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ARTICLES

An Experimental Design Approach for Optimization of Spectrophotometric Estimation of Mirabegron in Bulk and Pharmaceutical Formulations[1]

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Abstract—Three simple, low-cost, sensitive and diversely applicable UV-Vis spectrophotometric methods have been developed for the estimation of drug Mirabegron. Method A is based on the reaction of Mirabegron with ninhydrin in the presence of sodium molybdate at pH 5.5. Method B is based on the reaction of the drug with 1,2-napthaquinone-4-sulphonate and cetyltrimethyl ammonium bromide in an alkaline medium. Method C is based on a redox reaction of the drug with Folin–Ciocalteu reagent in sodium carbonate medium. Beer's law was obeyed in the concentration ranges of 2.5–22.5, 5–35, and 5–70 μg/mL for methods A, B, and C. The proposed methods can be applied to drug formulation and recommended for the routine analysis in quality control laboratories.

Keywords: ninhydrin, sodium 1,2-napthaquinone-4-sulphonate, cetyltrimethyl ammonium bromide, Folin–Ciocalteu, Mirabegron, pharmaceuticals

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Mirabegron, 2-(2-amino-1,3-thiazol-4-yl)-N-(4-2-{(2R)-2-hydroxy-2-phenylethyl)amino}ethyl)phenyl)acetamide], is a potent and selective β_3-adrenoreceptor agonist, the first of a new class of compounds for the treatment of overactive bladder [1] with a mode of action different from antimuscarinic agents. Mirabegron activates β_3-adrenoreceptor on the detrusor muscle of the bladder to facilitate filling of the bladder and urinary storage [2]. Mirabegron is currently at phase I of clinical trials for the treatment of overactive bladder [3]. A literature survey reveals only two methods developed and validated for the determination of Mirabegron and its metabolites in human plasma and their application to a clinical pharmacokinetic study: LC–MS/MS [4], and RP-HPLC [5]. However, the reported methods are time consuming, tedious, and dedicated to sophisticate and expensive analytical instruments. Spectrophotometric methods are the most convenient techniques because of their inherent simplicity, high sensitivity, low cost, and wide availability in quality control laboratories. Therefore, the development and validation of new spectrophotometric methods for the determination of Mirabegron that can overcome the disadvantages of the existing methods is essential.

The present investigation describes simple, sensitive, accurate and precise spectrophotometric methods for the determination of Mirabegron in bulk and pharmaceutical dosage forms. Scanning for the published methods for the determination of the cited drug showed no reports on a spectrophotometric method, which would be less expensive than the published LC–MS/MS and RP-HPLC methods. The proposed methods are not included in pharmacopoeias but were validated as per ICH guidelines and can be applied for the routine drug determination in quality control laboratories.

In this paper, we reported three new spectrophotometric methods based on the reaction of Mirabegron with ninhydrin and sodium molybdate at pH 5.5 in citrate buffer (method A), on the nucleophilic substitution reaction between 1,2-napthaquinone-4-sulphonate (**NQS**) and amino group of the drug along with cetyltrimethyl ammonium bromide (**CTAB**) in alkaline medium resulting in the formation of an orange colored product (method B) and on the redox reaction of the drug with Folin–Ciocalteu reagent in sodium carbonate medium (method C).

[1] The article is published in the original.

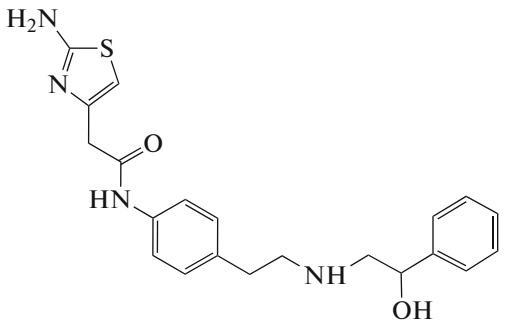
Ninhydrin, a well known carbonyl reagent, was applied in the pharmaceutical assay of different nitrogeneous compounds, amino acids, amines, amides, piperazines and cyanides, as it is known to form a condensation product of distinctive purple color that could be measured spectrophotometrically. Ruhemann's purple color condensation product was formed in the reaction of a primary or secondary amine with ninhydrin and sodium molybdate mixture at pH 5.5. This reaction was used in the determination of many drugs such as Isoniazid, Lisinopril dihydrate, Amoxicillin trihydrate, Ampicillin trihydrate, Glucosamine sulphate, Phenylpropanolamine hydrochloride, Gabapentin and Gentamycin [6], Cephalosporins (cefepime, cefazolin sodium and cefalothin sodium) [7], Cefatoxime sodium and Cefoperazone sodium [8], Gabapentin [9], Acyclovir [10], Armodafinil, Pramipexole and Terazosin [11], Pregabalin [12], and Amoldipine Besylate [13].

1,2-Napthaquinone-4-sulphonate proved to be a useful and sensitive analytical derivatizing agent for the determination of pharmaceuticals bearing a primary and secondary amino group; however, the use of NQS for spectrophotometric determination of Mirabegron was not reported. An orange colored product was formed in the reaction of NQS with primary amino group of the drug along with cetyltrimethyl ammonium bromide. Several pharmaceutical compounds have been determined through this approach such as Isoniazid [14], pharmaceutical amine [15], Paracetamol and Phenacitin [16], Moxifloxin [17], Albendazole [18], Stavudine [19], Etravirine [20], Finasteride [21], and Sulfanilamide [22].

Folin–Ciocalteu (**FC**) reagent is a colorimetric reagent specially used for the determination of phenolic compounds by utilizing its liability to be reduced into a blue colored product. The blue colored chromogen was formed in the redox reaction of the drug with FC reagent in Na_2CO_3 medium. Many drug substances such as Isoniazid [23], Dobutamine hydrochloride, Pyridoxine hydrochloride, Linezolid [24], Diclofenac sodium [25], Salbutamol [26], Minocycline [27], Trimetazidine [28], and Gliclazide [29] were determined on this basis. The structural feature of Mirabegron (**MRB**) allowed the use of the three described reagents for its assay.

EXPERIMENTAL

Instruments and reagents. A BL 198 Bio spectrophotometer (UV-Vis) with 1.0 cm matched cells was used for electronic spectral measurements. The pH measurements and adjustments were performed using a digital pH meter (Equiptronics, Mumbai, India, and Model EQ-614). Mirabegron was purchased from Manus aktteva Biopharma LLP, Ahmadabad, Gujarat, India; ninhydrin and sodium molybdate from Merck, Germany; 1,2 napthaquinone-4-sulphonate from BDH, UK; cetyltrimethyl ammonium bromide from Hopkins and Williams, UK; NaOH, Na_2CO_3, Folin-Ciocalteu from S.D. fine chem., Ltd, Mumbai, India. All reagents were of analytical grade and double distilled water was used throughout the experiment. The structure of studied drug is shown in Scheme 1.



Scheme 1. Structure of Mirabegron.

Preparation of standard solution. Stock solution of MRB (100 μg/mL) was prepared by dissolving 10 mg of the drug in a small amount of methanol and diluted to volume with distilled water in a 100 mL volumetric flask. The solution was further diluted quantitatively according to the linearity range.

Preparation of reagents. *Method A*. Citrate buffer of pH 5.5 was prepared by mixing 21.0 g of citric acid and 200 mL 1.0 M NaOH in water and making up the volume to 1000 mL with water. For the preparation of ninhydrin and sodium molybdate mixture (**NSM**), equimolar concentrations of 0.2 M ninhydrin and sodium molybdate were dissolved in citrate buffer at pH 5.5 and made up the volume of 25 mL. This solution was used within 8 h of preparation.

Method B. 1,2-Napthaquinone-4-sulphonate (0.02% solution) was prepared by dissolving 0.02 g of the compound in water and making up the volume to 100 mL with water. Aqueous solution of NQS was freshly prepared and protected from sunlight. Cetyltrimethyl ammonium bromide (1%) was prepared by dissolving 1 g of the compound in hot water and diluting to 100 mL with water. A 4% (w/v) NaOH solution was prepared by dissolving a suitable quantity in water.

Method C. Aqueous solution of FC reagent (1 : 1, v/v) was prepared by mixing 50 mL of reagent with 50 mL water. A 20% solution of sodium carbonate was prepared by dissolving 20 g compound in 100 mL of water.

Assay procedures. *Method A*. Aliquots of different working standard solutions of MRB (2.0–22.5 μg/mL) were transferred into a series of 10 mL calibrated flasks. To each flask 1.0 mL of NSM solution and 0.5 mL of citrate buffer (pH 5.5) were added and the flasks were heated on water bath at 100°C for 10 min. After cooling to room temperature, the solu-

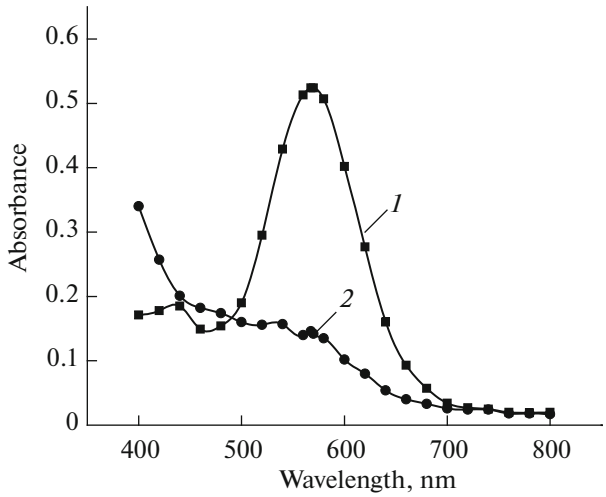


Fig. 1. Absorption spectra of the reaction product of MRB (12.5 μg/mL) with ninhydrin (*1*) and reagent blank (*2*) against distilled water.

tions were made up to the mark with distilled water. The absorbance of each solution was measured against reagent blank at 567 nm and the calibration graph was constructed by plotting absorbance versus concentration of the drug. No appreciable changes were observed if the order of addition of reagents was changed.

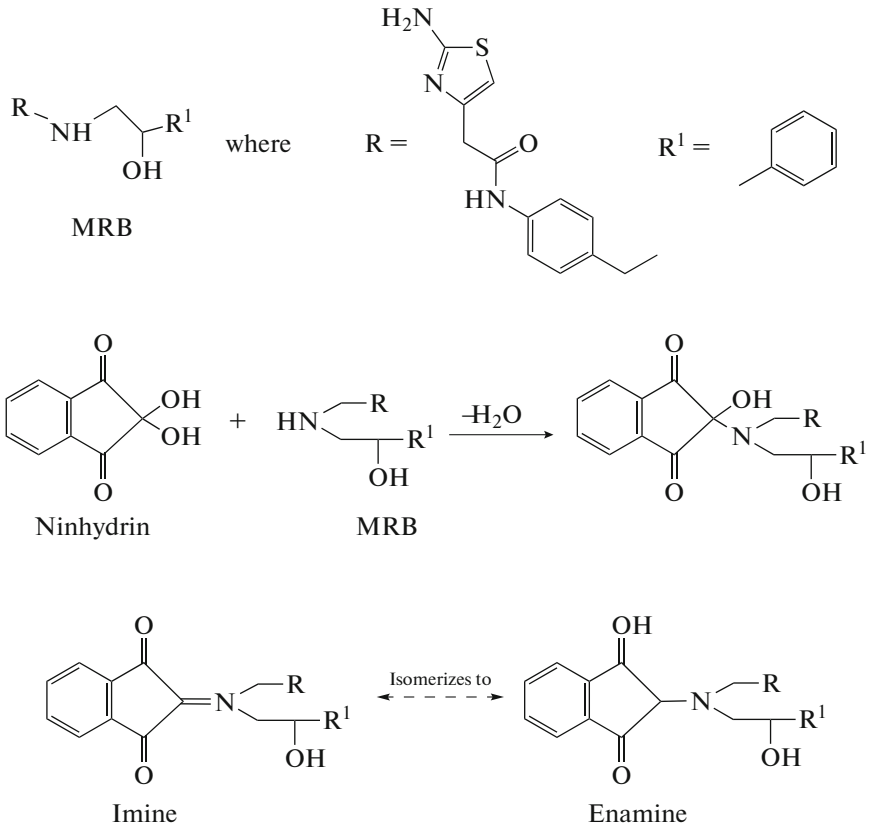
Method B. Into a series of 10 mL volumetric flasks, various aliquots of working standard solution of MRB concentration ranging from 5–35 μg/mL were transferred, followed by the addition of 1.5 mL of 0.02% NQS, 1.0 mL of 1% CTAB and 1.5 mL of 4% NaOH. The flasks were stoppered, the contents were mixed well, the volume was made up to the mark with water and absorbance of each solution was measured after 10 min at 535 nm against a reagent blank, similarly prepared in the absence of drug.

Method C. Different aliquots of standard MRB solution ranging from 5–70 μg/mL were transferred into a series of 10 mL calibrated flasks. To each flask, 1 mL of 1 : 1 FC reagent and 2.5 mL of 20% Na_2CO_3 solution were successively added by a micro burette. The flasks were stoppered; their contents were mixed well and kept at room temperature for 20 min. After 20 min, the volume was made up to the mark with water and the absorbance of each solution was measured at 747 nm against reagent blank prepared simultaneously without adding MRB. Standard graph was prepared by plotting the absorbance vs. MRB concentration, and the concentration of the unknown was computed from the respective regression equation derived using the absorbance–concentration data.

Assay of Mirabegron in pharmaceutical preparations. Twenty tablets were weighed and finely powdered. An accurately weighed quantity containing 10 mg of MRB was transferred to a 100 mL volumetric flask, a small amount of methanol was added, shaken well for 20 min, made up to the mark with water and filtered. Appropriate aliquots of the solution were taken and the proposed assay procedures were followed.

RESULTS AND DISCUSSION

Spectral characterization. Method A is based on the reaction of ninhydrin with drug molecules in the presence of sodium molybdate in citrate/citric acid buffer solution at pH 5.5 to give Ruhemann's purple color product having maximum absorption at 567 nm. Primary and secondary amine and amides react with ninhydrin in the presence of sodium molybdate as a catalyst. Here, the secondary amine group of the drug reacts with one molecule of ninhydrin in the presence of sodium molybdate in the 1st step (i.e., direct substitution). Imine is formed in the 2nd step, but it is unstable and immediately isomerizes to form enamine (stable form), i.e. Ruhemann's purple color product, which shows maximum absorbance at 567 nm. A tentative reaction mechanism of the drug with ninhydrin in the presence of sodium molybdate is shown in Scheme 2. The absorption spectrum of the colored product of Mirabegron and the reagent blank is shown in Fig. 1. The corresponding reagent blank had practically negligible absorbance at this wavelength. The unknown concentration of the drug can be calculated by knowing the absorbance at the λ_{max} using the regression equation.



Scheme 2. A proposed reaction mechanism of the drug with ninhydrin in the presence of sodium molybdate.

Method B is based on the nucleophilic substitution reaction of MRB with NQS in the presence of sodium hydroxide in an aqueous medium. In the 1st step, NQS reacts with MRB containing a primary amino group, it yields a pink-colored anionic compound produced by nucleophilic displacement of the sulfonic acid group of NQS in the presence of NaOH and shows maximum absorbance at 500 nm. Under the experimental conditions, the suggestion is that when the light yellow alkaline solution of NQS reacts with compounds containing two removable hydrogen atoms attached to one nitrogen atom, a pale pink colored anionic product results [14, 30, 31]. In the 2nd step, when cetyltrimethyl ammonium bromide was added to this solution, an intense orange colored product was obtained with a bathochromic shift of 35 nm (λ_{max} = 535 nm). Hence this wavelength was used for all subsequent measurements. The absorption spectra of the products and the reagent blank are shown in Fig. 2. A tentative reaction mechanism of the drug with NQS in the presence of NaOH is shown in Scheme 3.

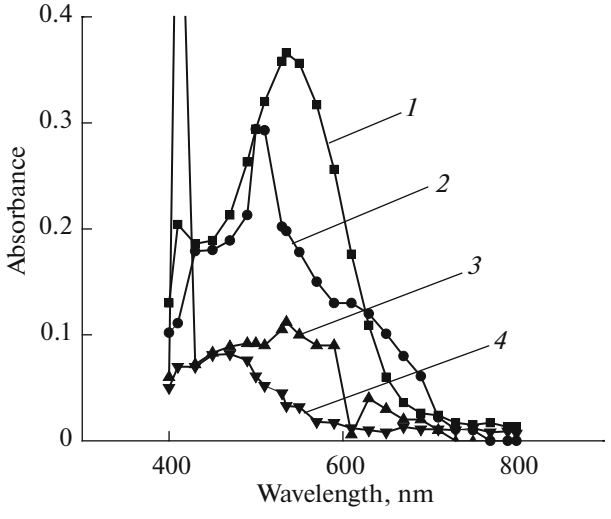


Fig. 2. Absorption spectra of MRB + NQS + CTAB (*1*), MRB + NQS product (*2*), NQS + CTAB reagent blank (*3*), NQS + reagent blank (*4*). MRB concentration: 20 μg/mL.

Method C is based on the formation of a blue colored chromogen, following the reduction of phosphomolybdotungstic mixed acid of the FC reagent [32] by the drug, in the presence of sodium carbonate, which could be measured at 747 nm. The mixed acids in the FC reagent are the final chromogen and involve the following chemical species:

$$3H_2O \cdot P_2O_5 \cdot 13WO_3 \cdot 5MoO_3 \cdot 10H_2O;$$

$$3H_2O \cdot P_2O_5 \cdot 14WO_3 \cdot 4MoO_3 \cdot 10H_2O.$$

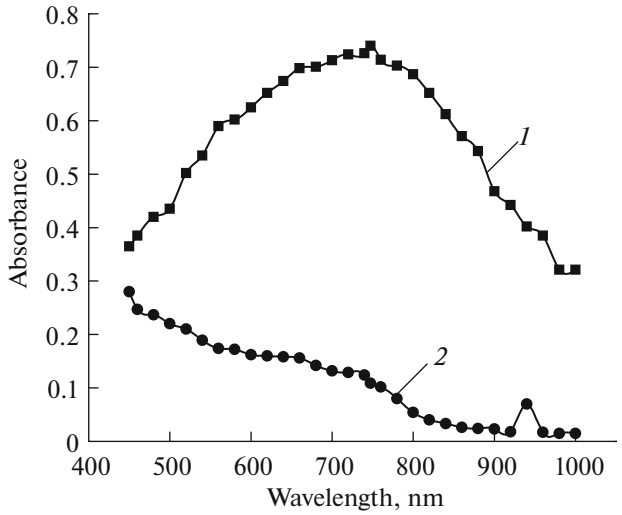


Fig. 3. Absorption spectra of MRB (50 μg/mL) with Folin–Ciocalteu reagent (*1*) and reagent blank (*2*) against distilled water.

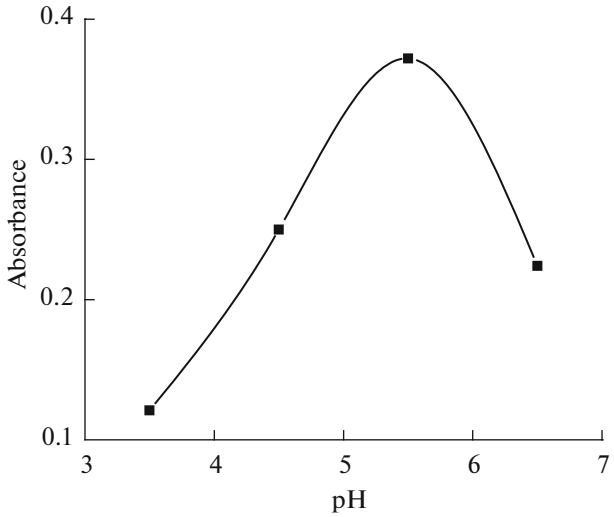
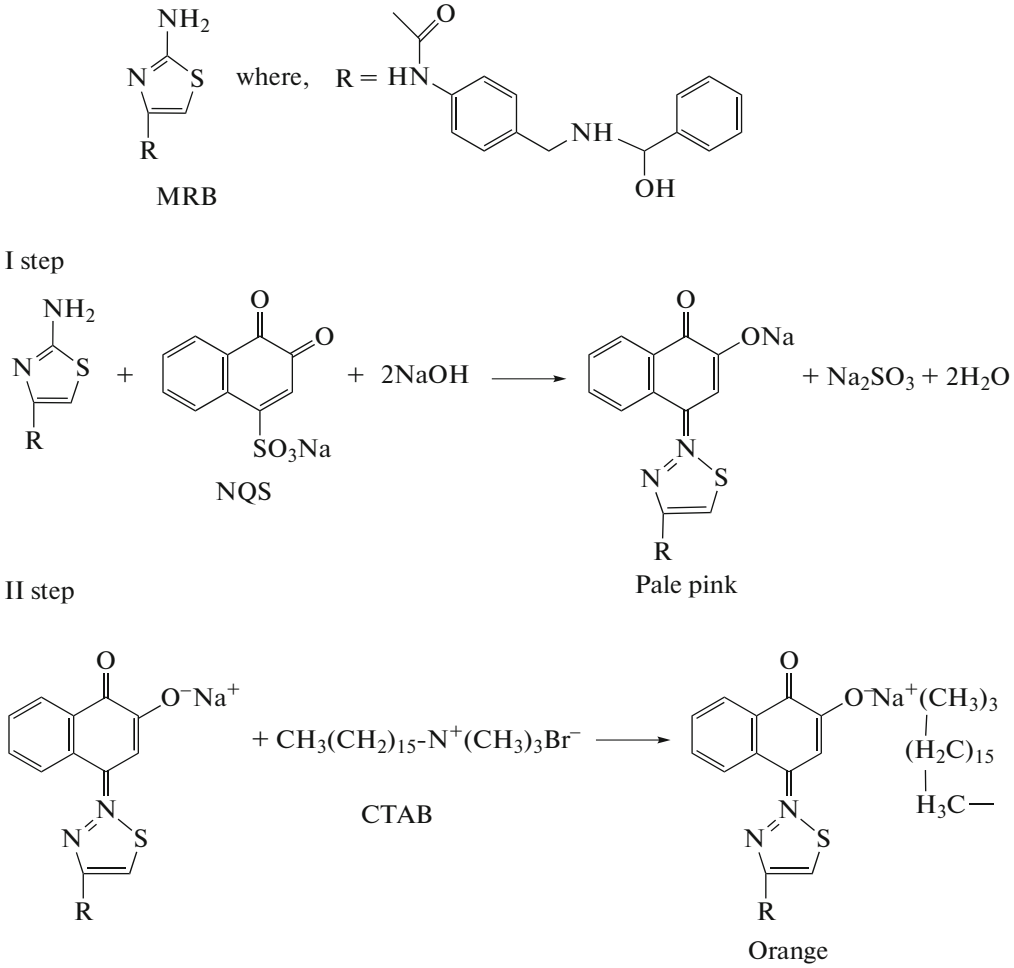


Fig. 4. Effect of pH on the absorbance of 10 μg/mL MRB with ninhydrin in the presence of sodium molybdate.



Scheme 3: A tentative reaction mechanism of the drug with NQS and CTAB in the presence of NaOH.

Drug probably affects the reduction of oxygen atoms from tungstate and/or molybdate in the FC reagent, thereby producing one or more reduced species which have characteristic blue color. The intensely

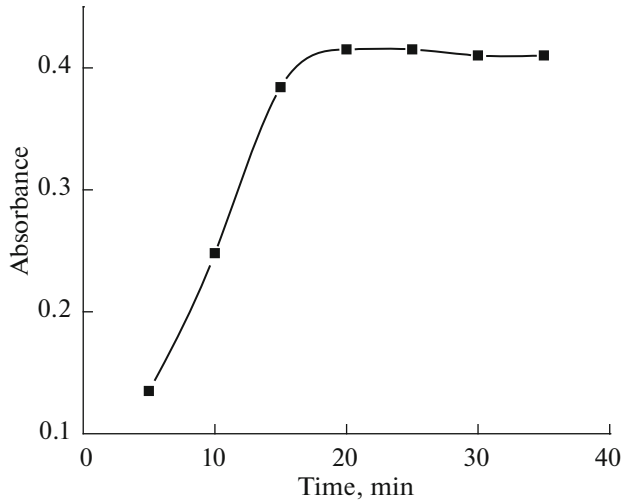


Fig. 5. Effect of reaction time on the absorbance of Folin–Ciocalteu reagent with MRB (30 μg/mL).

blue colored product (molybdenum-tungsten mixed acid blue) formed in this method exhibited maximum absorption at 747 nm. The absorption spectra of the blue colored product of Mirabegron with Folin–Ciocalteu and the reagent blank are shown in Fig. 3.

Optimization of reaction variables. Investigations were carried out to establish the most favorable condition to achieve maximum color development in the determination of the drug.

Method A. Effect of concentration of NSM. Different concentration combinations of ninhydrin and sodium molybdate in citrate buffer (pH 5.5) were attempted, which showed that concentration equivalent to 0.206 M of ninhydrin and sodium molybdate gave the best result. For Mirabegron, the volume ranged from 0.5–3.0 mL of NSM was tested. The maximum color development was obtained at 1.0 mL of NSM at 567 nm.

Effect of pH. The effect of pH on the absorption of the Ruhemann's purple color product formed by the reaction of MRB with ninhydrin in the presence of sodium molybdate was studied with different buffers such as acetate and citrate. The color development was obtained by using citrate buffer that was therefore studied at different pH range from 3.0–7.0. The maximum color development was achieved at pH 5.5 by using 1.0 mL of citrate buffer, as shown in Fig. 4.

Effect of reaction time and temperature. The optimum reaction time and temperature were determined by carrying out the reaction at different temperatures (25–100°C) and time intervals (0–25 min). Satisfactory maximum color intensity and reproducible λ_{max} values were obtained when the reaction mixture was heated at 95 ± 5°C for 15 min.

Stability of the colored product. The color was developed by heating the solution for about 15 min after the addition of all the reagents on a boiling water bath followed by cooling to room temperature. The color was stable for 2 h at room temperature.

Effect of solvent. Different solvents such as methanol, ethanol, acetonitrile and water have been used to get maximum intensity. Highest sensitivity was obtained when water was used.

Method B. Effect of reagent concentration. The study of NQS concentration revealed that the reaction was dependent on NQS reagent. The highest absorption was attained when the concentration of NQS was 0.02% in the range of 1.0–4.0 mL, and concentration of 1% CTAB in the range of 0.5–3.0 mL. The effect of volume of NQS and CTAB on the reaction product with MRB was studied. It was found that 1.5 mL of NQS and 1.0 mL of CTAB were optimum.

Effect of alkali. Various volumes of 4% NaOH were tried and the best results were obtained between 0.5–3.0 mL of NaOH. Below 0.5 mL, the color intensity decreased. Therefore, 1.5 mL of 4% NaOH was selected for all further studies.

Effect of reaction time and temperature. The reaction time was determined by following the color development at room temperature and thermostatically controlled water-bath at different temperatures. The system is stable in the temperature range of 0–50°C. The absorbance values remain constant for 4 h in the temperature range of 0–50°C. At higher temperatures, the absorbance decreases, indicating the dissociation of the product on prolonged heating. It was observed that the absorbance reached maximum after leaving the solution for 10 min at room temperature (25 ± 2°C). This reaction time and temperature were chosen for color development. The colored product was stable for 4 h at 27°C. The effect of reaction time and temperature were studied.

Method C. By varying one and keeping the other experimental parameters and amount of drug constant, the effect of FC reagent and Na_2CO_3 were tested.

Effect of FC reagent. Several experiments were carried out to study the influence of FC reagent concentration on the color development. It is apparent that 0.5 to 3.0 mL of reagent gave the maximum color intensity; thus, 1.0 mL of reagent was used throughout the investigation.

Selection of reaction medium and optimization of the base. To select a suitable medium for the reaction, different aqueous bases such as sodium hydroxide, sodium carbonate or bicarbonate, sodium acetate and sodium hydrogen phosphate were investigated. Better results were obtained with sodium carbonate. In order to determine the optimum concentration of Na_2CO_3, different volumes of 20% Na_2CO_3 solution (1.0–4 mL) were attempted at a constant concentration of MRB (30 μg/mL). It was found that different volumes

Table 1. Optical characteristics and statistical data of the regression analysis

Parameter	Optical characteristics		
	method A	method B	method C
Color	Purple	Orange	Blue
λ_{max}, nm	567	535	747
Beer's law limit, μg/mL	2.5–22.5	5–35	5–70
Molar absorptivity, L/(mol cm)	1.371×10^4	7.471×10^3	5.532×10^3
Sandell's sensitivity, μg/cm^2	0.028	0.053	0.071
Limit of detection, μg/mL	0.368	0.268	0.583
Limit of quantification, μg/mL	1.116	0.813	1.768
Regression equation[a]			
Slope (B)	0.034	0.016	0.012
Intercept (A)	0.076	0.029	0.039
Correlation coefficient (r)	0.992	0.998	0.998
Relative standard deviation[b]	0.02	0.04	0.02

[a] $Y = BX + A$, where X is the concentration of the measured solution in μg/mL and Y is the unit for absorbance.
[b] Average of five determinations; concentrations of MRB: 5, 10 and 15 μg/mL (method A), 10, 20 and 30 μg/mL (method B) and 10, 30, and 50 μg/mL (method C).

ranging from 1.0 to 3.0 mL of 20% Na_2CO_3 were optimum; thus 1.0 mL was used throughout the work

Effect of time. Maximum color development was obtained in 20 min after mixing the reactants, and the color was stable for 3 h. The sequence of order of addition of the reactants had significant effect on the absorbance value. So, the order used in the general procedure should be followed for maximum absorbance. The effect of reaction time on the absorbance of Folin–Ciocalteu reagent with MRB is as shown in Fig. 5.

Method validation. The validity of the proposed methods were tested regarding linearity, range, limits of detection, limits of quantification, accuracy, precision and specificity according to current ICH recommendations [33]. Beer's law range, molar absorptivities and sandell's sensitivities [34], regression equation, and correlation coefficients were evaluated and are given in Table 1. A linear relationship was found within the range of 2.5–22.5, 5.0–35, and 5.0–70 μg/mL for methods A, B and C, respectively. The proposed methods showed excellent linearity for the determination of drug with good correlation coefficients in the range of 0.9920–0.9988. High molar absorptivity in the range of 10^3–10^4 and low Sandell's sensitivity values (0.02–0.07) showed the methods are sensitive. The relative standard deviation (**RSD**) for the analysis of five replicates of each three different concentrations of MRB indicated that the methods are precise and accurate. Regression analysis of the Beer's law plots revealed a good correlation. The graphs showed negligible intercept, which was calculated by the least square method regression equation $Y = BX + A$, where, Y is the absorbance of solution, B is the intercept, A is the slope, and X is the concentration of the measured solution in μg/mL. The limits of detection (**LOD**) and quantification (**LOQ**) were calculated as $K\sigma/S$, where $K = 3.3$ for LOD and 10 for LOQ, σ is the standard deviation of reagent blank determination, and S is the slope of the calibration curve.

Interference studies. The effects of common excipients used in the pharmaceutical preparation were studied by analyzing synthetic sample solutions containing the quantity of drugs mentioned in Table 2 in presence of 100-fold concentrations of each excipient. The absorbance values of solution of the excipients alone were measured too, at 567, 535, and 747 nm,

Table 2. Recovery of drugs from solution with a 100-fold excess of various additives used as excipients

Excipient	Mean % recovery ± % RSD ($n = 3$)		
	method A	method B	method C
Dextrose	99.9 ± 0.2	99.9 ± 0.2	100.0 ± 0.1
Lactose	100.0 ± 0.1	100.0 ± 0.1	100.0 ± 0.1
Sucrose	99.8 ± 0.2	99.8 ± 0.2	99.9 ± 0.2
Starch	99.7 ± 0.5	99.7 ± 0.5	98.7 ± 0.4
Talc	98.7 ± 0.4	98.7 ± 0.4	99.7 ± 0.3
Magnesium stearate	99.7 ± 0.3	99.7 ± 0.3	99.9 ± 0.3

Mean ± % RSD ($n = 3$) – mean of three determinations; concentration of MRB used, μg/mL: 10 (method A), 20 (method B), 30 (method C).

Table 3. Intra- and inter-day precision data of determination of Mirabegron

Method	Amount taken, μg/mL	Intra-day		Inter-day	
		amount found, μg/mL	% recovery ± % RSD[a]	amount found, μg/mL	% recovery ± % RSD[b]
A	5.0	4.99	99.8 ± 0.9	4.998	100 ± 1
	10.0	10.01	100.0 ± 0.7	10.0	100 ± 1
	15.0	14.98	99.9 ± 0.8	14.87	99.1 ± 0.6
B	10.0	10.01	100 ± 2	9.99	100 ± 2
	20.0	19.98	100 ± 1	19.83	99 ± 1
	30.0	29.99	100 ± 1	29.87	100 ± 1
C	10.0	9.98	100 ± 2	9.99	100 ± 2
	30.0	29.99	100.0 ± 0.6	29.6	98.7 ± 0.6
	50.0	50.01	100 ± 1	49.8	99.6 ± 0.6

[a]Mean value of five determinations, [b]mean of five determinations performed over a period of five days.

showing no significant difference from the baseline. The tolerance limit was defined as the concentration which gave an error of ± 3.0% in the determination of drug. The common excipients such as starch, dextrose, lactose, talc, suc`rose and magnesium stearate had no effect in the analysis.

Precision studies. The short term precision (intra-day precision) was evaluated by measuring 5 independent samples at 3 different concentration levels 5, 10, 15 μg/mL, 10, 20, 30 μg/mL, and 10, 30, 50 μg/mL for methods A, B and C, respectively. Similarly, the assay for daily precision (inter-day precision) at the same concentration level was repeated for 5 consecutive days (Table 3). The percentage relative standard deviation values were ≤2% (intra-day) and ≤3% (inter-day), indicating good precision of the method. The available pharmaceutical dosage forms of the investigated drug were analyzed by the proposed methods.

Table 4. Analysis of drugs in pharmaceutical formulation Myrbetriq TM[a]

Method	Label claim, mg	% Recovery ± SD	
		proposed method[b]	RP-HPLC
A	10	99.9 ± 0.9 (t = 0.15, F = 1.19)	100.0 ± 0.8
B	10	100 ± 1 (t = 0.77, F =1.43)	100 ± 1
C	10	100.0 ± 0.7 (t = 0.24, F = 1.29)	100.7 ± 0.4

[a] MRB equivalent to 10 mg/tab (Astellas Pharma US) for methods A, B, and C.
[b] Mean of five determinations ± standard deviation, n = 5; the t- and F-values obtained after comparison to the reference method, which have the following theoretical values at 95% confidence limit: t = 2.44 and F = 5.05. After adding the pure drug to the fixed concentration of preanalyzed pharmaceutical formulations.

The precision of the methods were checked by taking 5 replicate measurements.

Application to formulations. The proposed methods were applied to the quantification of MRB in tablet dosage forms. The results in Table 4 showed that the methods are successful for the determination of MRB and that the excipients in the dosage forms do not interfere. The result obtained from the assay of MRB by the proposed methods and reference method [5] is presented in Table 4. The results agreed well with label claim and also were in agreement with the results obtained by the reference method. When the results were statistically compared with those of the reference method by applying the Student's t-test for accuracy and F-test for precision, the calculated t-value and F-value at 95% confidence level did not exceed the tabulated values [35] (t = 2.44, F = 5.05), respectively, for five degrees of freedom. Hence, no significant difference existed between the proposed methods and the reference method with respect to accuracy and precision.

Recovery studies. The reliability and accuracy of the proposed methods were ascertained through recovery studies using the standard addition method by adding different amount of standard drug to the pre-analyzed dosage forms such that the cumulative amount after adding the drug did not exceed their linearity range. The mean percentage recoveries relative to the labeled amounts ranged from 99.2 to 100.06 (Table 5).

CONCLUSIONS

The present paper described the evaluation of ninhydrin, NQS, and FC, as analytical reagents in the development of simple, sensitive, rapid, and reliable spectrophotometric methods for the determination of Mirabegron in pure or pharmaceutical dosage forms.

Table 5. Results of recovery study via standard addition method with tablet of Myrbetriq TM, 10 mg

Method	MRB in tablet, μg/mL	Pure MRB added, μg/mL	Total found ± SD, μg/mL[a]	Pure MRB recovered percent ± % RSD
A	3.0	4.5	7.45 ± 0.003	99 ± 1
		9.5	12.51 ± 0.003	100.0 ± 0.7
		14.5	17.48 ± 0.004	100.0 ± 0.7
B	4.0	1.0	4.96 ± 0.002	99 ± 2
		11.0	15.01 ± 0.003	100 ± 1
		21.0	25.08 ± 0.003	100.3 ± 0.7
C	5.0	15.0	19.92 ± 0.003	100 ± 1
		35.0	39.97 ± 0.002	99.9 ± 0.4
		55.0	60.02 ± 0.005	100 ± 1

[a] Mean value of five determinations.

No UV or spectrophotometric methods have been described for the determination of MRB. The described methods are superior to the previously reported LC–MS/MS and HPLC methods in terms of its simplicity and sensitivity. The proposed methods have comparable analytical performances and are devoid of any potential interference. The accuracy, reproducibility, and cost effectiveness of the methods suggest their application in quality control laboratories, where the modern and sophisticated instruments are not available. Therefore, the proposed methods can be recommended for the routine analysis of MRB in quality control laboratories.

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