrations on three different days by different operators. The peak area ratios of all injections were measured, and the standard deviation, percent relative standard deviation (RSD), was calculated.

Accuracy

Accuracy was assessed utilizing the standard addition method at three levels: 50, 100, and 150%. A known quantity of the standard drug was added to the blank sample at each level. The mean recovery of mirabegron and solifenacin was calculated.

Limit of Detection and Limit of Quantification

The calibration curve method was employed to determine the limit of detection (LOD) and limit of quantification (LOQ) of mirabegron and solifenacin. Mirabegron and solifenacin solutions were prepared in the linearity range and injected in triplicate. The concentration was plotted against the average peak area of three analytes. LOD and LOQ were calculated using the following equations:

$$\text{LOD} = 3.3 \sigma / S$$

$$\text{LOQ} = 10 \sigma / S$$

Where σ = the standard deviation of the blank measurements

S = the slope of the calibration curve

To assess the robustness of the analytical method, the HPLC conditions were slightly modified. The flow rate, column temperature, and mobile phase composition were altered (31).

To assess the robustness of the analytical method, the HPLC conditions were slightly modified. The flow rate, column temperature, and acetonitrile proportion in the mobile phase were modified.

Forced Degradation Study

Alkaline, acidic, oxidative stress, thermal, aqueous, and direct exposure to UV were conducted ³².

Alkali Hydrolysis:

To 10 ml of mirabegron and solifenacin stock solution, 4 ml of 1N sodium hydroxide was added, and the mixture was refluxed at 60°C for 30 minutes. The solution was cooled to room temperature, neutralized with 1N HCl, and then diluted to the target concentration with deionized water.

Acid Hydrolysis:

To 10 ml of stock solution of mirabegron and solifenacin, 4 ml of 1M hydrochloric acid was added, followed by 30 minutes of refluxing at 60°C. After cooling to room temperature, the solution was neutralized with 1N NaOH before being diluted to the target concentration with deionized water.

Oxidative Stress:

To 10 ml of stock solution of mirabegron and solifenacin, 1 ml of 20% hydrogen peroxide (H₂O₂) was added, and the solutions were maintained at 60°C for 30 minutes. The solution was cooled to room temperature before being diluted with deionized water to the desired concentration.

Thermal Degradation:

To study dry heat degradation, 10 ml of standard stock solution of drugs was transferred to a 100 ml volumetric flask and placed in an oven at 80°C for 6 hours. The solution was then cooled and diluted to the mark with deionized water to reach target concentration.

Hydrolytic Degradation:

10ml of standard stock solution of drugs was transferred to a 100ml volumetric flask. 10ml of deionized water was added, and the solution was heated in a water bath for 1 hour. Subsequently, the solution was cooled and diluted to the target concentration with deionized water.

RESULTS AND DISCUSSION

The developed HPLC method involves the separation of mirabegron and solifenacin on a Thermo C18 column (250 x 4.6 mm, 5 μ m) at ambient column temperature. The optimized mobile phase consists of 0.1% Diazanium sulphate buffer: Acetonitrile (60:40 v/v) with a flow rate of 1ml/min and UV detection at 246nm. The retention time was 3.05min for mirabegron and 4.21 min for solifenacin.

Results and Discussion:

The proposed method was validated according to the ICH guidelines for system suitability, specificity, recovery, precision, linearity, robustness, limit of detection (LOD) and limit of quantification (LOQ) (33).

System Suitability

Theoretical plate numbers exceeded 2000, and the percent relative standard deviation (RSD) of six standard injection areas was less than 2. These values under optimal conditions, the retention times for mirabegron and solifenacin were 3.05 minutes and 4.21 minutes, respectively. For both compounds, peak symmetries were 1.5, within the range permitted by International Conference on Harmonization (ICH) guidelines. The results are presented in Table 1.

Table 1: System suitability results

| Parameter | Mirabegron | Solifenacin |
|--------------------|------------|-------------|
| Peak area | 4348892 | 1237980 |
| Theoretical plates | 9139 | 6660 |
| Retention time | 3.05min | 4.21 min |
| Tailing factor | 1.16 | 1.15 |

Specificity:

To evaluate the method's specificity, interference from excipients in the placebo solution-formulated pharmaceutical dosage form was assessed. Figure 1 illustrates an optimized chromatogram of mirabegron and solifenacin. The chromatogram demonstrates the method's capacity to determine the concentration of the analyte in the presence of other components. excipients.

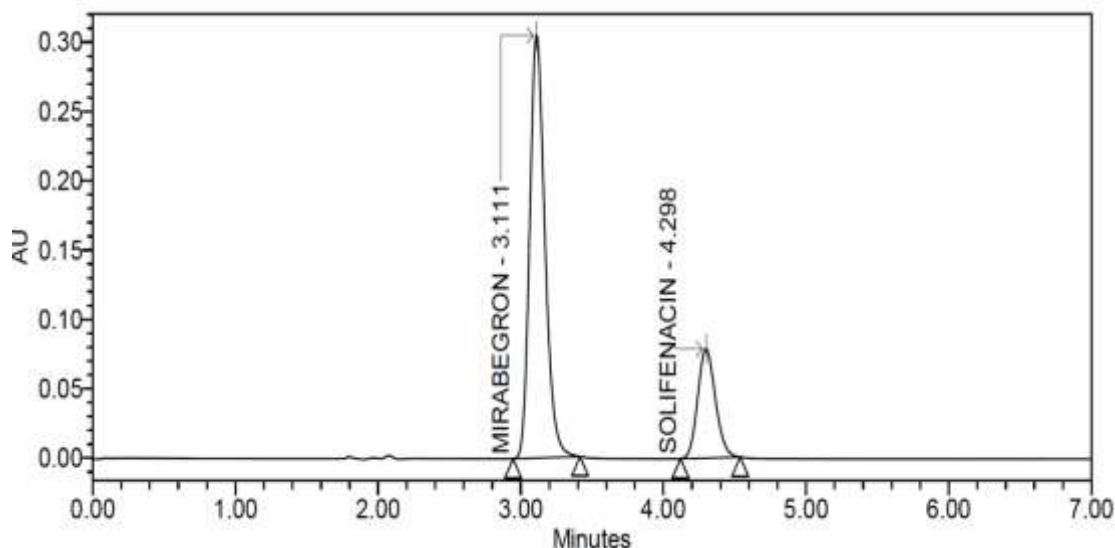


Figure 1: Optimized chromatogram of Mirabegron and Solifenacin

Linearity and Range:

The linearity was evaluated at concentration ranges of 25-75 µg/ml for mirabegron and 2.5-7.5 µg/ml for solifenacin. Table 2 presents the concentration of drugs and the corresponding area for the construction of calibration curves. Figures 3 and 4 illustrate the relationships between concentrations and peak area ratios. In each instance, a strong linear relationship was observed between concentration and peak area. The relationship is characterized by the linear equations $y = 43633x - 18350$ for mirabegron and $y = 12255x + 6446, 4$ for solifenacin, where X represents the drug concentration and Y the peak area. In all cases, the regression coefficient (R²) was 0.999. The R² value was in accordance with ICH recommendations.

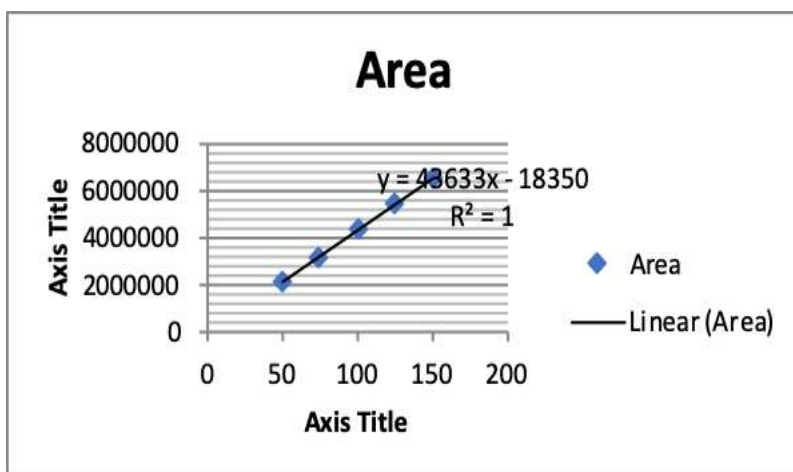


Figure 3: Linearity graph of Mirabegron

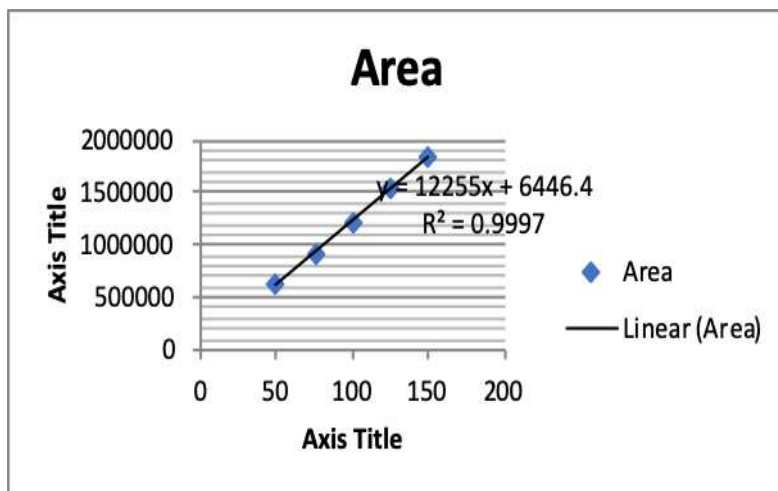


Figure 4: Linearity graph of Solifenacin

Table 2 : Linearity data

| Conc. of Mirabegron (µg/ml) | Peak area | Conc. of Solifenacin(µg/ml) | Peak area |
|-----------------------------|-----------|-----------------------------|-----------|
| 25 | 2164011 | 2.5 | 621628 |
| 37.50 | 3254872 | 3.75 | 931849 |
| 50.00 | 4340736 | 5 | 1220976 |
| 62.5 | 5438849 | 6.25 | 1532021 |
| 75 | 6526103 | 7.50 | 1853470 |

System Precision

A single dilution containing 50 ppm of Mirabegron and 5 ppm of Solifenacin was injected into the HPLC system in six replicates. The resultant data were within the acceptance limits (RSD<2), as presented in Table 3.

Table 3: System Precision data

| S. No | Mirabegron | | Solifenacin | |
|-------|-------------|-----------|-------------|-----------|
| | Conc. (ppm) | Peak area | Conc.(ppm) | Peak area |
| 1 | 50 | 4345274 | 5 | 1225044 |
| 2 | 50 | 4340448 | 5 | 1222257 |
| 3 | 50 | 4358624 | 5 | 1212768 |
| 4 | 50 | 4349004 | 5 | 1232716 |
| 5 | 50 | 4354277 | 5 | 1229487 |
| 6 | 50 | 4345242 | 5 | 1212820 |
| Avg | | 4348812 | | 1222515 |
| SD | | 6654.3 | | 8344.2 |
| %RSD | | 0.2 | | 0.7 |

Method Precision (Repeatability):

Six injections of a sample preparation with a known concentration of 50 ppm Mirabegron and 5 ppm Solifenacin were analyzed on the same day by introducing them into an HPLC column. The

calculated percent relative standard deviation (RSD) was determined to be within the acceptable range. The results of precision are presented in Table 4.

Table 4: Method Precision data

| S.No | Mirabegron | | Solifenacin | |
|------|------------|-----------|-------------|-----------|
| | Conc(ppm) | Peak area | Conc(ppm) | Peak area |
| 1 | 50 | 4345184 | 5 | 1225820 |
| 2 | 50 | 4340183 | 5 | 1222392 |
| 3 | 50 | 4358183 | 5 | 1212601 |
| 4 | 50 | 4349185 | 5 | 1232204 |
| 5 | 50 | 4354803 | 5 | 1229307 |
| 6 | 50 | 4345720 | 5 | 1212290 |
| Avg | | 4348876 | | 1222436 |
| SD | | 6648.4 | | 8411.3 |
| %RSD | | 0.2 | | 0.7 |

Accuracy:

A predetermined quantity of the standard drug was introduced to the blank sample at each concentration level. Satisfactory recovery of the spiked drugs was obtained at each added concentration, and the mean percentage recovery of mirabegron and solifenacin was determined to be between 100-101% and 100-102%, respectively. The results are presented in Tables 5 and 6.

Recovery data of Mirabegron

| Sample name | Amount added($\mu\text{g/ml}$) | Amount found($\mu\text{g/ml}$) | % Recovery |
|-------------|----------------------------------|----------------------------------|------------|
| S1:50% | 24.750 | 24.84 | 100 |
| S1:50% | 24.750 | 24.81 | 100 |
| S1:50% | 24.750 | 24.73 | 100 |
| S1:100% | 49.500 | 49.70 | 100 |
| S1:100% | 49.500 | 49.81 | 101 |
| S1:100% | 49.500 | 49.65 | 100 |
| S1:150% | 74.250 | 74.57 | 100 |
| S1:150% | 74.250 | 74.51 | 100 |
| S1:150% | 74.250 | 74.66 | 101 |

Table 6: Recovery data of Solifenacin

| Sample name | Amount added($\mu\text{g/ml}$) | Amount found($\mu\text{g/ml}$) | % Recovery |
|-------------|----------------------------------|----------------------------------|------------|
| S1:50% | 2.450 | 2.49 | 102 |
| S1:50% | 2.450 | 2.48 | 101 |
| S1:50% | 2.450 | 2.48 | 101 |
| S1:100% | 4.900 | 4.95 | 101 |
| S1:100% | 4.900 | 4.89 | 100 |
| S1:100% | 4.900 | 4.89 | 100 |
| S1:150% | 7.350 | 7.41 | 101 |
| S1:150% | 7.350 | 7.45 | 101 |
| S1:150% | 7.350 | 7.41 | 101 |

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The limits of detection and quantification were determined through serial dilutions of analyte stock solution to achieve signal-to-noise ratios of 3:1 for LOD and 10:1 for LOQ, respectively. The LOD values for mirabegron and solifenacin were determined to be 0.238 µg/mL and 0.0092 µg/mL, and the LOQ values were calculated to be 0.793 µg/mL and 0.307 µg/mL, respectively.

Robustness:

Robustness evaluation was conducted by varying flow rate, column temperature, and acetonitrile proportion in the mobile phase. The results were found to be within the range of 98.4-101.23% for both drugs.

Forced degradation studies: The development of a stability-indicating method necessitates an investigation of forced degradation to demonstrate specificity. A stability-indicating method is defined as one that accurately quantifies the active ingredient without interference from degradation products, excipients, and other potential impurities. Forced degradation studies were conducted under various conditions: acid hydrolysis (1M HCl heated at 60°C for 30 minutes), alkali hydrolysis (1 N NaOH heated at 60°C for 30 minutes), oxidative degradation (20% H₂O₂ heated at 60°C for 30 minutes), and thermal degradation (samples placed in an oven at 80°C for 6 hours). For hydrolytic degradation, samples were subjected to a hot water bath for one hour. Results are presented in Tables 7, 8.

Table 7: Forced Degradation studies of Mirabegron

| Sample Name | Recovery (%) | Degradation (%) | Purity Angle | Purity Threshold |
|-----------------------|--------------|-----------------|--------------|------------------|
| Water Degradation | 89.65 | 10.35 | 0.211 | 0.653 |
| Acid Degradation | 92.09 | 7.91 | 0.259 | 0.855 |
| Alkali Degradation | 93.56 | 6.44 | 0.290 | 0.753 |
| Peroxide Degradation | 90.71 | 9.29 | 0.341 | 0.645 |
| Thermal Stress Sample | 95.08 | 4.92 | 0.288 | 0.607 |
| Photo Stress Sample | 99.11 | 0.89 | 0.341 | 0.645 |

Table 8: Forced Degradation studies of Solifenacin

| Sample Name | Recovery (%) | Degradation (%) | Purity Angle | Purity Threshold |
|-----------------------|--------------|-----------------|--------------|------------------|
| Water Degradation | 90.10 | 9.9 | 0.310 | 0.584 |
| Acid Degradation | 94.84 | 5.16 | 0.323 | 0.785 |
| Alkali Degradation | 89.28 | 10.72 | 0.460 | 0.685 |
| Peroxide Degradation | 92.25 | 7.75 | 0.294 | 0.785 |
| Thermal Stress Sample | 98.45 | 1.55 | 0.307 | 0.808 |
| Photo Stress Sample | 91.76 | 8.24 | 0.294 | 0.785 |

CONCLUSION

In accordance with ICH guidelines, a reversed-phase high-performance liquid chromatography

(RP-HPLC) method for the simultaneous quantification of mirabegron and solifenacin in tablet dosage form was developed and validated. Linearity was established in the ranges of 25-75 µg/ml for mirabegron and 2.5-7.5 µg/ml for solifenacin, with correlation coefficients ($r^2 = 0.998$). The recovery percentages for mirabegron and solifenacin were between 100 and 102 percent, meeting the acceptance criteria. The relative standard deviation (RSD) percentage was not more than (NMT) 2%, demonstrating the accuracy of the developed method. The method developed is characterized as simple, sensitive, rapid, linear, rugged, precise, robust, and specific.

Disclosure Statement

The authors declare no conflicts of interest. The authors are solely responsible for the content and writing of this article.

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