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Research Article

A New Simple Method Development and Validation of Mirabegron in Bulk and Pharmaceutical Dosage Form by RP-HPLC

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ABSTRACT

A simple, accurate, and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of Mirabegron in bulk and pharmaceutical dosage forms. Mirabegron, a beta-3 adrenergic agonist used in the treatment of overactive bladder and neurogenic detrusor overactivity, was analyzed using an optimized mobile phase of Methanol: Acetonitrile: Potassium Dihydrogen Phosphate buffer (30:30:40 v/v) at pH 6.0. The method employed a UV detector set at 252 nm with a flow rate of 1.0 mL/min and a retention time of 3.926 minutes under isocratic elution. Validation studies conducted as per ICH guidelines demonstrated excellent linearity ($R^2 = 0.9997$) over the concentration range of 10–60 µg/mL, with limits of detection and quantification at 1.1493 and 3.4827 µg/mL, respectively. The method showed acceptable specificity, accuracy (99.95–100.02%), precision (RSD < 2%), and robustness against minor variations in chromatographic conditions. The study confirms that the proposed RP-HPLC method is simple, rapid, cost-effective, and suitable for routine quality control of Mirabegron in pharmaceutical formulations.

INTRODUCTION

To know about the composition and structure of matter, Analytical chemistry, a branch of science, is used, by acquiring, practicing and conveying information. It is not confined to definite compounds or reactions and it deals with the study of the natural and artificial materials. Geometrical features like molecular morphologies and species identity are constituted in the properties of analytical chemistry. The development of its various concepts and theories include safety and quality of food, pharmaceuticals and water, environmental monitoring, biomedical applications and also to support the legal processes (forensics) and diagnose diseases, etc., Analytical chemists play a vital role to support this. To

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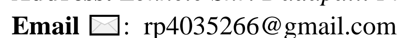
Email : rp4035266@gmail.com

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identify and measure the chemical species in a sample, analytical chemists use different techniques. By comparison of the known substance to a similar substance (whose concentration is known), which is called a standard reference material, and almost every technique will be carried out. In general, the drugs may be new or partially modified in structure of the existing ones with combinations releasing into the market annually. Frequently, from its introduction into the market to the inclusion of pharmacopoeias, it is being delayed, so there is a lack of analytical methods for these drugs, and for such drugs, this can lead to the development of newer analytical methods. In the literature, for the drugs, no appropriate analytical methods are available. Excipients cause interferences in drug formulation; hence no suitable methods are available for drug analysis. The use of some expensive reagents and solvents leads to the convolution of extraction and separation procedures, which may not be trustworthy.

Chromatography- an overview:

To resolve a multi-component mixture into its individual components, chromatography, is a new, well-known and a primary tool of separation and it can be applied both quantitatively and qualitatively. Despite, some other methods like IR spectroscopy, Nuclear Magnetic resonance spectroscopy or Mass spectroscopy etc. are required for the final identification and confirmation. Tswett. M, in 1806, in Warsaw, invented a new technique, while separating the plant pigments by a column of calcium carbonate, which acted as an adsorbent and the different substances get adsorbed to different extent and this give rise to the different coloured bands, at different positions on the column. In Greek, chroma means colour and graphos means writing. Hence, he termed the system of coloured bands as the chromatogram and the method as chromatography. To separate coloured as well as colourless substances, recent advances have been made there after. Thus, in general, a sample moves over a stationary phase through the mobile phase in chromatography. Chromatography is one of the best and most likely used analytical techniques, now-a-days and in foreseeable future. It is the cornerstone of molecular analytical chemistry. Recent advancements of chromatography have been introduced by A.P.J. Martin and R.L.M. Synge in 1941, made them noble prize winners.

High Performance Liquid Chromatography (HPLC):

HPLC is used to figure out the amount of specific compound in a solution. It supports reliable quantitative range to allow the determination of substances in a single run. This method is considered to be rapid, accurate, precise and specific and offers the ease of automation. It is because methods using HPLC have more advantages over the conventional methods.

Principle of HPLC:

A mixture of sample is dissociated into components for its identification, quantification and purification by HPLC, due to the differences in their relative affinities for the mobile phase and stationary phase used. Especially, RP-HPLC, relies on the principle of hydropohobic interactions, as the more non-polar the material is, longer it will be retained. Due to their low affinities and polar nature, most of the drugs elute at a faster rate through the column and so they are separated and detected easily. The optimization of laboratory resources is ensured by the effective method development, while methods meet the objectives required at each stage of drug development. To approve the drug, at certain

stages, method development is required by the regulatory agencies.

Types of HPLC:

There are following variants of HPLC, depending upon the phase system (stationary) in the process:

Normal Phase HPLC:

This method separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these. Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

1. Reverse Phase HPLC:

The stationary phase is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more nonpolar the material is, the longer it will be retained.

2. Size-exclusion HPLC:

The column is filled with material having precisely controlled pore sizes, and the particles are separated according to it's their molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later.

3. Ion-Exchange HPLC:

The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.

Application of HPLC:

The information that can be obtained by HPLC includes resolution, identification and quantification of a compound. It also aids in chemical separation and purification. The other applications of HPLC include:

- **Pharmaceutical Applications**

1. To control drug stability.
2. Tablet dissolution study of pharmaceutical dosages form.
3. Pharmaceutical quality control.

- **Environmental Applications**

1. Detection of phenolic compounds in drinking water.
2. Bio-monitoring of pollutants.

- **Applications in Forensics**

1. Quantification of drugs in biological samples.
2. Identification of steroids in blood, urine etc.
3. Forensic analysis of textile dyes.
4. Determination of cocaine and other drugs of abuse in blood, urine etc.

- **Food and Flavour**

1. Measurement of Quality of soft drinks and water.
2. Sugar analysis in fruit juices.
3. Analysis of polycyclic compounds in vegetables.
4. Preservative analysis.

- **Applications in Clinical Tests**

1. Urine analysis, antibiotics analysis in blood.
2. Analysis of bilirubin, biliverdin in hepatic disorders.
3. Detection of endogenous Neuropeptides in extracellular fluid of brain etc.

UV-VIS spectroscopy:

UV-VIS spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. This property is influenced by the sample composition, potentially providing information on what is in the sample and at what concentration. Since this spectroscopy technique relies on the use of light, let's first consider the properties of light. Light has a certain amount of energy which is inversely proportional to its wavelength. Thus, shorter wavelengths of light carry more energy and longer wavelengths carry less energy. A specific amount of energy is needed to promote electrons in a substance to a higher energy state which we can detect as absorption. Electrons in different bonding environments in a substance require a different specific amount of energy to promote the electrons to a higher energy state. This is why the absorption of light occurs for different wavelengths in different substances. Humans are able to see a spectrum of visible light, from approximately 380 nm, which we see as violet, to 780 nm, which we see as red.[1] UV light has wavelengths shorter than that of visible light to approximately 100 nm. Therefore, light can be described by its wavelength, which can be useful in UV-Vis spectroscopy to analyze or identify different substances by locating the specific wavelengths corresponding to maximum absorbance (see the Applications of UV-Vis spectroscopy section).

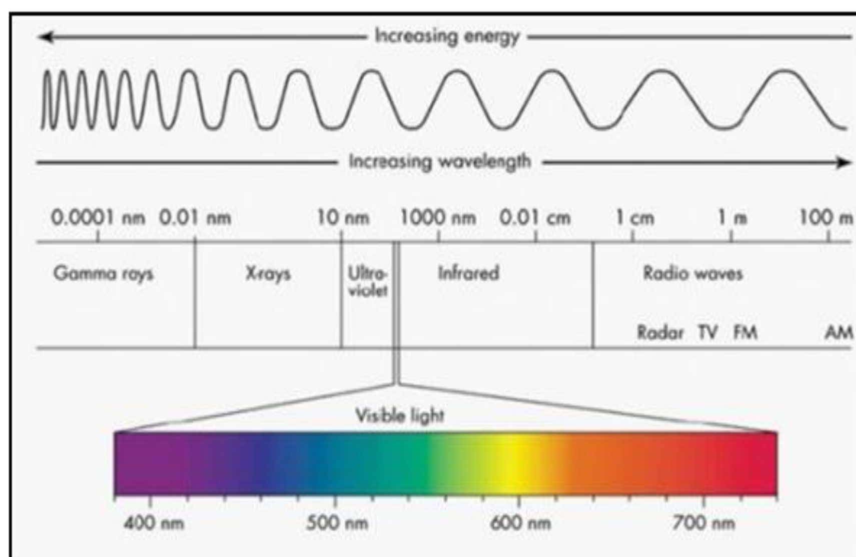


Figure 1.1: UV Radiations

Origen and Characteristics of UV-Visible Spectrum:

UV-VIS spectrum results from the interaction of electromagnetic radiation in the UV-Visible region with molecules, ions or complexes. It forms the basis of analysis of different substances such as, inorganic, organic and biomolecules. These determinations find applications in research, industry, clinical laboratories and in the chemical analysis of environmental samples. It is therefore important to learn about the origin of the UV-VIS spectrum and its characteristics.

Radiation and energy

Radiation is a form of transmitted energy Electromagnetic radiation is so-named because it has electric and magnetic fields that simultaneously oscillate in planes mutually perpendicular to each other and to the direction of propagation through space Electromagnetic radiation has the dual nature: its exhibits wave properties and particulate properties.

The nature of light

Light is a form of energy. Energy can be transferred from one point to another point either by particle motion or by wave motion. Accordingly, different theories on the nature of light have been proposed. The important theories are as follows: -

1. Particular like theory.
2. Electromagnetic wave theory.

The nature of electromagnetic radiation and spectral regions:

The electromagnetic spectrum is composed of a large range of wavelengths and frequencies (energies). It varies from the highly energetic gamma rays to the very low energy radio-waves. The entire range of radiation is commonly referred to as the electromagnetic spectrum. The major spectral regions of the spectrum are shown in figure below and the divisions are based on the methods required to generate and detect various types of radiations

Absorption and Emission of Radiation:

Electromagnetic radiation can interact with matter in a number of ways. If the interaction results in the transfer of energy from a beam of radiant energy to the matter, it is called "absorption". The reverse process in which a portion of the internal energy of matter converted into radiant energy is called "emission". In emission process, species in an excited state can emit photons of characteristic energies by returning to lower energy states or ground states. Part of the radiation which passes into matter, instead of being absorbed, may be scattered or reflected or may be re-emitted at the same wavelength or a different wavelength upon emerging from the sample. Radiation, which is neither absorbed nor scattered, may undergo changes in orientation or polarization as it passes through the sample

Absorption of radiation:

Absorption of radiation by matter always involves the loss of energy by the radiation and a corresponding gain in energy by the atoms or molecules of the medium. The energy absorbed from radiation appears as increased internal energy, or in increased irrational and rotational energy of the atoms and molecules of the absorbing medium. As a general rule, translational energy is not directly increased by absorption of radiation, although it may be indirectly increased by degradation of electronic energy or by conversion of rotational or vibration energy to that of translation by intermolecular collisions

Interaction of Matter with radiation:

The word spectroscopy is used to refer to the broad area of science dealing with the absorption, emission, or scattering of electromagnetic radiation by molecules, ions, atoms, or nuclei. Spectroscopic techniques are some of the most widely used analytical methods in the world today. These techniques are useful in determining both the identity of unknown substances and their concentration in solution. Different regions of the electromagnetic spectrum such as infrared, visible, ultraviolet, or, X-ray radiation can be used to

interact with matter The interaction of radiation with matter can cause redirection of the radiation and/or transitions between the energy levels of the atoms or molecules.

Absorption: A transition from a lower level to a higher level with transfer of energy from the radiation field to an absorber, atom, molecule, or solid.

Emission: A transition from a higher level to a lower level with transfer of energy from the emitter to the radiation field. If no radiation is emitted, the transition from higher to lower energy levels is called nonradioactive decay. The data that is obtained from spectroscopy is called a spectrum. Spectrum is a plot of the intensity of energy detected versus the wavelength (or mass or momentum or frequency, etc.) of the energy. A spectrum can be used to obtain information about atomic and molecular energy levels, molecular geometries, chemical bonds, interactions of molecules, and related processes. Often, spectra are used to identify the components of a sample (qualitative analysis).

Instrumentation:

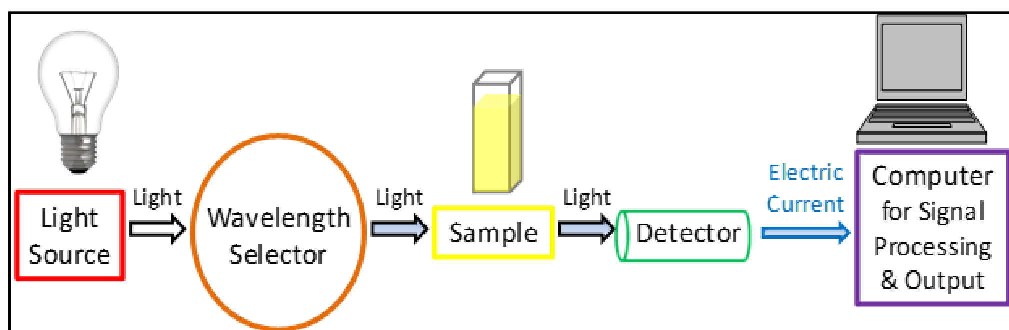


Figure 1.2: Components in a UV-Vis spectroscopy

Light source:

As a light-based technique, a steady source able to emit light across a wide range of wavelengths is essential. A single xenon lamp is commonly used as a high intensity light source for both UV and visible ranges. Xenon lamps are, however, associated with higher costs and are less stable in comparison to tungsten and halogen lamps. For instruments employing two lamps, a tungsten or halogen lamp is commonly used for visible light, whilst a deuterium lamp is the common source of UV light. As two different light sources are needed to scan both the UV and visible wavelengths, the light source in the instrument must switch during measurement. In practice, this switchover typically occurs during the scan between 300 and 350 nm where the light emission is similar from both light sources and the transition can be made more smoothly.

Wavelength selection:

In the next step, certain wavelengths of light suited to the sample type and analyte for detection must be selected for sample examination from the broad wavelengths emitted by the light source. Available methods for this include:

Monochromators:

A monochromator separates light into a narrow band of wavelengths. It is most often based on diffraction gratings that can be rotated to choose incoming and reflected angles to select the desired wavelength of light. The diffraction grating's groove frequency is often measured as the number of grooves per mm. A higher groove frequency

provides a better optical resolution but a narrower usable wavelength range. A lower groove frequency provides a larger usable wavelength range but a worse optical resolution. 300 to 2000 grooves per mm is usable for UV-Vis spectroscopy purposes but a minimum of 1200 grooves per mm is typical. The quality of the spectroscopic measurements is sensitive to physical imperfections in the diffraction grating and in the optical setup. As a consequence, ruled diffraction gratings tend to have more defects than blazed holographic diffraction gratings. Blazed holographic diffraction gratings tend to provide significantly better-quality measurements.

Method development of drugs:

The analyte is enough to know the information of the compound. One can select the most suitable HPLC method development by the physical and chemical characteristics and by a vast literature review. Information regarding the sample can be achieved by molecular weight, structure, functionality, Pka value, UV-spectra and solubility of the compound. By knowing whether the pure compound is organic soluble or water soluble, one can select the best mobile phase and column for HPLC method development. In many laboratories, typical detectors like Mass spectroscopy, UV-Visible detectors are used as they can detect a wide variety of compounds. Analytical methods are intended to establish the identity, purity, physical characteristics and potency of the drugs. To support drug testing against specifications that arise during manufacturing and quality release operators, as well as during long-term stability studies and for safety and characterization studies or evolution of drug performance, methods are developed. Before the final method optimization, all individual components should be investigated during the preliminary method development. By this, it is easy to evaluate the method performance in each component and streamline the final method optimization. Resolving power, specificity and speed are the major attributes of method development. By combining different factors like composition of solvent, type of stationary phase, mobile phase, P^H and buffers, selectivity can be achieved. For the chromatographic separation, changing solvents and stationary phases, proper range of P^H are the most suitable approaches. Better chromatographic resolution can be achieved by the P^H ranging from 1-12 and the development of the method depends upon column efficiency, selectivity and retention time. For chromatographic separation, mobile phase composition or strength plays a vital role. The most commonly used solvents in RP-HPLC are acetonitrile, methyl alcohol and Tetra hydro furan (having the wavelength of 190, 205 & 212nm). By the selection of right column temperature and changing the mobile phase, the separation of many samples can be enhanced.

Method validation of drugs:

According to ISO definition, validation is defined as “Verification, where the specified requirements are adequate for an intended use.” Method validation can be used for qualitative, semi-quantitative or quantitative methods. The scientific soundness of the measurement or characterization and also to varying extents throughout the regulatory submission process, the validation of analytical method is required. The method development includes the measurement of the correct substance, in correct amount and in appropriate range. The goal of method validation is to identify the critical parameters and to establish the acceptance criteria of system suitability. The effort done in method development and optimisation leads to the effective development of HPLC method and its final performance. For the method development of

samples in chromatographic separation, method validation is very important.

Method Validation

This process consists of establishments of the performance characteristics and the limitation of the method.

Method Performance Parameters are Determined using Equipment that is:

1. Within specification
2. Working correctly
3. Adequately calibrated

Method Validation is required when:

1. A new method is being developed
2. Revision of the established method
3. When established method are used in different laboratories and by different analysts etc.

4. Comparison of method

5. When quality control indicates method changes

Literature Survey

Determination and Validation of RP-HPLC Method for the Estimation of Mirabegron in Tablet Dosage Form.

Objective: A reversed phase liquid chromatography was determined and validated for the estimation of Mirabegron in tablet dosage form.

Methods: The validation study of RP-HPLC showed a simple, rapid, accurate, precise, reproducible results by using a stationary phase: Waters Acquity HSS T-3 C18 (100 × 2.1 mm, 1.7µm and Mobile Phase-Potassium di-hydrogen phosphate: acetone in the ratio (40:60 v/v) at PH6.0±0.02. Detection is carried out at 243 nm using UV detector.

Results: The total chromatographic analysis time per sample was about 6 min with Mirabegron eluting at a retention time of 2.754. Tailing factor obtained from the standard injection is 1.6. Theoretical Plates obtained from the standard injection is 2736.7. The flow rate is 1 ml/min and linearity in the concentration range of 30-70µg/ml (R2=0.999). Analytical Method Development and Validation for the Determination of Mirabegron in Pharmaceutical Dosage Form by RP-HPLC. This current study describes developing the novel, precise, simple analytical method suitable for determination of Mirabegron (MIRA) in a pharmaceutical dosage form. Reversed-Phae High-Performance Liquid Chromatography (RP-HPLC) method was utilized for method development and validation studies of MIRA. Chromatographic separation was carried out on Agilent technologies -1260 infinity system, eclipse XDB C18 column (4.6 mm i.d. × 250 mm, 5 µm particle size) at a flow rate of 1 ml/min and detection wavelength set at 251 nm. Mobile phase consists of methanol and acetonitrile were mixed in the ratio of 95:5 v/v. The retention time for MIRA was found to be 5.813 min. RP-HPLC Method Development and Validation for the Quantitative Determination of Potential Impurities of Mirabegron.

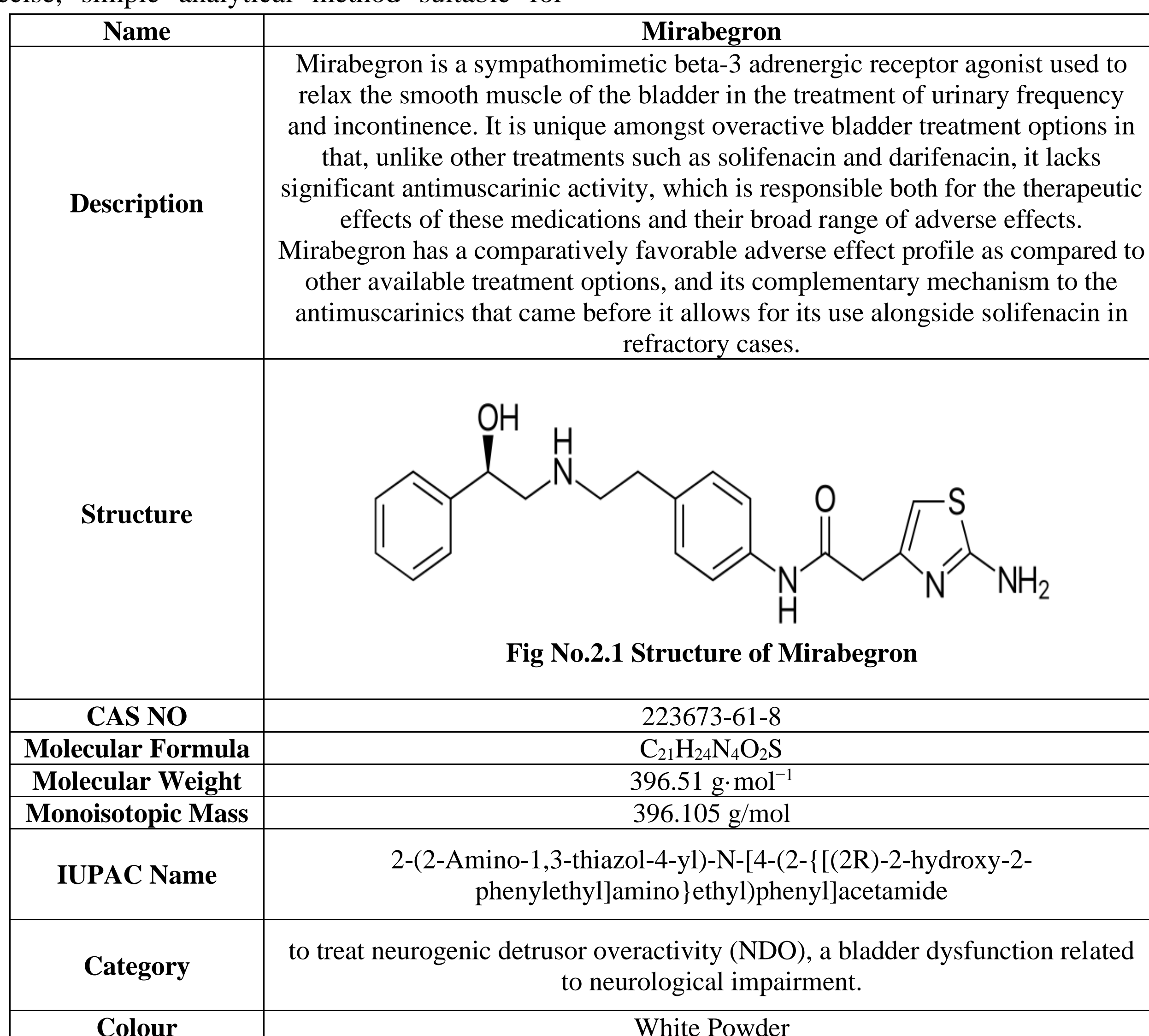
The objective of the study was to develop and evaluate the reverse phase high performance liquid chromatography (RP-HPLC) method for the quantitative determination of potential impurities of Mirabegron active pharmaceutical ingredient. The method uses Puratis C18 column (250 × 4.6mm, 5µm) with mobile phase A consisted, 20 mM Ammonium acetate, pH adjusted to 4.5 and mobile phase B consisted methanol with a gradient programme. The column temperature was

maintained at 25 °C and the detection was carried out at 247 nm. Efficient and reproducible chromatographic separation was achieved on C18 stationary phase in gradient elution profile. The newly developed HPLC method was validated according to ICH guidelines considering three impurities to demonstrate precision, linearity, accuracy and robustness of the method.

Analytical Method Development and Validation For The Determination Of Mirabegron In Pharmaceutical Dosage Form By RP-HPLC.

This current study describes developing the novel, precise, simple analytical method suitable for determination of Mirabegron (MIRA) in a pharmaceutical dosage form. Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) method was utilized for method development and validation studies of MIRA. Chromatographic separation was carried out on Agilent technologies -1260 infinity system, eclipse XDB C18 column (4.6 mm i.d. × 250 mm, 5 µm particle size) at a flow rate of 1 ml/min and detection wavelength set at 251 nm. Mobile phase consists of methanol and acetonitrile were mixed in the ratio of 95:5 v/v.

Drug Profile



Name	Mirabegron
Description	Mirabegron is a sympathomimetic beta-3 adrenergic receptor agonist used to relax the smooth muscle of the bladder in the treatment of urinary frequency and incontinence. It is unique amongst overactive bladder treatment options in that, unlike other treatments such as solifenacin and darifenacin, it lacks significant antimuscarinic activity, which is responsible both for the therapeutic effects of these medications and their broad range of adverse effects. Mirabegron has a comparatively favorable adverse effect profile as compared to other available treatment options, and its complementary mechanism to the antimuscarinics that came before it allows for its use alongside solifenacin in refractory cases.
Structure	**Fig No.2.1 Structure of Mirabegron**
CAS NO	223673-61-8
Molecular Formula	$C_{21}H_{24}N_4O_2S$
Molecular Weight	396.51 g·mol^{-1}
Monoisotopic Mass	396.105 g/mol
IUPAC Name	2-(2-Amino-1,3-thiazol-4-yl)-N-[4-(2-{[(2R)-2-hydroxy-2-phenylethyl]amino}ethyl)phenyl]acetamide
Category	to treat neurogenic detrusor overactivity (NDO), a bladder dysfunction related to neurological impairment.
Colour	White Powder

pKa	13.84 (strongest acidic) 9.62 (Strongest Basic)
Melting point	178 °C
Water Solubility	Aqueous solubility: 0.885 mg/mL (at 25 °C)
Storage	Store at room temperature away from light and moisture.
Uses	Mirabegron is used is in the treatment of overactive bladder. It works equally well to antimuscarinic medication such as solifenacin or tolterodine. In the United Kingdom it is less preferred to these agents. Mirabegron is also indicated to treat neurogenic detrusor overactivity (NDO), a bladder dysfunction related to neurological impairment, in children ages three years and older.
Mechanism of Action	M Mirabegron is a potent and selective agonist of beta-3 adrenergic receptors. The activation of beta-3 receptors relaxes detrusor smooth muscle during the storage phase of the urinary bladder fill-void cycle, which increases the bladder's storage capacity thereby alleviating feelings of urgency and frequency.
Absorption	The absolute bioavailability of orally administered mirabegron ranges from 29% at a dose of 25 mg to 35% at a dose of 50 mg. The Tmax for the extended-release tablet and suspension formulations are approximately 3.5 hours, while the Tmax for the granule formulation is 4-5 hours.
Distribution	Following intravenous administration, mirabegron has an apparent steady-state volume of distribution (Vd) of 1670 L indicating extensive distribution.
Metabolism	Mirabegron is extensively metabolized via a number of mechanisms, although unchanged parent drug is still the major circulating component following oral administration. Presumed metabolic pathways and their resultant metabolites include amide hydrolysis (M5, M16, M17), glucuronidation (mirabegron O-glucuronide, N-glucuronide, N-carbamoylglucuronide, M12), and secondary amine oxidation or dealkylation (M8, M9, M15), amongst others. The enzymes responsible for the oxidative metabolism of mirabegron are thought to be CYP3A4 and CYP2D6, while the UDP-glucuronosyltransferases responsible for conjugation reactions have been identified as UGT2B7, UGT1A3, and UGT1A8. Other enzymes that may be involved in the metabolism of mirabegron include butylcholinesterase and possibly alcohol dehydrogenase.
Protein Binding	Mirabegron is approximately 71% protein-bound in plasma, primarily to albumin and alpha-1-acid glycoprotein.
Route of elimination	Mirabegron is not metabolized but is eliminated rapidly by the kidney. Mirabegron crosses the placental but not the blood-brain barrier and is excreted in breast milk.
Clearance	Total plasma clearance following intravenous administration is approximately 57 L/h, with renal clearance accounting for roughly 25% at approximately 13 L/h.
Half Life	The mean terminal elimination half-life of mirabegron in adults being treated for overactive bladder is approximately 50 hours.6 In pediatric patients receiving the granule formulation for the treatment of neurogenic detrusor overactivity, the mean terminal elimination half-life is approximately 26-31 hours.
Toxicity	At doses of up to 400mg in healthy volunteers (~8x the recommended maximum), reported symptoms of overdose included palpitations and increased heart rate. Symptoms of chronic overdosage are similar in presentation and may also include a rise in systolic blood pressure. In cases of overdosage, employ standard symptomatic and supportive measures in addition to ECG monitoring.

Pharmacodynamic	Mirabegron exerts its pharmacologic effects by forcing bladder smooth muscle to relax, thereby expanding its capacity and relieving urgency. Mirabegron does not appear to adversely affect the mean maximum flow rate or mean detrusor pressure at maximum flow rate in patients with lower urinary tract symptoms and bladder outlet obstruction (BOO), but should be used with in patients with BOO due to reports of significant urinary retention.
Side effects	Mirabegron can cause mild or serious side effects. The following list contains some of the key side effects that may occur while taking Mirabegron. This list does not include all possible side effects.
Drug Interaction	Abacavir may decrease the excretion rate of Mirabegron which could result in a higher serum level. The serum concentration of Mirabegron can be increased when it is combined with Abametapir.

MATERIAL AND METHOD

1.1MATERIAL:

Table No. 1.1: Details of Marketed Formulation

Sr. No.	Brand name	Content	Mfg. company
1	MIRBEG (50mg)	Mirabegron 50 Mg Tablets	IPCA Pharmaceuticals Ltd. Mumbai

Table No. 1.2: Details of standard drug and manufacturer

Sr. No.	Content	Mfg. Company
1	Mirabegron Standard (98.94%)	Chemsar Research Private Limited, Pune

Table No. 1.3: List of Chemicals use in Research work

Sr. No.	Chemicals	Grade
1	Methanol	HPLC
2	Disodium Hydrogen Phosphate	GR
3	Potassium Dihydrogen Phosphate	GR
14	Sodium Chloride	GR
5	Ortho phosphoric acid	GR
6	Hydrochloric acid	GR
7	Sodium hydroxide	GR
8	Hydrogen peroxide	GR
9	Double distilled water	GR

1.2 Instruments

UV-visible spectrophotometers:

- Jasco V-630

High performance liquid chromatography system:

- Shimadzu HPLC LC2030 series chromatograph equipped with isocratic pump LC-10ADVP, UV detector.

pH Meter:

- EI, model No. 111E

Sonicator

- PCi mumbai, model No. 3.5L 100H

Weighing Balance

- Shimadzu AUX220
- Analytical CAS-44

Stability chamber:

- THERMOLAB, Sr. No. 398/10/09-10

Photostability chamber

- Hichon

Filtration

- Membrane filter
- Whattman filter paper of 0.45µ

Oven

- Tempo-model No. 1536
- Thermotech-TIC-4000N

Glassware's:

- Calibrated glasswares were used for the whole experimental work.

1.3 Method Development:

Chromatographic Conditions

During mobile phase optimization Methanol: Acetonitrile: Potassium Dihydrogen Phosphate (pH adjusted to 6.0 with orthophosphoric acid) in the ratio of 30: 30: 40 was found to be satisfactory for Mirabegron. The low-pressure gradient mode at a flow rate of 1.0ml/min at ambient temperature was used. The mobile phase was degassed and filtered through membrane filter (0.22µ). The injection volume was 10 µL and the run time for the analysis was 10 mins. The detection was carried out at 252 nm using UV detector. Water: ACN (50:50) was used as a diluent in the study.

Preparation of standard stock solution

Accurately weighed quantity of Mirabegron was transferred into two separate 100ml volumetric flask containing 60ml Diluent. The content was dissolved by sonication for 5 mins. The volume was made up to the mark with methanol. It was further diluted appropriately using diluent to get desired concentrations.

Preparation of Internal Standard solution

Accurately weighed quantity of Mirabegron (100 mg) was transferred to 100ml of volumetric flask containing 30ml methanol. The content was dissolved by sonication for 10mins. The volume was made up to 100ml with water and aliquot was further appropriately diluted using diluent to get a concentration of 10µg/ml.

Preparation of Sample solution and Assay of marketed formulation

Accurately weighed about 403mg of tablet powder (equivalent to 150mg of IRB and 12.5mg of HCTZ) was transferred to 100 ml of volumetric flask containing 60ml of methanol. The sample was dissolved by sonication for 15 mins and volume was made up to the mark with methanol. The resulting solution was filtered using membrane filter 0.22µ. The filtrate was further diluted appropriately with diluent to get 36 µg/ml

of IRB and 3 µg/ml of HCTZ. This diluted sample was then analyzed by HPLC.

Results and Trials:

Trial-1

Mirabegron:

- Mobile phase- Acetonitrile: Waterv(50:50)
- Flow rate- 1ml/min
- Run time – 12.00 min
- Injection Volume: 10microliter
- Column temperature: Room temperature
- λmax- 252nm
- Observation- Peak was not observed.

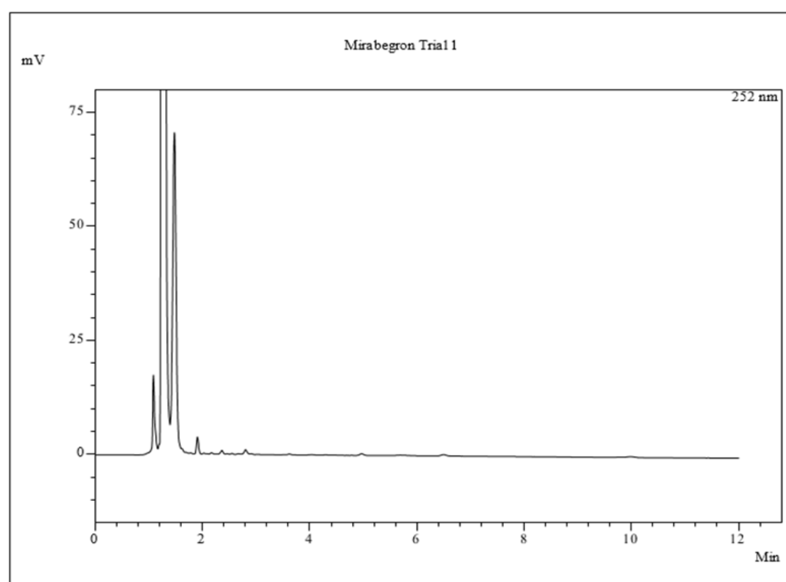


Fig no 1: Chromatogram 1 of Trail 1

Trial-2

Mirabegron:

- Mobile phase: Acetonitrile: methanol (60:40)
- Flow rate: 1ml/min
- Run time: 10:00 min
- Retention time: 6.103
- Injection Volume: 10microliter
- Column temperature: Room temperature
- λmax – 252nm
- Observation- Broadening & tailing observed of peak. Baseline is not proper. Peak properties not match with standards hence peak is rejected.

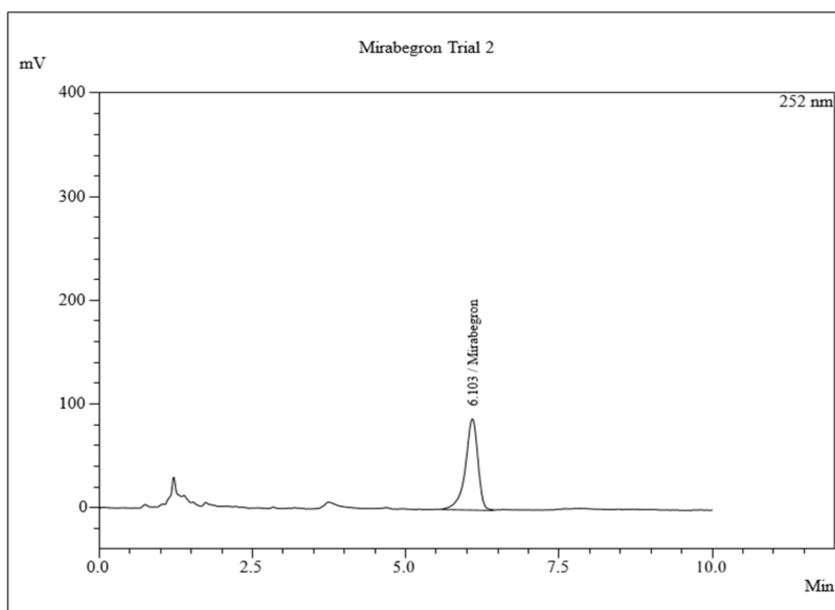


Fig No 2: Chromatogram 1 of Trail 2

Trial-3

Mirabegron:

- Mobile phase- Acetonitrile: methanol: water (20:20:60)
- Flow rate- 1ml/min
- tR – 8.893 min
- Run time: 12.00 min
- Injection Volume: 10microliter
- Column temperature: Room temperature
- λmax – 252nm
- Observation- Broadening & tailing of peak. More retention time. Baseline is not proper as per consideration. Peak properties not match with standards hence peak is rejected.

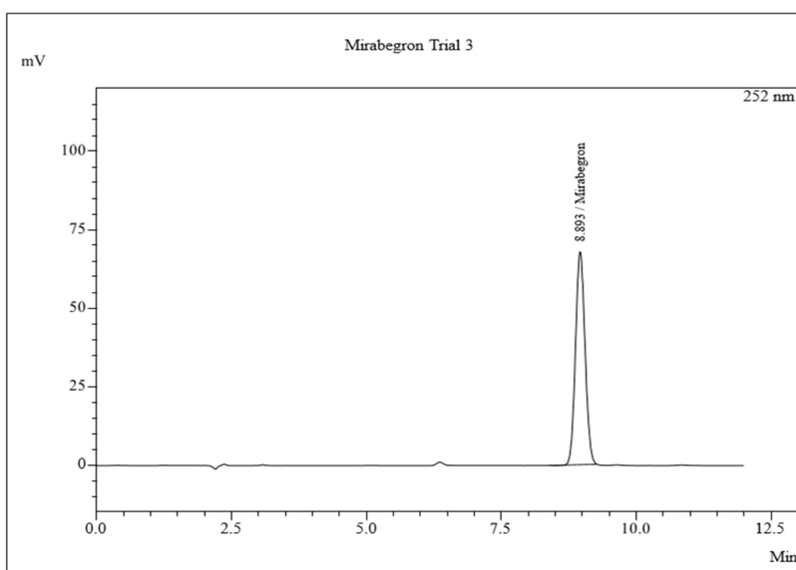


Fig no 3: Chromatogram 1 of Trail 3

Trial-4

Mirabegron:

- Mobile phase- Acetonitrile: water (42:58 v/v) pH 3.0
- Flow rate- 1ml/min
- Runtime: 8.00 min
- t_R – 4.627 min
- Injection Volume: 10microliter
- Column temperature: Room temperature
- λmax- 252nm
- Observation- Satisfactory Peak properties Observed. But retention time is more as we expected.

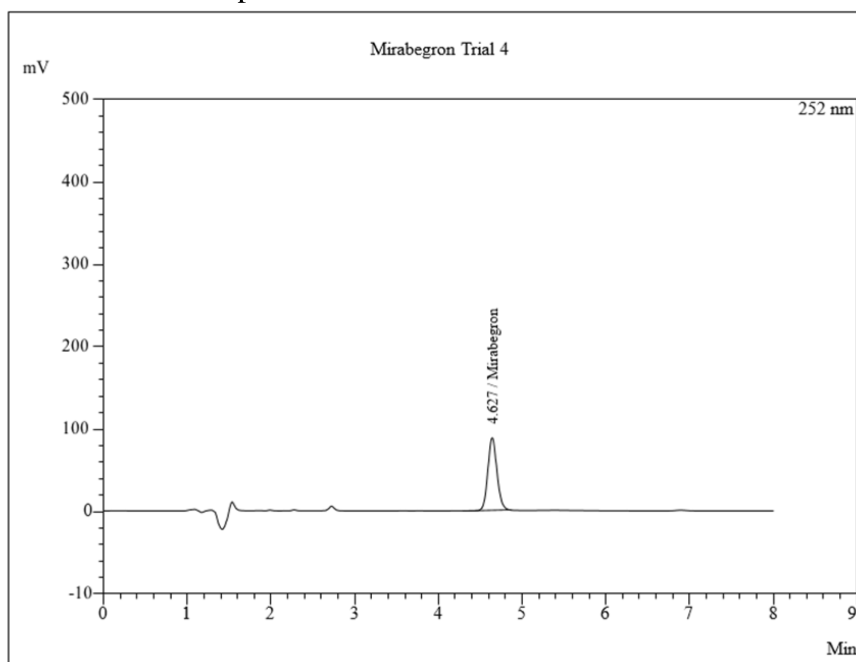


Fig no 4: Chromatogram 1 of Trail 4

Trial-5 (Optimized Trial)

Mirabegron:

- Mobile phase- Methanol: Acetonitrile: Potassium Dihydrogen Phosphate (pH adjusted to 6.0 with orthophosphoric acid) in the ratio of 30: 30: 40
- Flow rate- 1ml/min
- Runtime: 9.00 min
- t_R – 3.926 min
- Injection Volume: 10microliter
- Column temperature: Room temperature
- λmax- 252nm
- Observation- Satisfactory Peak detected in this chromatographic condition with very good shape, Resolution is good and also peak shape and good theoretical plates. No tailing observe and more theoretical plate as expected i.e. more than 2000. Resolution is good. Optimized chromatographic condition.

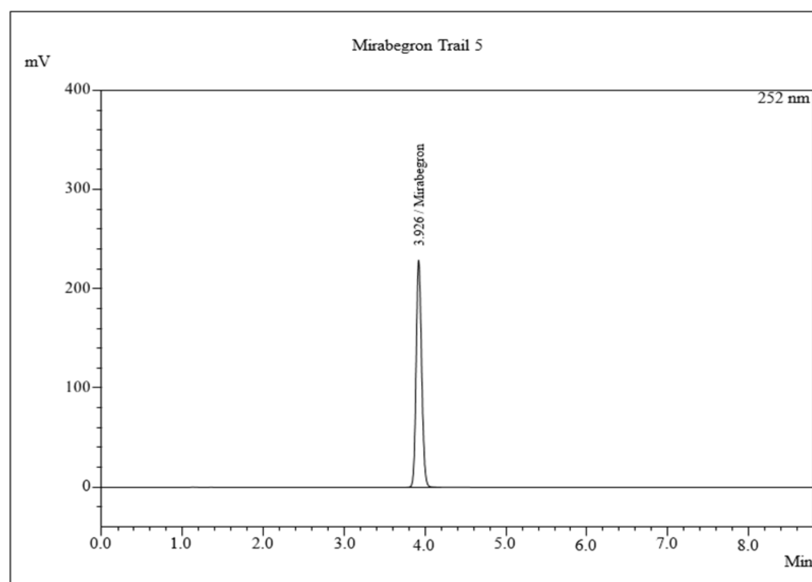


Fig no 5: Chromatogram 1 of Trail 5

Validation

The proposed HPLC method was validated in terms of system suitability, specificity, precision, accuracy and robustness as per the International Conference on Harmonization (ICH) guidelines.

1. Linearity:

The linearity of peak area response for Mirabegron was determined from 10 % to 50 % level of working concentration of Mirabegron. The stock solutions of standard Mirabegron was diluted to six different known concentrations. Linearity graph of concentration (as x-value) versus area (as y-value) were plotted and correlation coefficient, y-intercept and slope of the regression were calculated.

Table: 2.1 Linearity Result of Mirabegron

Linearity		
Sr. No	**Concentration (µg/mL)**	**Peak Area**
1	10	648217
2	20	1296434
3	30	1894651
4	40	2592868
5	50	3241085
6	60	3889302
Slope		64964.56
Standard Error		22625.31

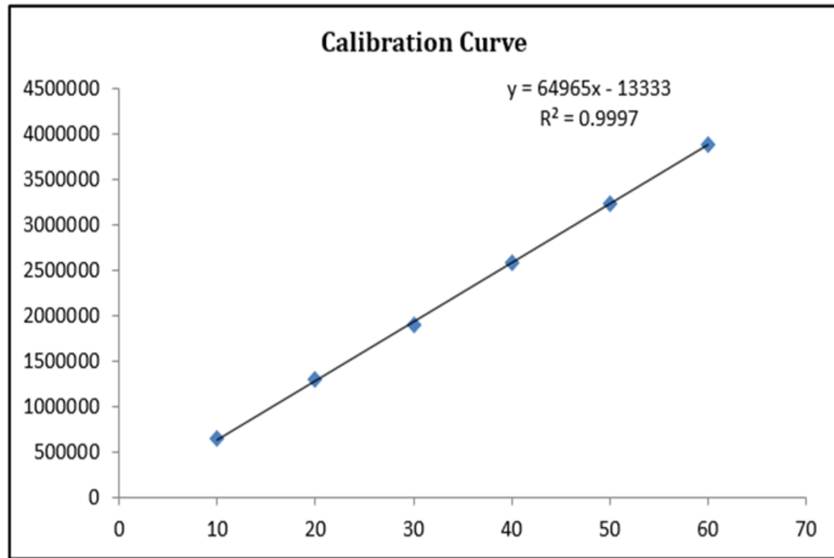


Figure: 6 Calibration Curve of Mirabegron

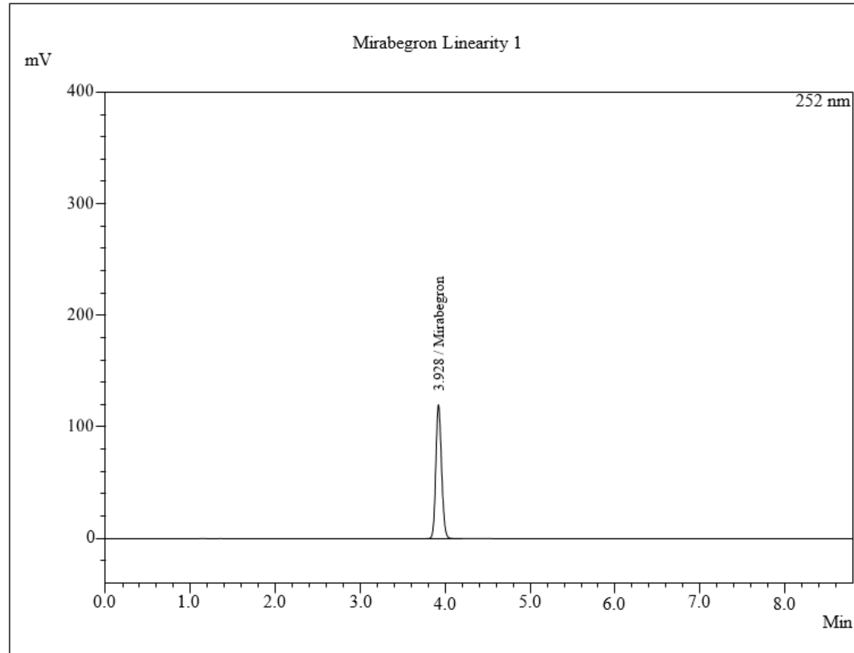


Figure 7: A typical Chromatogram of Linearity level 1

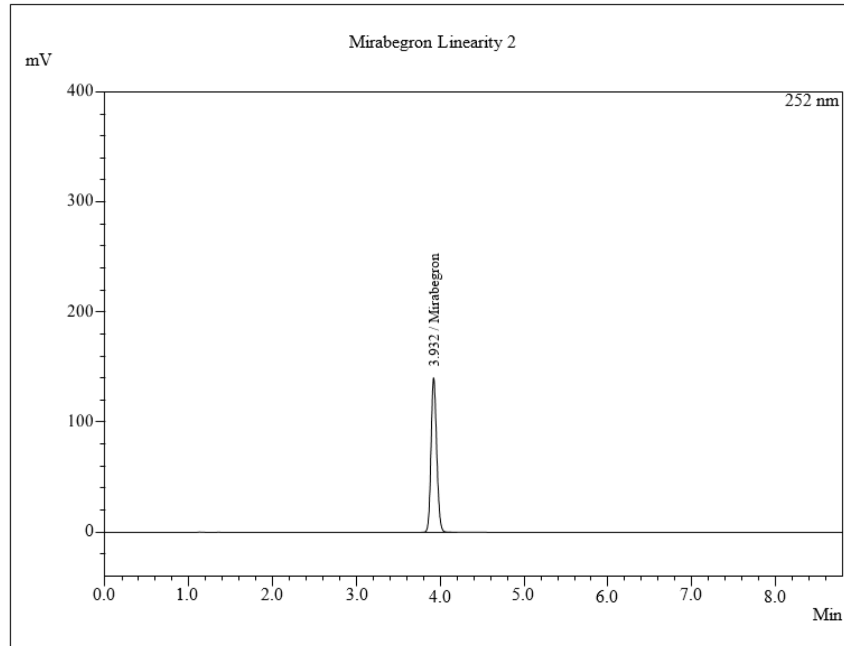


Figure 8: A typical Chromatogram of Linearity level 2

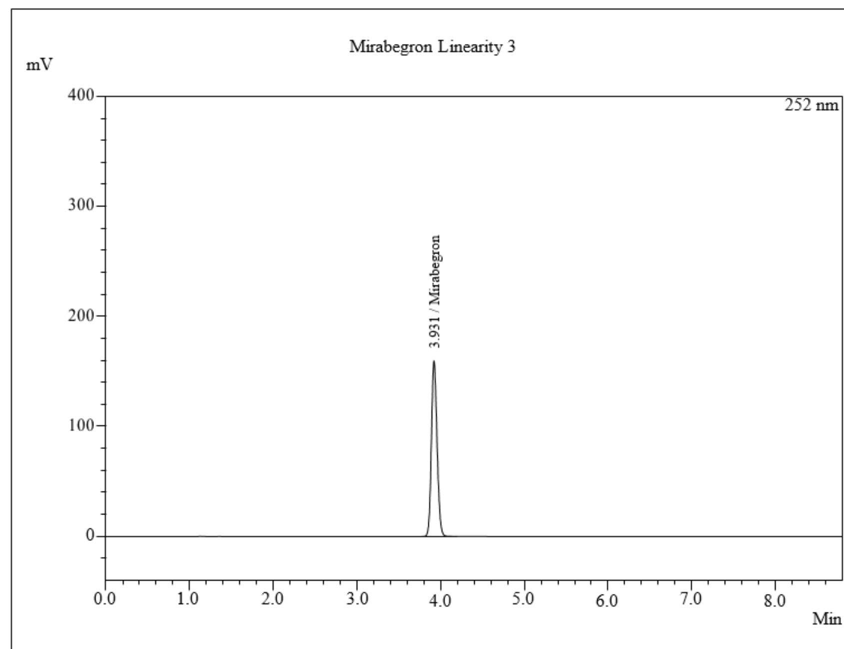


Figure 9: A typical Chromatogram of Linearity level 3

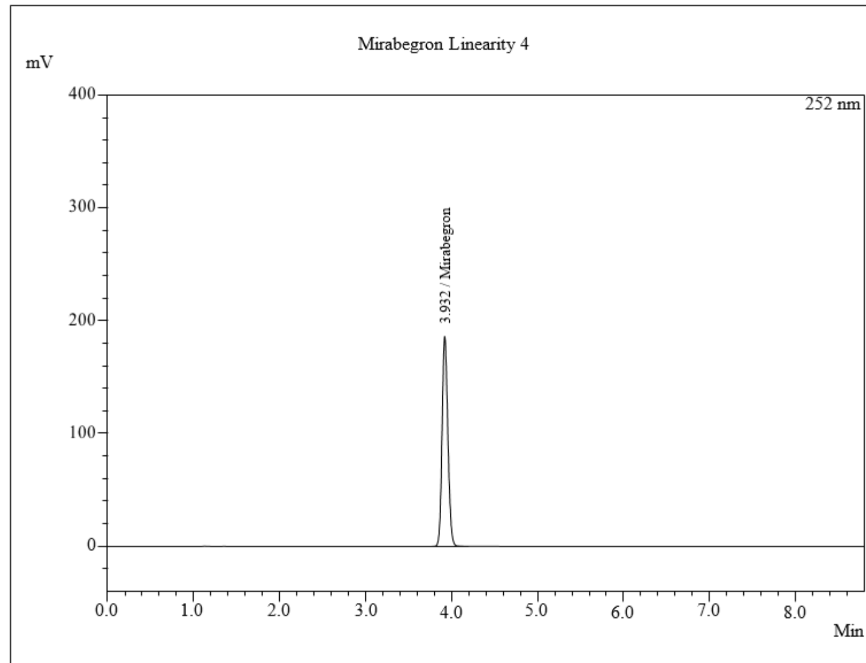


Figure 10: A typical Chromatogram of Linearity level 4

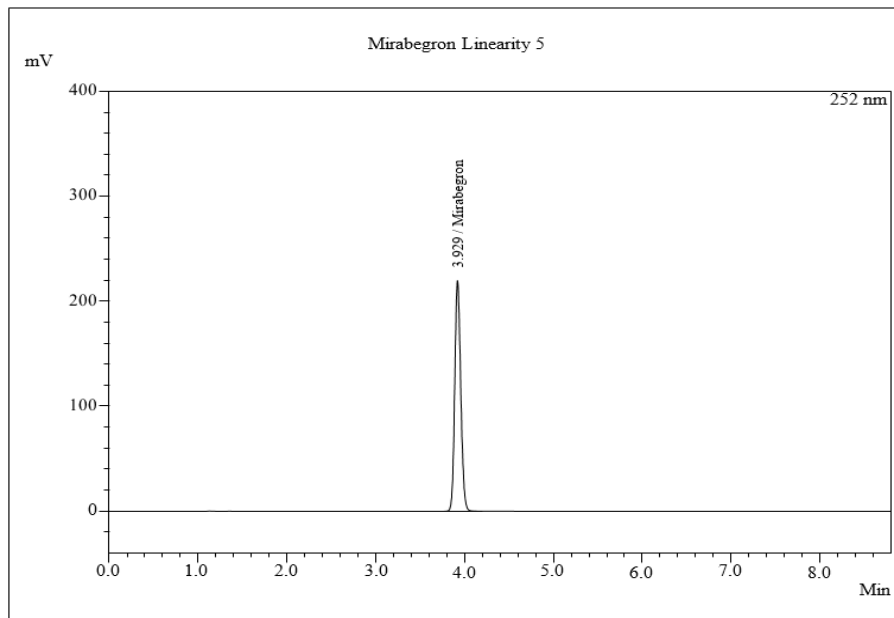


Figure 11: A typical Chromatogram of Linearity level 5

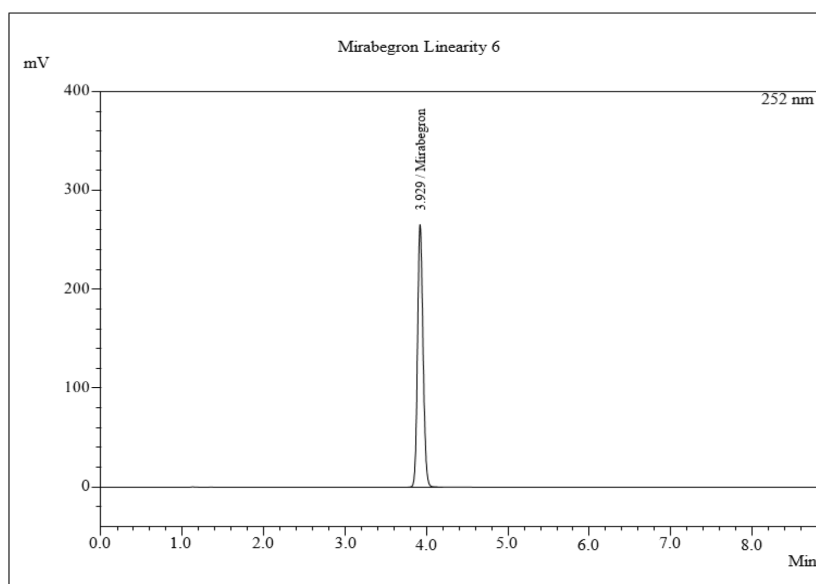


Figure 12: A typical Chromatogram of Linearity level 6

Table No. 2.2 Characteristic parameters of Mirabegron for the proposed HPLC method.

Parameter	**Result**
	Mirabegron
Calibration range (µg/ml)	10-60
Detection wavelength (nm)	252
Solvent (Acetonitrile: Methanol: Buffer)	30:30:40
Regression equation (y*)	Y = 64965x - 13333
Slope (b)	13333
Intercept (a)	64965
Correlation coefficient(r2)	0.9997
Limit of Detection (µg/ml)	1.1493
Limit of Quantitation (µg/ml)	3.4827

System Suitability:

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for ix replicate injections of the drug at a concentration of 20 µg/ml. The results which are given in Table No 7.15. were within acceptable limits.

Table No 2.3. System suitability studies of Mirabegron by HPLC method.

System Suitability Parameter		
Retention time (min)	**Concentration (µg/mL)**	**3.928**
Peak area	10	648217
Theoretical plates		3849
Asymmetric Factor		1.1

Formulation	
Name of Formulation	Mirabegron 50 Mg Tablets
Type of Formulation	Tablet

Concentration (mg)	50

Specificity:

Chromatogram of blank was taken as shown in Fig No.7.25. Chromatogram of Mirabegron showed peak at a retention time of 2.910 min. The mobile phase designed for the method resolved the drug very efficiently. The Retention time of Mirabegron was 2.910 ± 0.0078min. The wavelength 315 nm was selected for detection because; it resulted in better detection sensitivity for the drug. The peak for Mirabegron from the tablet formulation was Mirabegron.

Table No 2.4 Specificity of Mirabegron by HPLC method

Specificity				
Sample	**Label Claim (mg)**	**Amount Found**	**Recovery**	**Retention Time**
Tablet	50	49.91	99.82	3.928

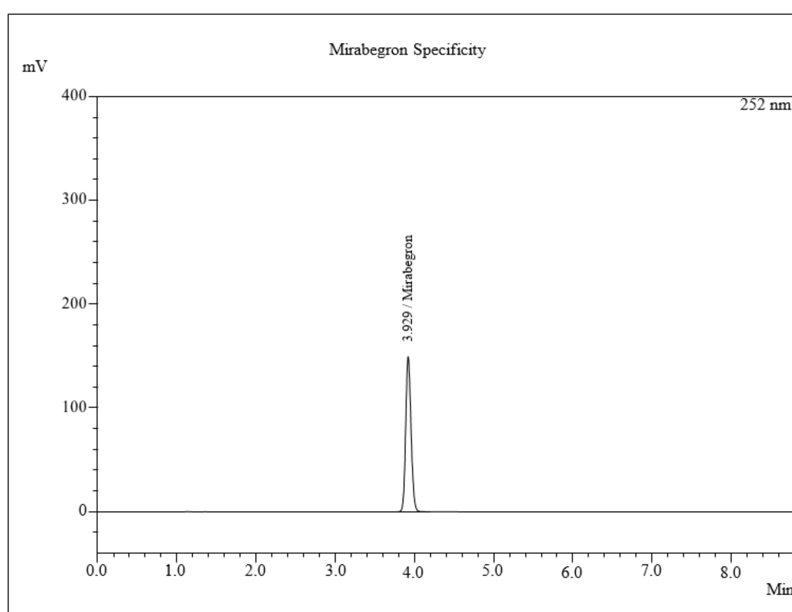


Figure 13: A typical chromatogram of Mirabegron [Concentration 20ug/ml]

Peak No.	Ret. Time	Name	Area	Area%	Tailing Factor	Theoretical Plates
1	4.085	Mirabegron	1001466	100.000	1.205	33049
Total			1001466	100.000		

Sensitivity:

The sensitivity of measurement of Mirabegron by use of the proposed method was estimated in terms of the limit of detection (LOD) and the limit of quantification (LOQ). The LOD and LOQ were calculated by the use of signal to noise ratio. In order to estimate the LOD and LOQ values, the blank sample was injected six times and the peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level, while ten times the noise value gave the LOQ. LOD and LOQ were found to be 1.01493 and 3.9586 respectively.

Precision:

Demonstration of precision was done under two categories. The injection repeatability (System Precision) was assessed by using six injections of the standard solution of Mirabegron and the % RSD of the replicate injections was calculated. In addition, to demonstrate the precision of method (Method Precision), six samples from the same batch of formulation were analyzed individually and the assay content of each sample was estimated. The average for the six determinations was calculated along with the % RSD for the replicate determinations. Both the system precision and method precision were subjected for inter-day and intra-day variations as reported in Table No 7.16. and 7.17 respectively.

Table No. 2.5 Repeatability of Mirabegron

Precision		
Repeatability		
Sr. No	**Concentration (µg/mL)**	**Peak Area**
1	40	2598086
2	40	2578086
3	40	2598099
4	40	2591212
5	40	2648086
6	40	2595874
Average		2601573.8
Standard Deviation		21905.7
RSD%		0.842

Table No. 2.6 Intraday Precision of Mirabegron

Precision			
Sr. No	**Concentration (µg/mL)**	**Intraday**	**Interday**
1	40	2598373	2648373
2	40	2591224	2658574
3	40	2595903	2719788
4	40	2577196	2735258
5	40	2648115	2745156
6	40	2595856	2755037
Average		2601111.2	2710364
Standard Deviation		22140.80	41712.85
RSD%		0.8512	1.539

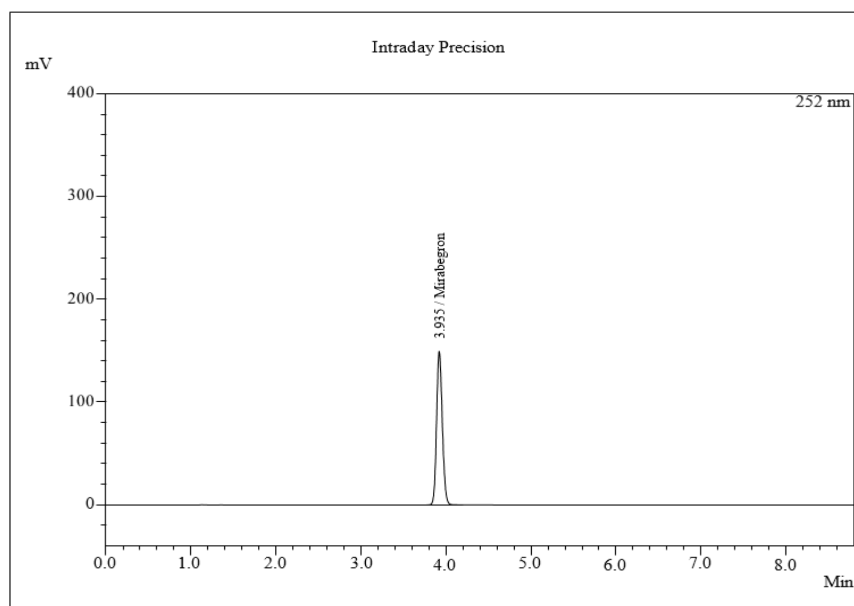


Figure 14: Intraday Precision of Mirabegron

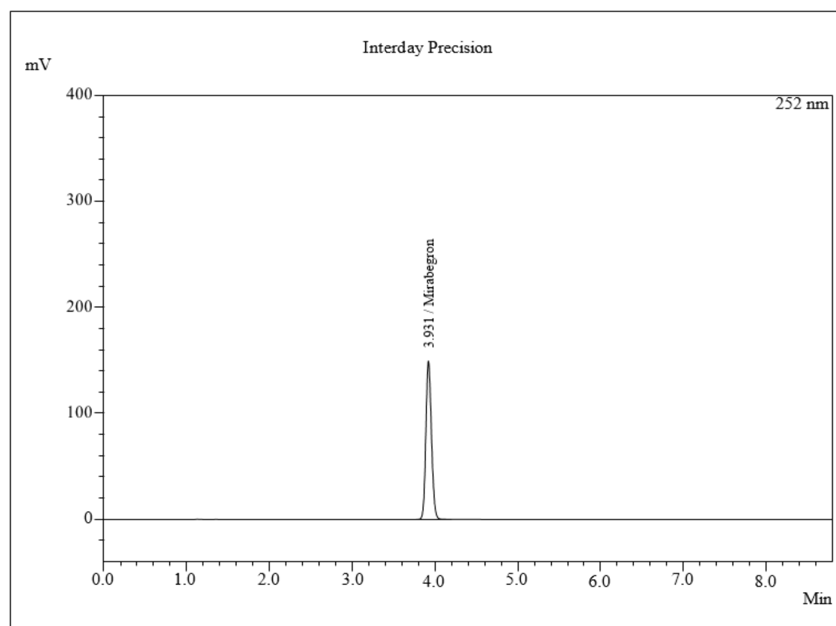


Figure 15: Interday Precision of Mirabegron

Accuracy:

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analysed samples of Mirabegron (20 µg/ml) were spiked with 80, 100, and 120 % extra Mirabegron standard and the mixtures were analysed by the proposed method. Standard deviation of the % recovery and % RSD were calculated and reported in Table No. 7.19.

Table 2.7: Recovery Table

Recovery				
Sr. No	**Amount of Sample**	**Amount of Drug Added**	**Amount of Drug Recovered**	**Recovery %**

	(µg/ml)	(µg/ml)	(µg/ml)	
1	40	20	19.989	99.95
2	40	40	39.99	99.98
3	40	60	60.01	100.02

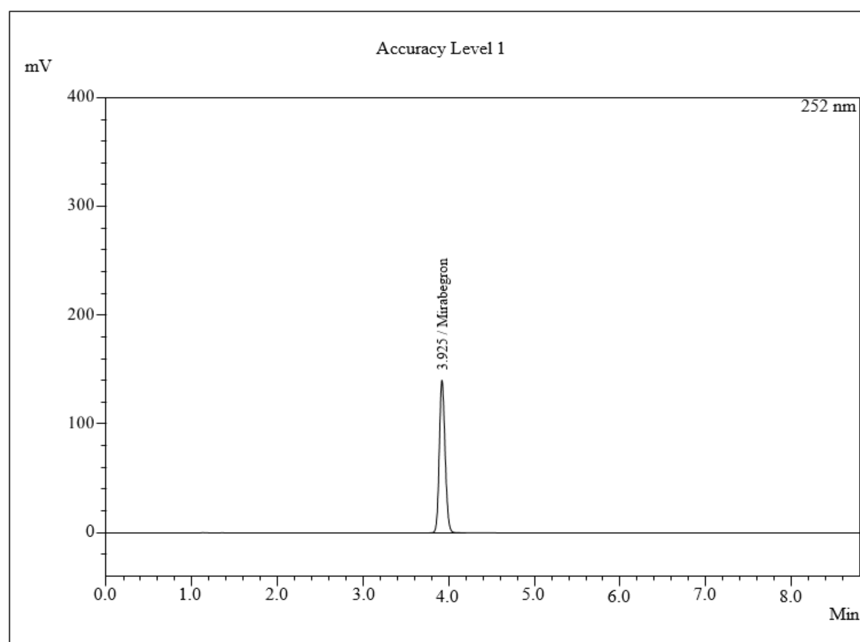


Figure 16: Chromatogram of Recovery level 1

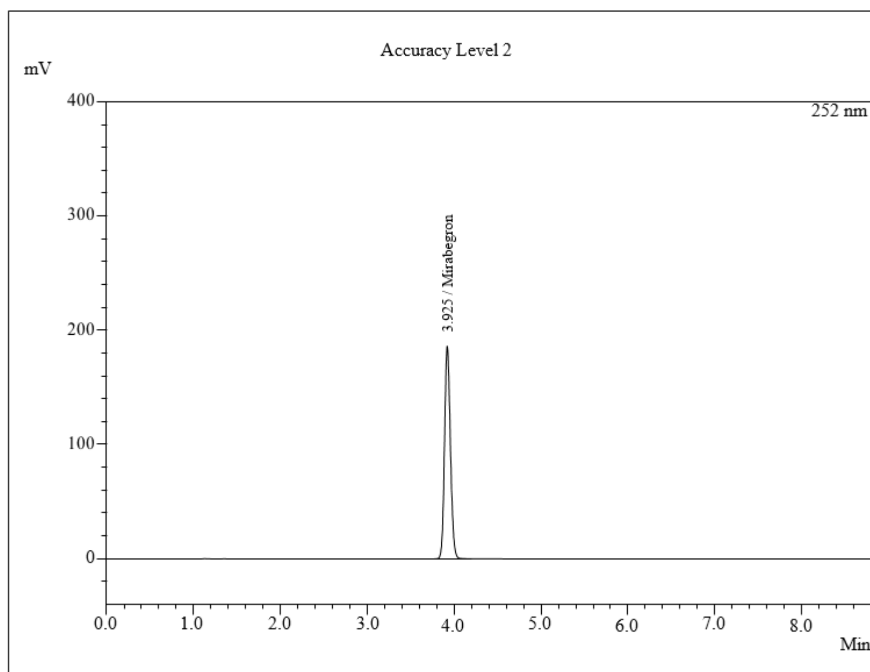


Figure 17: Chromatogram of Recovery level 2

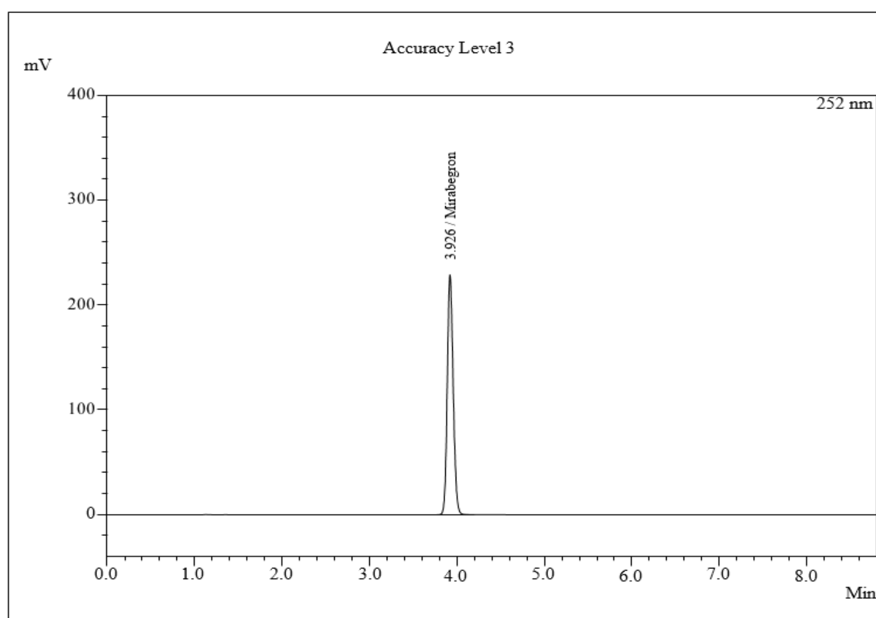


Figure 18: Chromatogram of Recovery level 3

Robustness:

Robustness is a measure of capacity of a method to remain unaffected by small, but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of mobile phase composition (Acetonitrile: Water and Acetonitrile: buffer) and mobile phase flow rate by 0.9 ml/min (0.8 and 1 ml/min) had no significant effect on the retention time and chromatographic response of the 20 µg/ml solution, indicating that the method was robust. The results are shown in Table No. 7.29.

Table No. 2.8 Robustness of Mirabegron at 252 nm

Robustness					
Sr. No	**Parameter**		**Response**	**Parameter**	**Response**
Acetonitrile: Potassium Phosphate Buffer			**Retention Time (min)**	**Detection Wavelength**	**Peak Area**
(V/V)				(nm)	
1	59	41	3.831	250	2534741
2	60	40	3.928	252	2593056
3	61	39	4.027	254	2626320
Average			3.929	Average	2584706
Standard Deviation			0.080	Standard Deviation	37850.36
RSD%			2.037	**RSD%**	1.464
Flow Rate			**Retention Time (min)**	**pH of Buffer**	**Peak Area**
(mL/min)				(mmol/L)	
1	0.9		4.059	5.8	2621128
2	1		3.928	6	2593229
3	1.1		3.9239	6.2	2517608

Average	3.970	Average	2577322
Standard Deviation	0.0627	Standard Deviation	43733.13
RSD%	1.580	**RSD%**	1.6968

CONCLUSION

For routine analytical purpose, it is always necessary to establish methods capable of analyzing huge number of samples in a short time period with due accuracy and precision. Mirabegron is official in Indian Pharmacopoeia. A very few analytical methods appeared in the literature for the determination of Mirabegron includes HPLC, HPTLC and UV- Visible spectrophotometric methods. In view of the above fact, some simple analytical methods were planned to develop with sensitivity, accuracy, precision and economical. In the present investigation HPLC method (Using Quality by Design) for the quantitative estimation of Mirabegron in bulk drug and per ICH guidelines pharmaceutical formulations has been developed. HPLC methods were validated as and results of linearity, precision, accuracy, Specificity, System suitability and robustness pass the limit. The HPLC method is more sensitive, accurate and precise compared to the previously reported method. There was no any interference of excipients in the recovery study. The low value of %RSD, molar extinction coefficient (L mol-1 cm-1) suggested that the developed methods are sensitive. The proposed high-performance liquid chromatographic method has also been evaluated over the accuracy, precision and robustness and proved to be convenient and effective for the quality control of Mirabegron. Developed method was found simple and cost effective for the quality control of Mirabegron. Moreover, the lower solvent consumption leads to a cost effective and environmentally friendly Spectroscopic procedure. Thus, the proposed methodology is rapid, selective, requires a simple sample preparation procedure, and represents a good procedure for Mirabegron.

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