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RP-HPLC Method Development and Validation for the Quantitative Determination of Potential Impurities of Mirabegron

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ABSTRACT

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 C_{18} 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. The developed HPLC method was found to be accurate and sensitive. The correlation coefficient values are greater than 0.99 for Mirabegron and its three impurities. Detection limit and quantitation limit was 0.04ppm and 0.14ppm respectively, indicating the high sensitivity of the newly developed method. Accuracy of the method was established based on the recovery obtained between 99.67% and 104.98% for all impurities. The result of robustness study also indicates that the method is robust and is unaffected by small variation in chromatographic conditions. The proposed HPLC method provides reliable, reproducible, accurate and sensitive for the quantification of Mirabegron related substances.

Keywords: Mirabegron; Impurities; RP-HPLC; Validation.

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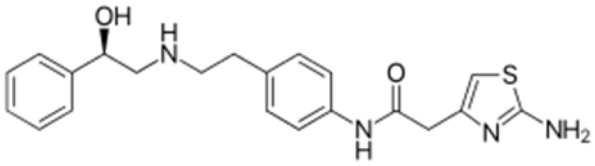
INTRODUCTION

Mirabegron is a medication used to treat overactive bladder. It is less preferred to antimuscarinic medication such as oxybutynin. It is taken by mouth. Common side effects include high blood pressure, headaches, and urinary tract infections. Other significant side effects include urinary retention, irregular heart rate, and angioedema. It works by activating the β3 adrenergic receptor in the bladder, resulting in its relaxation.

Mirabegron was approved for medical use in the United States in 2012. It is used is in the treatment of overactive bladder. It works equally well to antimuscarinic medication such as solifenacin or tolterodine. Mirabegron is a monocarboxylic acid amide obtained by formal condensation of the carboxy group of 2-amino-1,3-thiazol-4-ylacetic acid with the anilino group of (1R)-2-{[2-(4-aminophenyl) ethyl] amino}-1-phenylethanol.

Chemical structure of Mirabegron is given in figure 1.



Molecular weight = 396.51

Molecular formula = $C_{21}H_{24}N_4O_2S$

Figure 1: Structure of Mirabegron

Several analytical methods have been reported to determine Mirabegron in bulk drug, formulation and in biological matrices. These methods include spectrophotometry 13 to 15, , high performance thin layer chromatography (HPTLC) 12, high performance liquid chromatography (HPLC) 1-11, liquid chromatography tandem mass spectrometry (LC/MS) 16 and Ultra high performance liquid chromatography (UPLC) 17. Mirabegron is not an official drug in USP, EP, BP, and IP. Extensive literature survey reveals that no HPLC methods have been reported for the analysis of Mirabegron drug and its related substance. Hence it was felt necessary to develop an accurate, rapid and sensitive HPLC method for the determination of Mirabegron and its impurities.

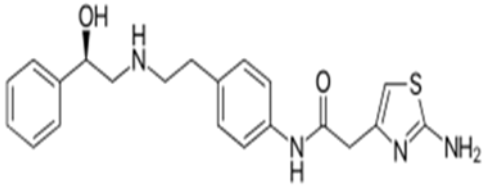
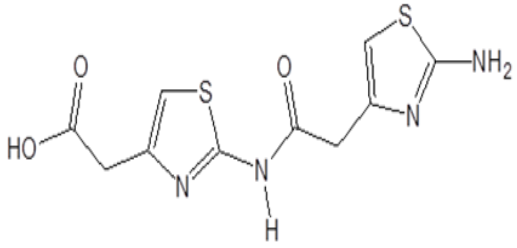
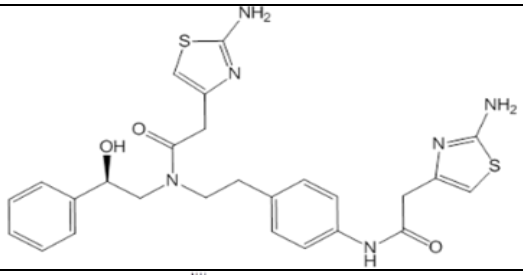
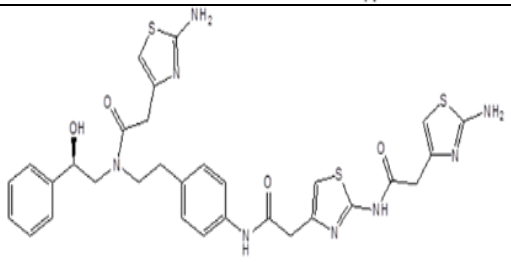
MATERIALS AND METHOD

Reagents and Chemicals

Samples of Mirabegron and standards of Imp-1, Imp-2 and Imp-3 (Table 1) were received from Analytica Chemie Inc, Bangalore, India. HPLC grade methanol and acetonitrile was purchased from Rankem, Mumbai, India. Deionized water was prepared using a Milli-Q plus water purification system from Millipore (Bedford, MA, USA). Analytical reagent grade of Ammonium

acetate, acetic acid, sodium hydroxide, hydrogen peroxide and hydrochloric acid were purchased from Merck India Limited (Mumbai, India).

Table 1: Potential impurities of Mirabegron

Sl. No.	Structure	Mol. Wt.	IUPAC name
1		396.506	2-(2-amino-1,3-thiazol-4-yl)-N-[4-(2-{[(2R)-2-hydroxy-2-phenylethyl] amino}ethyl)phenyl]acetamide
2		298.34	(R)-2-(2-Aminothiazol-4-yl)-N-(4-(2-(2-(2-(2-aminothiazol-4-yl)a cetamido)thiazol-4-yl)acetamido)phenethyl)-N-(2-hydroxy-2-phenylethyl)acetamide
3		536.67	(R)-2-(2-Aminothiazol-4-yl)-N-(4-(2-(2-aminothiazol-4-yl)acetamido)phenethyl)-N-(2-hydroxy-2-phenylethyl)acetamide
4		676.83	(R)-2-(2-Aminothiazol-4-yl)-N-(4-(2-(2-(2-(2-aminothiazol-4-yl)a cetamido)thiazol-4-yl)acetamido)phenethyl)-N-(2-hydroxy-2-phenylethyl)acetamide

Instruments

The LC method development and validation were done using Waters HPLC equipped with PDA detector. The data were collected and the peak purity of the Mirabegron peak was checked using Empower software.

Chromatographic conditions

The chromatographic separations were achieved on Puratis Eximius, C18 column (250 mm length × 4.6 mm ID with 5µm particle size. Mobile phase A consisted, 20 mM ammonium acetate, pH adjusted to 4.5 and mobile phase B consisted methanol with a gradient programme (T_{min}A:B)$T_0$90:10, T10.055:45, T20.010:90, T22.010:90, T25.090:10, T30.090:10 The column temperature was maintained at 25 ˚C and the detection was carried out at 247 nm. The flow rate

was set to 1.0 mL/min. The test concentration was about 100 µg/mL and the injection volume was 10µL. A degassed mixture of Methanol and water (8:2) was used as diluent for standard and sample preparations.

Buffers are recommended to control the pH stability of the mobile phase. Buffers like ammonium acetate, dipotassium hydrogen orthophosphate, potassium hydrogen phosphate, diammonium hydrogen orthophosphate, ammonium hydrogen phosphate, and its combination were studied for the Mirabegron HPLC method development. We have adjusted the pH of the buffer is 4.5 to get a better peak shape for all the experiments. Methanol was chosen as organic modifiers. The principle difference in the behavior of acetonitrile and methanol is that where acetonitrile forms a thick multi-molecular adsorbed layer on the surface of reverse phase adsorbent (C_1-C_{18} and phenyl phases), while methanol is adsorbed only in monomolecular fashion[14]. This brings a principal difference in the analyte retention mechanism in these two hydro-organic systems.

Column selection

The heart of a HPLC system is the column. Changing a column will have the greatest effect on the resolution of analytes during method development. Silica-based packing materials dominate in applications for RP separations in the pharmaceutical industry. The vast majority of RP LC separations take place on column that contain C_{18} bonded stationary phases due to their stability, retentively and reproducibility. In addition, these hydrophobic ligands provide the desired separation most of the time. However, screening several different types of stationary phases during method development for a particular separation is often useful because different columns usually have different selectivity for components in a sample.

Several experiments were conducted to get a baseline resolution between Mirabegron and impurities. water miscible organic solvent. Choosing a right buffer and pH is very critical for method development.

C_{18} were used in different mobile phases containing acetate buffers along with acetonitrile with pH 4.5 Use of methanol as an organic modifier shown significance improvement in resolution between Imp-2 and Imp-3.Use of RP_{18} column with a 250 mm length × 4.6mm ID column and 5µm particle size, use of mixture of 20 mM ammonium acetate, pH adjusted to 4.5 as mobile phase A and methanol as mobile phase-B was significant in achieving the desired resolution of Mirabegron and its impurities. After several trials for gradient profile, chromatographic conditions were finalized as described under section chromatographic conditions

Preparation of stock solutions for method validation

A test preparation of 1000 µg/mL of Mirabegron API sample was prepared by dissolving in diluent. A stock solution of impurities was prepared by dissolving 5 mg each of Imp-1, Imp-2, Imp-3 in diluent and made up to 10 mL with diluent. Transferred 2.5 mL of each individual stock solution into a 25 mL volumetric flask and made up to volume with diluent. From this stock solution, standard solution of 0.5 µg/mL to 3.0 µg/mL of each impurity and 2.0 µg/mL to 12.0 µg/mL of Mirabegron was prepared. A stock solution of Mirabegron was prepared by dissolving 25 mg made up to 25 mL with diluent. Transferred 5.0 mL of the stock solution into a 50 mL volumetric flask and made up to volume with diluent. From this stock solution, standard solution of 2.0 µg/mL to 12.0 µg/mL of each impurity

RESULTS AND DISCUSSION:

Method development

The determination of the suitability of a HPLC method is based upon the level of development. However, at a minimum HPLC method should provide baseline separation of starting materials, desired products, known impurities, and expected by-products. The chromatographic conditions should also be chemically compatible with the analytes. The main objective of the HPLC method development for Mirabegron was to achieve efficient separation of impurities and a short run time method.

Selection of wavelength

The optimum wavelength of detection is the wavelength that gives the highest sensitivity for the significant related substances and minimizes the difference in response factors between those of the active pharmaceutical ingredient and the related substances. Mirabegron and its impurities give good detector response at 247 nm, therefore the final absorption wavelength for detection was chosen at 247 nm.

Mobile phase selection

In reverse phase chromatography, the mobile phase consists of an aqueous buffer and a non-UV active

METHOD VALIDATION:

The newly developed method was validated for sensitivity, linearity, precision and accuracy, robustness and system suitability according to ICH guidelines 20. Validation study was carried out for Imp-1, Imp-2, Imp-3. The system suitability and selectivity were checked by injecting 12 µg/mL of Mirabegron solution containing 3µg/mL of all impurities monitored throughout the validation. Method validation results are summarized in Table 2.

Table 2: Method validation summary report

Parameter	Imp-1	Imp-2	Imp-3	MG
System suitability				
RT	10.81	18.65	19.42	14.87
RRT	0.72	1.25	1.30	1
Rs	-	22.01	19.76	3.95
N	49265	117444	153273	171111
T	1.03	1.07	1.05	1.04
Linearity				
r	0.9996	0.9993	0.998	0.9996
Slope	15568.64	24769.34	5823523.6	0939659.51
Detection limit (ppm)	0.07	0.07	0.02	0.01
Quantitation limit (ppm)	0.12	0.04	0.21	0.06
Precision				
% RSD (n 6)	2.01	1.09	1.43	0.22
Repeatability (intraday)				
% RSD (n 6)	3.82	0.98	1.98	1.57
Intermediate precision (inter day)				
% RSD (n 6)	2.97	0.72	0.93	0.35
Accuracy at 50% level (n 3)				
Amount added (%)	0.53	0.53	0.53	13.30
Amount recovered (%)	0.517	0.5131	0.5115	12.715
% Recovery	103.61	104.54	104.98	104.63
Accuracy at 100% level (n 3)				
Amount added (%)	1.05	1.05	1.05	25.95
Amount recovered (%)	1.03	1.02	1.023	25.36
% Recovery	102.26	102.94	103.24	102.34
Accuracy at 150% level (n 3)				
Amount added (%)	1.54	1.54	1.54	38.82
Amount recovered (%)	1.55	1.53	1.53	37.703
% Recovery	99.67	100.56	100.59	102.96

Stress condition	Time(min)	Temp(°C)	% Assay of active substance	% of Degradation products	% Area
Acid Hydrolysis (1N HCl)	120	60	104.2757	1.39	98.61
Basic Hydrolysis (1N NaOH)	120	60	90.62859	2.34	97.66
Oxidation (10% H_2O_2)	120	60	55.47937	25.70	74.30
Hydrolysis (60 °C)	120	60	101.2761	0.1	99.99
UV (254 nm)	3600	-	105.5835	0.1	99.99

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection and limit of quantitation were determined for trimipramine maleate and for each of the related substances as per ICH $Q2R_1$ guideline. The LOD and LOQ for Imp-1, Imp-2,

Imp-3 and Mirabegron were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively by injecting a series of diluted solutions with known concentration. The limit of detection and the limit of quantitation for Imp-1, Imp-2, Imp-3 and Mirabegron were about 0.07, 0.07 & 0.02ppm & 0.12, 0.04 & 0.21ppm respectively. Precision study was also carried at the LOQ level by injecting six individual preparations of all impurities and the relative standard deviation for LOQ concentration for all impurities were below 5%.

Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of all impurities. The range of the method was from LOQ to 0.21 µg/mL of the analyte concentration (100 µg /mL).

Precision

Precision of the method was studied for method precision and intermediate precision. Method precision was checked by injecting six individual preparations of (100 µg/mL) Mirabegron spiked with 0.5µg/mL of each impurity. In the intermediate precision study, the similar procedure of method precision was carried out by a different day. % RSD of areas of each impurity was within 5.0, confirming good precision at low level of the developed analytical method.

Accuracy

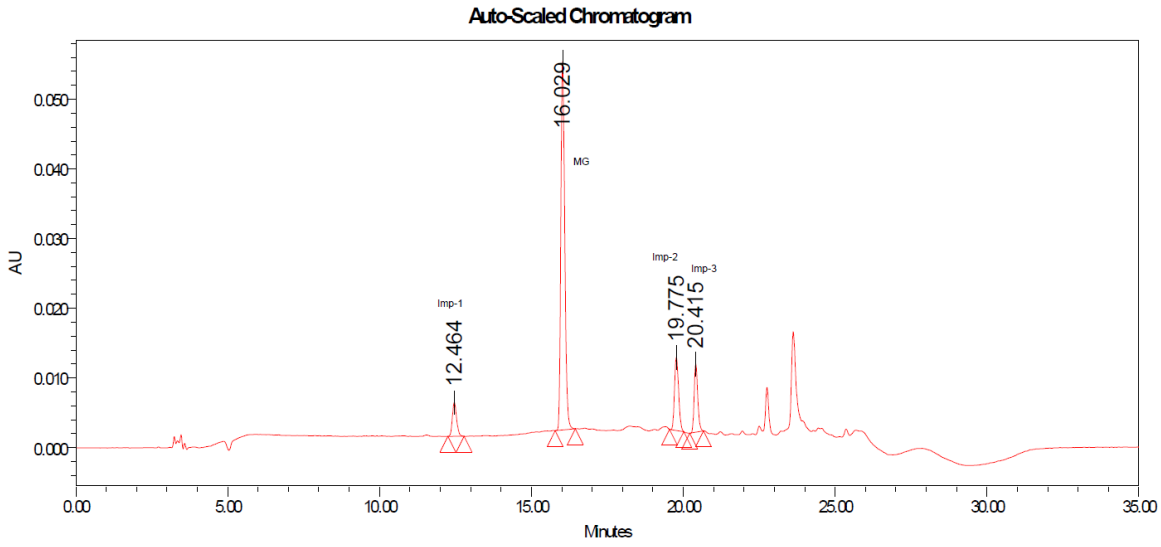


Figure 2: Chromatogram of Mirabegron spiked with impurities

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was evaluated in triplicate 50%level (0.51µg/mL), 100% level (1.02 µg/mL) and 150% level (1.53µg/mL). The percentage recovery of all impurities in drug substance has been calculated. Chromatogram of Mirabegron spiked with three impurities was

depicted in Figure 2. concentration of the analyte in the sample. A linearity test solution for related substance method was prepared by diluting the impurity stock solution to the required concentrations. was subjected to linear regression analysis method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The residuals and sum of the residual squares were calculated from the predicted responses. The correlation coefficient obtained was greater than 0.99 for all impurities. The result showed an excellent correlation between the peak and its impurities

Robustness

To determine the robustness of the method, experimental conditions were deliberately changed and the resolution between closely eluting peaks, Imp-2 and Imp-3 were evaluated. Close observation of analysis results of deliberately changed chromatographic conditions viz; flow rate (0.2±0.05 mL/min), pH 4.5±0.2 and column temperature (25± 2 °C) shown no significant change in relative retention time for all impurities in spiked sample illustrating the robustness of the method.

Solution stability and mobile phase stability

The solution stability of trimipramine maleate and its related impurities was carried out by leaving both spiked and unspiked sample solutions in tightly capped HPLC vials for 72 h in an auto sampler. Content of each impurity was determined against freshly prepared standard solution. No significant changes were observed in the content of any of the impurities. The solution stability and mobile phase stability experiment data confirms that the sample solutions and mobile phase used during related substance determination were stable for at least 72 hour.

CONCLUSION:

The developed HPLC method provides reliable, reproducible, accurate and sensitive for the quantification of trimipramine maleate related substances. This newly developed method has been validated as per regulatory requirements and has shown acceptable precision, accuracy and adequate sensitivity. This method can be used for the routine analysis of Mirabegron active pharmaceutical ingredient related substances.

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