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Diaryl pyrrolone fluorescent probing strategy for Mirabegron determination through condensation with ninhydrin and phenylacetaldehyde: Application to dosage forms, human urine and plasma

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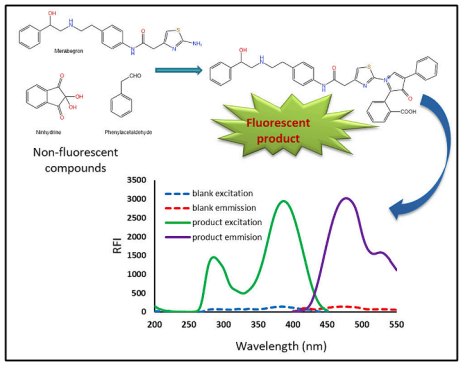
HIGHLIGHTS

- A new spectrofluorimetric method was designed to evaluate mirabegron.
- The method involved condensing the drug with ninhydrin and phenylacetaldehyde.
- The approach was used to evaluate the drug in combined pharmaceutical tablets.
- Additionally, the method was applied well for human plasma and urine analysis.
- The approach provides improved simplicity, greenness, and wide applicability.

GRAPHICAL ABSTRACT



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ABSTRACT

Mirabegron (MRB) is a β_3-adrenoceptor agonist used for managing overactive bladder syndrome. A cost-effective, environmentally friendly, and highly sensitive spectrofluorimetric method was suggested to serve the purpose of quantifying MRB in its pure state, pharmaceutical tablets, spiked human plasma and urine, and testing content uniformity. In the present study, ninhydrin and phenylacetaldehyde react with the amino group moiety of MRB in Teorell-Stenhagen buffer (pH 7.5) to generate a strongly fluorescent diaryl pyrrolone compound that emits fluorescence at a wavelength of 477 nm upon excitation at 385 nm. The obtained calibration curve showed a linear relationship with a high correlation coefficient ($r = 0.9997$) in the concentration range of 0.25 to 5.0 μg mL^{-1}. Limits of detection (LOD) and quantitation (LOQ) were 0.082 and 0.248 μg mL^{-1} respectively. The procedure was verified in accordance with the ICH guidelines. The suggested approach could be utilized for the selective analysis of MRB in its pharmaceuticals, either containing a single drug or co-formulated with solifenacin succinate. The greenness of the suggested method was confirmed using different green analytical metrics.

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1. Introduction

Overactive bladder syndrome is a medical problem characterized by urgency, frequent urination, and urine incontinence. Mirabegron (MRB) had been approved by the FDA in 2012 for managing overactive bladder syndrome [1]. It is used either alone or in combination with solifenacin Succinate. MRB causes the relaxation of the smooth muscles in the detrusor during the urinary bladder's storage phase hence enhancing bladder capacity [2]. It may have further uses, such as the treatment of heart failure or metabolic diseases [3]. The adverse effect profile of MRB is better than that of older antimuscarinic drugs, even though its effectiveness is equivalent [4]. The chemical nomenclature for MRB is 2-(2-amino-1,3-thiazol-4-yl)-*N* −[4-[2-[[(2*R*)-2-hydroxy-2-phenylethyl] amino]ethyl]phenyl]acetamide (Fig. 1).

From the literature, it was found that numerous analytical techniques have been documented for the evaluation of MRB. These methods include; spectrophotometric [5–8], spectrofluorimetric [9,10], TLC [11,12], HPLC-UV [13–15], UPLC-MS [16–18], and electrochemical methods [19]. Although the chromatographic techniques possess the benefit of exhibiting remarkable selectivity and can be applied for detecting the degradation products and studying the pharmacokinetics of drugs, they had certain limitations. HPLC techniques are time-consuming, require costly sophisticated instruments as well as large amounts of extremely pure organic solvents which are expensive and have high risk for environmental pollution. In addition, some HPLC methods involved the use of the costly MS detector [16–18].

Spectrofluorimetry is a simple and highly sensitive analytical technique which could be regarded as environmentally safe technique if the reagent and solvent are carefully chosen [20]. However, only two spectrofluorimetic methods were reported for MRB determination. The first one is indirect involving multiple components reagents [10] while the other utilized more hazardous reagent [9]. Therefor the attention in the present study was drawn to establish a spectrofluorimetric method that could overcome these limitations.

The aim of the present research was to design an environmentally friendly, precise, rapid, and cost-effective method for assessing pharmaceutical formulations that include MRB, either individually or in combination with solifenacin succinate. To achieve this, the study involved the reaction of MRB's primary amino group with ninhydrin and phenylacetaldehyde in a buffered system, resulting in the production of a strongly fluorescent substance with a concentration-dependent relationship to the drug. Therefore, a new spectrofluorometric method was developed using this reaction to determine MRB. The approach could selectively determine MRB in tablet formulations without any interference from their excipients and the solifenacin. Moreover, the components of human plasma and urine did not affected the results of MRB analysis. Furthermore, owing to its simple procedure, the method was effectively employed for content uniformity testing. The procedure was verified in accordance with the standards established by ICH guidelines [21].

2. Experimental

2.1. Instrumentation

Measurement of the fluorescence was conducted with a JASCO FP-8350 Spectrofluorometer (Hachioji, Tokyo, Japan). The fluorimeter was equipped with 150 W Xe-arc lamp. The photomultiplier tube (PMT) was set to 400 v. For the excitation and emission monochromators, the slit width was 5 nm, and the speed of scanning was set at a rate of 1000 nm per minute. A water purification system was used to produce double-distilled wate using Aquatron Water Still a4000d (Cole-Parmer in Staffordshire, UK). Jenway 3510 pH meter was used to adjust pH (Staffordshire, UK) and Mettler toledo 5-digit balance was used (Greifensee, Switzerland).

2.2. Materials and reagents

Amoun Pharmaceuticals (El-Obour City, Egypt) graciously provided the pure powder of MRB, which had a purity of 99.5 %. Eva Pharma (Giza, Egypt) graciously provided the standard for solifenacin succinate, which had a purity of 99.8 %. Flowadjust® tablets, manufactured by Amoun Pharmaceuticals (El-Obour City, Egypt), was labeled to have 25 mg of MRB per tablet, and Bladogra®, manufactured by Apex Company (Cairo, Egypt), labeled to contain 50 mg of MRB were purchased from a local pharmacy.

Spectroscopic grade methanol, acetonitrile, citric acid, ninhydrin and phenylacetaldehyde were provided by Merck (Darmstadt, Germany), while analytical grade sodium hydroxide and dimethyl formamide (DMF) were purchased from Fischer Scientific (Loughborough, U. K). Additionally, analytical grade acetone, ethanol, phosphoric acid, and hydrochloric acid were obtained from El Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt).

Ninhydrin (0.1 % w/v) was made as a fresh preparation daily using double-distilled water. Phenylacetaldehyde (0.02 % v/v) was prepared weekly in ethanol. Citric acid, sodium hydroxide, hydrochloric acid, and phosphoric acid were used as aqueous solutions to make the Teorell-Stenhagen buffer solution pH (5–9) [22].

2.3. Preparation of standard solution

For the preparation of the initial stock standard solution (500 μg mL^{-1}), a quantity of 50 mg of MRB was dissolved in 20 mL of methanol and further diluted to a total volume of 100 mL using double-distilled water. To get the working standard solutions, the primary stock standard solutions were diluted with double-distilled water to the required

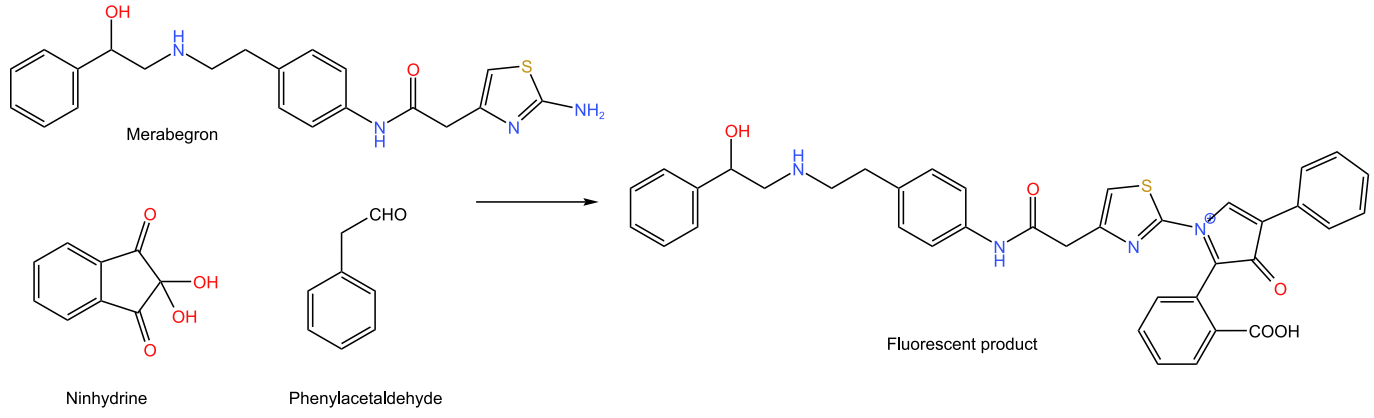

Fig. 1. Suggested reaction mechanism between MRB, ninhydrin, and phenylacetaldehyde.

concentrations.

2.4. Procedures for general assay

In a series of 15-milliliters test tubes, 1 mL of working standard MRB solutions in the range of 2.5–50 μg mL^{-1}, 1.2 mL Teorell-Stenhagen buffer (pH = 7.5), 2 mL of ninhydrin and 0.8 mL of phenylacetaldehyde were transferred and well-mixed. The test tubes underwent heating in a water bath at 87 °C for a duration of 16 min. Subsequently, they were allowed to gradually cool in an ice bath. The tubes contents were subsequently moved to a volumetric flask with a capacity of 10 ml and diluted with ethanol to the appropriate volume. The fluorescence intensity was measured at 477 nm (ex, 385 nm). The same procedure was used to prepare the blank experiment excluding the addition of MRB solution.

2.5. Procedure for tablet formulation

The contents of 20 MRB tablets (Flowadjust® tablets 25 mg/ tablet or Bladogra® 50 mg/ tablet) were weighed and finely ground. A quantity of the resulting powder, equivalent to 300 mg of MRB, was then transferred into a 100-mL volumetric flask and mixed well with 30 mL of methanol. Subsequently, the flask's contents underwent a 30-minutes sonication, after which the volume was adjusted to 100 mL using double-distilled water. The resulting solution underwent filtration, and the initial part of the filtrate was discarded. Following this, 1.0 mL of the filtered solution was quantitatively moved to a 100 mL volumetric flask, and its volume was adjusted to 100 mL using double-distilled water. The general assay procedure was applied using 1.0 mL of the final solution as mentioned earlier in five replicates.

2.6. Procedure for testing content uniformity

Content uniformity testing for MRB tablets was carried out in accordance with the standards specified in the USP guidelines [23]. An individual assay for each of ten Flowadjust® 25 mg tablets and ten Bladogra® 50 mg tablets was performed employing the aforementioned extraction and general assay procedures under "Procedure for tablet formulation".

2.7. Procedure for estimating MRB in biological human samples

2.7.1. Procedure for estimating MRB in spiked human plasma

Blood samples were drawn from the forearm vein of a healthy volunteer at Sohag University Hospital and were placed in a series of heparin-treated tubes. The participant received written consent to utilize the samples collected from them after being informed on the experiment's objectives. The procedure for gathering plasma from individual of good health was in accordance with the guidelines outlined in the Declaration of Helsinki [24]. A 5 ml portion of blood underwent centrifugation at 4000 revolutions per minute (rpm) for a duration of 30 min to separate the plasma component. The separated plasma was then gathered and preserved in Eppendorf tubes at a temperature of −20 °C. Subsequently, a 1.0 mL aliquot of the stored plasma was combined in a clean tube with 1.0 mL of MRB standard solution (25.0–500.0 μg mL^{-1}) then 8.0 mL methanol was added. The tube underwent mixing by vortex for a duration of 60 s before centrifugation at 4000 rpm for 10 min. One milliliter of the transparent solution was transferred to a clean tube, and the general assay procedure was performed. A blank was prepared in a similar manner without adding the drug to the plasma.

2.7.2. Procedure for estimating MRB in spiked human urine

A portion of 1.0 mL of urine obtained from 28 years old healthy volunteer, was combined with an equal volume of MRB standard solution (25.0–500.0 μg mL^{-1}) and 8.0 mL methanol was added to the tube content. The tube underwent mixing by vortex for a duration of 60 s before centrifugation at 4000 rpm for 10 min. One milliliter of the transparent solution was relocated to a clean tube, and the general assay procedure was implemented. A blank was prepared in a similar manner without adding the drug solution.

3. Results and discussion

It is widely recognized that primary amines and amino acids have the ability to participate in condensation reactions when reacted with ninhydrin and phenylacetaldehyde. This interaction results in the formation of a significantly fluorescent diaryl pyrrolone product. Many drugs containing primary amine groups have previously been quantified with spectrofluorimetric technique using this derivatization protocol [25–29].

MRB possesses a primary amine group capable of readily undergoing condensation reactions with ninhydrin and phenylacetaldehyde that serve as the fluorogenic reagent. Following reaction condition optimization, the produced fluorescence intensities were monitored at 477 nm after being excited at 385 nm (Fig. 2). This interaction was designed to tailor a novel spectrofluorimetric method for MRB determination. The suggested approach stands out as an exceptionally sensitive, straightforward, consistently replicable, and user-friendly analytical method, making it a preferred choice for the assaying of MRB in tablets, content uniformity testing, spiked human urine, and plasma.

3.1. Optimization of the experimental conditions

Various experimental variables affecting the effectiveness of the proposed approach were explored and adjusted. These parameters include; buffer pH, buffer volume, concentrations of ninhydrin and phenylacetaldehyde reagents, reaction temperature, reaction time, and diluting solvent.

3.1.1. Influence of buffer pH and buffer volume

The impact of varying pH levels on the fluorescence intensity of the resultant reaction product was investigated through the utilization of Teorell-Stenhagen buffer solutions with a pH ranging from 5.0 to 9.0 (Fig. 3). It was observed that alterations in pH significantly influenced the fluorescence intensity of the reaction product, with the highest level of fluorescence occurring at approximately pH 7.5 ± 0.2. Lower or higher pH apart from this rang significantly reduce the fluorescence. At lower pH, the $–NH_2$ group may be protonated and that would decrease the fluorescence efficiency. While, at higher pH (alkaline condition), –COOH group will be ionized and that would also affect the fluorescence efficiency.

In addition, the low fluorescence of the formed product at lower and

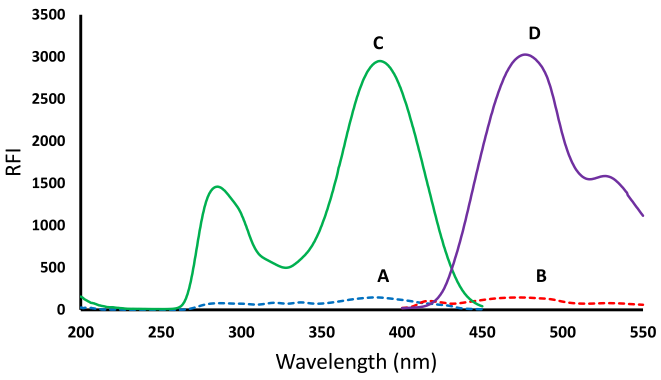

Fig. 2. Fluorescence spectra: (A) and (B) are the excitation and emission spectra of the reagent blank containing ninhydrin (0.1 % w/v) and phenylacetaldehyde (0.02 % v/v), while (C) and (D) are excitation and emission spectra of the reaction product of MRB (3.0 μg mL^{-1}). In all cases, Teorell-Stenhagen buffer (pH 7.5) was used.

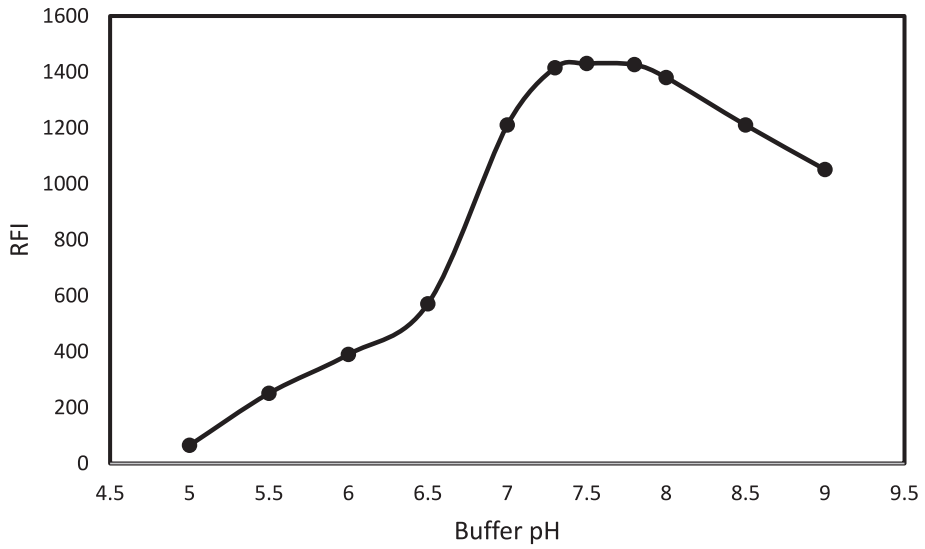

Fig. 3. Effect of the pH of Teorell-Stenhagen buffer on the relative fluorescence intensity of the formed condensation product between MRB (2.0 μg mL^{-1}), ninhydrin (0.1 % w/v) and phenylacetaldehyde (0.02 % v/v).

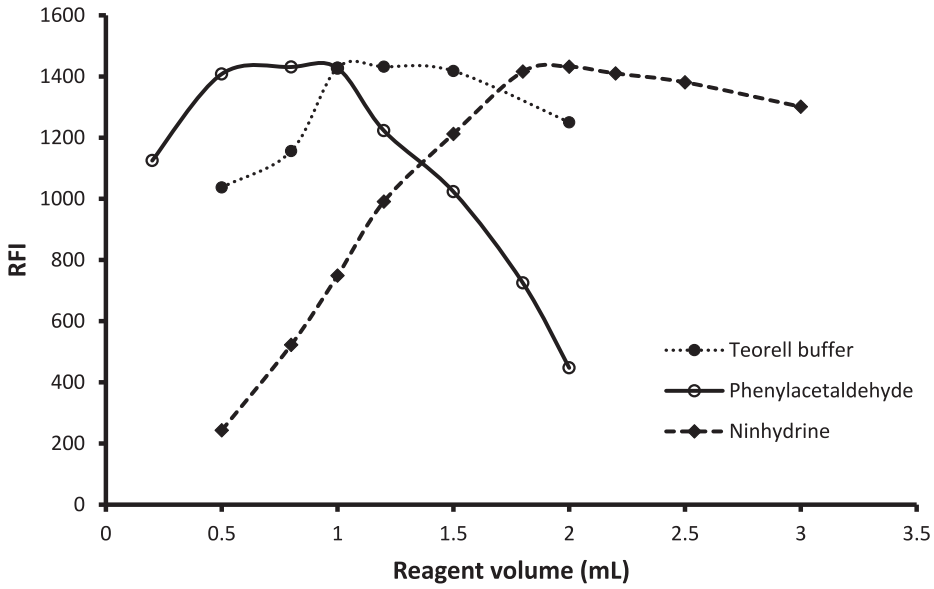

Fig. 4. Effect of the reagent volumes (ninhydrin 0.1 % w/v (–◆-) and phenylacetaldehyde 0.02 % v/v (–○-) and Teorell-Stenhagen buffer (–●-) on the relative fluorescence intensity of their condensation product with MRB (2.0 μg mL^{-1}).

higher pH values maybe linked to changes that occur in the structure of the formed product (Fig. 5). In strongly alkaline medium, 2-hydroxy-pyrrolinone derivative would be formed upon nucleophilic attack of the electron-rich group, hydroxide ions, on the electron-deficient carbon atom at position 2 of the product. Meanwhile, in highly acidic medium, the product is converted into lactone form. Both 2-hydroxy-pyrrolinone derivative and lactone form are non-planar and have less conjugated system. Thus, these forms have low fluorescence intensity [27]. On the other hand, the diaryl pyrrolone derivative has planner structure and contains more conjugated structure.

Furthermore, the influence of different volumes of the buffer solution (pH 7.5) was investigated within the range of 0.5 to 2.0 mL, as depicted in Fig. 4. The highest fluorescence intensity was recorded at approximately 1.2 ± 0.2 mL. As a result, 1.2 mL of Teorell-Stenhagen buffer solutions (pH 7.5) was adopted for all subsequent experiments.

3.1.2. *Influence of phenylacetaldehyde and ninhydrin volumes*

The impact of different of ninhydrin (0.1 % w/v) volumes (0.5–3.0 mL) on the intensity of fluorescence was investigated as seen in Fig. 4. A maximal fluorescence intensity was obtained when using ninhydrin volumes in the range of 1.8–2.5 mL. As a result, 2 mL of ninhydrin was employed for the subsequent procedures.

The impact of different volumes of phenylacetaldehyde (0.02 % v/v) within the range of 0.2 to 2.0 mL was investigated as presented in Fig. 4 to assess its influence on the emission intensity of the resulting solution. The highest emission signal was achieved when employing phenylacetaldehyde volumes between 0.6 to 1.0 mL. Consequently, a volume of 0.8 mL of phenylacetaldehyde was selected for subsequentexperiments.

3.1.3. *Influence of reaction temperature and time*

The influence of both reaction temperature and duration on the fluorescence intensity was examined by conducting the reaction at different temperature and time durations. The highest fluorescence

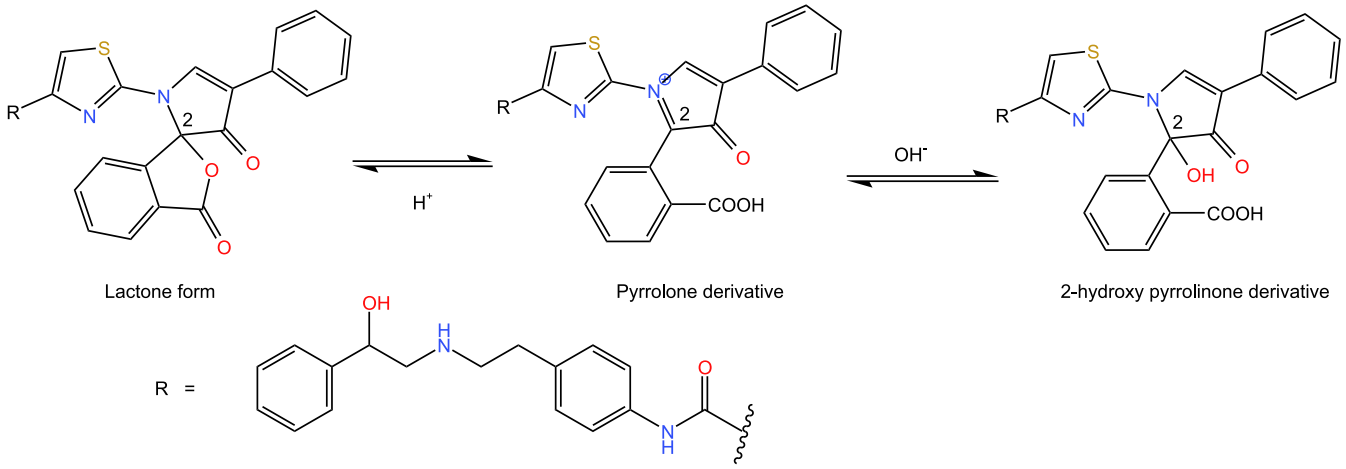

Fig. 5. Effect of pH on the chemical structure of the formed product between.

intensity was achieved when the reaction was heated for a period ranging from 14 to 18 min, while the most practical temperature was found between 85 and 90 °C (Fig. 6). So, the reaction was performed at 87 °C for 16 min.

3.1.4. Impact of dilution solvent

Different organic solvents (acetone, acetonitrile, DMF, ethanol and methanol) in addition to water were employed in the process of choosing the most appropriate solvent for diluting the reaction product (Fig. 7). A significant reduction in the fluorescence was obtained in the case of acetone, water, DMF and acetonitrile. Meanwhile, upon using ethanol and methanol maximum fluorescence was obtained. Because of its low toxicity, ethanol was chosen as solvent for dilution of the formed reaction product.

3.2. Method validation

The proposed spectrofluorimetric approach underwent assessment and validation in accordance with the principles outlined by the ICH guidelines [21].

3.2.1. Linearity and range

At the ideal reaction conditions, several standard solutions were analyzed using the general assay procedure. A linear relationship was established within the concentration range of 0.25 to 5.0 μg mL^{-1}, yielding a high correlation coefficient (r 0.9997) for the fluorescence intensity versus MRB concentration. Table 1 presents the regression analysis results along with various validation parameters for the suggested spectrofluorometric method.

3.2.2. Limit of detection (LOD) and limit of quantification (LOQ)

The method's sensitivity was assessed through calculations of LOD and LOQ. To determine the LOD and LOQ, the formulas recommended by the ICH guidelines were applied, specifically LOD = 3.3 SD/b and LOQ = 10 SD/b, where "b" represents the slope and "SD" stands for the standard deviation of the intercept. LOD was estimated to be 0.082 μg mL^{-1}, while the LOQ was 0.248 μg mL^{-1} (Table 1). These results demonstrate that the proposed method exhibits a high level of sensitivity when analyzing MRB.

3.2.3. Accuracy and precision

Based on the calculation of the mean percent recovery, the method's

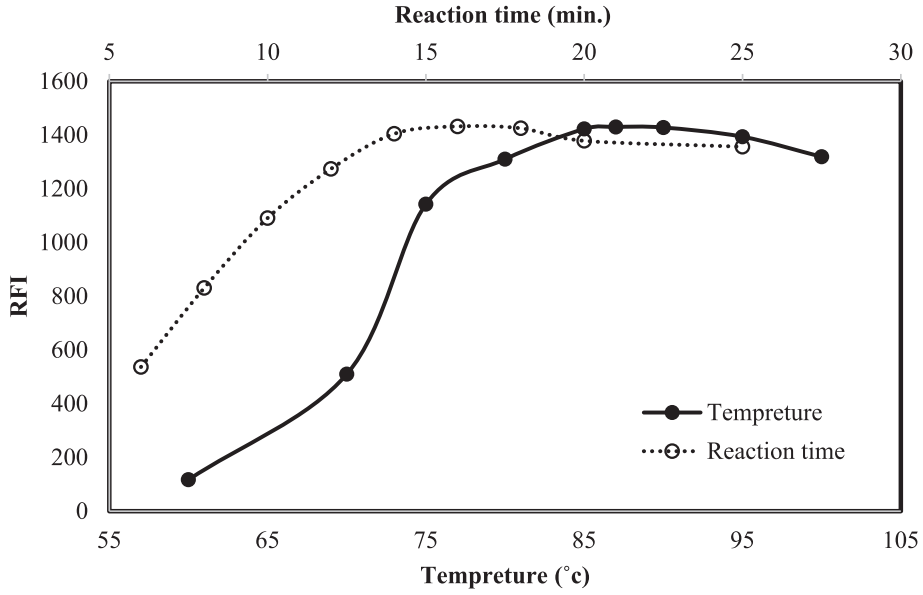

Fig. 6. Effect of reaction temperature (−●-) and reaction time (−○-) on the relative fluorescence intensity of the formed condensation product between MRB (2.0 μg mL^{-1}), ninhydrin (0.1 % w/v), and phenylacetaldehyde (0.02 % v/v).

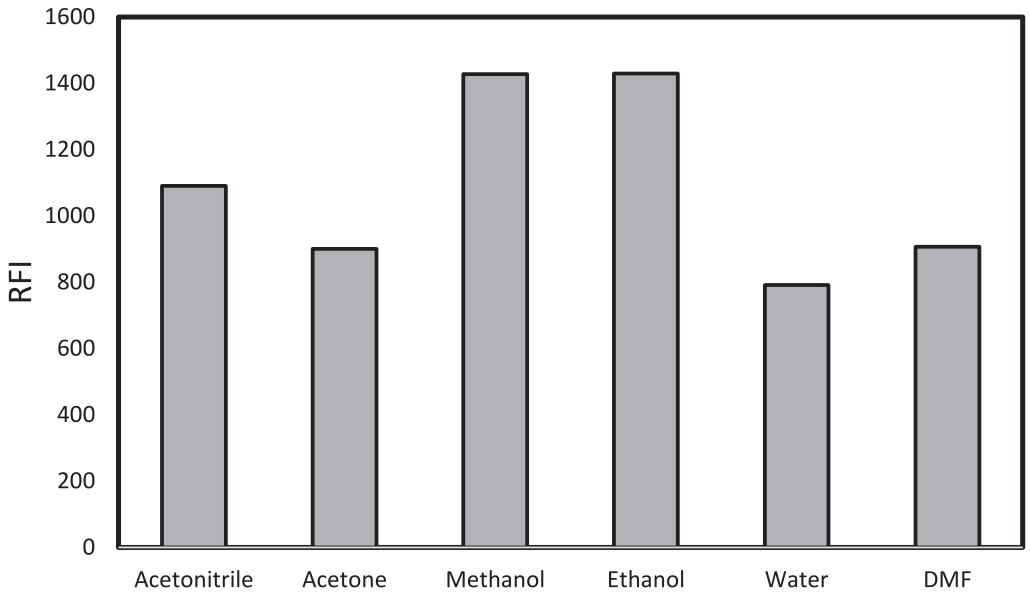

Fig. 7. Effect of diluting solvent on the relative fluorescence intensity of the formed condensation product between MRB (2.0 μg mL^{-1}), ninhydrine (0.1 % w/v), and phenylacetaldehyde (0.02 % v/v).

Table 1
The regression and validation parameters for the proposed spectrofluorometric method.

Parameter	Proposed Method
Linear range (μg mL^{-1})	0.25 – 5.00
Regression equation	Y = 727.32X −26.04
Slope	727.32
SD of slope (S_b)	6.72
Intercept	−26.04
SD of intercept (S_a)	18.02
Correlation Coefficient	0.9997
Coefficient of determination	0.9995
SD of residuals ($S_{y,x}$)	30.16
LOD (μg mL^{-1})	0.08
LOQ (μg mL^{-1})	0.25

accuracy was evaluated using standard addition method. Five different concentrations (0.2, 0.7, 1.7, 2.7, and 4.7 μg mL^{-1}) of MRB standard solution were added to a previously analyzed MRB samples (0.3 μg mL^{-1}) obtained from Bladogra® 50 tablets. The analysis was performed for each concentration in triplicate. The % recoveries shown in Table 2 were in the range of 99.46–100.52 % indicating the closeness of obtained results to the true value which confirmed the proposed method accuracy.

To evaluate the precision of the proposed method, five concentrations (0.3, 1.0, 2.0, 3.0, and 5.0 μg mL^{-1}) covering the drug linearity range were determined by applying the general procedures in triplicate.

Table 2
Accuracy of the proposed spectrofluorometric method using the standard addition method.

Amount taken from Bladogra® 50 mg (μg mL^{-1})	Amount added (μg mL^{-1})	Amount found (μg mL^{-1})	% Recovery [a] ± SD
0.30	0.00	0.300	100.08 ± 0.70
0.30	0.20	0.502	100.47 ± 0.50
0.30	0.70	0.995	99.46 ± 1.07
0.30	1.70	2.003	100.17 ± 0.95
0.30	2.70	3.016	100.52 ± 0.50
0.30	4.70	5.013	100.26 ± 0.57

[a] Mean of three determinations.

The assessment involved conducting analysis over a single day to assess intra-day precision and over three successive days to access inter-day precision. The results, as displayed in Table 3, demonstrate that the relative standard deviations for the tested drug were below 2 %, affirming the method's high precision in both intra-day and inter-day measurements.

3.2.4. *Robustness*

The robustness of the suggested approach was tested to assess the impact of a slight alteration in the reaction conditions on the method performance. The examined parameters included; pH (7.5 ± 0.2), Teorell-Stenhagen buffer volume (1.2 mL ± 0.2), ninhydrin volume (2.0 mL ± 0.2), phenylacetaldehyde volume (0.8 mL ± 0.2), reaction temperature (87.0 ℃ ± 2.0) and reaction time (16.0 min. ± 2.0). As demonstrated in Table 4, it was observed that none of the examined factors had a substantial impact on the recovery percentages of the medication. This proves the reliability of the suggested technique when routinely applied to analyze the specified drug. Consequently, the method can be deemed robust.

3.2.5. *Selectivity*

To test the selectivity of the current method, the impact of various tablet additives employed in tablet manufacturing and the presence of the co-formulated drug, Solifenacin Succinate, were investigated. The extent of how much they influenced the outcomes of the suggested approach was evaluated through calculating the recovery percentages and standard deviation. The study included; glucose, lactose, magnesium stearate, starch, talc, and zinc oxide, in addition to solifenacin succinate. Each interfering compound was mixed separately by adding

Table 3
Evaluation of the intra-day and inter-day precision for the proposed spectrofluorometric method.

Conc. level	% Recovery ± RSD [a]	
μg mL^{-1}	Intra-day precision	Inter-day precision
0.30	100.85 ± 0.91	100.90 ± 1.31
1.00	101.24 ± 0.75	100.31 ± 1.19
2.00	99.32 ± 0.82	99.79 ± 1.03
3.00	99.82 ± 0.48	100.14 ± 0.97
5.00	100.59 ± 0.83	100.39 ± 1.00

[a] Mean of three determinations.

Table 4
Evaluation of the robustness of the proposed spectrofluorometric method.

Parameter		% Recovery ± SD [a] 3 μg mL^{-1}
Buffer pH	7.3	99.09 ± 0.75
	7.7	99.82 ± 0.82
Buffer volume (mL)	1.0	99.96 ± 0.60
	1.4	99.27 ± 0.97
Ninhydrin volume (mL)	1.8	99.13 ± 0.89
	2.2	98.72 ± 1.08
Phenylacetaldehyde volume (mL)	0.8	99.56 ± 0.87
	1.2	99.61 ± 1.50
Temperature (c)	85	98.57 ± 0.80
	89	99.98 ± 0.83
Reaction time (min.)	14	99.36 ± 1.03
	18	99.78 ± 0.87

[a] Mean of three determinations.

certain amounts of these excipients or solifenacin succinate to a solution having constant MRB concentration. The outcomes summarized in Table 5, revealed that these compounds had no notable impact on the specificity of the recommended technique. The lack of interference liability from these compounds is due to the absence of the primary amino group in the tablet excipients and solifenacin succinate. Moreover, the previously mention standard addition presented in accuracy evaluation gives a further prove that the method has high specificity in determining MRB in commercial tablets.

3.3. Pharmaceutical application

The developed method suitability for the analysis of MRB in Flowadjust® tablets 25 mg and Bladogra® 50 mg was investigated. Upon utilizing the suggested approach, there was no matrix effect from tablets' excipients as indicated by the obtained good % recoveries (Table 6). The outcomes generated through the proposed approach were subjected to comparison with the results obtained from the reported method [6], utilizing F- and t-tests at a 95 % confidence level. The calculated F- and *t*-test values were found to be below the values listed in the reference tables, indicating that there is no substantial discrepancy in the levels of accuracy and precision between the suggested technique and reported method. The obtained results demonstrate the ability of the suggested method to analyze MRB precisely and accurately in pharmaceutical formulation.

Additionally, interval hypothesis was investigated, and the true bias implements on the recovery study was estimated using the following quadratic equation [30]:

$$\theta^2\left(\bar{x}_1^2 - S_p^2t^2/n_1\right) - 2\theta\bar{x}_1\bar{x}_2 + \left(\bar{x}_2^2 - S_p^2t^2/n_2\right) = 0$$

The lower (θ_L) and upper (θ_U) limits of the confidence interval were calculated according to the following formulae:

$$\theta_U = \frac{-b + \sqrt{b^2 - 4ac}}{2a}$$

Table 5
Evaluation of the selectivity for the proposed spectrofluorometric method.

Substance added	Amount added μg mL^{-1}	Drug taken μg mL^{-1}	% Recovery ± SD [a]
Talk	30	3	101.17 ± 0.90
Zinc oxide	30	3	99.33 ± 0.28
Magnesium stearate	30	3	100.16 ± 0.37
Starch	30	3	99.59 ± 0.32
Glucose	30	3	100.63 ± 0.31
Lactose	30	3	99.84 ± 1.09
Solifenacin Succinate	10	3	100.10 ± 0.52

[a] Mean of three determination.

Table 6
Application of the proposed methods for the determination of MRB in Flowadjust® 25 mg and Bladogra® 50 mg tablets (n = 5).

Dosage form	% Recovery ± SD Reported method [6]	Proposed method	t-value [a]	F-value [a]	θ_U [b]	θ_L [b]
Flowadjust® 25 mg tablets	101.05 ± 0.94	100.11 ± 0.61	1.88	2.33	0.9816	0.9999
Bladogra® 50 mg tablets	100.53 ± 0.86	100.18 ± 0.55	0.77	2.51	0.9881	1.0050

[a] Tabulated value at 95 % confidence limit; t = 2.306 and F = 6.338.
[b] Bias based on recovery of ± 2 % is acceptable.

$$\theta_L = \frac{-b - \sqrt{b^2 - 4ac}}{2a}$$

Where;$a = \left(\bar{x}_1^2 - S_p^2t^2/n_1\right)$

$b = 2\bar{x}_1\bar{x}_2$

$c = \left(\bar{x}_2^2 - S_p^2t^2/n_2\right)$

Where, $\bar{x}_1^2$ and $\bar{x}_2^2$ are the percentage recovery means for the suggested and reported methods, respectively. n_1 and n_2 are their number of determinations. *Sp* is the pooled standard deviation and *t*- is the one-sided tabulated *t*-value at 95 % confidence level. The values of θ_L and θ_U for analyzing MRB in the two dosage forms are presented in Table 6. For analyzing pharmaceutical formulations, it is accepted if the value of bias does not exceed ± 2. Results revealed that the proposed method has acceptable accuracy and precision as the calculated true bias values for both the investigated pharmaceutical formulations are less than ± 2 %,

Furthermore, comparing the suggested approach with the two previously reported spectrofluorometric techniques [9,10] revealed a superior level of the suggested method in terms of simplicity, sensitivity, eco-friendliness and applications as presented in Table 7.

3.4. Test the content uniformity (CU)

When the active ingredients in the tablet formulation comprise less than a quarter (25 %) of the tablet's overall weight or if the content of the active component drops below 25 mg, it is essential to perform the CU test for the tablet dosage form units [23]. The MRB percentage of total tablet weight is 8.89 % in Flowadjust® 25 mg tablets and 20.41 % in Bladogra® 50 mg tablets. Owing to its simple procedure, the spectrofluorometric technique was employed for the assessment of MRB uniformity in their commercially available tablets. If the calculated accepted value (AV) is equal to or less than the specified maximum acceptable value (L1), then, the active ingredient content in the analyzed pharmaceutical tablets could be considered consistent. AV can be calculated using the following equation:

$AV = KS + |M - \bar{X}|$.

In the context of this formula, S denotes the standard deviation, K signifies the acceptability constant, specifically set at 2.4, M denotes a reference value, while $\bar{X}$ represents the mean value of the tablet contents. The calculated values of AV achieved by the suggested method for assessing Flowadjust® 25 mg and Bladogra® 50 mg tablets did not exceed values of L1 approving that the investigated tablet dosage form units were uniform (Table 8).

3.5. Spiked human plasma and urine application

The maximum plasma (C_{max}) level of MRB ranged from 31.0 ng

Table 7
A comparison of two reported spectrofluorometric methods and the proposed method.

No.	Reagent	Application	Linear range (μg mL^{-1})	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	Ref.
1	Ninhydrin and phenylacetaldehyde	Tablets, human urine and plasma	0.25–5.00	0.08	0.25	This work
2	Acetoxymercuric fluorescein	Tablets only	1.00–5.00	0.14	0.43	[9]
3	Tyrosine and	Tablets only	2.00 – 20.00	0.136	0.49	[10]
	L-tryptophan	Tablets only	1.00 – 30.00	0.234	0.709	[10]

mL^{-1} for 50 mg daily dose to 720.0 ng mL^{-1} for 400 mg daily dose [31]. MRB is eliminated in the urine (55 %) with unchanged MRB accounting for 45 % [1]. Because of the significant sensitivity of the proposed approach, MRB levels in human plasma and urine that were spiked with various MRB concentrations could be estimated in the predetermined range. The MRB concentration was calculated through the utilization of its corresponding regression equation (RFI = 727.32x − 26.04). The resultant mean recovery values were (94.06–95.02 %) ± (0.48–1.04) for human plasma and (94.34–96.77 %) ± (0.88–1.35) for human urine (Table 9). These average recoveries confirmed the method's ability for analyzing MRB in spiked human plasma and urine.

3.6. Evaluation of method greenness

When it comes to safeguard people and the environment from dangerous substances and the waste that produced by chemicals and pharmaceuticals industries, analysts should have a great responsibility [32]. Thus, it is necessary to regularly develop and improve green analytical chemistry metrics [33]. Modern tools, such as the eco scale ratings [34], the Green Analytical Procedure Index (GAPI) [35], and the Analytical Greenness Calculator (AGREE) [36] are employed to assess the environmental merit of analytical techniques [37,38]. The suggested methodology's greenness was assessed using the eco-scale ratings. The output of the eco-scale assessment is a number which could be obtained by subtracting the total penalty points from 100. These penalty points refer to the risks that could be encountered during performing the research. The more environmentally friendly the process, the greater the rating, denoted by a high numerical value. The proposed method consumed lower than 0.1 kW/h of energy per sample and did not include any extracting steps. The estimated eco-scale for the proposed method is 86 (Table 10), that obviously demonstrating the environmental friendliness of the proposed approach.

GAPI is a three-colored symbol comprising 15 pictograms, each representing a stage in the analytical process. All analytical steps, from sample preparation to final analysis, are assessed and divided into three segments: red, yellow, and green. These segments correspond to environmental impact levels of high, moderate, and low. The GAPI approach allows analysts to assess the environmental impacts of established analytical procedures, facilitating the adoption of more environmentally friendly practices. In the case of the present spectrofluorimetric method, among all the pictograms, 9 are green, 5 are yellow, and only one is red. This suggests that the spectrofluorimetric method is environmentally green (Fig. 8).

The software known as AGREE is a user-friendly tool that utilizes the twelve-significance principle of green analytical chemistry as its input criterion. Each of these twelve inputs receives a score on a standard scale ranging from 0 to 1, mirrored on an intuitive red-yellow-green color scale. Additionally, the importance of each input criterion is considered in the process, and this is reflected by the width of its corresponding segment. The output takes the form of a clock-like graph, featuring the overall score and color representation in the center. A perfect analysis achieves a score of one, represented by a dark green color. According to the AGREE pictogram (Fig. 8), the present spectrofluorimetric method attained an outstanding green analysis with a score of 0.68.

Table 8
Application of the proposed methods for the content uniformity testing of MRB in Flowadjust® 25 mg and Bladogra® 50 mg tablets.

Tablet number	Flowadjust® 25 mg	Bladogra® 50 mg
1	98.68	97.12
2	98.81	98.95
3	100.74	97.71
4	96.34	100.74
5	102.34	101.84
6	96.84	102.66
7	103.40	96.8
8	99.91	99.64
9	101.47	102.8
10	100.78	103.12
Mean $\bar{X}$	99.93	100.14
S	2.28	2.44
AV*	6.13	6.59
L1*	15	15

*L1: maximum allowed acceptance value, AV: acceptance value.

Table 9
Determination of MRB in spiked human plasma and urine by the proposed spectrofluorometric method.

Conc. Level	% Recovery ± RSD [a]			
μg mL^{-1}	Spiked plasma		Spiked urine	
	Intra-day precision	Inter-day precision	Intra-day precision	Inter-day precision
0.50	94.06 ± 0.48	94.16 ± 0.97	95.71 ± 1.26	95.36 ± 1.35
2.00	94.11 ± 0.70	94.37 ± 1.04	96.77 ± 1.21	95.69 ± 1.22
4.00	95.02 ± 0.53	95.01 ± 0.94	94.87 ± 0.88	94.34 ± 0.90

[a] Mean of three determinations.

Table 10
Evaluation of the greenness of the proposed spectrofluorometric method using the eco-scale score approach.

Parameters	PP sign
Reagents	
Ethanol	0
Ninhydrin	1
phenylacetaldehyde	1
Buffer pH 7.8	0
Instruments	
spectrofluorometer	
Energy	0 [≤ 0.1 kWh/sample]
Water bath (87 °C)	2
Cooling	2
Occupational hazard	3
Waste	3
Total penalty points	Σ 12
Analytical eco-scale total score [a,b]	88 Excellent green analysis

If the score is > 50, it signifies acceptable green analysis.
If the score is < 50, it signifies inadequate green analysis.
[a] Analytical eco-scale total score = 100 − total penalty points.
[b] If the score is > 75, it signifies excellent green analysis.

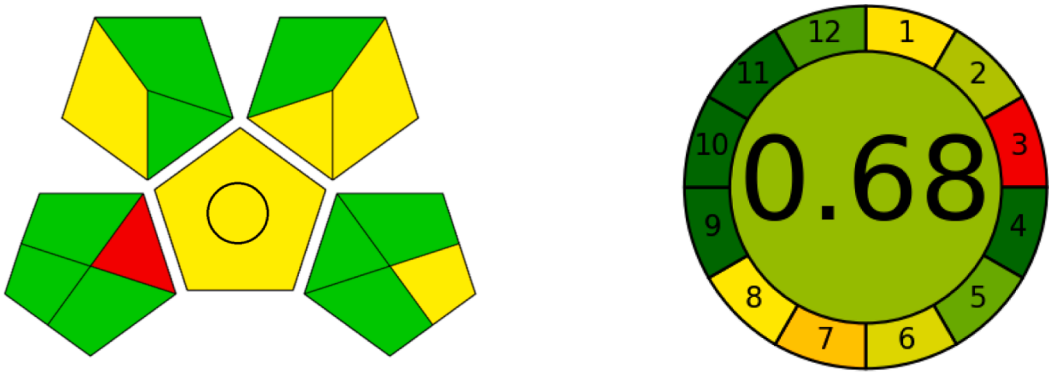

Fig. 8. Greenness evaluation of the proposed spectrofluorimetric methods using (A) GAPI and (B) AGREE.

4. Conclusion

A straightforward, highly sensitive, and environmentally friendly, spectrofluorimetric technique was designed to assess MRB, a newly approved medication by the FDA designed for managing the condition of overactive bladder syndrome. The method is based on the condensation of ninhydrin and phenylacetaldehyde with the primary amine group of MRB to produce a highly fluorescent product that can be used to detect MRB over the range of 0.25 – 5.00 μg mL^{-1}. The detection and quantification limits were 0.082 and 0.248 μg mL^{-1}, respectively. The percentage recoveries for analysis of the drug in spiked human plasma were (94.06–95.02 %) ± (0.48–1.04) and the values were (94.34–96.77 %) ± (0.88–1.35) for human urine. The ICH guidelines were used in validating the proposed spectrofluorimetric method. The approach has been effectively utilized for the assessment of MRB in tablets, without encountering any interference from tablet additives or solifenacin succinate. Additionally, the method was also used for the quantitative measurement of MRB in spiked human plasma and urine using simple extraction procedures with no interference from the plasma proteins or urine components. Compared to methods described in earlier reports, the proposed approach offers numerous benefits for the assessment of MRB, including enhanced simplicity, heightened sensitivity, environmentally greener and broader application capability.

CRediT authorship contribution statement

Sayed M. Derayea: Writing – review & editing, Supervision, Project administration, Conceptualization. **Ahmed S. Ahmed:** Writing – original draft, Methodology, Formal analysis. **Mohamed A. Abdelshakour:** Validation, Formal analysis, Data curation. **Mohamed Oraby:** Writing – original draft, Visualization, Resources. **Khalid M. Badr El-Din:** Writing – original draft, Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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