METHOD DEVELOPMENT AND VALIDATION OF SIMPLE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF *MIRABEGRON* IN BULK AND ITS FORMULATIONS

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Abstract— *Mirabegron* is a sympathomimetic beta-3 adrenergic agonist used to release the smooth muscle of the bladder in the treatment of urinary frequency and incontinence. A novel simple, accurate, precise reproducible UV spectrophotometric method has been developed and validated for the estimation of *MIRABEGRON* in bulk and its formulations. After determining the solubility, we had selected 0.1N HCL as a solvent and the mirabegron has absorbance at the wavelength of maximum λ max 249nm. The drug was characterized by the melting point test, proposed method was précised with RSD less than 2% linearity test was approved within the range of 0.5-3.0µg/ml with the correlation coefficient (R^2) of 0.9999, accuracy was 1000.3%, and LOD and LOQ were 0.777 µg/ml and 0.235 µg/ml. Hence developed method can be used for routine quantitative analysis of Mirabegron.

Index Terms— UV spectroscopy, 0.1N HCL, λmax, Solubility, Linearity, LOD and LOQ, Calibration curve, Standard deviation, Relative standard deviation.

INTRODUCTION:

One of the most effective methods for examining atomic and molecular structure is spectroscopy, which is employed in the examination of a variety of materials. Drugs are estimated using a variety of techniques, including the calibration curve, area under the curve, derivative spectroscopy, absorption ratio, absorption correction, and others. A number of techniques have been developed for estimating the new medication Mirabegron, which is used to treat the condition known as overactive bladder syndrome. According to the literature review, UV spectroscopy, including the zero-order method and derivative spectroscopy techniques, which primarily used methanol or concentrated solution as a solvent, has been used to estimate the drug from bulk and commercial formulations. The suggested study demonstrates three novel UV-spectrophotometric techniques that are quick, easy, and affordable for measuring Mirabegron in bulk and its tablets by using 0.1N HCL is the solvent. The developed method was tested for the accuracy, precision, sensitivity.

Drug profile:

Mirabegron is the first clinically available beta-3 agonist with approval for use in adults with Overactive bladder syndrome disease. Mirabegron was approved for medical use in the United States and in the European Union in 2012. In 2020, it was the 160th most prescribed medication in the United States, with more than 3 million prescriptions It is available as a generic medication.

- **Structure of Mirabegron:**

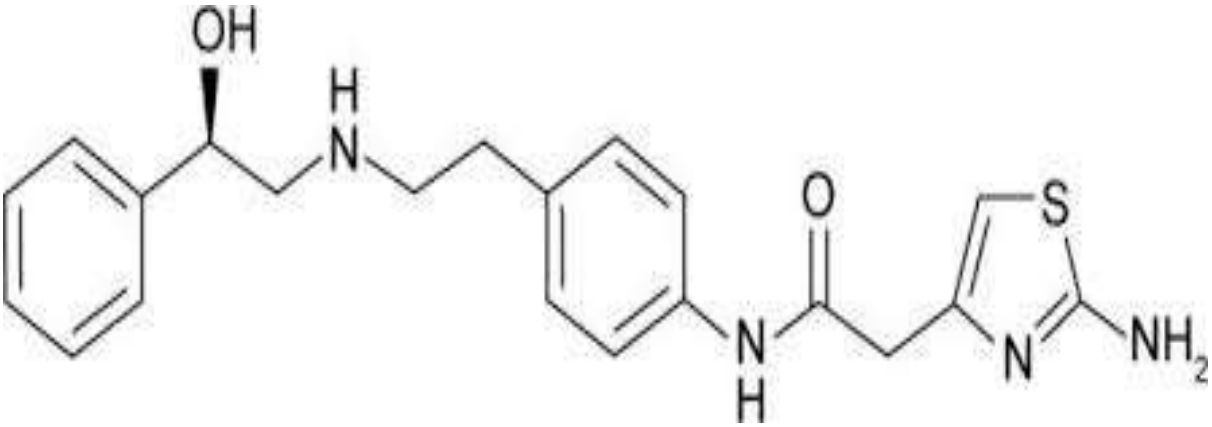

Figure 1: Structure of Mirabegron

- Chemical Name: (R)-2-(2-aminothiazol-4-yl)-N-(4-(2-((2-hydroxy-2-phenylethyl) amino) ethyl) phenyl) acetamide.
- Category: Beta 3 - adrenergic agonist
- Molecular Formula: - C21H24N4O2S
- Molecular weight: - 396.506 g/mol
- pka value: - 4.5
- Brand: Mirago (Sun Pharma), Mirbeg (IPCA Laboratories Limited), Bladmir (Alembic Pharmaceuticals), vesi-beta (Cipla Limited)), lupin-Mira (Lupin Pharmaceuticals).
- Pharmacokinetic Data Bioavailability-29% - 35% Metabolism – Majorly in liver Elimination half-life – 50 hours Maximum plasma concentrations (Cmax) at approximately 3.5 hours. Excretion - Urine and bile (90%)

MATERIALS AND METHODS:

Chemical and Reagent:

- Pharmaceutical grade Mirabegron standard was obtained as a gift sample. Solvents like Methanol, Ethanol, 0.1N HCL, Distilled water was used.

Instruments

- UV-Spectrophotometer: Analytical (2080) Double beam SL ultraviolet Spectrometer
- Sonicator: PCI Mumbai, Model No.3.5L 100H
- Weighing balance: Shimadzu IN-201L and Analytical Balance

Selection of solvent:

- The ability of a solid substance (solute) to dissolve in solvent and to form a solution.
- The sample is soluble in 0.1N HCL, methanol, ethanol and slightly soluble in chloroform and insoluble in water. based on solubility and economical parameters, 0.1N HCL selected as solvent.

Solvent	Solubility status
Water	Insoluble
Methanol	Soluble
Ethanol	Very soluble
0.1N HCL	Freely soluble
N-Hexane	Soluble

Table.1: Selection of solvent

Preparation of standard stock solution:

- Accurately weigh 100mg of Mirabegron transferred to 100ml volumetric flask and 3/4th of 0.1N HCL was added and sonicated for 15min remaining volume is made up with g/ml)0.1N HCL and labelled as standard stock solution.

Preparation of working standard solution:

- Dilute the standard solution 10ml into 100ml (100µg/ml)
- From this pipette out 10ml and makeup up to 100ml with 0.1N HCL (10µg/ml) which is used as working standard solution.

Preparation of sample stock solution:

- 20 tablets were weighed, and the average weight of each tablet was calculated then the equivalent weight to 100mg was transferred into100ml volumetric flask 3/4th of diluent was added and sonicated for 15min further the volume was made up with diluent and filtered by What man filter papers.
- From this filtrate 10ml diluted to 100ml (100 µg/ml) from this 10ml diluted to 100ml

Selection of detection wavelength:

To determine the λmax of Mirabegron 10µg/ml of working standard solution was scanned in UV wavelength range of 200-400nm using 0.1N HCL as a blank .it was observed that the drug shown maximum absorbance at 249 nm.

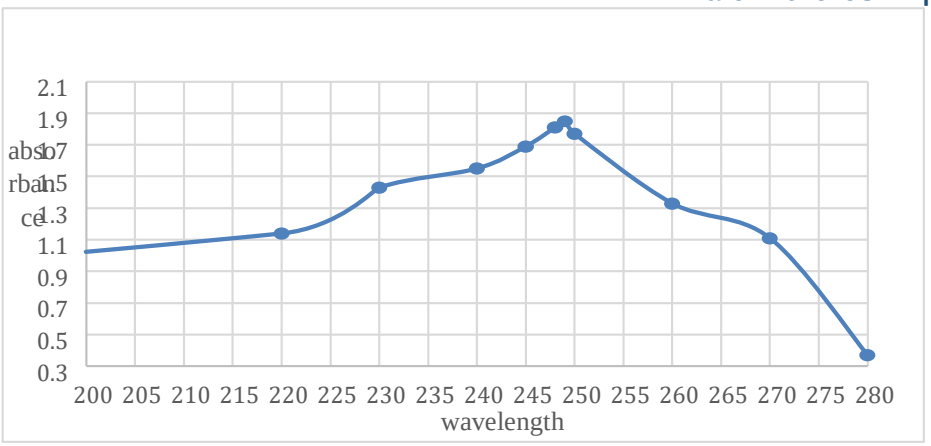

FIG. 2 Determination of λ max

preparation of calibration curve:

A calibration curve was plotted over a concentration range of 0.5-3µg/ml for Mirabegron by taking Mirabegron stock solution 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml was shifted to a series of 10ml volumetric flask and make up the volume with 0.1N HCL up to the mark. Calibration curve was prepared by taking readings at λ max 249nm and plotted a graph by taking the Mirabegron concentration on x-axis and their representative absorbance on y axis calibration data

- From the absorbance values a calibration curve was plotted in the desired concentration range.
- The curve obtained was linear with co-relation coefficient 0.9995 which represented

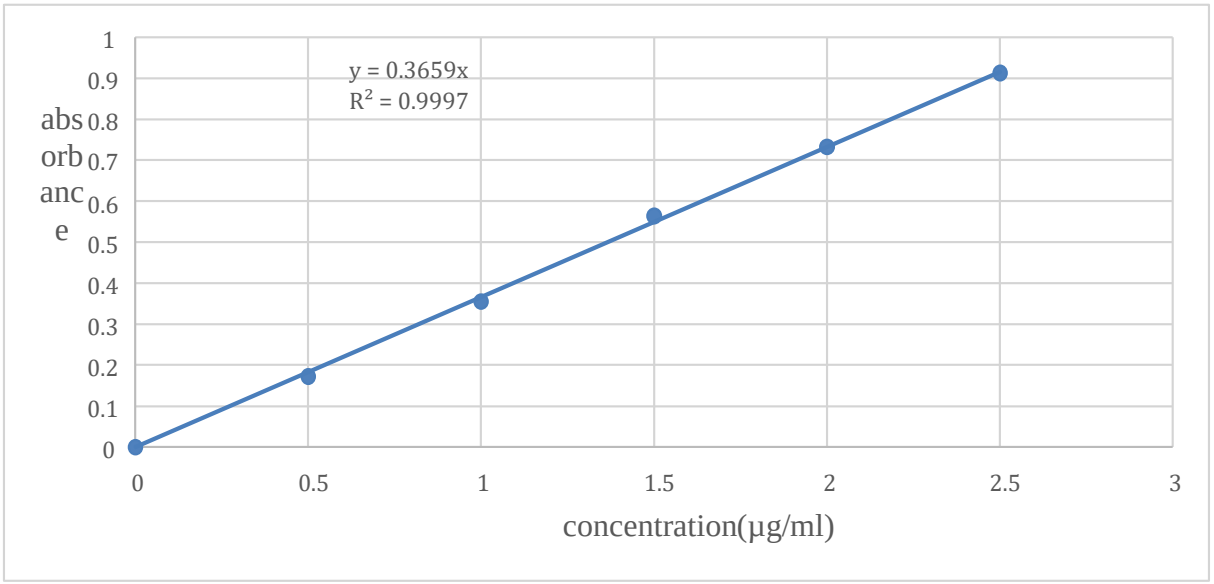

FIG 3 Calibration Curve of Mirabegron

Formulation linearity:

Mirabegron 20 tablets were weighed accurately and powdered by using mortar and pestle. The powder equivalent to100 mg of drug is taken into 100ml volumetric flask and add 3/4th volume of 0.1N HCL. The solution is sonicated for 15 min and solution was made up to 100ml by using 0.1N HCL from the solution pipette out 10ml and make up to 100ml volumetric flask (working standard) and the solution from the filtrate pipette out 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml into a series of 10ml volumetric flask and make up the volume

with 0.1N HCL giving solution concentration 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0µg/ml were prepared. The absorbance values of these were measured at 249nm.

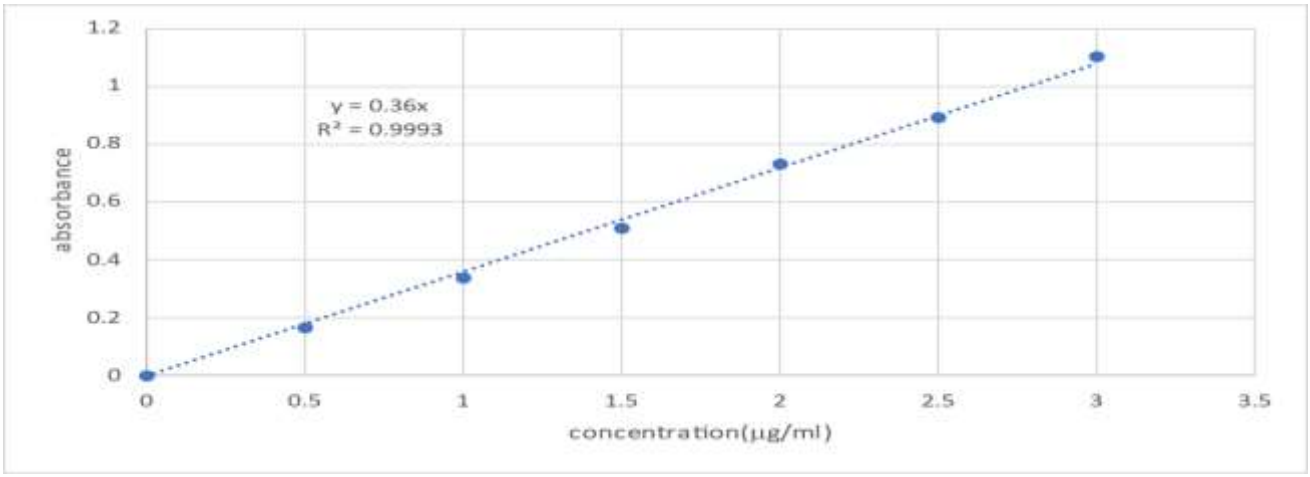

FIG 4: Linearity Curve-1

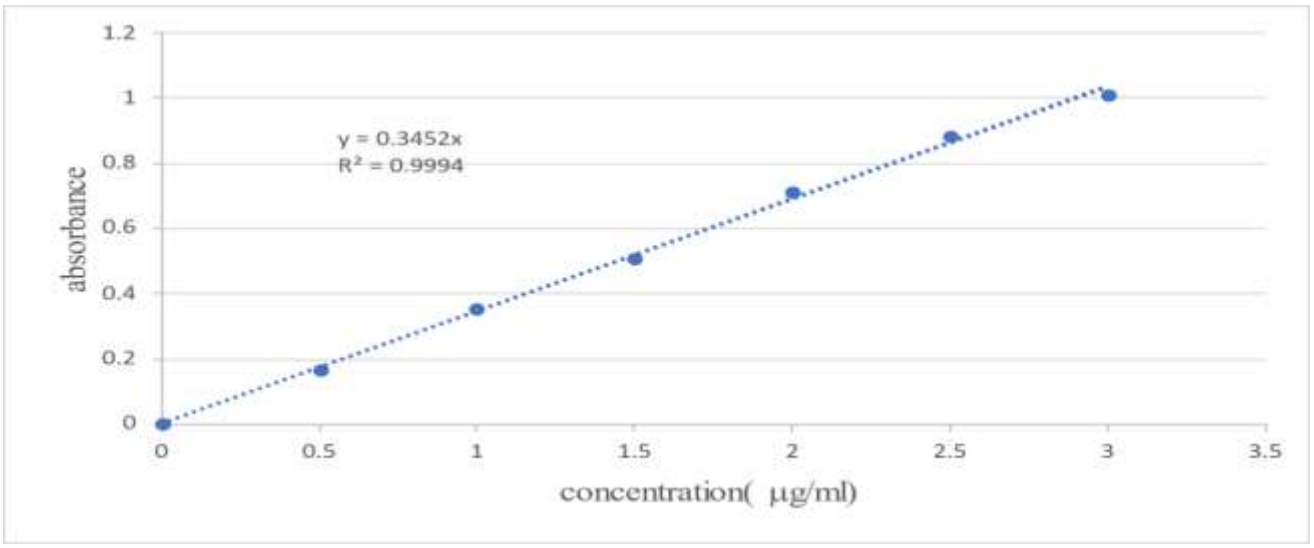

FIG 5: Linearity Curve-2

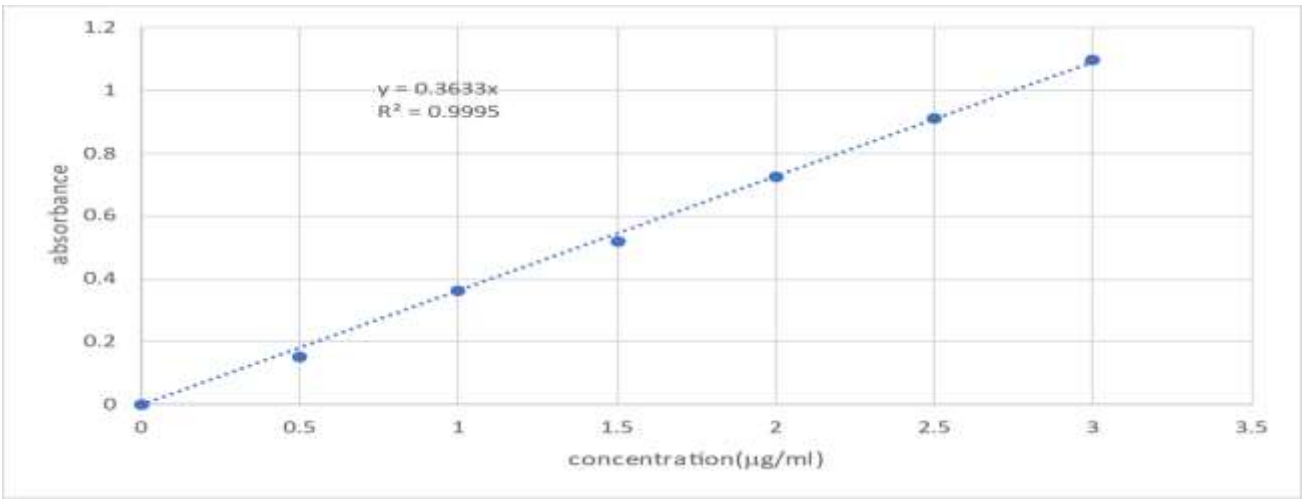

FIG 6: Linearity Curve-3

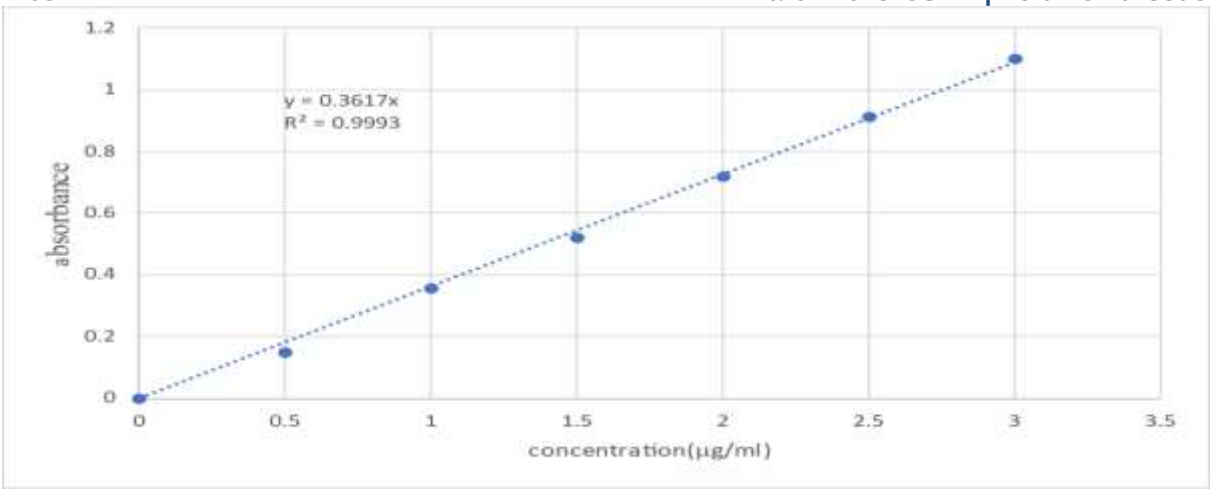

FIG 7: Linearity Curve-4

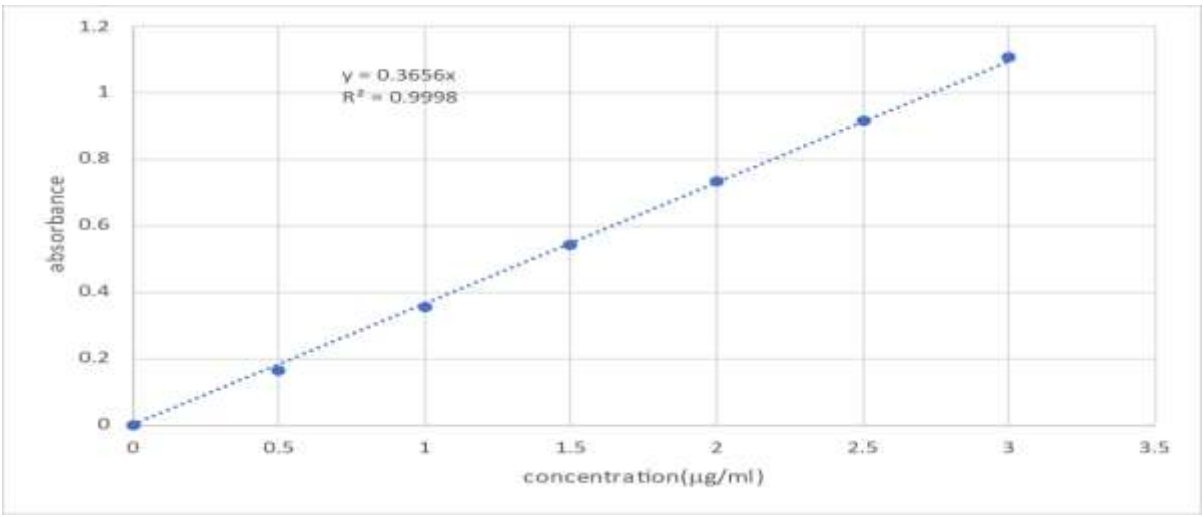

FIG 8: Linearity Curve-5

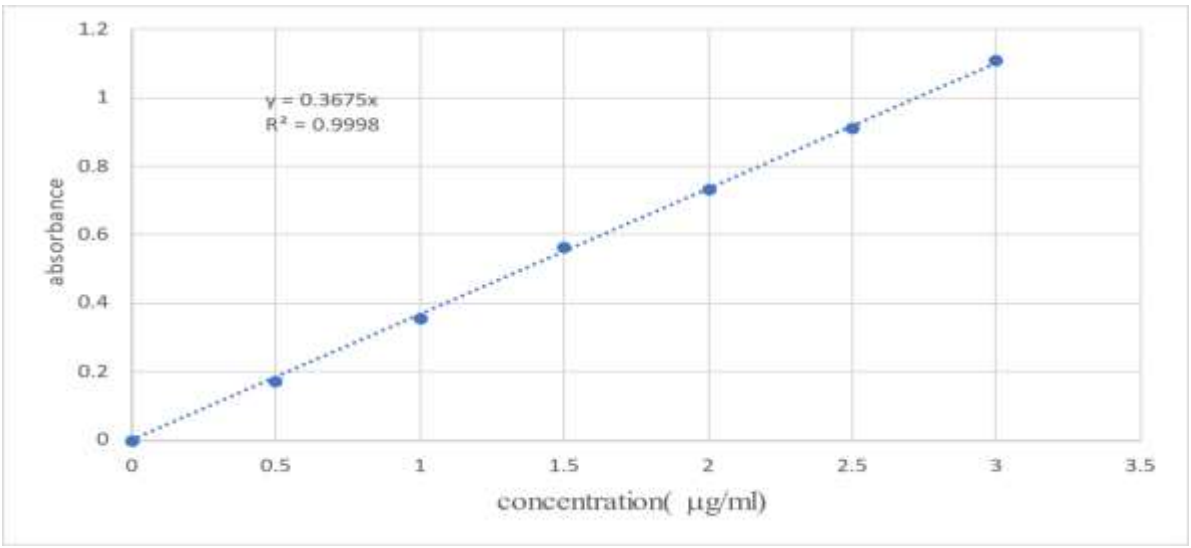

FIG 9: Linearity Curve-6

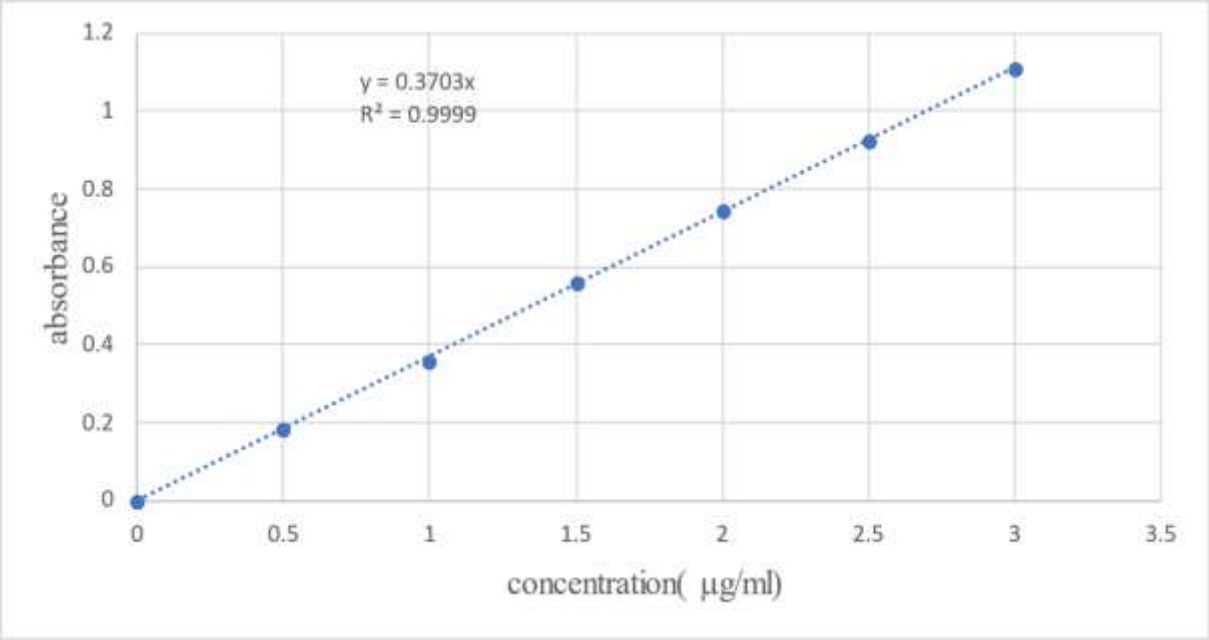

FIG 10: Formulation linearity curve

Accuracy and precision of proposed Methods:

Accuracy:

To check the accuracy of the developed method and to study interference of formulation excipients, analytical recovery studies were conducted by taking 1.5µg/ml solution of formulation in each of three 10ml volumetric flask and then adding 0.5,1.0,1.5µg/ml of standard mirabegron and make up to the mark with 0.1N HCL. the solution was prepared in triplicate. The readings taken and the amount recovered calculated by using formula Y=mx+c and the percentage recovery

Precision:

To check the precision of the proposed method the recovery studies performed three times on the same day (intra-day) and recovery studies between days (inter-day) were analysed. The relative standard deviation of intra-day and inter-day values were calculated. The precision is expressed in the form of percent relative standard deviation.

Limit of Detection (LOD) And Limit of Quantification (LOQ) of Proposed Methods.

Limit of Detection is the lowest concentration in a sample that can be detected but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of an analyte in a sample that can be determined. LOD and LOQ were obtained from the slope and the standard deviation of the interception from three calibration curves determined by a linear regression line as defined by ICH.

Limit of Detection (LOD)

Limit of detection can be calculated using the following equation as per ICH guidelines.

$LOD = 3.3*(N/S)$ ……1

Where N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve

Limit of Quantification (LOQ)

Limit of quantification can be calculated using following equations as per ICH guidelines.

$LOD = 10*(N/S)$ ……2

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

LOD and LOQ:

Sl. No.	Slope	LOD (µg/ml)	LOQ (µg/ml)	SD
1	0.3441	0.0777	0.2354	±0.008
2	0.3677			
3	0.3704			
4	0.3713			
5	0.3714			
6	0.3705			

TABLE 2: LOD AND LOQ

Recovery

Total 1.5ml of sample solution(10µg/ml) added to 0.5, 1.0, 1.5ml of working standard solution (10µg/ml) in a 10ml volumetric flask and make up the volume with 0.1N HCL mixed well and taken readings at 249nm. By taking absorbance values amount recovered calculated by using Y=mx+c formula and percentage recovery

Trials	Amount of drug present in (µg/ml)	Amount added (µg/ml)	absorbance	Amount recovery (µg/ml)	Percentage (%)	SD	RSD
1	1.5	0.5	0.7524	0.5056	101	±0.26	0.548
2	1.5	0.5	0.7512	0.5024	100.4		
3	1.5	0.5	0.7516	0.5035	100.1		
4	1.5	1.0	0.9378	0.9779	100		
5	1.5	1.0	0.9358	0.9840	97.7		
6	1.5	1.0	0.9342	0.9820	99.5		
7	1.5	1.5	1.1296	1.5210	101		
8	1.5	1.5	1.1224	1.5010	100		
9	1.5	1.5	1.1192	1.4930	99.5		

TABLE 3: recovery of MIRABEGRON

Trial	Amount of drug present in (µg/ml)	Amount added (µg/ml)	absorbance	Amount recovery (µg/ml)	Percentage (%)	SD	RSD
1	1.5	0.5	0.7524	0.5056	101	±0.26	0.548
2	1.5	0.5	0.7512	0.5024	100.4		
3	1.5	0.5	0.7516	0.5035	100.1		
4	1.5	1.0	0.9378	0.9779	100		
5	1.5	1.0	0.9358	0.9840	97.7		
6	1.5	1.0	0.9342	0.9820	99.5		
7	1.5	1.5	1.1296	1.5210	101		
8	1.5	1.5	1.1224	1.5010	100		
9	1.5	1.5	1.1192	1.4930	99.5		

TABLE 4: INTRA DAY-1 Recovery of MIRABEGRON

7 Trials	Amount of drug present in (µg/ml)	Amount added (µg/ml)	absorbance	Amount recovery(µg/ml)	Percentage (%)	SD	RSD
1	1.5	0.5	0.7514	0.5029	100.5	±0.33	0.868
2	1.5	0.5	0.7520	0.5045	100.9		
3	1.5	0.5	0.7523	0.5053	101		
4	1.5	1.0	0.9313	0.9873	99		
5	1.5	1.0	0.9346	0.9962	99		
6	1.5	1.0	0.9369	1.0024	100.2		
7	1.5	1.5	1.1297	1.5215	100.1		
8	1.5	1.5	1.1254	1.5099	100.6		
9	1.5	1.5	1.1238	1.5056	100.3		

TABLE 5: INTRA DAY-2 Recovery of MIRABEGRON

Trials	Amount of drug present in (µg/ml)	Amount added (µg/ml)	absorbance	Amount recovery(µg/ml)	Percentage (%)	SD	RSD
1	1.5	0.5	0.7498	0.4989	99.7	±0.33	0.900
2	1.5	0.5	0.7513	0.5026	100.5		
3	1.5	0.5	0.7516	0.5035	100.5		
4	1.5	1.0	0.9338	0.9940	99.4		
5	1.5	1.0	0.9341	0.3652	99.4		
6	1.5	1.0	0.9396	1.0096	101		
7	1.5	1.5	1.1295	1.5210	101.4		
8	1.5	1.5	1.1285	1.5183	101.2		
9	1.5	1.5	1.1182	1.4905	99.3		

TABLE 6: INTRA DAY-3 Recovery of MIRABEGRON

Trials	Amount of drug present in (µg/ml)	Amount added (µg/ml)	absorbance	Amount recovery(µg/ml)	Percentage (%)	SD	RSD
1	1.5	0.5	0.7524	0.5056	101	±0.26	0.548
2	1.5	0.5	0.7512	0.5024	100.4		
3	1.5	0.5	0.7516	0.5035	100.1		
4	1.5	1.0	0.9378	0.9779	100		
5	1.5	1.0	0.9358	0.9840	97.7		
6	1.5	1.0	0.9342	0.9820	99.5		
7	1.5	1.5	1.1296	1.5210	101		
8	1.5	1.5	1.1224	1.5010	100		
9	1.5	1.5	1.1192	1.4930	99.5		

TABLE 7: INTER DAY-1 Recovery of MIRABEGRON

Trials	Amount of drug present in (µg/ml)	Amount added (µg/ml)	absorbance	Amount recovery(µg/ml)	Percentage (%)	SD	RSD
1	1.5	0.5	0.7498	0.4989	99.7	±0.33	0.900
2	1.5	0.5	0.7513	0.5026	100.5		
3	1.5	0.5	0.7516	0.5035	100.5		
4	1.5	1.0	0.9338	0.9940	99.4		
5	1.5	1.0	0.9341	0.3652	99.4		
6	1.5	1.0	0.9396	1.0096	101		
7	1.5	1.5	1.1295	1.5210	101.4		
8	1.5	1.5	1.1285	1.5183	101.2		
9	1.5	1.5	1.1182	1.4905	99.3		

TABLE 8: INTER DAY-2 Recovery of MIRABEGRON

Trials	Amount of drug present in (µg/ml)	Amount added (µg/ml)	absorbance	Amount recovery(µg/ml)	Percentage (%)	SD	RSD
1	1.5	0.5	0.7498	0.4986	99.7	±0.47	1.805
2	1.5	0.5	0.7499	0.4989	99.7		
3	1.5	0.5	0.7512	0.5024	100.4		
4	1.5	1.0	0.9418	1.0156	101.5		
5	1.5	1.0	0.9359	0.9997	99.9		
6	1.5	1.0	0.9343	0.9954	99.5		
7	1.5	1.5	1.1297	1.5215	101.4		
8	1.5	1.5	1.1225	1.5021	101.1		
9	1.5	1.5	1.1193	1.4935	99.5		

TABLE 9: INTER DAY-3 Recovery of MIRABEGRON

SUMMARY

A UV-Spectrophotometric method has been developed and validated for determination of Mirabegron in pure form and its pharmaceutical dosage forms. The process was done by using 0.1N HCL as a solvent with the detection wavelength set at 249nm. Mirabegron was checked for its stability in the chosen solvent and found to be stable. The method was linear with correlation coefficient 0.9995 in the concentration range of 0/.5-3.0µg/ml. The limit detection and limit quantification were 0.0777 µg/ml and 0.23547 µg/ml, respectively. The intra and inter-day precisions were satisfactory; the relative standard deviations did not exceed 2%. The accuracy of the method is high as can be seen from the mean recovery values of Mirabegron which were in

the range of 0.5- 3µg/ml the method met the ICH regulatory requirements. The results of validation are summarized in table.

Sl. No.	parameter	Result
1	Detection of wavelength	249nm
2	Beer- Lambert law (µg/ml)	0.5-3(µg/ml)
3	Regression equation(y=mx+c)	0.981
4	Slope	0.3750
5	Accuracy (%mean recovery)	100.03%
6	LOD	0.0777
7	LOQ	0.23547
8	Standard deviation	±0.008
9	Relative standard deviation	0.1014

TABLE 10: Results

CONCLUSION

A simple, novel, economical, rapid, precise, and accurate UV spectrophotometric method was developed for the estimation of Mirabegron in bulk and pharmaceutical formulations. The method was developed by using 0.1N HCL as solvent. The developed method was validated for parameters via accuracy, precision, and linearity, limit of detection and limit of quantification as per ICH guidelines. All the parameters were found to be within the acceptance limits. The results indicated that the proposed method for the estimation of Mirabegron is very accurate and cost effective and can be employed in routine sample analysis of Mirabegron in bulk and pharmaceutical formulations.

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