DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR THE ASSAY OF MIRABEGRON IN BULK AND PHARMACEUTICAL FORMULATIONS **

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Simple, sensitive, precise, and validated spectrophotometric methods have been developed for the assay of Mirabegron in bulk and pharmaceutical dosage forms. The techniques are premised on the oxidation of Mirabegron with slight excess of N-bromosuccinimide (NBS), and estimating the unconsumed oxidant by assessing the amount of unreacted NBS by amaranth dye (method A), safranin dye (method B), aniline blue (method C), and rhodamine B (method D) at λ_{max} = 530, 530, 610, and 560 nm, respectively. Under optimum conditions, Beer's law was obeyed in the concentration range of 5–30, 10–60, 20–45, and 1–15 μg/mL for methods A, B, C, and D, respectively. The proposed methods were validated in terms of specificity, linearity, range, precision, and accuracy. Furthermore, the limit of detection (LOD) and limit of quantification (LOQ) values were also calculated. The recommended methods were successfully applied to the determination of drug in pure as well as in dosage forms, without any interference from the common excipients present in pharmaceutical formulations.

Keywords: *mirabegron, N-bromosuccinimide, spectrophotometry, pharmaceutical preparations.*

РАЗРАБОТКА И ВАЛИДАЦИЯ СПЕКТРОФОТОМЕТРИЧЕСКИХ МЕТОДОВ АНАЛИЗА МИРАБЕГРОНА В ОБЪЕМНЫХ И ФАРМАЦЕВТИЧЕСКИХ СОСТАВАХ

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Разработаны простые, чувствительные, точные и валидированные спектрофотометрические методы для анализа мирабегрона в нефасованной фармацевтической продукции и в лекарственных препаратах. Методы основаны на окислении мирабегрона с небольшим избытком N-бромсукцинимида (NBS) и оценке нерастворенного окислителя путем определения количества непрореагировавшего

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NBS с помощью амарантного красителя (метод A), сафранинового красителя (метод B), анилинового синего (метод C), родамина B (метод D) при λ_{max} = 530, 530, 610 и 560 нм соответственно. В оптимальных условиях закон Бэра соблюдался в диапазоне концентраций 5–30, 10–60, 20–45 и 1–15 мкг/мл для методов A, B, C и D соответственно. Методы прошли тестирование с точки зрения специфичности, линейности, рабочего диапазона определяемых концентраций, точности и достоверности. Найдены пределы обнаружения и количественной оценки. Рассматриваемые методы успешно применены для определения лекарственного вещества как в чистом виде, так и в лекарственных формах. Влияния вспомогательных веществ, обычно присутствующих в фармацевтических препаратах, не обнаружено.

Ключевые слова: *мирабегрон, N-бромсукцинимид, спектрофотометрия, фармацевтические препараты.*

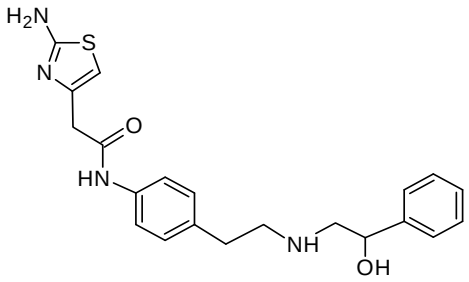
Introduction. Mirabegron (MRB) [2-(2-amino-1,3-thiazol-4-yl)-N-(4-2-{(2R)-2-hydroxy-2-phenylethyl)amino}ethyl)phenyl)acetamide] is the first of a new class of compounds, a potent and selective β_3-adrenoreceptor agonist used for the treatment of overactive bladder [1], with a mode of action that is different from antimuscarinic agents. MRB activates β_3-adrenoreceptor on the detrusor muscle of the bladder to facilitate filling of the bladder and urinary storage [2]. Currently, MRB and solabegron are in phase I and phase II clinical trials for the treatment of overactive bladder [3]. A literature survey reveals that only three methods were developed and validated for the determination of MRB. The above techniques comprise LC-MS/MS [4], RP-HPLC [5], and spectrophotometry. Nevertheless, the reported methods, except spectrometry, necessitate expensive and sophisticated instruments and are tedious to perform in all quality control laboratories. They may not be within reach of most laboratories. Spectrophotometry is one of the most convenient analytical methods due to their simplicity, high sensitivity, cost effectiveness, and wide availability in all quality control laboratories. Therefore, the development and validation of new spectrophotometric methods for the determination of MRB that can overcome the drawbacks of the presented methods are essential.

The present paper illustrates rapid, simple, sensitive, accurate, and precise spectrophotometric methods for the determination of MRB in bulk and pharmaceutical dosage forms, followed by the evaluation of their biological activities. Only one spectrophotometric method was reported by Roopa et al. [6] for the validation of MRB. In continuation of our work on the pharmaceutical and biological importance of drugs such as cefepime, cefazolin sodium, and cefalotin sodium [7], risperidone [8], pyridoxine hydrochloride, dobutamine hydrochloride, and linezolid Form-1 [9], dobutamine hydrochloride [10], phenylephrine hydrochloride and pyridoxine hydrochloride [11], cephalosporins [12], and lamotrigine [13], we developed new elegant spectrophotometric methods for the estimation of MRB in pharmaceutical dosage forms.

NBS (N-bromosuccinimide) is widely employed as a good oxidizing agent for many organic compounds. We have previously demonstrated the applicability of NBS as a valuable reagent for the assay of drugs dexmedetomidine hydrochloride [14], phenylephrine hydrochloride, and pyridoxine hydrochloride [15]. NBS was also used in the determination of many drug substances such as pizotifen maleate [16], metoprolol tartrate [17], ofloxacin [18], pantoprazole sodium [19], gemifloxacin mesylate and moxifloxacin HCl [20], fluoroquinolone [21], and tramadol [22]. The present work extends the utility of NBS as an oxidimetric reagent for the assay of MRB in pharmaceutical formulations. The methods were based on the oxidation of MRB with known excess of NBS, and unconsumed NBS has been determined by its reaction with four dyes such as amaranth, safranin, aniline blue, and rhodamine-B. The developed methods are more susceptible than the reported methods and are free from experimental variables such as heating or extraction and can be successfully applied for the routine drug analysis in quality control laboratories. It is not official in any of the pharmacopoeia. The methods were validated according to ICH guidelines.

Experimental. *Materials and methods*. A double beam BL 198 Bio spectrophotometer (UV-Vis) with 1 cm matched quartz cells was used for all absorbance measurements.

MRB was procured from Manus aktteva Biopharma LLP, Ahmadabad, Gujarat, India. Analytical grade amaranth dye (0.1%), safranin (0.05%), aniline blue (0.02%), and rhodamine-B (0.01%) were purchased from S.D fine chemicals PVT. Ltd, Mumbai, India. These solutions were prepared in the requisite amount of distilled water. N-bromosuccinimide (Merck, Germany), H_2SO_4 (Ranbaxy fine chemical, India, 0.2 M), and hydrochloric acid (Ranbaxy fine chemical, India, 1 M) were used. Analytical reagent grade chemicals and double de-ionized water was used throughout the analysis. The structure of the studied drug is as shown below:



Mirabegron

Preparation of standard solution. A stock solution of the drug (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 100 mL standard flask. The solution was further diluted quantitatively according to their linearity range.

Standardization of N-bromosuccinimide. NBS was prepared by dissolving 0.02 g of the chemical in hot water and diluting to 100 ml water and standardized [23]. The NBS solution was stored in a refrigerator when not used.

Procedure for pharmaceutical formulations. Different aliquots of the drug were transferred from stock solution to 10 ml volumetric flasks, which could be diluted quantitatively to obtain 5–30, 10–60, 20–45, and 1–15 μg/mL for methods A, B, C, and D, respectively. To each flask containing the drug, in the order mentioned above, 1.0 ml of 0.02% NBS, 0.4 mL of 0.1% amaranth dye after 5 min (method A), 0.4 mL of 0.03% safranin dye (method B), 0.5 mL of 0.2 M H_2SO_4, and 0.5 mL of 0.02% aniline blue dye (method C), and 0.5 mL of 1 M HCl and 1.0 mL of 0.01% rhodamine-B (method D) were added. The flasks were stoppered, the contents mixed well, the volumes made up with distilled water, and the absorbance of each sample against the corresponding reagent blank at λ_{max} = 530, 530, 610, and 560 nm measured.

Twenty tablets were weighed, powdered, and mixed thoroughly. A quantity equivalent to 10 mg of MRB was transferred to a 100 mL volumetric flask, dissolved in a small amount of methanol, shaken well for 20 min, sonicated, and made up to the volume with water. The resultant solution was filtered and analyzed as described under recommended procedures.

Results and discussion. *Spectral characteristic.* The absorption spectra of the reaction product with drug shows the maximum absorption at λ_{max} = 530, 530, 610, and 560 nm for methods A, B, C, and D, respectively. The blank solution was colorless and exhibited negligible absorbance at the λ_{max} in which the drug was analyzed. Thus, the color formed was stable for more than 24, 3, 3, and 18 h for methods A, B, C, and D, respectively. The absorption spectra of the reaction product and the corresponding reagent blank for methods A, B, C, and D are as shown in Fig. 1. Beer's law was obeyed in the concentration range 5–30, 10–60, 20–45, and 1–15 μg/mL for methods A, B, C, and D, respectively. The curves were found to be linear with different slopes for all the methods and an excellent correlation coefficient.

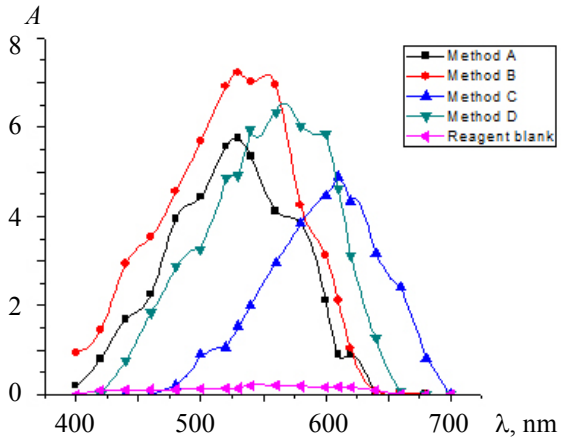
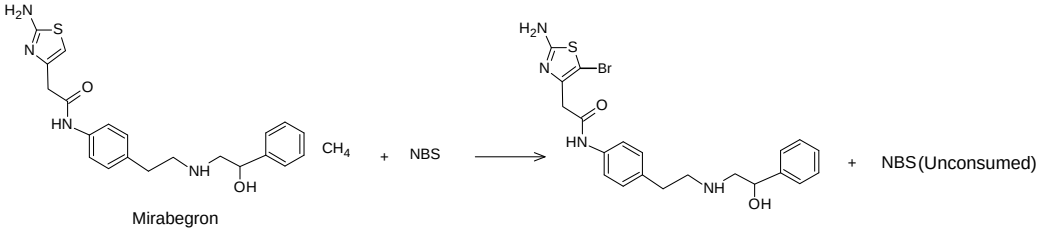
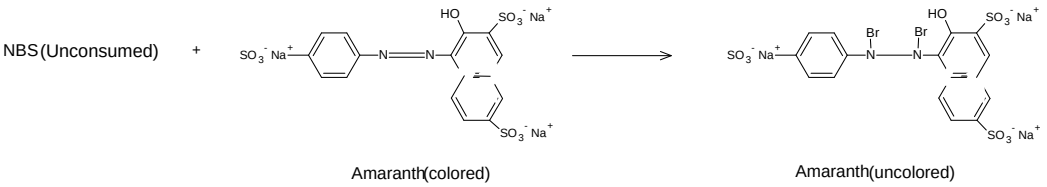


Fig. 1. Absorption spectra of MRB with amaranth dye (25 μg/mL) (method A), safranin dye (60 μg/mL) (method B), aniline blue (40 μg/mL) (method C), and Rhodamine-B (11 μg/mL) (method D) against reagent blank.

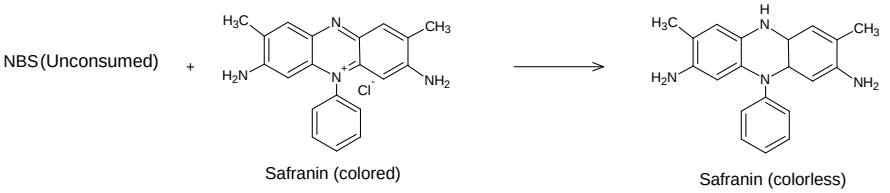
Reaction sequence. The developed spectrophotometric methods were based on the redox reaction between the drug, dye, and NBS (methods A and B) and between the drug, dye, and NBS in acidic medium (methods C and D) at room temperature. In the proposed methods, NBS acts as an oxidizing agent. In all the developed methods, the drug was reacted with a known excess of NBS, with the subsequent determination of unreacted oxidant NBS by reacting with amaranth dye or safranin dye (methods A and B) and aniline blue or rhodamine-B in acidic medium (methods C and D), followed by absorption measurement at 530, 530, 610, and 560 nm. The absorbance increased linearly with increasing concentration of the drugs when increasing amounts of the drug were added to a fixed amount of NBS. The latter was consumed, and a concomitant decrease in the concentration of NBS occurred. When a fixed amount of dye was added to a decreasing concentration of NBS, a concomitant increase in the concentration of dye was obtained, which in turn is directly proportional to the concentration of the drug. The suggested reaction mechanism is as shown in Scheme:



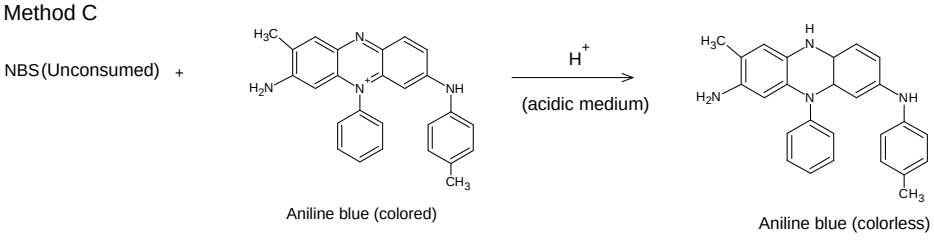
Method A



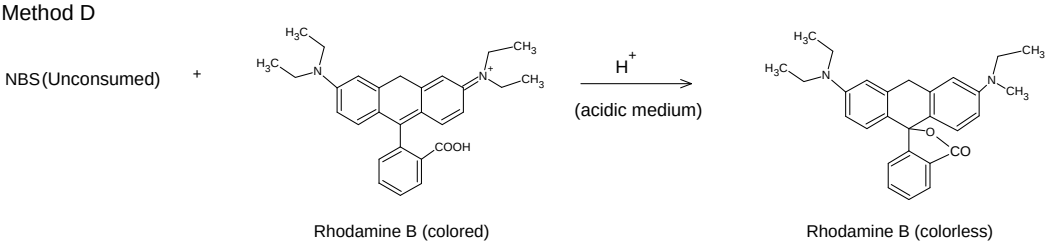
Method B



Method C



Method D



Optimization of reaction variables. *Effect of varying reagent concentration.* The effect of NBS and dye concentrations has been reviewed, and it was found that 0.02% NBS was optimum for the oxidation of the drug. The order of addition of reagents plays a major role in the formulation of drug. Maximum absorbance was obtained by the addition of the drug, followed by the dyes.

Effect of time and temperature. The reaction was carried out at room temperature (25 ± 30°C). Maximum color intensity was obtained at room temperature, and it was found that 15 min was essential for the drug oxidation after the addition of dyes; 2–5 min was required for bleaching. The colored products were stable for more than 24, 3, 3, and 18 h for methods A, B, C, and D, respectively.

Validity of the proposed methods. The methods were validated according to the procedures described in current ICH guidelines [24]. The Beer's law range, molar absorptivities, Sandell's sensitivities [25], the regression equation, and correlation coefficients were evaluated and are given in Table 1.

TABLE 1. Analytical and Regression Parameters of the Proposed Method

Parameters	Regression Parameters			
	Method A	Method B	Method C	Method D
Color	pink	pink	blue	pink
λ_{max}, nm	530	530	610	560
Beer's law limit, μg/mL	5–30	10–60	20–45	1–15
Molar absorptivity, L/mol cm	1.0228×10^4	3.0432×10^3	3.821×10^3	2.2937×10^4
Sandell's sensitivity, μg/cm^2	0.0387	0.11	0.1037	0.01728
Limit of detection [LOD], μg/mL	0.05495	0.1149	0.08058	0.049
Limit of quantification [LOQ], μg/mL	0.16652	0.34822	0.24419	0.1510
Regression equation $Y = BX+A$				
Slope [B]	0.01988	0.0135	0.01878	0.05252
Intercept [A]	0.06622	–0.1343	–0.27566	0.06118
Correlation coefficient [r]	0.98323	0.95158	0.99702	0.99515
Relative standard deviation*	0.011	0.019	0.0169	0.013

N o t e. X is the concentration of the measured solution in μg/ml and Y is the unit for absorbance.
* Average of five determinations (concentrations of 10, 20, and 30 μg/ml (method A); 20, 40, and 60 μg/ml (method B); 25, 35, and 45 μg/ml (method C); 7, 11, and 15 μg/ml (method D) for MRB, respectively).

Limits of detection (LOD) and quantification (LOQ). The limits of detection (LOD) and quantification (LOQ) were calculated according to the ICH guidelines using the formulae LOD = 3.3S/b, LOQ=10S/b, where S is the standard deviation of blank absorbance values and b is the slope of the calibration curve.

A linear relationship was found within the range 5–30, 10–60, 20–45, and 1–15 μg/mL for methods A, B, C, and D, respectively. The proposed methods showed excellent linearity for the determination of the drug, with good correlation coefficients in the range of 0.95158–0.99702. High molar absorptivity and low Sandell's sensitivity values showed that the methods are more 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. Beer's law curves of MRB with the dyes are shown in Fig. 2 for methods A, B, C, and D.

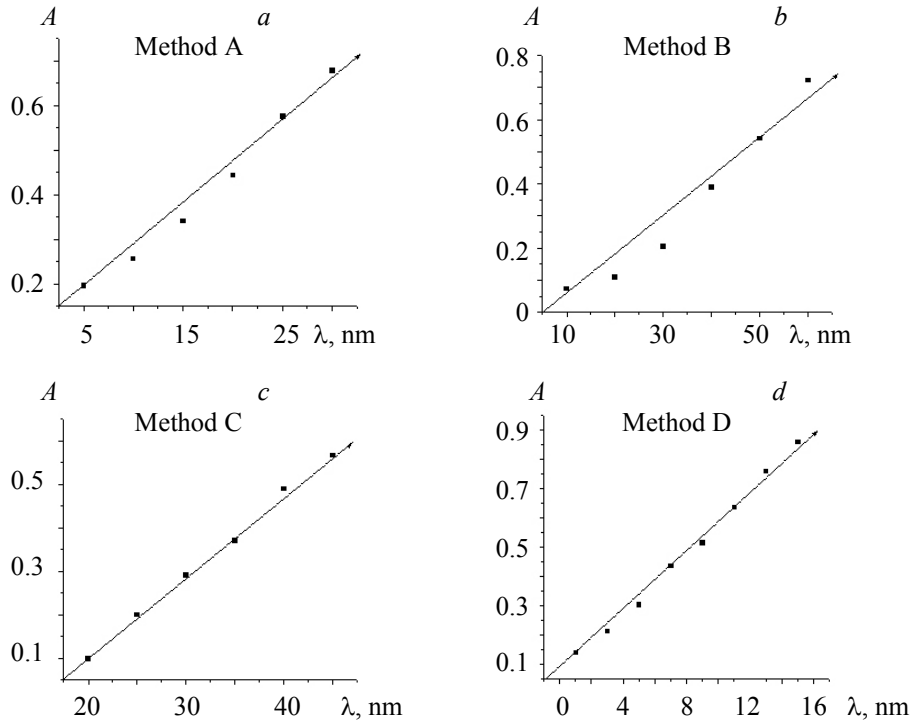


Fig. 2. Beer's law curve of MRB with amaranth (a), safranin (b), aniline blue (c), Rhodamine-B (d).

Interference studies. The effect of common excipients used in the pharmaceutical preparation was studied by analyzing synthetic sample solutions containing the quantity of drug as mentioned in (Table 2) in the presence of a 100-fold greater concentration of each excipient. The tolerance limit was defined as the concentration giving an error of ±3.0% in the determination of drug. Common excipients such as magnesium stearate, starch, dextrose, lactose, and talc had no effect on the analysis.

TABLE 2. Recovery of Drug from Solution in the Presence of 100-fold Concentration of Various Additives Used as Excipients in Formulation

Excipients	% Recovery ± %RSD[a]			
	Method A[b]	Method B[c]	Method C[d]	Method D[e]
Lactose	99.8±0.3	99.7± 0.2	99.9± 0.3	99.8 ± 0.3
Sucrose	98.7 ± 0.4	98.7 ± 0.4	99.7 ± 0.3	99.6 ± 0.2
Dextrose	99.8 ± 0.2	99.8 ± 0.2	99.9 ± 0.2	99.7± 0.3
Talc	99.8 ± 0.2	99.7 ± 0.2	99.9 ± 0.2	98.9 ± 0.2
Starch	99.8 ± 0.3	99.8 ± 0.3	99.9 ± 0.2	99.7± 0.3
Magnesium stearate	100.0 ± 0.2	100.0 ± 0.1	100.0 ± 0.1	100.0 ± 0.2

[a] Mean ± % R.S.D, $n = 3$, mean of three determinations.
[b] Concentration of MRB used 20 µg/mL.
[c] Concentration of MRB used 30 µg/mL.
[d] Concentration of MRB used 20 µg/mL.
[e] Concentration of MRB used 9 µg/mL.

Precision studies. The precision of the methods was calculated in terms of intermediate precision (intra-day and interday) by taking five replicate measurements at three different concentration levels within the same day and five consecutive days (Table 3). The available pharmaceutical dosage form of the investigated drug was analyzed by the proposed methods. The accuracy of analytical methods shows the close agreement between the reference value and the found value.

TABLE 3. Evaluation of Interday and Intraday Accuracy and Precision

Method	Intraday			Interday	
	Amount taken, µg/mL	Amount found, µg/mL	% Recovery± % RSD[a]	Amount found, µg/mL	% Recovery ± % RSD[b]
A	10	9.99	99.9 ± 0.89	10	100 ± 0.90
	20	20.01	100 ± 1.6	19.97	99.98 ± 1.5
	30	29.98	99.93 ± 0.67	30	100.0 ± 0.72
B	20	20.01	100.0 ± 1.99	19.90	99.5 ± 2.0
	40	39.98	99.95 ± 1.39	39.7	99.2 ± 1.28
	60	59.97	99.98 ± 2.0	60.0	100.0 ± 1.9
C	25	24.98	99.92 ± 1.8	24.72	98.88 ± 2.0
	35	35.01	100 ± 0.99	34.93	99.8 ± 1.0
	45	44.99	99.97 ± 0.79	40.1	100.2 ± 0.81
D	7	7.01	100.1 ± 0.97	7.0	100 ± 0.96
	11	10.99	99.90 ± 0.93	10.85	98.63 ± 1.1
	15	14.99	99.93 ± 0.29	14.98	99.86 ± 0.3

[a] Mean value of five determinations.
[b] Mean of five determinations performed over a period of 5 days.

TABLE 4. Analysis of Drug in Pharmaceutical Formulation and Statistical Comparison of the Results with the Official Method

Method	Drug formulations	Label claimed	% Recovery ± SD	
			Proposed method [a]	Reference method [*](RP-HPLC)
A	[b]Myrbetriq TM	10 mg	99.98 ± 0.97 t = 0.05 F = 1.18	100.01 ± 0.89
B	[b]Myrbetriq TM	10 mg	99.9 ± 1.1 t = 0.13 F = 1.19	99.99 ± 1.2
C	[b]Myrbetriq TM	10 mg	99.9 ± 1.65 t = 0.26 F = 2.83	100.1 ± 0.98
D	[b]Myrbetriq TM	10 mg	100.0 ± 1.05 t = 0.69 F = 1.27	100.4 ± 0.93

[a] Mean of five determinations ± standard deviation. n = 5; t- and F-values obtained after comparison with 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 pre-analyzed pharmaceutical formulations.
[b] MRB equivalent to 10 mg/tablet (Astellas Pharma US**)** for methods A, B, C, and D.

Application to formulations. The proposed methods were applied to the assay of MRB in tablet and pharmaceutical dosage forms. The results obtained by the proposed methods and the official method [5] for the dosage forms were compared statistically by means of Student's t-test for accuracy and F-test for precision at 95% confidence level. The calculated t- and F-values did not exceed the tabulated values [26] (t = 2.44, F = 5.05), and there was no significant difference between the proposed methods and the reported method (Table 4), which shows the excellent agreement between the proposed methods and official method.

Recovery studies. The reliability and accuracy of the developed methods were further ascertained through recovery studies using the standard addition technique by adding different amounts 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 recovery of the pure drug added was quantitative, and the co-formulated substances magnesium stearate, starch, talc, dextrose, and lactose did not interfere in the determination. The results of recovery study are compiled in Table 5.

TABLE 5. Results of Recovery Study of 10 mg Myrbetriq TM via Standard Addition Method

Method	Amount of drug taken in tablet, µg/mL	Amount of pure drug added, µg/mL	*Total found, µg/mL	% Recovered ±% RSD
A	5.0	5.0	10.01	100.1 ± 0.97
	5.0	15	19.99	99.95 ± 0.42
	5.0	25	29.98	99.99 ± 0.96
B	10	10	19.98	99.9 ± 2.1
	10	30	40.01	100.0 ± 1.46
	10	50	59.88	99.8 ± 1.1
C	5.0	20	25.1	100.4 ± 2.0
	5.0	30	35.01	100 ± 1.65
	5.0	40	44.94	99.86 ± 0.60
D	2.0	5.0	6.99	99.85 ± 1.05
	2.0	9.0	10.98	98.81 ± 1.21
	2.0	13	15.01	100.0 ± 0.93

*Mean of five determinations.

Conclusions. The present paper illustrated the evaluation of N-bromosuccinimide as a good oxidizing agent in the development of a simple, fairly sensitive, rapid, economical with high degree of precision, and reliable spectrophotometric methods for the determination of mirabegron in pure and pharmaceutical dosage forms. The described methods are superior in its simplicity and sensitivity than the previously reported methods like LC-MS/MS and HPLC. The methods experience no instability of colors as the bleaching of the dye is involved. The entire analysis was carried out in a short period of about 15–20 min. Also, the procedures do not involve critical reaction conditions or tedious sample preparation steps. Thus, these recommended methods are well suited for the assay and evaluation of the drug in pharmaceutical preparations.

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