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A NEW RP-HPLC METHOD FOR ESTIMATION OF MIRABEGRON IN PHARMACEUTICAL DOSAGE FORM WITH FORCE DEGRADATION STUDIES

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ABSTRACT

A simple and selective HPLC method is described for the determination of Mirabegron in tablet dosage forms. Chromatographic separation was achieved on a Waters Acquity C18 (50mm x2.1 mm ID) 1.8µm using mobile phase consisting of a mixture of 70 volumes of Potassium di-hydrogen phosphate and 30 volumes of methanol with detection of 254nm. Linearity was observed in the range 50-120 µg /ml for Mirabegron (r^2 =0.998) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Key Words: Mirabegron, tablet dosage form, HPLC method

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INTRODUCTION

Quality investigation plays a very important role in quality specification establishment of chemical drugs. The number of drugs introduced into the market every year .very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. Hence, standards and analytical procedures for these drugs may not be available in the pharmacopoeias.

It becomes necessary, therefore to develop newer analytical methods for such drugs. Analytical method development provides the support to track the quality of the product from batch to batch. Method development involves considerable trial and error procedures. The most difficult problem usually is where to start, what type of column is worth trying with what kind of mobile phase. Single dosage forms with combination of drugs are widely used today due to their advantages and their simultaneous estimation of individual component is a challenging task.

Chromatography is a non-destructive procedure for resolving a multi-component mixture of traces, minor or constituents in to individual fractions. It is a method of separating a mixture of components in to individual components through a porous medium under the influence of solvent.

HPLC was derived from classical column chromatography and has found an important place in analytical techniques. It's a physical separation technique in which a sample dissolved in a liquid is injected into a column packed with small particles and it is separated into its constituent components. It is probably the most important and widely used analytical technique for quantitative analysis of organics and biomolecules, also applicable to much kind of samples. HPLC utilizes a liquid mobile phase to separate the components of a mixture. The stationary phase can be a liquid or a solid phase. These components are first dissolved in a solvent, and then forced to flow through a chromatographic column under a high pressure. In the column, the mixture separates into its components. The amount of resolution is important, and is dependent upon the extent of interaction between the solute components and the stationary phase. The stationary phase is defined as the immobile packing material in the column. The interaction of the solute with mobile and stationary phases can be manipulated through different choices of both solvents and stationary phases. As a result, HPLC acquires a high degree of versatility not found in other chromatographic systems and it has the ability to easily separate a wide variety of chemical mixtures. Non-polar compounds in the mixture will tend to form attractions with the hydrocarbon groups because of Vander Waals dispersion forces. They will also be less soluble in the solvent because of the need to break hydrogen bonds as they squeeze in between the solvent molecules, for example, water or methanol. They therefore spend less time in the solvent and this will slow them down on their way through the column. That means, now it is the polar molecules that will travel through the column more quickly. Reversed phase HPLC is the most commonly used form of HPLC

Mirabegron is a drug for the treatment of overactive bladder. It was developed by Astellas Pharma and was approved in the United States in July 2012. Mirabegron activates the β_3 adrenergic receptor in the detrusor muscle in the bladder, which leads to muscle relaxation and an increase in bladder capacity. Mirabegron is a potent and selective agonist for beta-3 adrenergic receptors. Once beta-3 receptors are activated, the detrusor smooth muscle relaxes to allow for a larger bladder capacity. At higher doses (200 mg), there is a potential for mirabegron to activate beta-1 and beta-2 adrenergic receptors. Mirabegron has little effect on the mean maximum flow rate or mean detrusor pressure at maximum flow rate in patients with lower urinary tract symptoms and bladder outlet obstruction. Furthermore, mirabegron increases blood pressure in a dose dependent manner. However, this effect is reversible when mirabegron is discontinued. Mirabegron also increases heart rate in a dose dependent manner. The dose in which half-maximal efficacy is demonstrated is 25 mg. Comparatively, the dose in which maximal efficacy is demonstrated is 100 mg. The absolute bioavailability increases from 29% at a dose of 25 mg to 35% at a dose of 50 mg. Mean Cmax and AUC increase more than dose proportionally. This relationship is more apparent at doses above 50 mg. Females generally have a lower magnitude of increase of Cmax and AUCtau compared to males when doses of mirabegron doubles or quadruples. Steady state concentrations are achieved within 7 days of once daily dosing with mirabegron. After once daily administration, plasma exposure of mirabegron at steady state is approximately double that seen after a single dose. Tmax, oral dose, healthy subjects= 3.5 hours (7).

Aim is to develop a new RP-HPLC method for estimation of Mirabegron in pharmaceutical dosage form with force degradation studies.

MATERIALS AND METHODS

Determination of Working Wavelength (λmax)

Preparation of standard stock solution of mirabegron (8-10)

10 mg of Mirabegron was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Assay

Preparation of standard solution

Weigh accurately 10 mg of mirabegron in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10 µg/ml mirabegron is prepared by diluting 1.0 ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (Mirago 25mg Extended Release Tablets) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solution of mirabegron were prepared by dissolving weight equivalent to 10 mg of mirabegron and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 10ug/ml of mirabegron was made by diluting 1.0 ml to 10ml with mobile phase.

Stability Studies

Peroxide degradation

Sample solution of Mirabegron (10µg/ml) and 1 ml of 20% hydrogen peroxide (H2O2) was mixed. For HPLC study, 10 µl were injected into the system and the chromatogram was recorded to assess the stability of sample.

Photolytic degradation

The photochemical stability of the drug was studied by exposing the 50µg/ml solution to UV light by keeping the beaker in UV chamber for 7 days.

For HPLC study, the resultant solution 10µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

Acidic degradation

Take 1 tablet, powdered and place in a 50ml volumetric flask and dissolve in mobile phase up to 75% then sonicate it for 10 minutes then add 1 ml of 0.1N HCl then kept in oven at 60^0c for 1 hour then cool and add 1 ml of 0.1N NaOH it then make up the volume up to 50ml with mobile phase, then place the sample in the vial and measure the chromatogram.

Alkaline degradation

Take 1 tablet, powdered and place in a 50ml volumetric flask and dissolve in mobile phase up to 75% then sonicate it for 10 minutes then add 1 ml of 0.1N NaOH then kept in oven at 60^0c for 1 hour then cool it and add 1 ml of 0.1N HCl then make up the volume up to 50ml with mobile phase, then place the sample in the vial and measure the chromatogram.

Thermal degradation

Sample solution of Mirabegron (10µg/ml) was placed in oven at 105^0C for 6 hr to study dry heat degradation. For HPLC study, the resultant solution was injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The absorption curve shows characteristic absorption for mirabegron.

The percentage purity of mirabegron found to be within the limits that is 98-102% (Table-1).

Table-1 Results of assay

MIRABEGRON		
	Standard Area	**Sample Area**
Injection-1	102102014	102262822
Injection-2	102433207	101950528
Injection-3	101915558	101670222
Injection-4	101721781	101998023
Injection-5	101795941	101853817
Average Area	101993700.2	101947082.4
Assay(%purity)	**99.95**	

The % RSD for the retention times and peak area of mirabegron were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit (Table-2).

Table-2 Results for system suitability of mirabegron

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.497	105011137	2197	1.32
2	1.497	104237044	2209	1.28
3	1.497	104909445	2213	1.28
4	1.497	104025812	2216	1.32
5	1.497	103574883	2230	1.28
Mean	1.497	104351664.2	-	-
SD	0.00154	26716.59	-	-
%RSD	0.103	0.082	-	-

The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparations of mirabegron is 0.998. The relationship between the concentration and area of mirabegron is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits (Fig-1).

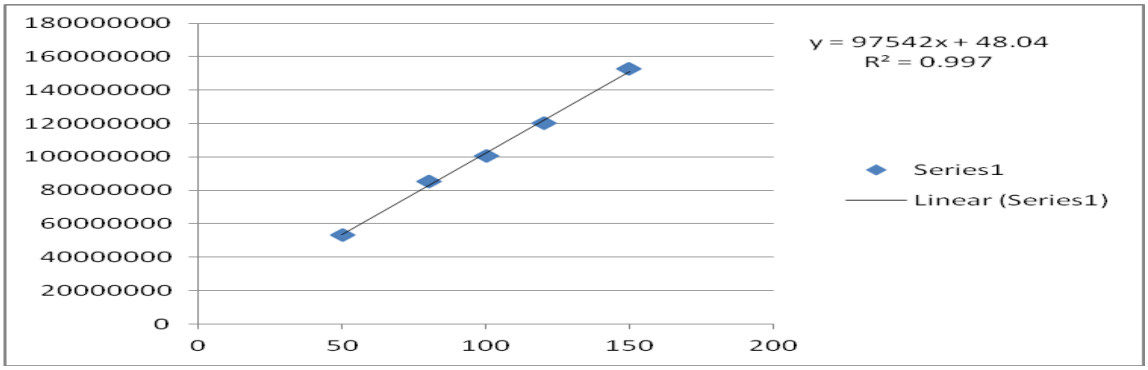

Fig-1 Linearity graph of mirabegron

Stability Studies

Sample solution of Mirabegron (10µg/ml) was tested for forced degradation studies under the conditions such as peroxide degradation, photolytic degradation, acidic degradation, alkaline degradation, thermal degradation and the chromatograms (Fig-2-6) were recorded to assess the stability of the sample

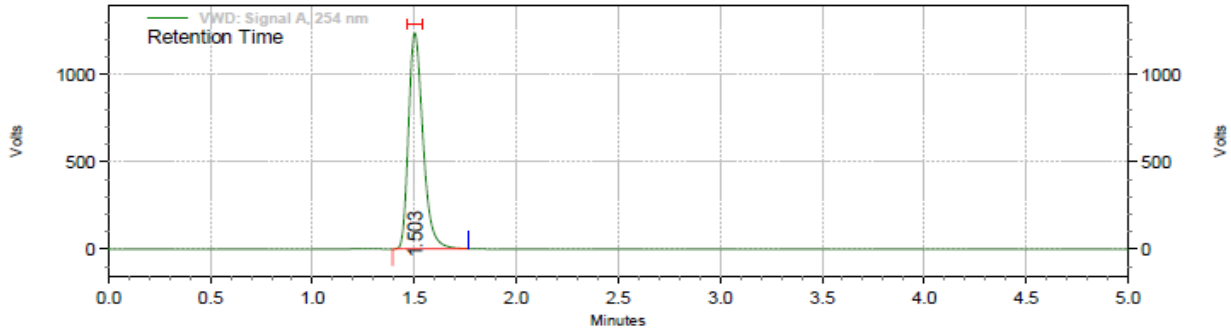

Fig-2 chromatogram of peroxide degradation

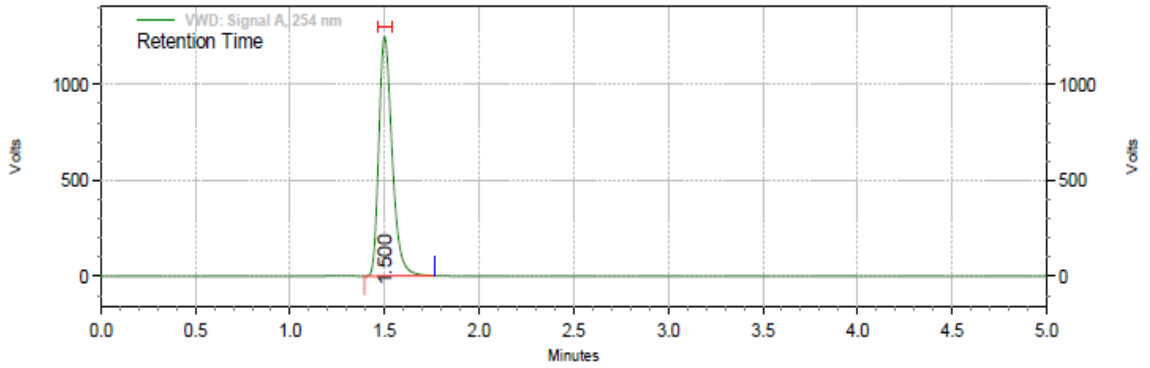

Fig-3 chromatogram of photolytic degradation

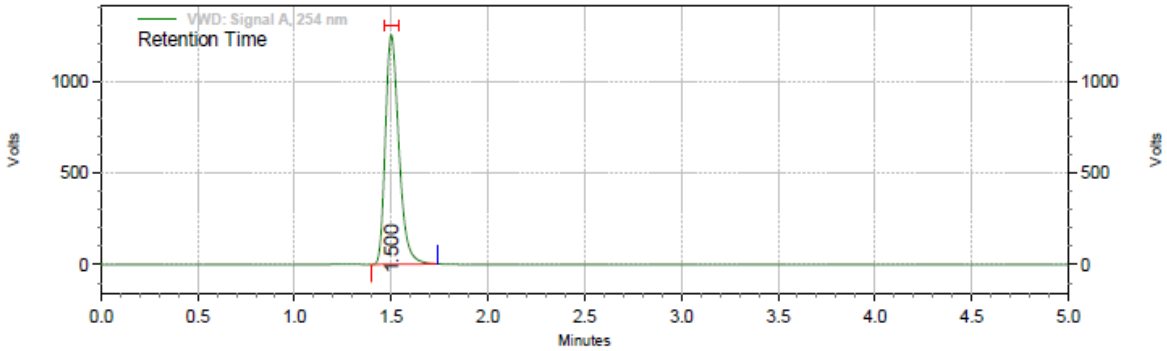

Fig-4 chromatogram of acidic degradation

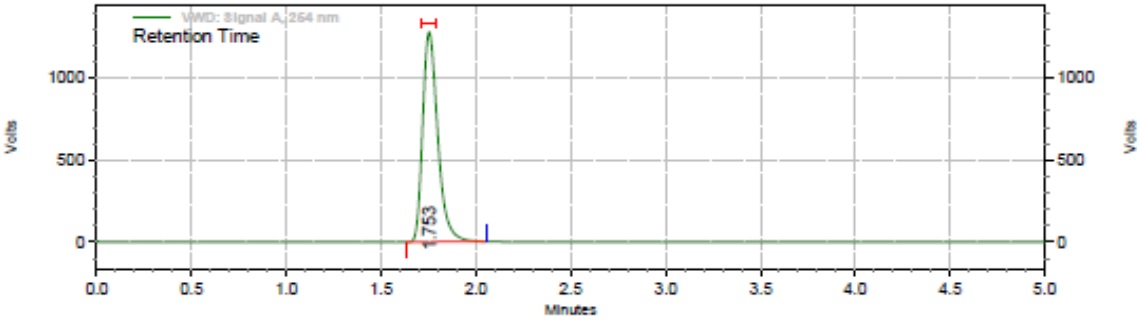

Fig-5 chromatogram of alkaline degradation thermal degradation

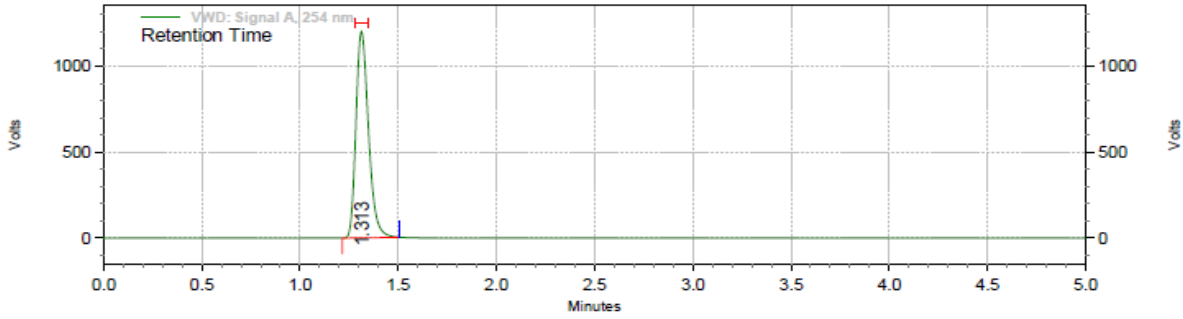

Fig-6 chromatogram of thermal degradation

CONCLUSION

The above experimental results and parameters it was concluded that, this newly developed method for the estimation of Mirabegron was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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