Chemical Methodologies 4(2020) 340-358

Chemical Methodologies

Journal homepage: http://chemmethod.com

Original Research article

Quantification of A β Adrenergic Receptor Drug Mirabegron by Stability Indicating LC Method and Uv–visible Spectroscopic Method in Bulk and Pharmaceutical Dosage Form

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ARTICLE INFORMATION

Received: 06 April 2019
Received in revised: 19 November 2019
Accepted: 18 December 2019
Available online: 01 May 2020

DOI: 10.33945/SAMI/CHEMM.2020.3.11

KEYWORDS

Validation
Mirabegron
Forced degradation study
HPLC
UV–visible spectroscopy
Adrenergic

ABSTRACT

In this work, an accurate, sensitive, reproducible and precise stability indicating high performance liquid chromatographic (HPLC) method and ultra violet (UV)-visible spectroscopic method were established for the quantification and validation of β adrenergic drug mirabegron in bulk and its pharmaceutical dosage form. High performance liquid chromatography is a physical separation technique for a mixture of compounds that are dissolved in solution. Mirabegron is in a class of medications called diuretic. The HPLC method was developed with proposed chromatographic condition with mobile phase containing acetonitrile: water (50:50, v/v) adjusted pH 9 with 1 mL of 1% TEA. Accomplishment of UV-visible spectroscopic determination was done at wavelength maxima of 247 nm using methanol as a solvent. The linearities were in the range of 2-18 µg/mL for UV-visible spectroscopic method and 0.01-20 µg/mL for HPLC method, respectively. Validation of proposed method has been accomplished with respect to linearity, accuracy, precision, specificity and robustness. Forced degradation study has been performed under different conditions like acid and alkali hydrolysis, chemical oxidation, dry heat degradation and photolytic degradation study by use of stock solution of mirabegron and quantification has been achieved by proposed reverse phase-liquid chromatography (RP-LC) method. Mirabegron is susceptible to acid and base hydrolysis, chemical oxidation, dry heat and photolytic degradation studies; found that degradants are well resolve from parent drug peak of mirabegron. Due to the sensitivity, promptness and accuracy of methods, we rely on that the both intended methods will be useful for the regular quality control analysis and quantification of drug in bulk and pharmaceutical dosage form.

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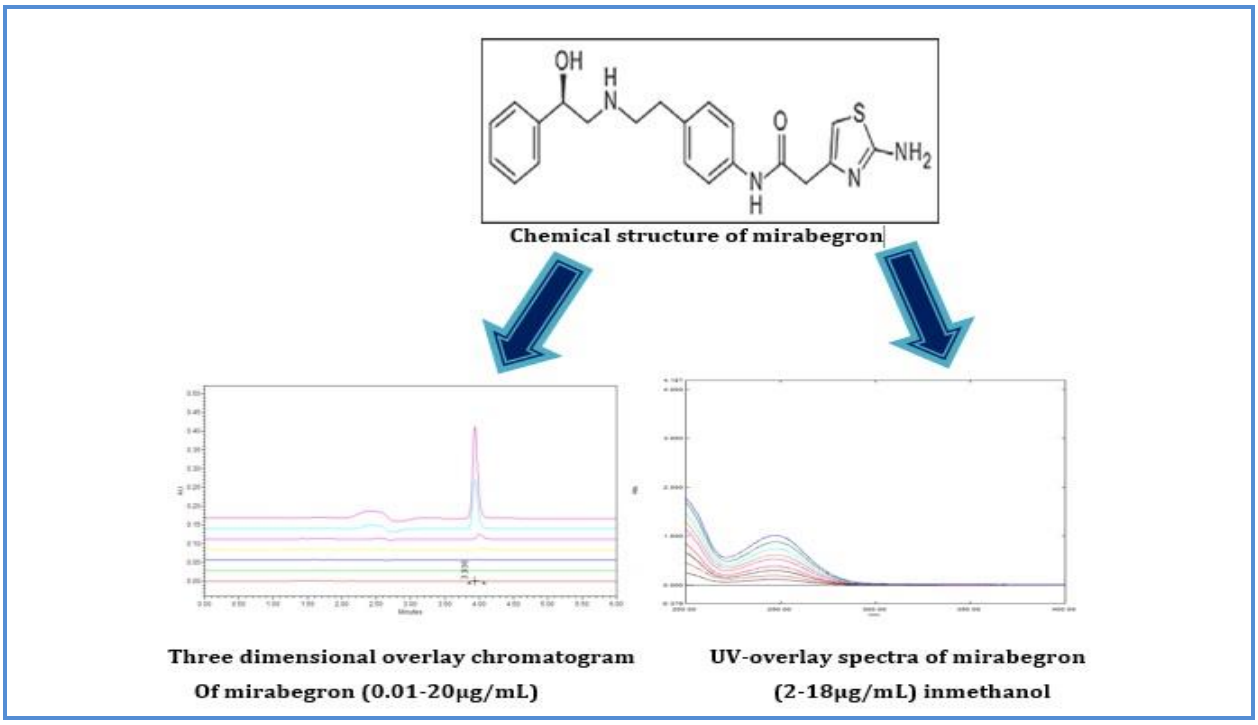
Graphical Abstract



Introduction

Mirabegron is a beta-3 adrenergic agonist. The chemical name is 2-(2-aminothiazol-4-yl)-*N*-[4-(2-{[(2R)-2-hydroxy2-phenylethyl]amino}ethyl)phenyl]acetamide having an empirical formula of $C_{21}H_{24}N_4O_2S$ and a molecular weight of 396.51 [1]. Mirabegron is a potent and selective agonist for beta-3-adrenergic receptors. Once beta-3 receptors are activated, the drug smooth muscle relaxes to allow for larger bladder capacity. At higher dose (200 mg), there is a potential for mirabegron to activate beta-1 and beta-2 adrenergic receptors [2]. The most frequent adverse events for the 25 mg or 50 mg dose were nausea, headache, hypertension, diarrhea, constipation, dizziness and tachycardia. Mirabegron is a white powder. It is practically insoluble in water (0.082 mg/mL). It is soluble in methanol and dimethyl sulfoxide [1]. Structure of mirabegron is demonstrated in Figure 1.

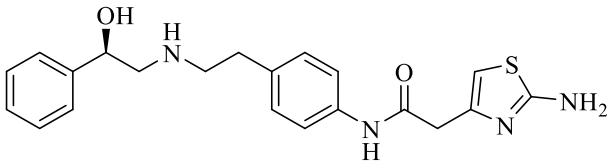


Figure 1. Structure of mirabegron

Extensive literature survey reveals that several analytical techniques have been accounted for the estimation of mirabegron which incorporates spectroscopy [3-4], high performance liquid chromatography [5-8]. A major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This decreases the time and cost of examination, minimizes exposure risks, and significantly reduces disposal problems of toxic organic solvents, thereby reducing the possibilities of environment pollution. In the proposed investigation, attempt has been made to develop sensitive stability indicating HPTLC technique for the estimation of mirabegron in bulk and pharmaceutical dosage form. The proposed technique was approved by ICH guidelines [9] and its refreshed global tradition.

Experimental

Development and validation of HPLC method

Selection of solvent

Solvents are chosen based on solubility, stability and UV-visible cutoff wavelength. Mirabegron is slightly soluble in aqueous solvent and soluble in organic solvent. Methanol was chosen as a solvent for preparation of stock solution as it is freely soluble.

Selection of analytical wavelength

The solution of mirabegron was processed in methanol at a concentration of 10 µg/mL. It was examined in the wavelength range of 400-200 nm. Analytical wavelength of 247 nm was preferable for perseverance of mirabegron.

Optimization of mobile phase

Several solvent compositions tried like methanol, acetonitrile and water for selection of mobile phase, acetonitrile: water (ACN: water) (50:50, v/v) adjusted pH 9 with 1 mL of 1% triethylamine (TEA) selected as mobile phase and showed satisfactory results at a flow rate of 1 mL/min. Mirabegron shows 3.93 min retention time where total time of analysis was 7 min.

Preparation of mobile phase

Mobile phase was prepared by taking acetonitrile: water (50:50, v/v) in 500 mL reservoir and adjusted pH 9 with 1 mL of 1% triethylamine. The reservoir was sonicated for 20 min for degassing the mobile phase prior to use. This mixture of solvent was used as mobile phase.

Preparation of standard stock solution (100 µg/mL)

Accurately weigh 10 mg of mirabegron and transfer to 10 mL volumetric flask containing 5.0 mL of methanol and makeup the volume up to the mark with methanol to obtained final concentration of stock solution (1000 µg/mL) of mirabegron. From the above solution, pipette out 1.0 mL of aliquot and transferred it in another 10 mL volumetric flask and dilute up to mark with methanol obtained working standard stock solution (100 µg/mL) of mirabegron.

Calibration curve

Pipette out appropriate volume of aliquot from working standard stock solution (100 µg/mL) and transferred to different volumetric flask of 10 mL and volume was adjusted with the mark with the mobile phase to give a solution containing 0.01, 0.05, 0.1, 0.5, 1, 10, and 20 µg/mL of mirabegron. Each solution was analyzed by the proposed method and the chromatogram was recorded. Calibration curve were constructed by plotting concentration *v/s* peak area and regression equation were computed.

Validation of HPLC method

Validation of the proposed developed HPLC method was performed out as per the international conference on harmonization (ICH) guidelines Q2 (R1) [9].

Linearity and range

Linearity was studied by preparing standard solution of 7 different concentrations of 0.01, 0.05, 0.1, 0.5, 1, 10 and 20 µg/mL for mirabegron. Each concentration was repeated 5 times and linearity was assessed in terms of slope, intercept and correlation coefficient of mirabegron. The calibration curves were developed by plotting concentration *v/s* peak area (n=5).

Accuracy

The accuracy was determined by calculating recovery of mirabegron by standard addition method. Known amount of mirabegron (80%, 100%, and 120%) were added to pre quantified sample solution and the amount of mirabegron were estimated by putting the value of peak area to the straight line equation of calibration curve.

Precision

Precision was calculated in terms of intraday and interday precisions. Intraday precision was determined by analyzing sample solution of mirabegron (0.01, 0.5 and 20 µg/mL) at three levels

covering low, medium and high concentration of the calibration curve three times on the same day (n=3). Now, interday precision were determined by analyzing sample solution of mirabegron (0.01, 0.5 and 20 µg/mL) at three levels covering low, medium and high concentration over a period of three days (n=3). The peak areas obtained were used to calculate mean and %RSD values. The repeatability studies were carried out by estimating the response of 0.5 µg/mL of mirabegron 6 times and result are reported in terms of %RSD.

Sensitivity

The limit of detection (LOD) is construed as the lowest concentration of an analyte that can reliably be distinguished from background levels. The limit of quantification (LOQ) of a discrete analytical procedure is the lowest quantity of analyte that can be quantitatively examined with suitable precision and accuracy.

As per ICH guidelines, LOD and LOQ were calculated using the following equation:

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

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

Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Robustness

Small change in the detection wavelength, flow-rate introduced and the mobile phase ratio on the results were examined for the concentration of 0.5 µg/mL. The mean and %RSD of peak were calculated.

Solution stability

The stock solution of mirabegron (0.5 µg/mL) was prepared and store in room temperature analyzed by RP-LC at regular intervals of 0, 4, 8 and 24 h.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically these might include impurities, degradants, preservatives *etc*. The drug was analyzed from prepared tablet using proposed chromatographic method.

Forced degradation study

Force degradation study was performed with acid and alkali hydrolysis, oxidation by use of chemical, photolytic degradation and dry heat degradation were accomplished and interruption of the degradation products was examined. Accurately weight 10.0 mg of mirabegron was added to 10 mL volumetric flasks and expose to various stress conditions.

Heat induced alkali hydrolysis

To study forced degradation in basic medium 10.0 mg of mirabegron was transferred to 10 mL volumetric flask and 2 mL of 0.1 M NaOH was added to the flask. The content of the flask was heated at 80 °C for 30 min and allowed to cool to room temperature. Solution was neutralized with 0.1 M HCl using pH strip and volume was adjusted to the mark with methanol.

Heat induced acid hydrolysis

To study forced degradation in acidic medium 10.0 mg of mirabegron was transferred to 10 mL volumetric flask and 2 mL of 0.1 M HCl was added to the flask. The flask was kept at room temperature for 30 min. Solution was neutralized with 0.1 M NaOH using pH strip and volume was adjusted to the mark with methanol.

Oxidative stress degradation

To perform oxidative stress degradation study, 10.0 mg of mirabegron was transferred to 10 mL volumetric flask and 2 mL of 3% hydrogen peroxide was added. The flask was kept at room temperature for 30 min and volume was adjusted to the mark with methanol. Aliquot (1 mL) was pipetted out into 10 mL volumetric flask, diluted with methanol to obtain concentration of 100 μg/mL of mirabegron.

Study photolytic degradation

Analytically pure 10 mg of drug weighed and kept in petri dish under exposed to UV light for 48 hrs. The solids were allowed to cool and transferred to volumetric flask (10 mL) and dissolve in few mL of methanol. Volume was made up to the mark with the methanol.

Dry heat degradation

To study dry heat degradation, 10.0 mg of mirabegron was transferred to 10 mL volumetric flask and was exposed in oven at 80 °C for 3 h. The solid was allowed to cool and dissolved in few mL of

methanol. Volume was made up to the mark with the methanol.

From each of the above forced degradation studies, Aliquot (1.0 mL) was pipetted out into 10 mL volumetric flask, diluted with methanol to obtain concentration of 100 μg/mL of mirabegron. Solution was further diluted with the mobile phase to obtain final concentration of 10 μg/mL of mirabegron. The solution was analysed under proposed chromatographic conditions and chromatogram recorded. The amount of mirabegron was computed using regression equation.

System suitability

The system-suitability tests are integral part of gas and liquid chromatography. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests are based on concept that the equipment, electronics, analytical operations, and sample to be analysed constitute an integral system that can be evaluated as such. The system suitability parameters like capacity factor, theoretical plates and asymmetric factor were calculated and compared with standard values.

Analysis of marketed formulation

Twenty tablets were weighed and powdered; powder contain equivalent to 25 mg of mirabegron was transferred in to 25 mL volumetric flask containing 5 mL of methanol and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. This will produce sample solution containing mirabegron 1000 μg/mL. From the above solution, 1 mL of aliquot was pipetted out and transfers into another 10 mL volumetric flask and diluted to the mark with methanol to obtained sample solution containing mirabegron 100 μg/mL. From the above solution, 1 mL aliquot was pipette out into another 10 mL volumetric flask and diluted upto the mark with methanol. This will produce sample solution containing mirabegron 10 μg/mL. The Rt of the solution was measured at 247 nm and the quantification was carried out by keeping this values to the straight line of calibration curve.

Results and discussion

Optimization of mobile phase

An ideal wavelength is the one that gives good response of detection wavelength. Therefore, analytical wavelength of 247 nm was selected for estimation of mirabegron. The standard solution containing 10 μg/mL of mirabegron was chromatographed with use of different composition of mobile phases.

Mobile phase acetonitrile: HPLC grade water (50:50, v/v pH adjusted to 9) with 1 mL of 1% TEA gave sharp peak and good asymmetric factor so it was selected as a mobile phase for determination of mirabegron. The flow rate was maintained at 1 mL/min and the asymmetric factor was found to be 1.06 (Figure 2). Overlay chromatograms of mirabegron (0.01-20 µg/mL) are shown in Figure 3.

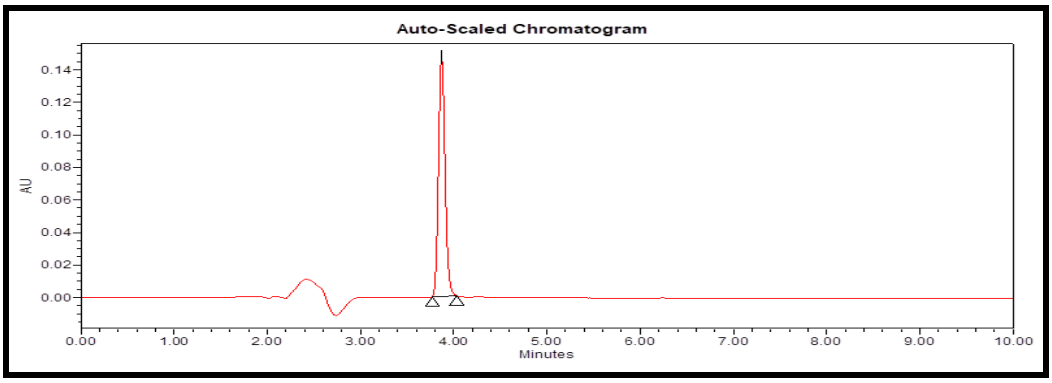


Figure 2. Chromatogram of mirabegron (10 µg/mL) using ACN: water (50:50, v/v pH adjusted to 9.0) with 1 mL 1% TEA

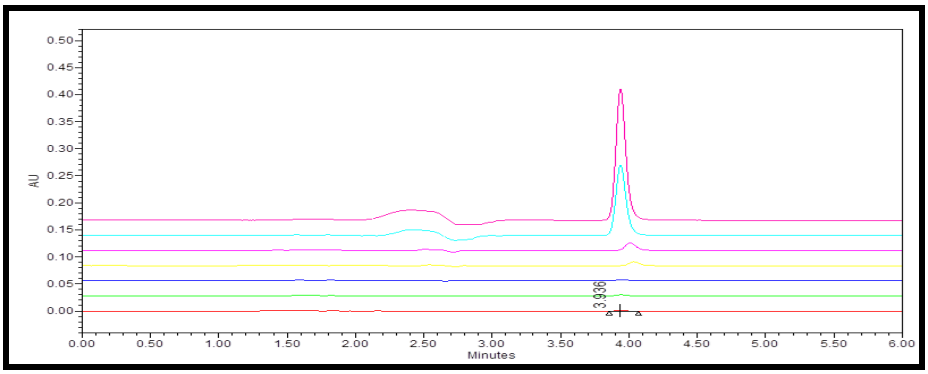


Figure 3. Overlain chromatogram of mirabegron (0.01-20 µg/mL) at 247 nm

Method validation

Linearity and calibration curve

The calibration curve of mirabegron was found to be between 0.0.1-20 µg/mL having correlation coefficient of 0.9975. The data of calibration curve and regression analysis of calibration curve are reported in Table 1.

Precision

The intra-day and inter-day precision were carried out and it was found to be 0.32-1.01 and 0.33-1.57 %RSD for mirabegron. Instrumental precision was determined by performing injection repeatability test and the %RSD was found to be 0.33%.

Table 1. Regression analysis of calibration curve

Parameters	Values
Linearity (µg/mL)	0.01- 20
Regression coefficient (R^2)	0.9975
Slope	63887
Standard deviation of slope	133.63
Intercept	11686
Standard deviation of intercept	66.25
Limit of detection (LOD)	0.006
Limit of quantification (LOQ)	0.01

Accuracy

The accuracy of the method was determined by calculating recoveries of mirabegron, where a known amount of standard was spiked into pre-analyzed sample solutions. The recoveries were found to be 99.39-101.27% for mirabegron Table 2.

Table 2. Result of accuracy study

Amount of drug from formulation (µg/mL)	Amount of standard drug spiked (µg/mL)	Amount of drug found (n=3) (µg/mL)	% Recovery± SD
8	0	7.99	99.89 ±0.04
8	6.4	14.45	100.70 ±0.11
8	8	16.02	100.31 ±0.07
8	9.6	17.70	101.27±0.44

Limit of detection and limit of quantification

The LOD and LOQ were carried out by visual method, where LOD for mirabegron was found to be 0.006 µg/mL and also LOQ was found to be 0.01 µg/mL. The summary of validation parameters are shown in Table 3.

Table 3. Summary of validation parameters of HPLC

Parameters	Mirabegron
Range (µg/mL)	0.01-20
Retention time	3.93
Detection limit (µg/mL)	0.006
Quantitation limit (µg/mL)	0.01
Accuracy (%)	99.39-101.27
Precision (%RSD)	
Intra-day (n=3)	0.32 - 1.01
Inter-day (n=3)	0.33 - 1.57
Instrument precision (%RSD)	
Repeatability (n=6)	0.33
Specificity	Specific

Specificity

The specificity was carried out to check the interference of excipients used in formulation. The developed method was found to be specific.

Robustness

In the robustness study, the % relative standard deviation was found to be less than 2% after introducing small, deliberate changes in instrumental parameters like change in flow rate, mobile phase ratio and detection wavelength in the developed HPLC method which shows the proposed method is robust.

System suitability test was carried out as per procedure mentioned in pharmacopeias and parameters also mentioned in experimental section.

Solution stability

Stability of standard and sample solution of mirabegron was evaluated at room temperature and test was carried out after time intervals. The drug was found to be stable for 48 hours with percentage amount of drug found more than 98%.

Analysis of marketed formulation

The proposed method is applied to the determination of mirabegron in their dosage form. The % amount of drug found to be more than 98%.

Forced degradation study

Chromatogram of base hydrolysis performed at 80 ºC for 30 min reflux indicated degradation of mirabegron with degradation item peak at retention time (Rt) 1.90, 3.37, 4.83, 6.25 (Figure 4). Chromatogram of acid hydrolysis performed at room temperature for 30 min demonstrated degradation of mirabegron with degradation peaks at retention time (Rt) 1.58, 1.92 (Figure 5).

The chromatogram of oxidized mirabegron with 3% hydrogen peroxide at room temperature for 30 min indicated degradation of mirabegron with degradation peaks at retention time (Rt) 0.63, 1.71, 3.02, 3.20 (Figure 6). The chromatogram of mirabegron open to UV light for 48 hrs indicated degradation of mirabegron with degradation peaks at retention time (Rt) 1.08, 3.35, 6.29, 12.04 (Figure 7). The chromatogram of mirabegron presented to dry heat at 80 ºC for 3 hrs indicated degradation of mirabegron with degradation peaks at retention time (Rt) 1.87 (Figure 8).

The degradation study about in this manner demonstrated that mirabegron was susceptible to base hydrolysis, acid hydrolysis, oxidation (3% hydrogen peroxide), photo degradation and dry heat and data are shown in Table 4. The degradation peaks were all around settled from the drug peak and no degradation product from various stressed conditions influenced estimation of mirabegron which show that the technique is selective and specific.

Table 4. Summary of data derived from forced degradation study by proposed HPLC method

Conditions	Time (h)	% Amount of drug found	Rt value of degradants of mirabegron
Base 1 N NaOH	30 min	81.73	1.90, 3.37, 4.83, 6.25
Acid 1 N HCl*	30 min (RT)	82.57	1.58, 1.92
3% Hydrogen peroxide	30 min (RT)	88.70	0.63, 1.71, 3.02, 3.20
UV-visible light	48 hrs	80.81	1.08, 3.35, 6.29, 12.04
Dry heat*	3 hrs	88.94	1.87

* Samples were heated at 80° for specified period of time

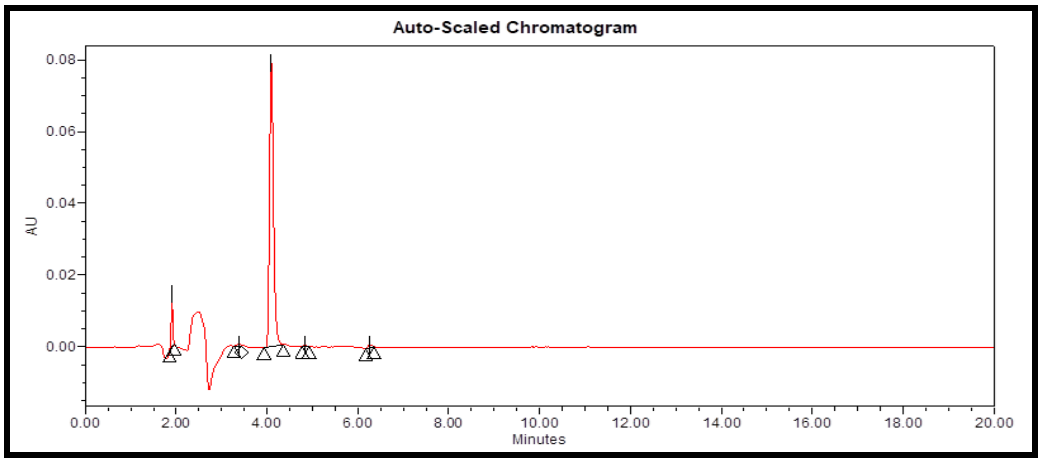


Figure 4. Chromatogram of alkali treated (0.1 N NaOH) mirabegron (10 µg/mL) reflux for 30 minutes at 80 °C

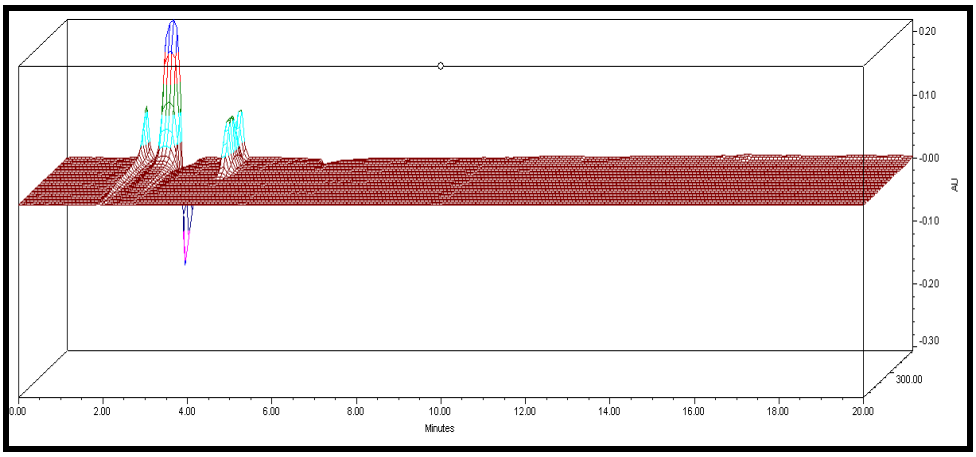


Figure 4a. 3D chromatogram of alkali treated (0.1 N NaOH) degraded mirabegron (10 µg/mL) reflux at 80 °C for 30 min

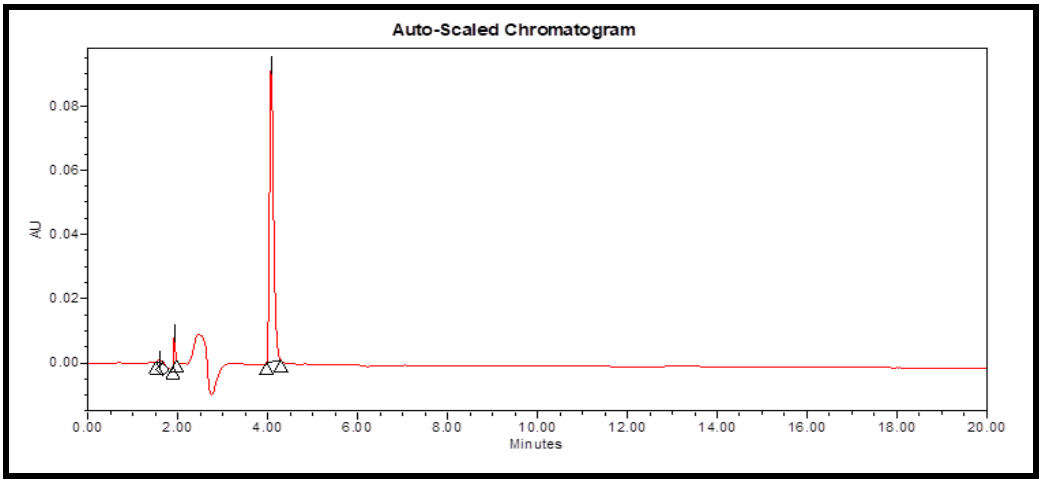


Figure 5. Chromatogram of acid treated (0.1 N HCl) degraded mirabegron (10 µg/mL) kept at room temp for 30 min

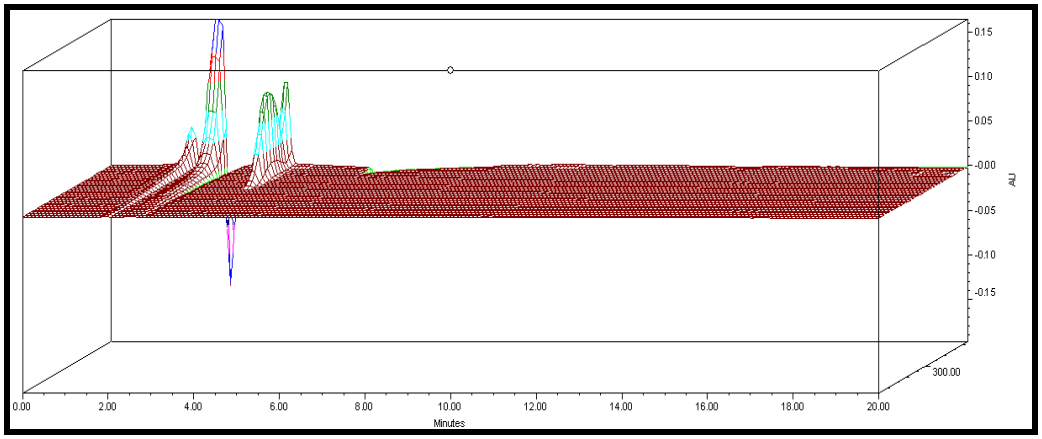


Figure 5a. 3D chromatogram of acid (0.1 N HCl) degraded mirabegron (10 µg/mL) kept at room temp for 30 min

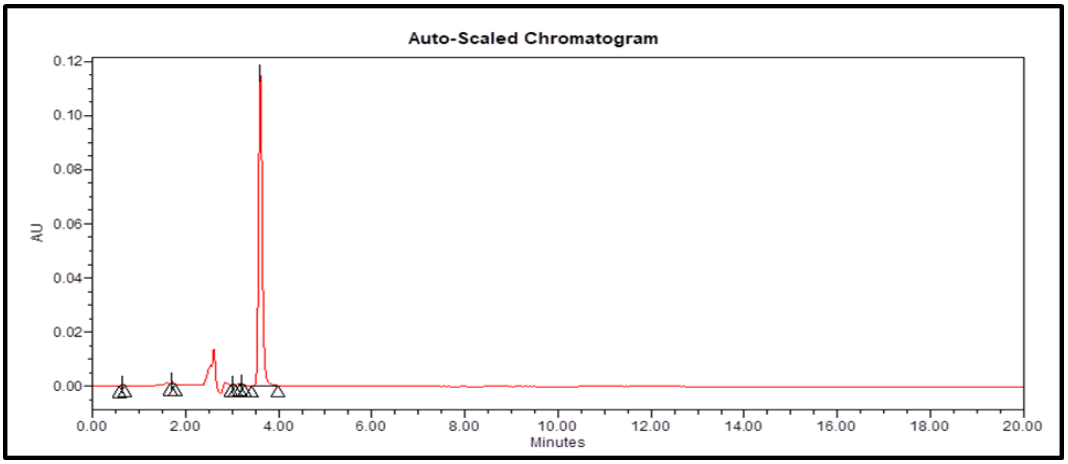


Figure 6. Chromatogram of chemical oxidation (3% H_2O_2) degraded mirabegron (10 µg/mL) kept at room temp for 30 min

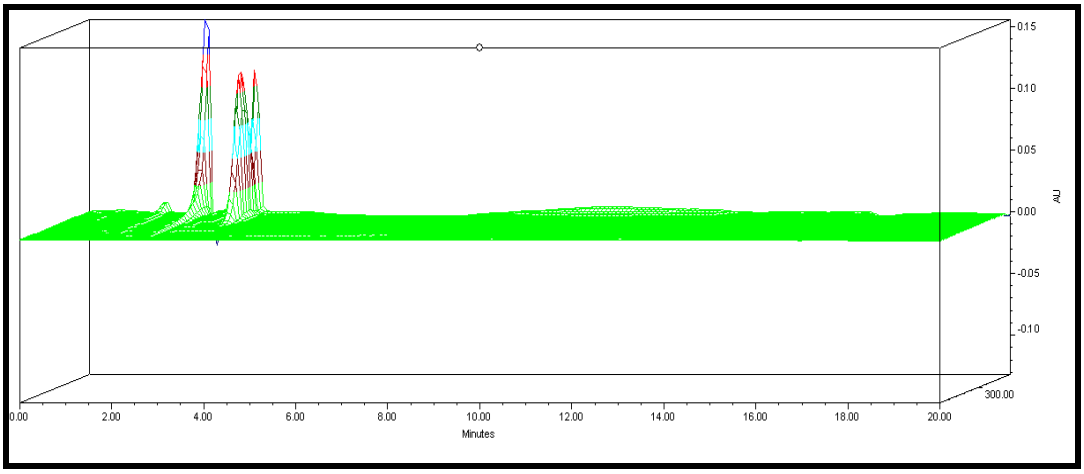


Figure 6a. 3D chromatogram of chemixal oxidation (3% H_2O_2) degraded mirabegron (10 µg/mL) kept at room temp for 30 min

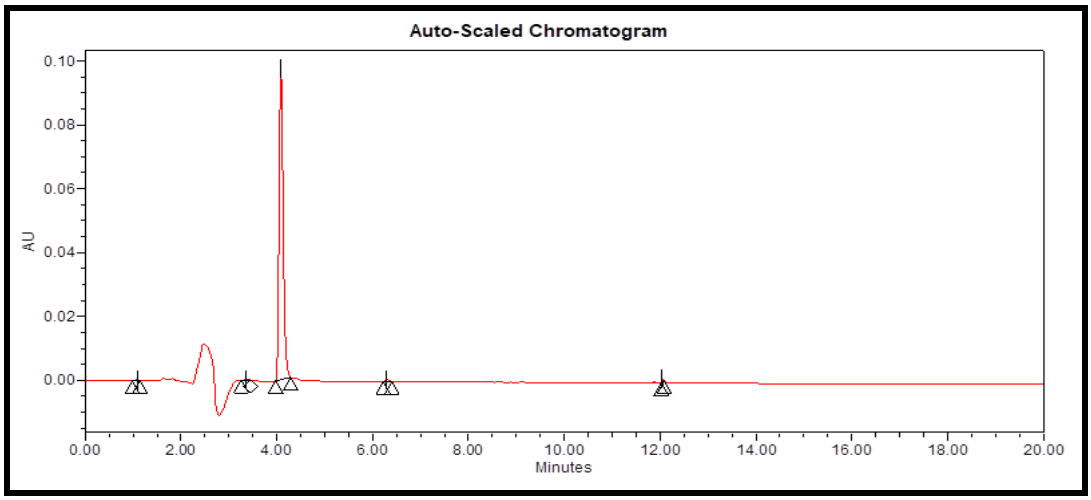


Figure 7. Chromatogram of photolytic degradation study of mirabegron (10 µg/mL) for 48 hrs

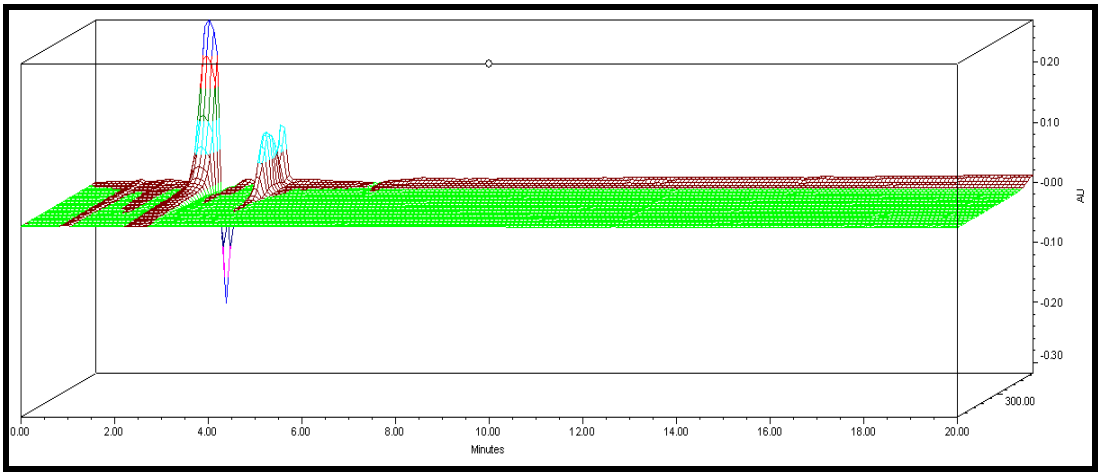


Figure 7a. 3D chromatogram of photolytic degradation study of mirabegron (10 µg/mL) for 48 hrs

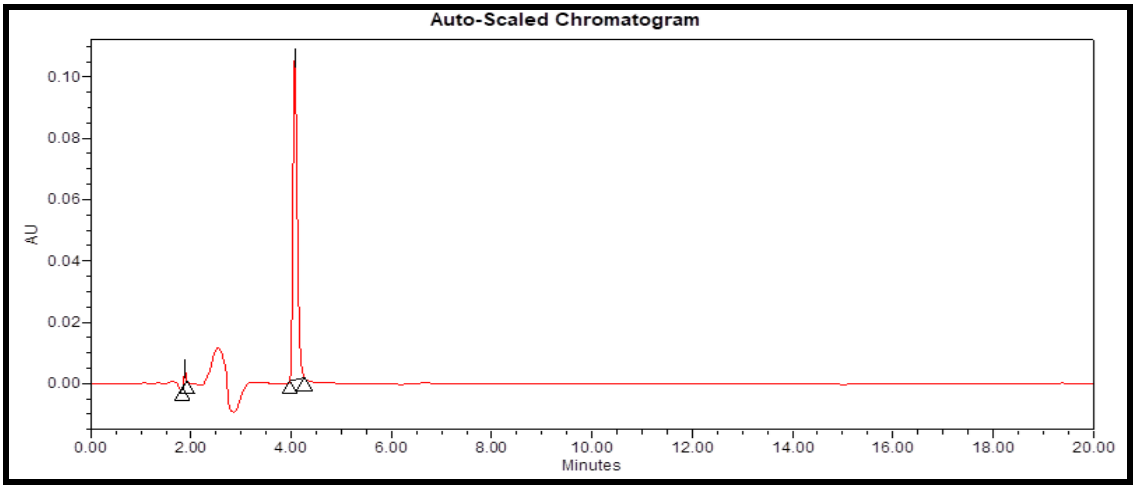


Figure 8. Chromatogram of dry heat degradation of mirabegron (10 µg/mL) at 80±5 °C for 3 hrs

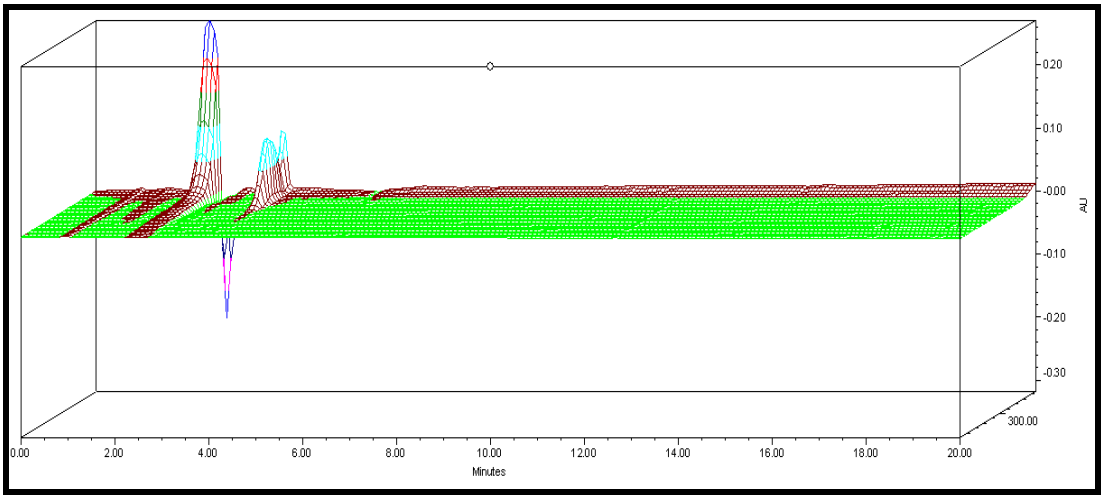


Figure 8a. 3D chromatogram of dry heat degradation study of mirabegron (10 µg/mL) at 80±5 °C for 3 h

Development and validation with result and discussion of UV-visible spectroscopic method

Selection of solvent

Solvents are chosen based on solubility, stability and UV-visible cutoff wavelength. Mirabegron is slightly soluble in aqueous solvent and soluble in organic solvent. Methanol was chosen as a solvent for preparation of stock solution as it is freely soluble.

Preparation of standard stock solution

For UV-visible spectrophotometric method, 10 mg of mirabegron was weighed accurately and transferred into a 10 mL volumetric flask containing little methanol (5 mL) and swirl to dissolve. Methanol was added up to the mark to produce a stock solution containing 1000 µg/mL of

mirabegron. Form the above solution pipette out 1 mL of aliquot and transfer into another 10 mL volumetric flask and diluted up to the mark with methanol to obtain standard stock solution of 100 µg/mL.

Selection of analytical wavelength

Standard solutions of mirabegron was scanned between 200-400 nm in UV-visible spectrophotometer and the solutions showed λ_{max} at 247 nm which was selected as the analytical wavelength.

Calibration curve

Standard stock solution was pipette out into nine separate 10 mL volumetric flask and volume was adjusted to the mark with distilled water to obtain final concentration of 2, 4, 6, 8, 10, 12, 14, 16 and 18 µg/mL.

Validation of UV- visible method

Validation of the developed UV-visible method was accomplished according to the international conference on harmonization (ICH) guidelines Q2 (R1) [9].

Linearity and range

The linearity of method was evaluated by analyzing calibration curves prepared at nine concentrations of mirabegron in range of 2-18 µg/mL for UV-visible spectrophotometric method and respective solutions of mirabegron. Linear regression equation was obtained over the concentration range (y = mx+c). Limit of detection (LOD) and limit of quantification (LOQ) were calculated from standard deviation of response and slope of calibration curve (n=6). Statistical data of mirabegron are shown in Table 5.

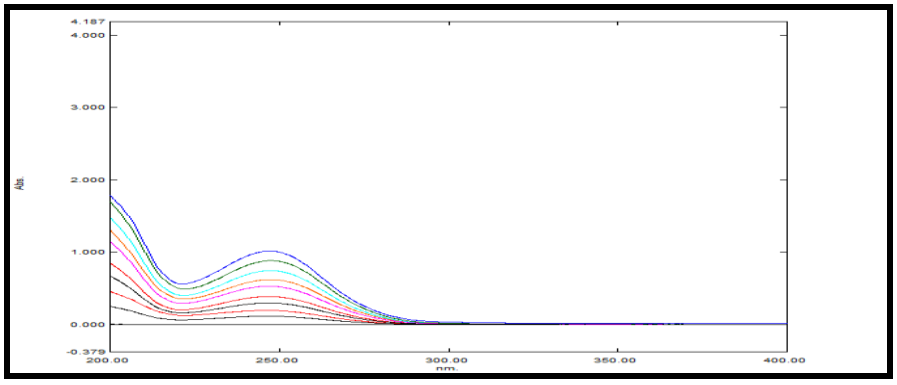


Figure 9. Overlay spectra of mirabegron (2-18 µg/mL) in water

Table 5. Statistical data for mirabegron by spectrophotometry method

Parameters	Mirabegron
Range (µg/mL)	2-18
Regression coefficient (r^2)	0.9955
Slope of regression equation	0.0524
Standard deviation of slope	0.0011
Intercept of regression	0.0178
Standard deviation of intercept	0.015

Accuracy

In order to check the accuracy of the developed methods, recovery studies were carried out from pre-analyzed sample at different levels of standard addition 0%, 80%, 100% and 120%. The percentage (%) Recovery was the average of determinations at each standard addition level which proved that the methods were nearly accurate Table 6.

Table 6. Accuracy data for mirabegron by the proposed UV–visible spectroscopic method

Quantity of drug taken from drug sample (µg/mL)	Quantity of standard drug spiked (µg/mL)	Quantity of drug found (µg/mL) (n=3)	% Recovery± SD (n=3)
8	0	7.94	99.28±0.38
8	6.4	14.66	101.82±0.21
8	8	16.21	101.35±1.52
8	9.6	17.94	101.94±0.43

Precision

Repeatability

Standard solution of mirabegron (10 µg/mL) was prepared and spectra were recorded. Absorbance was measured at 247 nm using water as a blank. The absorbance of the same concentration solution was measured six times and % relative standard deviation (%RSD) was calculated.

Intra and inter day precision

Variation of results of three different concentrations (6, 12 and 18 µg/mL) within the same day (intra-day) and variation of results between different days (inter-day) were analyzed. Reproducibility of the methods was checked by performing intra-day precision (three times a day) and inter-day precision (repeated triplicates for three consecutive days).

Limit of detection

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. Then LOD was measured by using mathematical expressions given in section. Calculation of limit of detection (LOD) of the drug by using the following equations adopted by international conference on harmonization (ICH) guideline:

$$LOD=3.3\ X\sigma/S$$

Where, σ = the standard deviation of the response at *y*–intercept, S = slope of the calibration curve.

Limit of quantification

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. Then LOQ was measured by using mathematical expressions given in section. Calculation of limit of quantification (LOQ) by using the following equations adopted by international conference on harmonization (ICH) guideline:

$$LOQ=10\ X\sigma/S$$

Where, σ = the standard deviation of the response at *y*-intercept S = slope of the calibration curve

Ruggedness

It is the degree of reproducibility of test results acquired by analyzing the same sample under variety of normal test conditions such as different analysts, instruments, days, reagents and make of columns. Ruggedness study was accomplished by analyzing drug using different analysts and different instruments means in laboratory two different UV-visible spectroscopy instrument used.

Solution stability

The stock solution of mirabegron (10 µg/mL) was prepared and stored in the room temperature, analyzed by UV at regular intervals 0, 12, 24 and 48 h. Summary of validation parameters are presented in Table 7.

Analysis of marketed formulation

Twenty tablets were weighed and powdered; powder equivalent to 25 mg of mirabegron was transferred in to 25 mL volumetric flask containing few mL of methanol and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol.

Table 7. Summary of validation parameters of spectrophotometry

Parameters	Mirabegron
Range (µg/mL)	2-18
Detection limit (µg/mL)	0.078
Quantitation limit (µg/mL)	0.24
Accuracy (%) (n=3)	99.28-101.94
Precision (%RSD)	
Intra-day (n=3)	0.32-1.56
Inter-day (n=3)	0.38-1.79
Repeatability (n=6)	1.09

The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. This will produce sample solution containing mirabegron 1000 µg/mL. From the above solution, 1 mL aliquot was pipetted out and transfers into another 10 mL volumetric flask and diluted to the mark with methanol to obtained sample solution containing mirabegron 100 µg/mL (stock solution). From the stock solution 1 mL was transferred into another 10 mL volumetric flask and volume was made up to the mark with water to give a solution containing 10 µg/mL mirabegron. The absorbance of solution was measured at 247 nm and the quantification was carried out by keeping these values to straight line equation of calibration curve. The percentage amount of drug was found to be more than 98%.

Comparison between LC and UV-visible spectroscopic method

The proposed two analytical methods were correlated using statistical analysis and student's *F*–test was exercised which does not reveal noteworthy difference between the experimental values acquired in the assay of sample analysis by the two methods. The calculated *F*-value (F_{cal}= 0.32) was found to be not more than the critical *F*-value (t_{cri}= 5.14) at 95% remarkable level hence it was concluded that both the methods do not differ appreciably. Therefore, both these methods can be conveniently used for routine quality control analysis of mirabegron in bulk and its pharmaceutical dosage form.

Conclusion

Acquainting RP-LC and UV-visible spectroscophotometric method in pharmaceutical analysis serve as a crucial step in quality assurance. The proposed study reveals that the stability indicating RP-LC and UV-visible spectroscophotometric methods used for the quantification of *β* adrenergic receptor mirabegron in bulk and its pharmaceutical dosage form. The methods were validated and found to be sensitive, accurate and precise. Statistical analysis concluded that method was repeatable and selective for the analysis of mirabegron deprived of any interruption from the excipients. Mirabegron was found to be

susceptible under alkali and acid hydrolysis, chemical oxidative conditions, dry heat and photolytic conditions. As the chromatographic method separate the drugs from its degradent products, can be applied for the analysis of sample acquired during accelerated stability experiments to anticipate expiration dates of pharmaceuticals.

Acknowledgements

All authors appreciate the SICART and Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, for providing necessary facilities to carry out research work.

Conflict of Interest

We have no conflicts of interest to disclose.

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How to cite this manuscript: Preeti N. Yadav, Usmangani K. Chhalotiya*, Kesha M. Patel, Jinal N. Tandel, Quantification of A *β* Adrenergic Receptor Drug Mirabegron by Stability Indicating Lc Method and Uv–visible Spectroscopic Method in Bulk and Pharmaceutical Dosage Form. Chemical Methodologies 4(3), 2020, 340-358. DOI:10.33945/SAMI/CHEMM.2020.3.11.