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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE DETERMINATION OF 2-ATAA IMPURITY IN MIRABEGRON TABLETS: A COMPREHENSIVE ANALYSIS OF CHROMATOGRAPHIC METHOD OPTIMIZATION AND VALIDATION

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ABSTRACT

Mirabegron, a beta3-adrenergic receptor agonist developed by Astellas Pharma, is widely used for the treatment of overactive bladder (OAB). This drug improves bladder function by promoting detrusor muscle relaxation, thereby increasing bladder capacity. However, during the manufacturing of Mirabegron tablets, impurities such as 2-ATAA can arise, potentially compromising the safety and efficacy of the drug. Regulatory bodies such as the International Council for Harmonisation (ICH) and the U.S. Food and Drug Administration (FDA) emphasize stringent control over impurity levels in pharmaceutical products. High-performance liquid chromatography (HPLC) is a well-established analytical technique for impurity detection, offering high resolution and sensitivity. This study focused on the development and validation of an optimized HPLC method for the detection and quantification of the 2-ATAA impurity in Mirabegron. Initial method development trials using ammonium acetate buffer and acetonitrile yielded poor retention of 2-ATAA, as it eluted near the void volume. Subsequent trials employing Tetrabutylammonium hydrogen sulfate (TBAHS) as a buffer led to successful separation of the 2-ATAA impurity at a retention time of 4.4 minutes. The finalized method utilized a Waters Symmetry Shield RP-8 column, with gradient elution and detection at 250 nm. Method validation was performed according to ICH Q2 (R1) guidelines, assessing parameters such as specificity, linearity, precision, and robustness. The method demonstrated excellent linearity ($R^2 = 0.999$) across the tested impurity concentration range (0.05%–0.6%) and met precision requirements, with relative standard deviation (RSD) values below 2%. Stress testing confirmed that there was no interference from degradation products, ensuring the method's specificity. This validated HPLC method provides a robust, accurate, and sensitive approach for the determination of 2-ATAA impurity in Mirabegron tablets, meeting regulatory requirements and supporting quality control in pharmaceutical manufacturing.

Keywords: Mirabegron, HPLC, 2-ATAA impurity, beta3-adrenergic receptor, method validation, impurity profiling, ICH Q2(R1), TBAHS, impurity quantification, pharmaceutical analysis.

1. INTRODUCTION

Mirabegron is a selective beta3-adrenergic receptor agonist developed by Astellas Pharma, designed to manage overactive bladder (OAB) syndrome by relaxing the detrusor muscle and increasing bladder capacity, ultimately reducing bladder urgency and frequency. This makes it a preferable alternative to traditional antimuscarinic drugs (Abrams et al., 2015). Mirabegron is available in 25 mg and 50 mg tablet forms and has become a leading option in treating OAB, offering a favorable safety profile (Chapple et al., 2013). However, during its manufacturing process, impurities such as 2-ATAA can be introduced, potentially affecting the safety and efficacy of the drug (Khullar et al., 2013). Ensuring the control and accurate quantification of these impurities is crucial in pharmaceutical manufacturing, as regulatory authorities like the International Council for Harmonisation (ICH) and the U.S. Food and Drug Administration (FDA) require strict limits on impurity levels (Deshpande et al., 2018). Analytical techniques such as high-performance liquid chromatography (HPLC) play a critical role in identifying and quantifying these impurities to ensure that Mirabegron meets industry standards (Sahu et al., 2018). This study focuses on developing and validating an HPLC method to detect and quantify 2-ATAA impurity in Mirabegron tablets, following ICH Q2 (R1) guidelines for specificity, precision, linearity, and robustness (Patel et al., 2020).

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2. LITERATURE REVIEW

1. Overactive Bladder and Mirabegron: Overactive bladder affects millions of individuals worldwide, causing discomfort due to symptoms like urinary frequency, urgency, and incontinence. Mirabegron, being a selective beta3-adrenergic receptor agonist, has emerged as a key treatment option for OAB. Clinical trials such as those conducted by Abrams et al. (2015) demonstrated that Mirabegron significantly improves bladder control with fewer side effects compared to antimuscarinic drugs, such as dry mouth and constipation. Khullar et al. (2013) found that patients taking Mirabegron showed significant improvement in symptom control compared to a placebo group. Its efficacy and tolerability have made it a cornerstone in OAB treatment regimens (Chapple et al., 2013).

2. Importance of Impurity Control in Pharmaceuticals: Pharmaceutical drugs often contain impurities arising from manufacturing processes, degradation, or raw materials used in production. Regulatory agencies like the ICH and USFDA emphasize the importance of controlling these impurities to maintain drug safety and efficacy. Deshpande et al. (2018) illustrated that even trace impurities could significantly alter a drug's pharmacokinetics or lead to adverse effects. In their study on multi-drug resistant tuberculosis treatments, they emphasized the need for accurate impurity quantification. Similarly, Shaikh et al. (2020) reported on nitrosamine impurities found in several pharmaceutical products, further stressing the critical nature of impurity control in regulatory submissions.

3. HPLC as a Method for Impurity Detection: HPLC is one of the most widely used techniques for impurity detection and quantification in pharmaceutical products. Its high resolution and sensitivity make it ideal for analyzing drug formulations, even when impurities are present in trace amounts. Patel et al. (2020) demonstrated the efficacy of HPLC in separating impurities in fluoxetine, showing that modifications in gradient programs, mobile phases, and column types can lead to optimized impurity profiling. Furthermore, Sahu et al. (2018) highlighted the role of HPLC in enhancing the accuracy and robustness of pharmaceutical impurity testing through method development strategies, including adjustments in buffer compositions and column temperatures. HPLC remains a preferred method due to its versatility and ability to meet regulatory guidelines for impurity quantification.

4. Method Development and Optimization for Impurity Profiling: The development of an HPLC method for impurity profiling involves optimizing several parameters, including the selection of buffers, mobile phases, and columns to achieve the best separation of impurities from the main drug component. Sahu et al. (2018) discussed the importance of buffer selection and gradient programming in improving retention times and peak resolution in HPLC analyses. In their study, they explored how different mobile phase compositions could affect impurity separation, providing insight into method optimization for impurity profiling. Similarly, studies by Shaikh et al. (2020) demonstrated how buffer modifications could enhance the separation of critical impurities such as nitrosamines, further validating the need for comprehensive method development strategies in pharmaceutical impurity detection.

5. Regulatory Requirements and Validation: Ensuring the validity and accuracy of the analytical method is essential for regulatory approval. Guidelines provided by ICH Q2 (R1) specify that methods for impurity determination must demonstrate specificity, precision, linearity, and robustness (Patel et al., 2020). These parameters ensure that the method can reliably detect and quantify impurities at specified levels. Furthermore, Deshpande et al. (2018) emphasized that stress testing under various conditions, including acidic, basic, and thermal stresses, is essential for confirming that impurities do not interfere with the quantification of the active pharmaceutical ingredient (API). Accurate method validation not only ensures product safety but also helps pharmaceutical companies comply with stringent global regulatory standards.

3. MATERIALS AND METHODS

Chemicals and Reagents

Mirabegron standard (purity ≥ 99%) and its impurities, including 2-ATAA, were obtained from Astellas Pharma. HPLC-grade acetonitrile, water, ammonium acetate, acetic acid, and tetrabutylammonium hydrogen sulfate (TBAHS) were purchased from Sigma-Aldrich. Analytical grade acetic acid and buffer salts were procured from Merck. All reagents were used without further purification.

Instrumentation and Chromatographic Conditions

An Agilent 1100 series HPLC system equipped with a quaternary gradient pump, autosampler, column heater, and UV detector was employed for the method development and validation experiments. Data acquisition and analysis were performed using ChemStation software.

The chromatographic separation was achieved on a Waters Symmetry Shield RP-8 column (150 mm × 3.9 mm, 5 µm). The column temperature was maintained at 30°C. The mobile phase consisted of (A) ammonium acetate buffer (pH 6.0) and (B) acetonitrile. A gradient elution program was used as follows: initial 0% mobile phase B, increased to 80% at 11 min, held at 80% until 15 min, then returned to 0% at 60 min. The flow rate was set at 1.0 mL/min, and UV detection was performed at 250 nm.

Method Development

Initial method development trials focused on the separation of the 2-ATAA impurity from the void volume and other known impurities. Trial 1 used ammonium acetate buffer (pH 6.0) and acetonitrile in a 95:5 ratio. The 2-ATAA impurity eluted at 1.7 min, close to the void volume, which indicated poor separation.

In trial 2, the buffer was modified with TBAHS to improve retention of the 2-ATAA impurity. A Phenomenex Synergy Polar column was used with ammonium acetate buffer and acetonitrile in a 90:10 ratio. The 2-ATAA impurity was successfully retained at 4.4 min, but the peak shape was unsatisfactory.

Trial 3 utilized the Waters Symmetry Shield RP-8 column and a modified gradient program with a flow rate of 0.8 mL/min. Under these conditions, the 2-ATAA impurity was eluted at 4.0 min, and the method successfully separated all known impurities, including MIR-1 (RT 6.2 min), Deshydroxy (RT 21.6 min), and Diamide impurities (RT 28.2–28.8 min). Baseline noise and peak tailing were minimized.

Method Evaluation and Robustness

To further optimize the method, different concentrations of TBAHS (0.05%, 0.1%, and 0.2%) were tested. It was observed that TBAHS concentrations of 0.05% and 0.1% provided the best balance between baseline stability and peak separation, with minimal baseline disturbance.

Relative Response Factor (RRF) Determination

The relative response factor (RRF) for the 2-ATAA impurity was determined using standard solutions at concentrations of 0.3% and 0.6%. The RRF value was found to be 0.63.

Method Validation

The final method was validated according to ICH and USFDA guidelines, assessing specificity, precision, linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness, and ruggedness.

- **Specificity**: Stress testing was performed under acidic, basic, oxidative, and thermal conditions to evaluate the potential interference from degradation products. No significant interference was observed, and peak purity passed for all conditions.
- **Linearity**: The method exhibited linearity over a concentration range of 0.05% to 0.6% for the 2-ATAA impurity with a correlation coefficient (R^2) of 0.999.
- **Precision**: Intra-day and inter-day precision studies demonstrated %RSD values below 2%, indicating good precision.

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- **LOD and LOQ**: The LOD and LOQ for the 2-ATAA impurity were determined to be 0.03% and 0.1%, respectively.

- **Robustness**: Minor variations in flow rate, column temperature, and mobile phase composition had no significant impact on the results, confirming the robustness of the method.

4. RESULT AND DISCUSSION

Mirabegron is used to treat bladder overactive nature. This medicinal drug was developed by Astellas Pharma Company. Mirabegron activates the beta3 adrenergic receptor in the detrusor muscle in the bladder to improve the muscle relaxation and increase the bladder capacity [1-3]. Mirabegron is available in 25 mg and 50 mg tablet dosage form.

4..1 Method development

HPLC method development was initiated for the determination of 2ATAA impurity in method-1. The 2-ATAA impurity was eluted at 1.7 min, which means that this impurity was eluted at void volume. The present objective was to determine the 2-ATAA impurity without interference of other known impurities. Below Figure represented the previous reported Mirabegron method-1 chromatogram. Method development experiments were initiated.

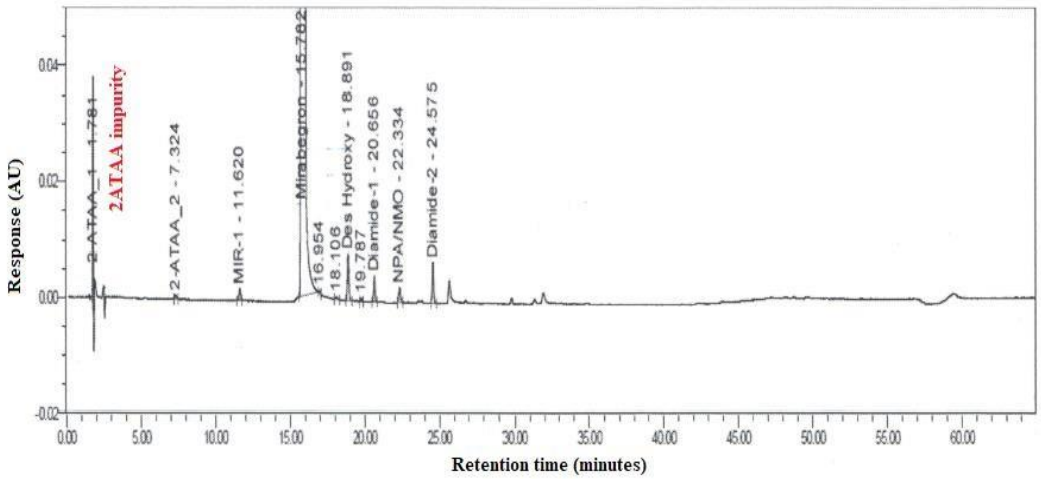

Figure 4.1: Previous reported method (Chapter-2 / method-1)

Development trial-1

Ammonium acetate buffer (3.8 g in 1liter water, pH 6.0 with acetic acid) and acetonitrile in 95:5 v/v ratio. Waters symmetry shield RP-8, 150 x 3.9 mm,5 µ; 1.0 mL/min; 30°C column oven temperature were used. Figure 4.2 represented the experiment chromatogram.

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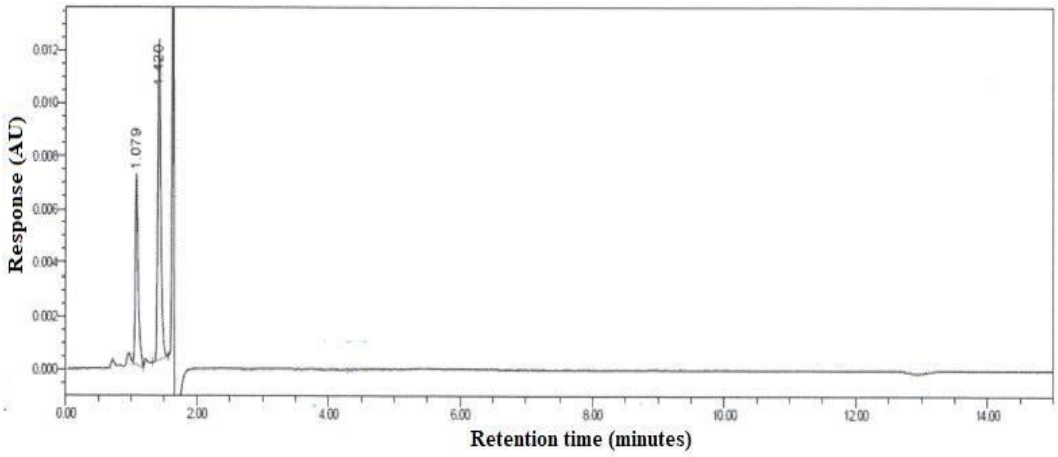

Figure 4.2: Method development trial chromatogram

Observations: 2-ATAA impurity was eluted before 2 min. Ion pair buffer salt may give good results to retain this impurity.

Development trial-2

Phenominex synergy polar, pH 6.0 ammonium acetate and acetonitrile (90:10), other conditions are same as experiment-1. Different salt buffers were checked and finally Tetrabutyl ammonium hydrogen sulphate (TBAHS)given better results. Below Figure shows the chromatogram of 2-ATAA impurity elution.

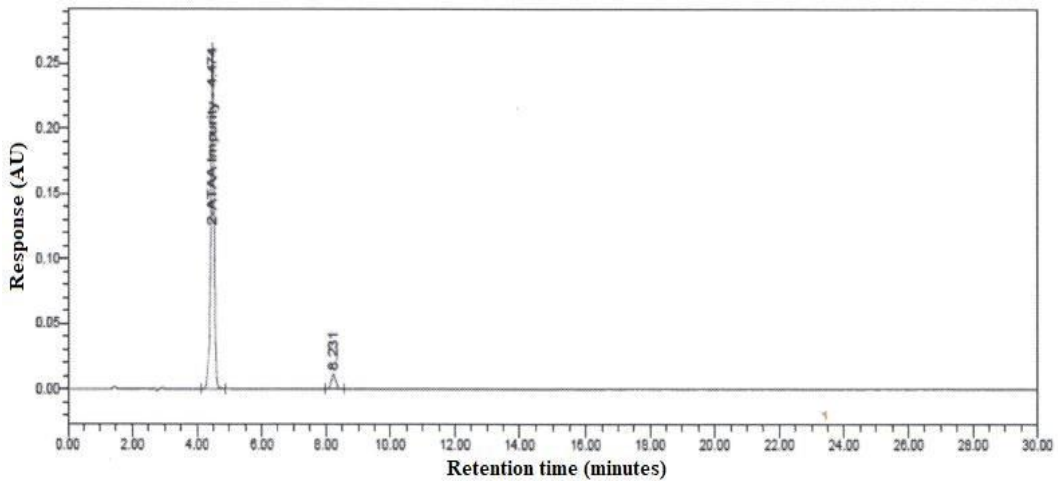

Figure 4.3: Development trial chromatogram

Observations: 2-ATAA impurity is retained about 4.4 min and it's separated from the void volume. But peak shape is not good.

Development trial-3

Waters symmetry shield RP-8 150 x3.9 mm, 5µ, flow rate 0.8 mL/min, 30°C column oven temperature, 250 nm, 10 µL injection volume were used.

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Mobile phase A is buffer and B acetonitrile. Gradient program is at 0 min M.P-B 0 %, at 10 min 0 %, at 11 min 80 %, at 15min 80 %, at 20 min 50 %, at 38 min 50 %, at 40 min 90 %, at 45 min 90 %, at 60 min 0 %, at 60 min 0 %. Different gradient programs were checked to get the better base line.

Observation: MIR-1 impurity RT 6.2 min; 2-ATAA impurity was eluted around 4.0min; Deshydroxy impurity RT 21.6 min; Diamide-2 RT 28.8 min and Diamide- 1 RT 28.2 min. Base line noise and peak tailing were less.

4..2 Method evaluation experiment

Different concentration of TBAHS salt was evaluated with 0.05 %, 0.1 %, 0.2 % with 6.0 pH solutions. Inertsil C8, 150 x 4.6 mm, 3 µ, acetonitrile as organic modifier, buffer-acetonitrile (95:5). Below Figure represented the 2-ATAA impurity elution.

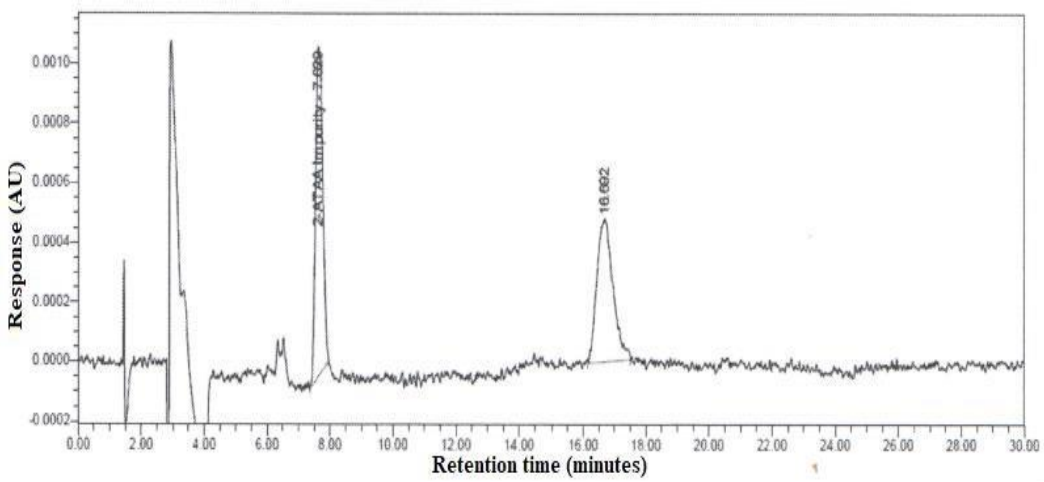

Figure 4.4: 2-ATAA impurity chromatogram.

Observations: Retention time not varying with concentration of TBAHS, but base line disturbance is increasing with TBAHS concentration. At 0.05% and 0.1% concentration base line was good.

RRF values

RRF values were established with 0.3 % and 0.6 % concentrations. RRF value for 2-ATAA impurity found to be 0.63.

4..3 Method validation

Method validation was performed with finalized method as per the ICH, USFDA industry guidance. Validation was executed with precision, linearity, accuracy, specificity, LOD, LOQ, ruggedness and robustness.

Specificity

Specificity was performed to confirm the interference from diluent, placebo and degradation products. Stress study was performed and confirmed that no interference and peak purity pass results. Below Figure 4.5 to 3.28 represent the blank, placebo, spiked test solution and force degradation studies chromatograms and peak purity plots. Below table summarized the force degradation conditions and % of 2-ATAA impurity degradation results.

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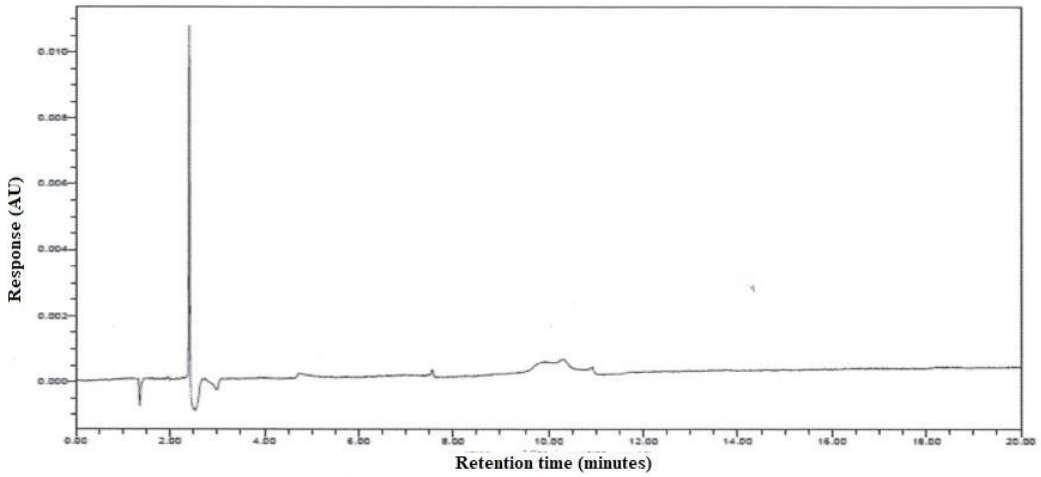

Figure 4.5: Blank chromatogram

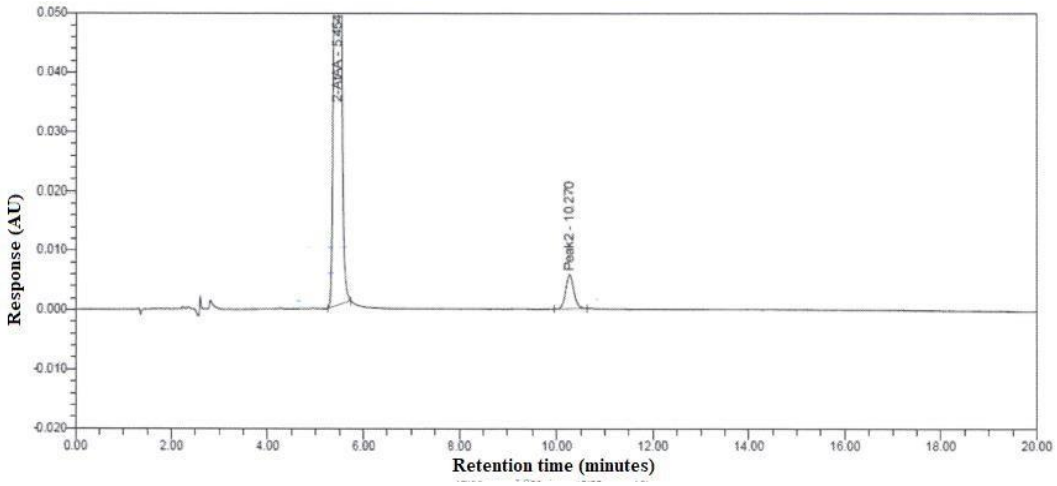

Figure 4.6: 2ATAA impurity chromatogram

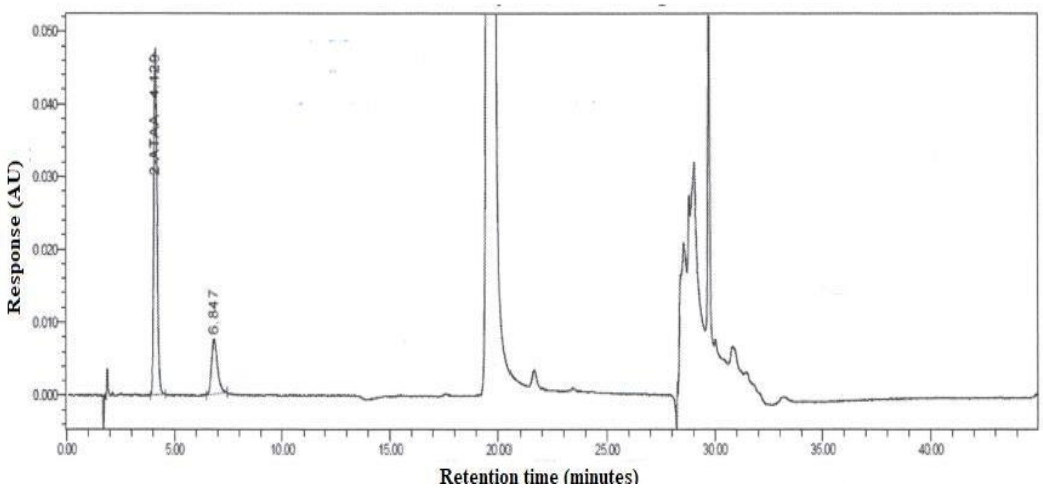

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Figure 4.7: Impurity spiked chromatogram

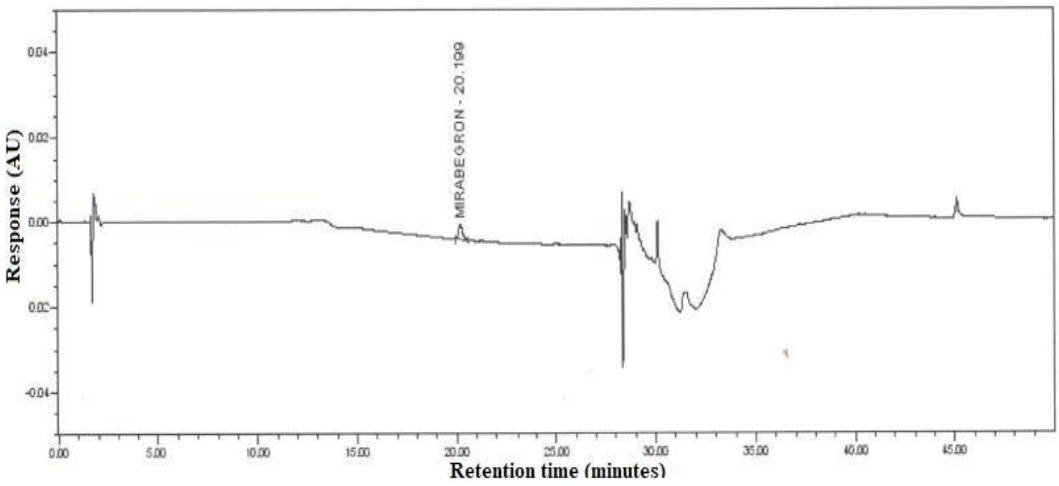

Figure 4.8: Standard solution chromatogram

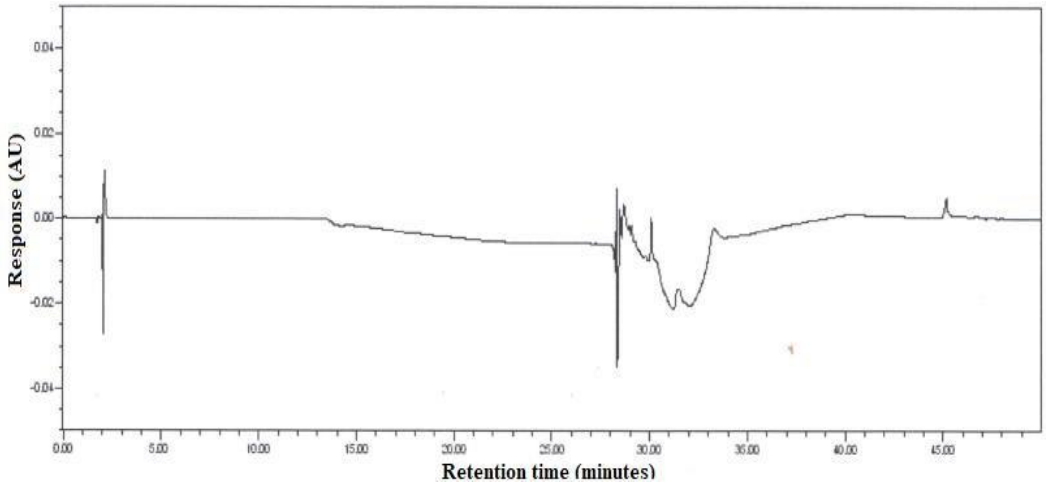

Figure 4.9: Blank chromatogram

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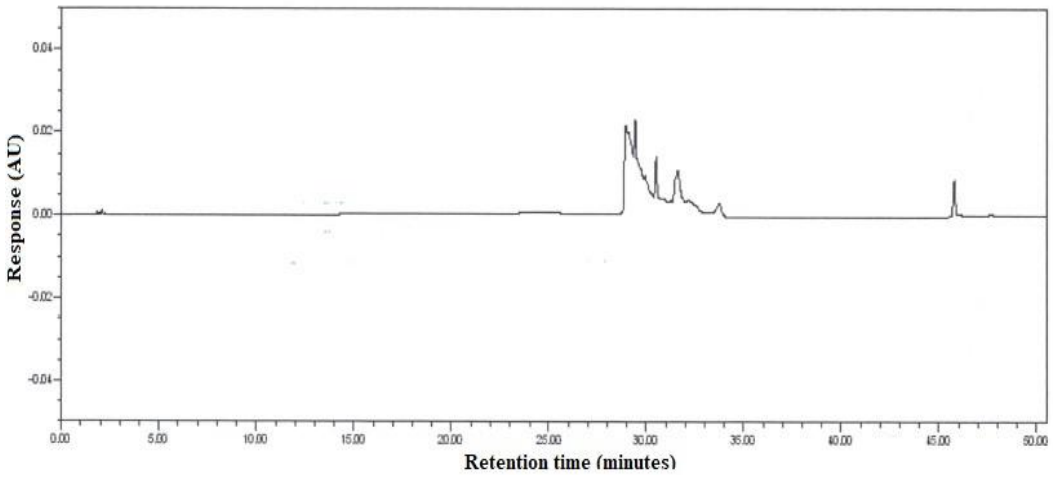

Figure 4.10: Placebo chromatogram

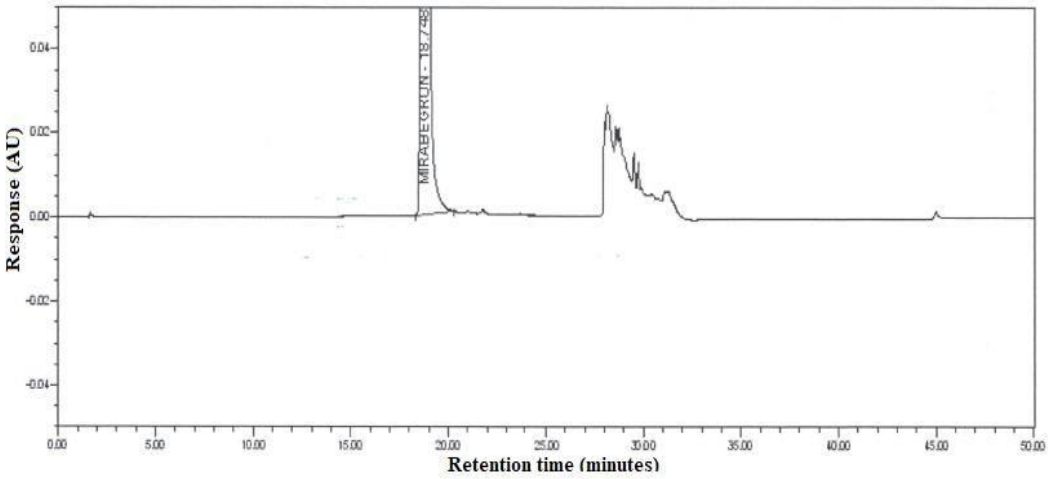

Figure 4.11: Test solution chromatogram

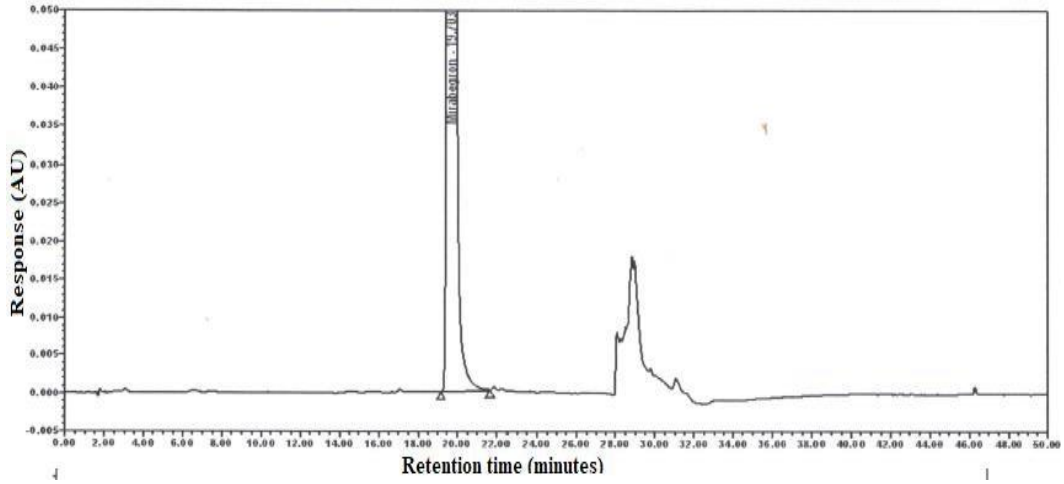

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Figure 4.12: Unstressed sample chromatogram

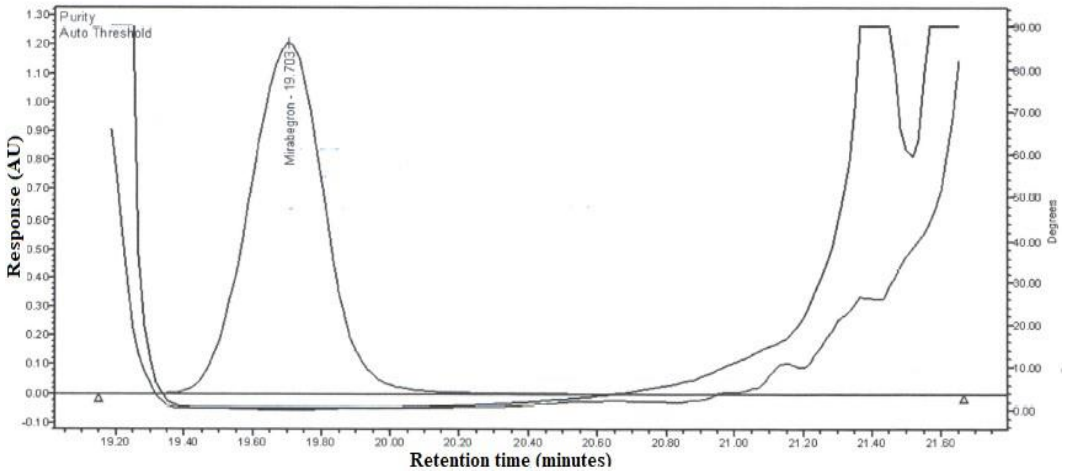

Figure 4.13: Peak purity plot for unstressed sample

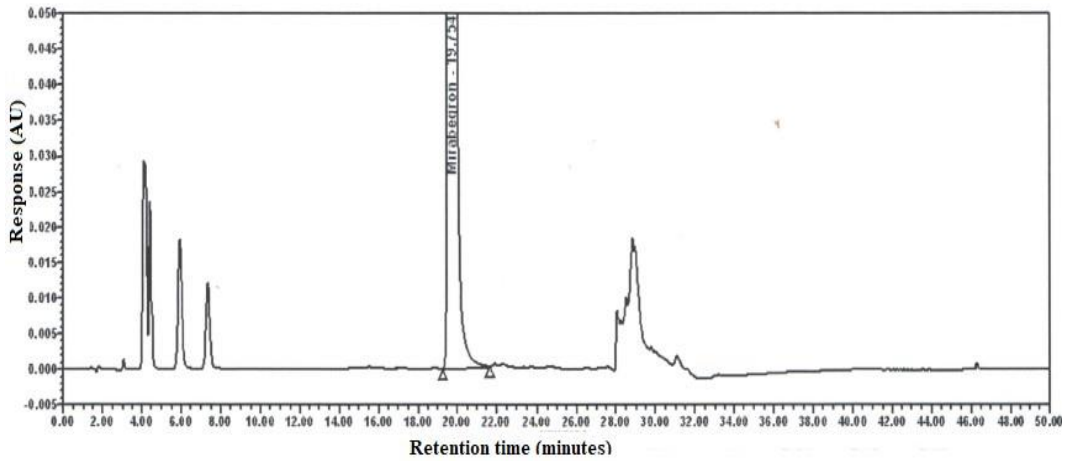

Figure 4.14: Acid stress sample chromatogram

5. CONCLUSION

The method development and validation for the determination of impurities, particularly the 2-ATAA impurity in Mirabegron, was successfully carried out with various chromatographic trials. Initial development showed that the 2-ATAA impurity eluted at the void volume, prompting further method optimization. After multiple trials using different buffers, mobile phases, and column configurations, an effective method was established with optimal retention time and minimal peak interference. The use of TBAHS buffer provided good peak separation, although baseline disturbances were observed at higher concentrations. Method evaluation indicated that 0.05% and 0.1% TBAHS concentrations yielded the best results with good baseline and clear separation. The RRF value for 2-ATAA was determined to be 0.63. Further method validation was executed according to ICH and USFDA guidelines, confirming precision, linearity, accuracy, specificity, LOD, LOQ, and robustness. Stress testing demonstrated no significant interference from degradation products, and peak purity analysis showed satisfactory results under different stress conditions.

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