DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING HPLC METHOD FOR THE ESTIMATION OF IMPURITIES IN MIRABEGRON TABLETS

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ABSTRACT

This study focuses on the development and validation of a stability-indicating High-Performance Liquid Chromatography (HPLC) method for the estimation of impurities in Mirabegron tablets. The method was optimized for detecting impurities formed during storage, ensuring that mirabegron remains safe and effective throughout its shelf life. Key parameters such as system suitability, precision, accuracy, linearity, and robustness were validated following ICH guidelines. The method was also successfully applied to monitor stability under accelerated and long-term conditions. Results confirmed that the method is precise, reliable, and applicable for routine quality control of mirabegron tablets.

Keywords: Mirabegron, HPLC, impurity profiling, stability studies, β3-adrenoceptor agonist, method validation, pharmaceutical stability, ICH guidelines, precision, robustness.

1. INTRODUCTION

Pharmaceutical stability is a fundamental aspect of drug development, ensuring that medications remain effective, safe, and of high quality throughout their intended shelf life. Stability studies are necessary to determine how various factors, such as environmental conditions (temperature, humidity, light) and the drug's formulation, impact the potency and purity of a pharmaceutical product over time. Drugs may undergo physical, chemical, or microbiological changes during storage, which can lead to degradation or the formation of impurities that may compromise their efficacy or safety. Therefore, stability studies are critical in predicting the behavior of a drug under normal and extreme storage conditions. For the treatment of overactive bladder (OAB), mirabegron has emerged as an important pharmaceutical. As a β3-adrenoceptor agonist, mirabegron works by relaxing the bladder muscle, improving its ability to store urine and reducing the frequency of bladder contractions. It is commonly prescribed in two dosage forms—25 mg and 50 mg—and is known to be well-tolerated by most patients, as indicated by clinical trials conducted by Abrams et al. (2015) and Chapple et al. (2014). In particular, Japanese studies, like those from Yamaguchi et al. (2014), have shown that mirabegron is safe and effective in reducing symptoms of OAB, with fewer side effects compared to alternative therapies.

Despite its clinical benefits, the stability of mirabegron, especially in terms of impurity formation during storage, remains a critical concern. The presence of impurities, either from degradation of the active pharmaceutical ingredient (API) or during the manufacturing process, is a significant factor that can affect a drug's safety and efficacy. Impurities can arise due to environmental stress, chemical reactions, or interaction with excipients in the formulation. Regulatory bodies such as the International Council for Harmonisation (ICH) and the US Food and Drug Administration (FDA) set stringent guidelines for impurity profiling in pharmaceuticals, emphasizing the need for robust analytical methods to detect and quantify impurities in drug products. High-Performance Liquid Chromatography (HPLC) has become the gold standard for impurity profiling due to its ability to offer precise, sensitive, and reliable separation of complex mixtures (Shaikh et al., 2020).

2. LITERATURE REVIEW

The concept of pharmaceutical stability testing has undergone significant evolution over the past few decades. Initially, stability testing in pharmaceuticals was largely based on physical observations such as changes in color, dissolution rate, or the formation of precipitates. However, as analytical technologies advanced, the focus shifted towards more precise detection and quantification of chemical changes, including the formation of degradation products and impurities. This shift was largely driven by the need for improved safety and efficacy in pharmaceuticals, as outlined

by Alves et al. (2017), who demonstrated the importance of using advanced chromatographic techniques like Ultra-High-Performance Liquid Chromatography (UHPLC) for detecting impurities in fluoxetine formulations.

One of the most notable advancements in stability testing was the introduction of HPLC, which offered unprecedented accuracy in the separation and quantification of pharmaceutical compounds and their impurities. HPLC allows for the detection of trace-level impurities that may arise during manufacturing or storage. For example, Ravisankar et al. (2016) developed and validated a UV-spectrophotometric method specifically designed for quantitative determination of mirabegron, demonstrating its efficacy in detecting degradation products at very low concentrations. The use of HPLC in this context is highly advantageous, as it allows for robust separation of impurities from the main drug product, ensuring accurate profiling.

The International Council for Harmonisation's (ICH) guidelines on impurity profiling, specifically ICH Q3A(R2) and Q3B(R2), require that pharmaceutical products undergo rigorous testing to detect any impurities that may arise during synthesis or storage. These guidelines mandate specific limits for impurities based on the drug's daily dosage and safety profile. In cases where impurities exceed the prescribed limits, they must be identified and their potential impact on patient safety assessed. HPLC, as noted by Shaikh et al. (2020), plays a pivotal role in ensuring compliance with these regulatory standards by providing accurate quantification of even trace-level impurities in pharmaceutical formulations.

In the case of mirabegron, numerous studies have focused on its pharmacological efficacy, but fewer have addressed the stability of the drug during long-term storage. Studies by Yamaguchi et al. (2014) and Nitti et al. (2013) primarily focus on the clinical outcomes associated with the drug, but also highlight the need for rigorous stability testing to ensure that the therapeutic effects of the drug are maintained over time. As environmental factors such as temperature, humidity, and light can influence the degradation of the drug, an HPLC method capable of tracking these changes is crucial for maintaining product safety. Furthermore, the role of impurities in influencing drug stability has gained increasing attention. For instance, studies on other β3-adrenoceptor agonists and similar compounds have shown that degradation can lead to the formation of potentially harmful by-products, necessitating the development of precise analytical methods to monitor these changes (Ravisankar et al., 2016). This underscores the importance of method validation in ensuring that the HPLC technique is robust, reliable, and suitable for routine quality control of mirabegron tablets.

3. RESEARCH METHODOLOGY

This section outlines the detailed methodology used for the development and validation of a stability-indicating HPLC method for the estimation of impurities in mirabegron tablets. The methods used in this study align with the guidelines set by the International Council for Harmonisation (ICH) and the US Food and Drug Administration (FDA) for pharmaceutical stability testing and impurity profiling.

3.1 Chemicals and Reagents

- **Mirabegron Reference Standard:** The reference standard of mirabegron (99.5% purity) was obtained from a certified manufacturer.
- **Impurities Reference Standards:** The identified impurities, including Deshydroxy, Diamide-1, Diamide-2, and MIR-1, were sourced from standard pharmaceutical vendors.
- **Solvents:** HPLC-grade acetonitrile and methanol were purchased from Merck and Rankem. Milli-Q water was used for all aqueous preparations.
- **Buffers:** Ammonium acetate (Sigma-Aldrich) was used for buffer preparation in the mobile phase. The buffer pH was adjusted using acetic acid and ammonium hydroxide.

3.2 Instrumentation

- **HPLC System:** The analysis was performed using a Waters Alliance HPLC system equipped with a UV-Vis detector set to 240 nm for maximum absorbance detection.
- **Column:** Chromatographic separation was achieved using an Inertsil C8 (150 x 4.6 mm, 3.0 µm particle size) analytical column.
- **pH Meter:** A digital pH meter (Eutech Instruments) was used to adjust the pH of the buffer solutions.
- **Spectrophotometer:** UV/Vis spectrophotometer (Shimadzu) was used for wavelength absorbance determinations.

3.3 Preparation of Solutions

- **Mobile Phase:** The mobile phase was composed of 50 mM ammonium acetate buffer (pH 6.0) and acetonitrile. The gradient elution was prepared by adjusting the ratio of the mobile phase A (buffer) and mobile phase B (acetonitrile).
- **Standard Solution:** The mirabegron standard solution (0.5 mg/mL) was prepared by dissolving the reference standard in methanol. Impurities standards were also prepared by dissolving the reference standards in a mixture of water and acetonitrile (60:40 v/v).
- **Test Solution:** Mirabegron tablets were finely powdered, and an appropriate amount equivalent to 25 mg or 50 mg of mirabegron was dissolved in methanol, sonicated, and filtered through a 0.45 µm membrane filter.

3.4 HPLC Method Development

The method development process was carried out systematically by optimizing chromatographic conditions such as the mobile phase composition, flow rate, injection volume, column temperature, and wavelength detection.

- **Column Selection:** Inertsil C8 column was chosen based on preliminary trials that demonstrated good separation of mirabegron and its impurities.
- **Mobile Phase Composition:** The mobile phase was optimized using a gradient elution technique to ensure the efficient separation of mirabegron from its related impurities. Various buffer compositions and acetonitrile ratios were evaluated.
- **Flow Rate and Injection Volume:** The flow rate was set at 1.0 mL/min, and the injection volume was fixed at 15 µL.
- **Temperature:** The column oven temperature was maintained at 30°C during the trials.

3.5 Method Validation

The HPLC method was validated in accordance with ICH Q2(R1) guidelines, which cover aspects of system suitability, precision, accuracy, linearity, specificity, robustness, and detection limits.

3.5.1 System Suitability

The system suitability was tested by running six replicate injections of a standard solution to ensure the resolution, retention times, tailing factor, and column efficiency met the acceptance criteria. The relative standard deviation (RSD) of the peak area was calculated for both mirabegron and impurities.

3.5.2 Precision

- **Repeatability:** Six replicate injections of the sample and standard solutions were performed to evaluate repeatability. The %RSD for the peak areas and retention times of mirabegron and its impurities were calculated.

- **Intermediate Precision:** Precision was further assessed under different conditions, including changes in analysts, instruments, and days.

3.5.3 Accuracy

Accuracy was determined by recovery studies, where known amounts of mirabegron and impurities were spiked into the sample matrix. The recovery percentage was calculated by comparing the measured amount with the expected value.

3.5.4 Linearity

Linearity was assessed by injecting solutions of mirabegron and its impurities at five different concentrations, ranging from 50% to 150% of the target concentration. Calibration curves were plotted, and correlation coefficients (R^2) were calculated to confirm linearity.

3.5.5 Detection and Quantification Limits (LOD and LOQ)

The limits of detection (LOD) and quantification (LOQ) were determined based on the signal-to-noise ratio. LOD was defined as the concentration that produced a signal-to-noise ratio of 3:1, while LOQ was defined as the concentration that produced a ratio of 10:1.

3.5.6 Robustness

Robustness was evaluated by introducing small variations in the method parameters, such as changes in the mobile phase composition, flow rate, and column temperature. The effects of these changes on the peak resolution and retention times were monitored.

3.5.7 Specificity

Specificity was assessed by analyzing the placebo solution, where no interference from excipients was observed. The method was found to be selective for mirabegron and its related impurities.

3.6 Stability Studies

Stability studies were conducted under accelerated (40°C, 75% RH) and long-term (25°C, 60% RH) conditions. Samples were collected at predetermined time intervals (0, 3, 6, and 12 months) and analyzed using the validated HPLC method to monitor the formation of degradation products and impurities.

4. RESULT AND DISCUSSION

For the treatment of overactive bladder symptoms such as urge urine incontinence, urgency, and urinary frequency, mirabegron is used as a beta-3 adrenergic agonist. Two dosage forms of Mirabegron, 25 mg and 50 mg, are commercially marketed. After two weeks of taking 25 mg and 50 mg of Mirabegron, respectively, researchers evaluated for tolerance using 4 mg of Tolterodine extended release once daily for 12 weeks, and the findings validated tolerability [1-3]. Mirabegron, a β3-adrenoceptor agonist, was shown to be effective and safe in Japanese patients, with less adverse effects reported with the 50 mg dosage [4-7].

The colour of mirabegron powder ranges from white to light yellow. It dissolves well in dimethyl sulfoxide and has a neutral to slightly acidic solubility in water, methanol, and acidic pH solutions. The aqueous medium has a pH of 7.5. We have pKa values of 4.5 and 8.0.

4.1.1 Method development

Mirabegron and impurities solubility was evaluated with different pH solutions. Mirabegron is soluble in methanol, dimethyl sulfoxide, partially soluble in ethyl acetate. Mirabegron has high solubility in 0.1N Hydrochloric acid and pH 4.5 buffer. Mirabegron pKa value is 4.5.

UV absorbance

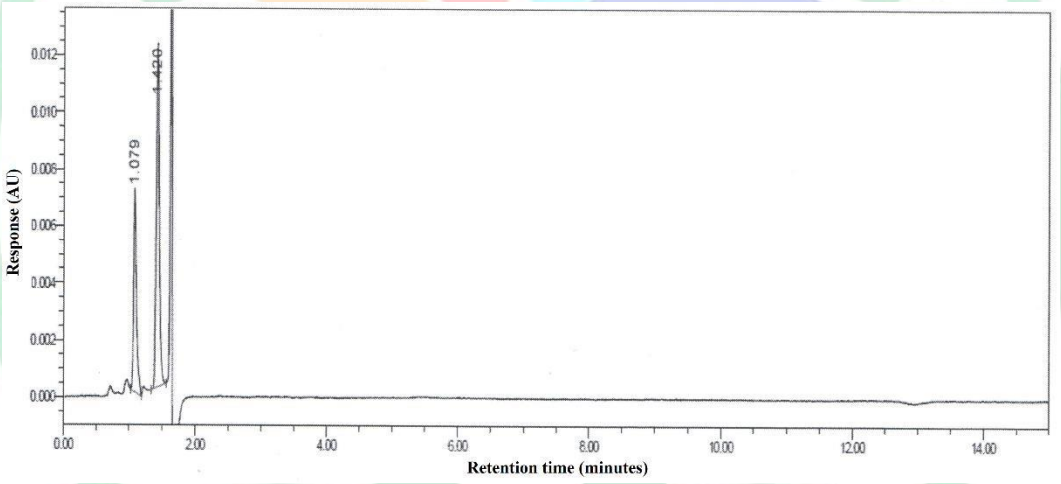
Wavelength absorbance for Mirabegron and all impurities were evaluated with UV/Visible spectrophotometer. Mirabegron and other impurities have maximum absorbance at 250 nm except MIR-1 impurity. MIR-1 impurity has maximum absorbance at 240 nm. Based on the maximum absorbance of the all analytes and MIR-1 impurity, 240 nm was selected for method optimization. There is no considerable absorbance change for all impurities.

Initial method development

HPLC method was started based on the above evaluations such as solubility, solutions pH, and UV absorbance and pKa values. Initial method conditions ammonium acetate, C18 150 mm column, acetonitrile, 240 nm was selected and performed the method development trial. 2-ATAA impurity was eluting at void volume, Des hydroxy impurity was eluting at the retention time of Mirabegron product peak.

Development trial-1

Waters Symmetry shield RP8 150*3.9 mm, 5.0 µ column, 30°C column oven temperature, 1.0 mL/min, 15 µL injection volume and 240 nm were used. Mobile phase composed with 50 mM Ammonium acetate with pH 6.0 and acetonitrile in the ratio of 95:5 v/v. Diluent: water and acetonitrile 60:40 v/v. Figure 4.1 represented the development chromatogram.



"Figure 4.1: Method development tria-1 chromatogram Observation: 2-ATAA impurity was eluted at void volume about 1.42 min.

Development trial-2

Inertsil C8 150 x 4.6 mm, 3.0 µ column, 30 °C column oven temperature, 1.0 mL/min, 15 µL injection volume and 240 nm were used. Mobile phase composed with 50mM CH3OONH4 Ph6.0 with 1.0% Tetra butyl ammonium hydrogen sulphate (TBAHS) and acetonitrile in the ratio of 95:5 v/v. Diluent: water and acetonitrile 60:40 v/v. Figure 4.2 represented the development chromatogram.

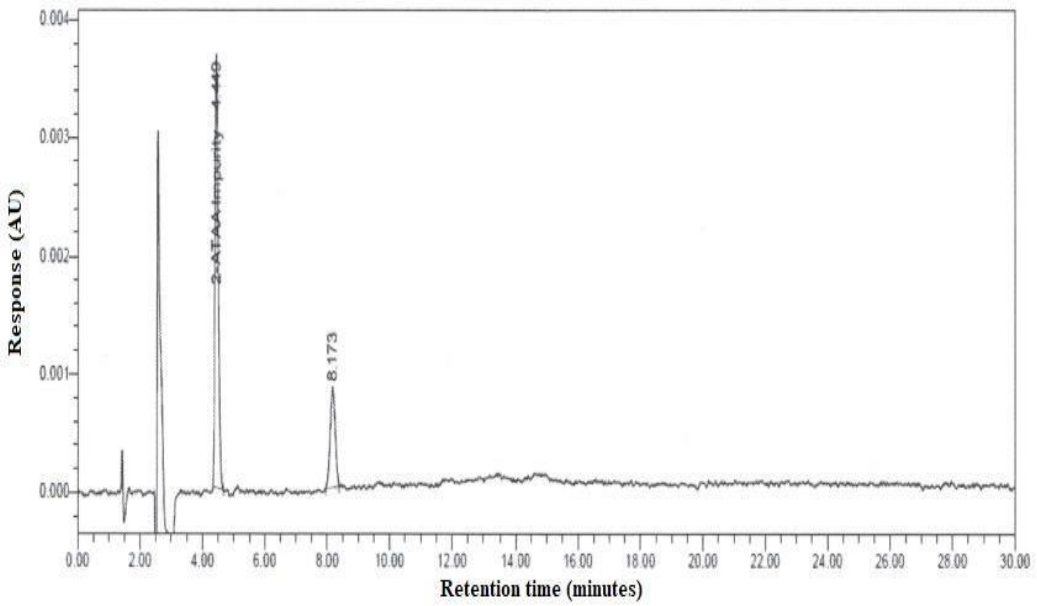


Figure 4.2: Method development trial 2 chromatogram Observation: 2-ATAA impurity was eluted at 4.4 min of retention time (RT). 2- ATAA impurity degradation peak was observed at 8.1 min RT.

Development trial-3

Inertsil C8 150 x 4.6 mm, 3.0 µ column, 30 °C column oven temperature, 1.0 mL/min, 15 µL injection volume and 240 nm were used. Mobile phase composed with sol-A: 50mM CH3OONH4 pH6.0 with 1.0 % TBAHS buffer, solution-B:

acetonitrile. Diluent: water and acetonitrile 60:40 v/v. Gradient elution program-1 was at 0min solution-B 10 %; at 7min solution-B 10 %; at 15min solution-B 35 %; at 25min solution-B 50 %; at 30min solution-B 50 %; at 40min solution-B 60%; at 45min solution-B 90 %; at 55min solution-B 90 %; at 56min solution-B 10%; at 70min solution-B 10 %. Gradient elution program-2 was at 0min solutionB 20 %; at 45min solution-B 80 %; at 55min solution-B 80 %; at 56min solutionB 10 %; at 70min solution-B 10 %. Figure 4.4 represented the development chromatogram.

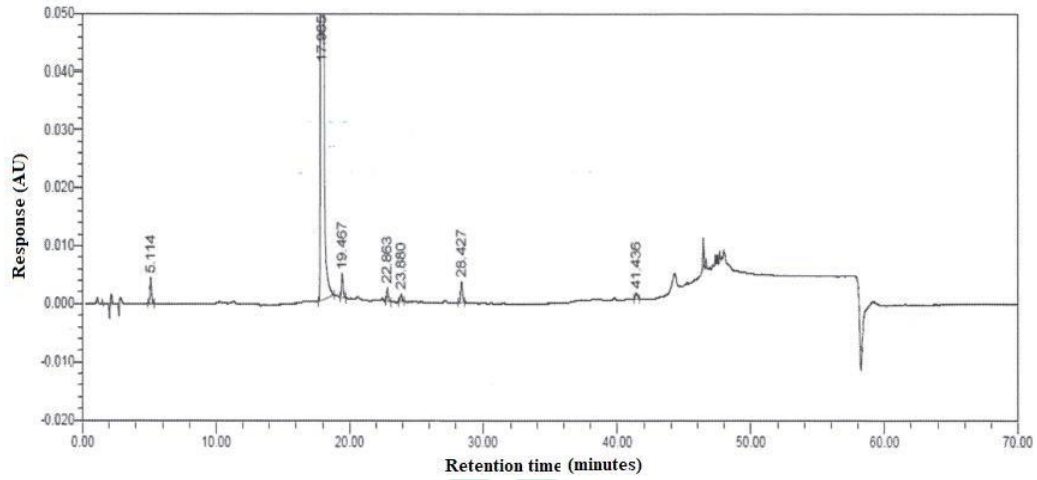


Figure 4.3: Method development trial 3 chromatogram

Observation: Des hydroxy impurity was separated from the Mirabegron peak but baseline noise was observed. Diluent

peaks were eluted at impurities retention time.

Development trial-4

Inertsil C8 150 x 4.6 mm, 3.0 µ column, 30 °C column oven temperature, 1.0 mL/min, 15µL injection volume and 240 nm were used. Buffer was 50 mM Ammonium acetate with 1.0 % TBAHS. Mobile phase composed with solution-A: buffer: acetonitrile 90:10 v/v; solution-B: buffer: acetonitrile 10:90 v/v. Diluent: water and acetonitrile 60:40 v/v. Gradient elution program was at 0 min solutionB 0 %; at 15 min solution-B 20 %; at 25 min solution-B 40 %; at 30 min solutionB 45 %; at 40min solution-B 50 %; at 45min and 55 min solution-B 90 %; at 56 min and 60 min solution-B 0%. Figure 4.3 represented the development chromatogram.

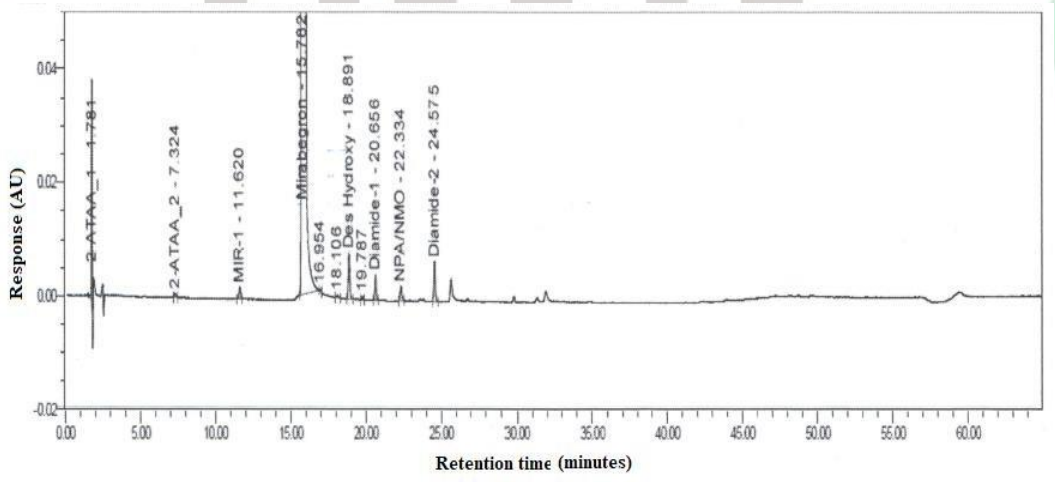


Figure 4.4: Method development trial 4 chromatogram

Observation: the 2-ATAA impurity was retained about 1.78 min. other degradant peak of 2-ATAA impurity was eluted at 7.3 min.

Development trial-5

Inertsil C8 150 x 4.6 mm, 3.0 µ column, 30 °C column oven temperature, 1.0ml/min, 15 µL injection volume and 240 nm were used. Buffer was 50 mM CH3OONH4 pH6.0 with 1.0% TBAHS. Mobile phase composed with solution-A: buffer: acetonitrile 90:10 v/v; solution-B: buffer: acetonitrile 20:80 v/v. Diluent: water and acetonitrile 60:40 v/v. Gradient elution program-1 was at 0 min solution-B 5 %; at 15 min solution-B 20 %; at 30 min and 36 min solution-B 40 %; at 44 min solution-B 50 %; at 47 min and 55 min solution-B 90 %; at 56 min and 65 min solution-B 5 %. Gradient elution program-2 was at 0min solution-B 5 %; at 15 min solution-B 20 %; at 30 min and 40 min solution-B 40 %; at 50 min solution-B 50 %; at 51min and 60 min solution-B 90 %; at 61min and 70 min solution-B 5 %. Figure 4.4 represented the development chromatogram.

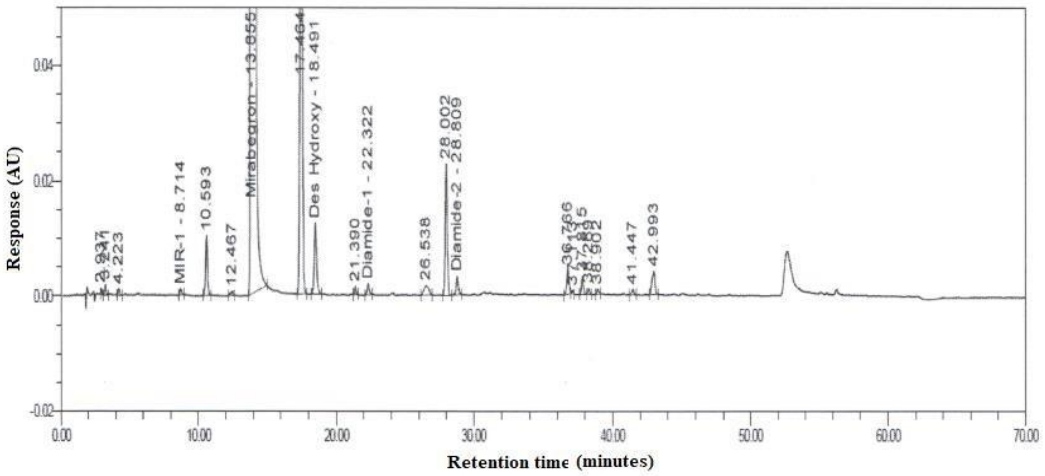


Figure 4.5: Method development trial 5 chromatogram Observation: Mirabegron and impurities were separated.

Further chromatographic conditions were eluted to finalize the method.

4.1.2 Method evaluation experiment

Method chromatographic conditions were evaluated with small change in pH, column oven temperature, and slight change in the gradient program, acetonitrile grade and water. These evaluations were used to finalize the HPLC method before method validation.

Column oven temperature 30°C and 40°C, buffer pH 5.5 and 7.0, milli-Q water Vs HPLC grade water (Rankem, Merck), Acetonitrile grades (Rankem, Merck) were evaluated. Method evaluation results were tabulated.

Table 4.1: Method evaluation results

HPLC method conditions	Observations
Column oven temperature 30°C / 40°C	40°C column temperature given well separation and good peak shape.
pH 6.0 / 7.0	pH 6.0 is suitable for all analytes with high resolution.
Water (Water filtered through Milli-Q system/ HPLC grade /Rankem or Merck)	Retention time 2.38 min unknown peak was observed due to Water filtered through Milli Q water. Merck grade was suitable and no unknown peak at 2.38 min.
Acetonitrile (Rankem / Merck)	Both grades were given good peak shape and resolutions between adjacent peaks.

RRT and RRF values

Relative retention time change was evaluated from the above all changes experiments and method development trials. Relative response factor for all impurities were established with 0.3 % and 0.6 % concentration standard solutions.

Figure 4.7 represented the RRF values establishment solution chromatogram. RRT, RRF values were tabulated below.

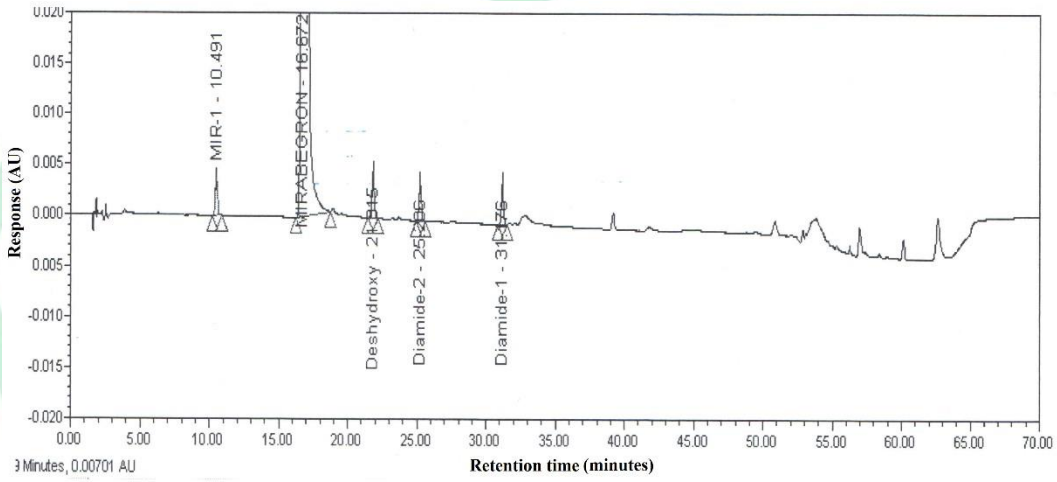


Figure 4.6: RRF values establishment solution chromatogram

Table 4.2: RRT and RRF values

Analyte name	RRT values	RRF values
MIR-1 impurity	0.63	0.74
Mirabegron	1.00	1.00
Des Hydroxy impurity	1.33	0.97
Diamide 1 impurity	1.56	0.70
Diamide 2 impurity	1.98	0.86

4.1.3 Method validation

HPLC method validation was performed as per the ICH, FDA, EMA guidance's. Method validation parameters precision, linearity, accuracy, LOD, LOQ, ruggedness and robustness were performed.

Precision

Precision with newly prepared norm and test solutions has been carried out. Figures 4.7 to 10 reflected blank, placebo, norm and test solution. There have been six preparations. Medium accuracy was achieved with various HPLC, columns and analysts. Table 4.4 and 2.5 showed the results.

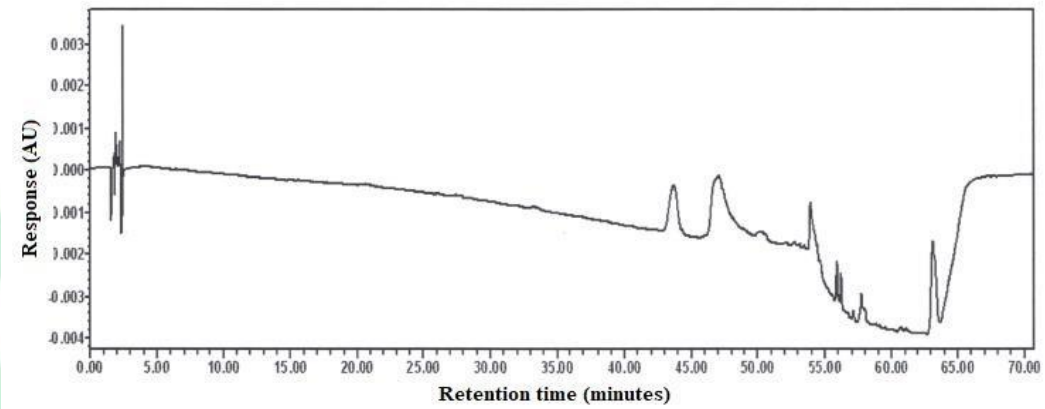


Figure 4.7: Blank chromatogram

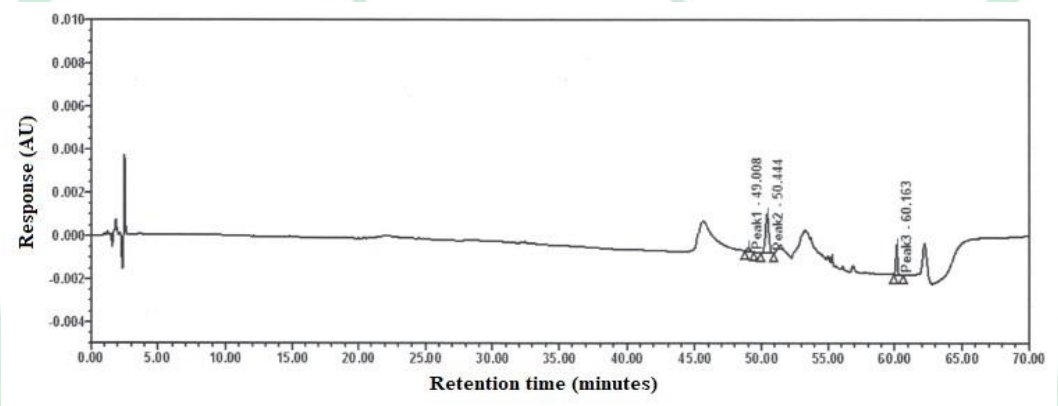


Figure 4.8: Placebo chromatogram

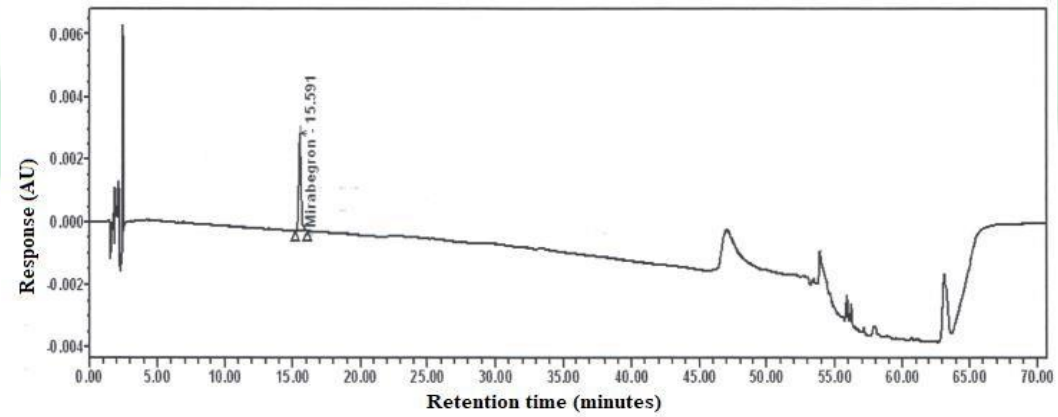


Figure 4.9: Standard solution chromatogram

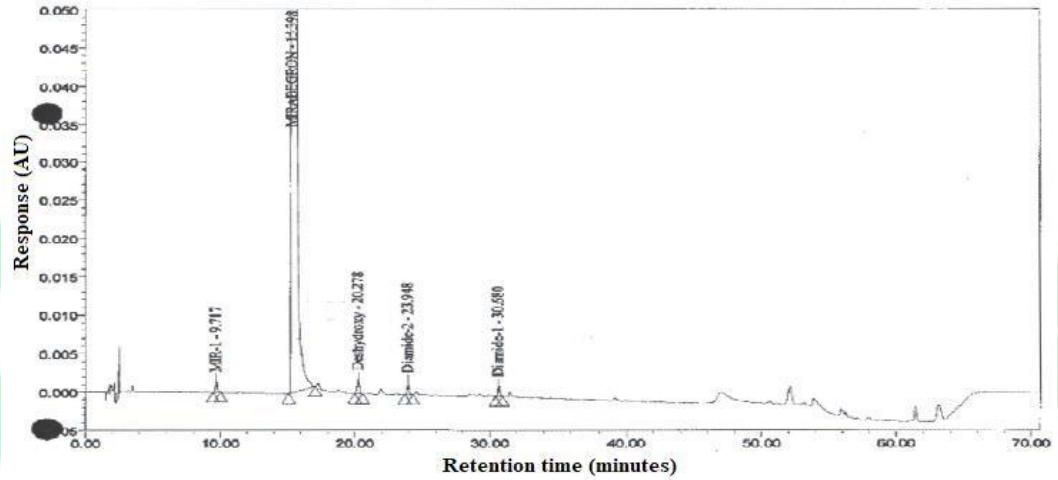


Figure 4.10: Mirabegron tablets 25 mg test solution chromatogram

Table 4.3:

S.No.	Percentage Impurities (%)				
	MIR-1	**Deshydroxy**	**Diamide-2**	**Diamide-1**	**Mirabegron**
1	0.160	0.194	0.185	0.153	0.217
2	0.159	0.184	0.184	0.152	0.213
3	0.152	0.187	0.187	0.153	0.215
4	0.153	0.185	0.185	0.152	0.210
5	0.153	0.184	0.184	0.152	0.208
6	0.153	0.186	0.186	0.153	0.212
Avg.	0.161	0.185	0.185	0.153	0.213
% RSD	2.6	0.6	0.6	0.4	1.5
Confidence interval at 95%	0.153 & 0.159	0.184 & 0.191	0.184 & 0.186	0.153 & 0.153	0.210 & 0.216

Precision results

S.No.	Percentage Impurities (%)				
	MIR-1	**Deshydroxy**	**Diamide-2**	**Diamide-1**	**Mirabegron**
1	0.158	0.181	0.195	0.156	0.2057

2	0.156	0.184	0.193	0.155	0.2072
3	0.159	0.184	0.194	0.155	0.2012
4	0.160	0.183	0.196	0.156	0.2033
5	0.160	0.185	0.196	0.157	0.2118
6	0.156	0.184	0.196	0.155	0.2086
Avg.	0.158	0.184	0.195	0.156	0.2063
% RSD	1.2	0.7	0.6	0.5	1.8
Confidence interval at 95%	0.157 & 0.159	0.183 & 0.185	0.194 & 0.196	0.155 & 0.157	0.2033 & 0.2093

Table 4.4: Intermediate precision results

Ruggedness

Ruggedness of the method was evaluated at room temperature storage and refrigerator conditions. Day-1, 2 and 6 were confirmed for room temperature and day-0 and 6 were evaluated for refrigerator conditions. Table 4.11 represented the ruggedness results.

Table 4.11: Ruggedness results

Time in day	MIR-1 impurity				Deshydroxy impurity			
	Test-1	Test-2	Difference		Test-1	Test-2	Difference	
			Test-1	Test-2			Test-1	Test-2
Initial	0.1568	0.1573	NA	NA	0.1873	0.1893	NA	NA
Day-1	0.1588	0.1615	0.00	0.00	0.1910	0.1901	0.00	0.00
Day-2	0.1609	0.1596	0.00	0.00	0.1908	0.1911	0.00	0.00
Day-6	0.2847	0.2602	0.13	0.10	0.1848	0.1928	0.00	0.00
	Diamide-2 impurity				Diamide-1 impurity			
Initial	0.1981	0.1993	NA	NA	0.1582	0.1582	NA	NA

Day-1	0.2002	0.1991	0.00	0.00	0.1623	0.1601	0.00	0.00
Day-2	0.2028	0.2000	0.00	0.00	0.1621	0.1601	0.00	0.00
Day-6	0.1885	0.1970	0.01	0.00	0.1523	0.1626	0.01	0.00
	Total impurities				MIR-1 impurity Refrigerator stability			
Initial	1.1085	1.1113	NA	NA	0.1568	0.1573	NA	NA
Day-1	1.1465	1.1548	3.4	3.9	NA			
Day-2	1.1578	1.1459	4.4	3.1				
Day-6	1.4192	1.1094	28.0	0.2	0.1591	0.2104	0.00	0.05
	Deshydroxy Refrigerator stability				Diamide-2 impurity Refrigerator stability			
Initial	0.1873	0.1893	NA	NA	0.1981	0.1993	NA	NA
Day-6	0.1897	0.1940	0.00	0.00	0.1987	0.2007	0.00	0.00
	Diamide-1 impurity Refrigerator stability				Total impurities Refrigerator stability			
Initial	0.1582	0.1582	NA	NA	1.1085	1.1113	NA	NA
Day-6	0.1622	0.1635	0.00	0.01	1.1428	1.1825	3.1	6.4

Robustness

Robustness was evaluated for mobile phase flow rate, column oven temperature, mobile phase pH, mobile phase B aqueous and mobile phase A organic solvent ratio variations. Filter validation was performed PVDF and NYLON filters. Table 4.12 and 4.13 represented the robustness results.

Table 4.12: Results of Effect of variations

Condition	**Flow rate**			**Column temperature**		
	0.8 ml/min	**1.0 ml/min**	**1.2 ml/min**	**35°C**	**40°C**	**45°C**
Tailing factor	1.1	1.1	1.1	1.1	1.1	1.1
% RSD	0.3	1.1	0.7	0.3	1.1	0.2

	Mobile phase pH			M.P.-B aqueous solution ratio		
	pH 5.8	pH 6.0	pH 6.2	90%	100%	110%
Tailing factor	1.2	1.1	1.2	1.0	1.1	1.0
% RSD	4.7	1.1	2.4	0.8	1.1	0.1
	Mobile phase A acetonitrile ratio					
	90%	100%	110%			
Tailing factor	1.1	1.1	1.0			
% RSD	0.5	1.1	0.5			

Table 4.13: Filter Variability results

Impurity name	Centrifuged		Nylon filter				PVDF filter			
	% impurity		% impurity		% Difference		% impurity		% Difference	
	Spl-1	Spl-2	Spl-1	Spl-2	Spl-1	Spl-2	Spl-1	Spl-2	Spl-1	Spl-2
MIR-1	0.403	0.399	0.404	0.399	0.00	0.00	0.415	0.399	0.01	0.00
Deshydroxy	0.445	0.444	0.453	0.446	0.01	0.00	0.446	0.445	0.00	0.00
Diamide-2	0.453	0.448	0.457	0.451	0.00	0.00	0.452	0.452	0.00	0.00
Diamide-1	0.472	0.463	0.473	0.468	0.00	0.01	0.468	0.469	0.00	0.01
MSUI	0.074 (RRT 1.42)	0.080 (RRT 1.42)	0.077 (RRT 1.42)	0.068 (RRT 1.42)	NA	NA	0.076 (RRT 1.42)	0.073 (RRT 1.42)	NA	NA
Total imp.	2.195	2.164	2.223	2.192	0.02	0.01	2.223	2.192	0.03	0.03

5 CONCLUSION

Stability indicating simple, accuracy and robustness HPLC method was developed and validated for the estimation of known and unknown impurities in Mirabegron tablet dosage form. Mirabegron synthetic and formulation related

impurities were evaluated and analyzed. Method validation was performed for precision, accuracy, linearity, LOD, LOQ, robustness and ruggedness. Precision results confirmed the %RSD NMT 2.0 % and tailing factor NMT 2.0 results. Linearity results correlation coefficient above 0.999, accuracy (recovery) results 97 % to 103 %, S/N ratio for LOD and LOQ were confirmed the method intended usage.

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