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Development and Validation of UV-Spectrophotometric Method for Macitentan Bulk Drug and Formulation

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Abstract

A simple, accurate, precise, inexpensive, rapid, and sensitive UV visible spectrophotometric method has been developed for the determination of Macitentan in drug substances. The developed method was validated according to ICH guidelines. Drugs were analyzed by UV/Vis spectrophotometry and validated for linearity, precision, precision and specificity, limits of detection, and limits of quantitation. The solvent used was 0.1N HCl and the wavelength corresponding to the maximum absorbance of the drug was 274 nm. The linear response over the concentration range of Macitentan from 10 to 70 µg/ml was plotted with y=0.0114x-0.0042 and regression coefficient r2=0.9991. Accuracy ranged from 93.83 to 104.2%. Intraday and intraday accuracy was found to be within limits. To determine the sensitivity of the method, the limit of detection (LOD) and limit of quantification (LOQ) were determined and were 7.78µg/ml and 21.25 µg/ml, respectively. Percent drug content was determined to be 101.6 by assay. The drug was confirmed by interpretation of the UV spectrum. Therefore, the proposed method has been validated and can therefore be used for routine analysis of Macitentan in pharmaceutical dosage forms.

Keywords: Macitentan, Intraday, Concentration, Spectrophotometry

Introduction

Macitentan is a chemical compound with the molecular formula [5-(4-bromophenyl)-6-[(5-bromo-pyrimidin-2-yl) oxy] ethoxypyrimidin-4-yl] sulfamoyl (propyl) amine].[1]

By preventing ET-1 from binding to ET receptors, Macitentan prevents the ET1-dependent increase in intracellular calcium. Because there are more ETA receptors than ETB receptors in pulmonary arterial smooth muscle cells, inhibiting the ETA receptor subtype appears to be more important in the therapy of PAH than blocking the ETB receptor subtype.[2-4]

According to a review of the literature, Macitentan has been the subject of studies using RP-HPLC, first order Derivative UV Spectroscopy, UP-HPLC, LC MS/MS, and stability indicating analytical methods. There are no analytical methods at all, according to a literature review. Therefore, it was deemed worthwhile to develop a technique for estimating Macitentan from bulk drug and marketed formulations.[5-15]

Therefore, it was thought to be of interest to develop a precise, accurate, sensitive, and selective spectrophotometric method for estimation of Macitentan in tablet dosage form, which will provide valuable information that, can be used to estimate, ultimately to improve formulation and manufacturing process. The objective of the work was to develop a

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simple UV-visible spectrophotometric method and validate it for the dosage form of Macitentan tablets.[16-18]

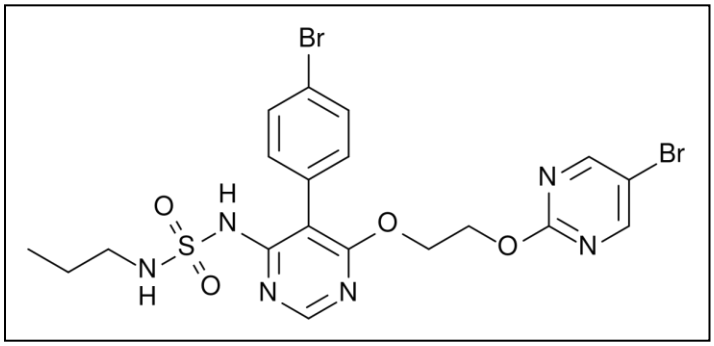

Figure No 1: Structure of Macitentan

Materials and method

Chemicals and Reagents: All the chemicals used were of analytical grade. All the solutions were freshly prepared in 0.1N HCl and Phosphate Buffer pH 6.8. Authentic of Macitentan were obtained as gift samples from Mylan Laboratories Limited, Hyderabad

Instruments: Sonicator, Weighing balance, UV-visible spectrophotometer.

Marketed Formulation: Mecitent 10 mg Tablet (MSN Laboratories)

Methods

Construction of Standard Curve of Macitentan In 0.1N HCl

Preparation of Stock Solution

Stock solution 100µg/ml of Macitentan was prepared in 0.1N HCl solution. This solution was approximately diluted with 0.1N HCl to obtain a concentration of 10µg/ml. The resultant solution was scanned in the range of 200- 400 nm using UV double beam spectrophotometer (Lab India UV-2000)

Selection of Detection Wavelength

Drug solution was scanned over the range of 200- 400 nm. The wavelength of Macitentan was determined to be 274 nm.

Standard Calibration of Macitentan In 0.1N HCl

100 mg of Macitentan was accurately weighed and dissolved in100 ml of 0.1N HCl to obtain a concentration of 1000 µg/ml. From the above 10 ml was withdrawn and diluted to 100 ml to obtain a concentration of 100µg/ml. From this stock solution aliquots were diluted in 10 ml volumetric flask with phosphate buffer to give concentrations in range of 10µg/ml to 70µg/ml respectively, absorbance was measured at 274 nm.

Construction of Calibration Curve

Pipette out 1,2,3,4,5,6,7, and 8 ml of working solution and transfer into separate 10 ml volumetric flasks. Dilute all of them to 10 ml with water to get solution of concentrations to 2, 4, 6,8,10,12,14,16 µg/ml respectively.

UV Method Validation[19-20]

The ultraviolet spectrophotometric method was verified for robustness, accuracy, linearity, and precision.

Linearity

Appropriate aliquots of Macitentan working standard solutions were taken in different 10 ml volumetric asks and diluted up to the mark with distilled water to obtain final concentrations of 10-70µg/ml. Calibration curves were constructed by plotting absorbance versus concentrations and regression equations were calculated for both the drugs.

Range

The Range of the analytical method was decided from the interval between the upper and lower level of the calibration curve by plotting curve.

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Precision

Intraday precision was determined by analyzing the drugs at concentrations (4µg/ml) and each concentration for three times, on the same day. Inter-day precision was determined similarly, but the analysis is carried out daily, for two consecutive days. Repeatability (intraday) of the method was determined by analyzing six samples of the same concentrations of the drug (4µg/ml). The absorbance of each was measured and reported in terms of relative standard deviation to obtain the variation.

Accuracy

The accuracy of the method was determined by calculating recoveries of Macitentan by method of standard additions at three different levels 50, 100 and 150 %. Mean percentage recovery was determined. Recovery values were calculated and shown in table.

Table No 1: Calibration Curve Data for Macitentan in 0.1 N HCl

Sr. No.	Drug Conc. (µg/ml)	Stock solution of Macitentan (ml)	0.1 N HCl (ml)	Mean absorbance ±SD (n=3)	% RSD
1	1	1	9	0.0981±0.0004	0.69
2	2	2	8	0.241±0.001	0.6
3	3	3	7	0.364±0.001	0.38
4	4	4	6	0.508±0.001	0.45
5	5	5	5	0.591±0.002	0.46
6	6	6	4	0.683±0.001	0.33
7	7	7	3	0.841±0.002	0.35
8	8	8	2	0.881±0.001	0.23

Detection Limit

The Detection Limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The detection limit (LOD) may be expressed as. LOD= 3.3σ/S Where σ = Relative standard deviation of the response. S = the slope of the calibration curve (of the analyte).

Quantitation Limit

The Quantitation limit of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

Quantitation Limit (LOQ) may be expressed as: LOQ = 10σ/S Where

σ = Relative standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

Assay Marketed Formulation

Weighed and transferred 10 Macitentan tablets powder after crushing (equivalent to 10mg of Macitentan) into 100ml volumetric flask, added about 40 ml of 0.1N HCl and sonicated for 5 minutes with intermittent shaking, further added 60 ml 0.1N HCl . Pipette out 1 ml of filtered solution into 10 ml volumetric flask, and made the volume up to the mark with water and mixed well. 1 ml of the above was again diluted with distilled water to obtain 10 µg/ml of Macitentan.

Results & Discussion

Analytical Method for Drug Concentration Measurements (UV/VIS Method)

Selection of detection wavelength: The wavelength of Macitentan was determined to be 274 nm as shown in figure 2.

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Calibration Curve of Macitentan

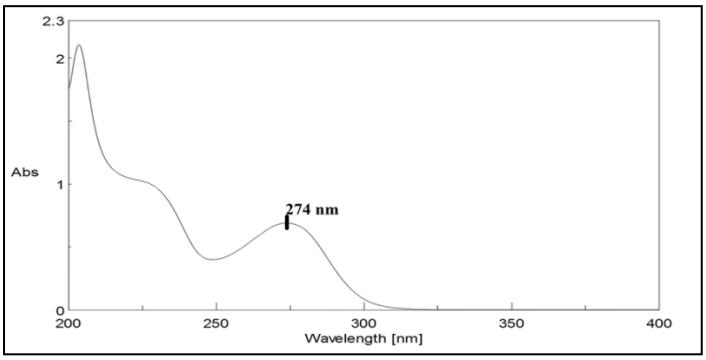

Figure No 2: λ_{max} of Macitentan in 0.1N HCl

Preparation of standard plot for Macitentan: Absorbance of the resultant solution was measured at 274 nm using blank. A graph was plotted between the concentrations and their respective absorbance. The response of the drug was found linear in the entire investigational range of 10 to 70µg/ml as shown in table 2. The calibration curve showed the linear equation as, y = 0.0114x - 0.0042, with a correlation coefficient, R^2 = 0.9991, where y represents absorbance (optical density) and x represents the concentration (µg /ml) as shown in figure 3.

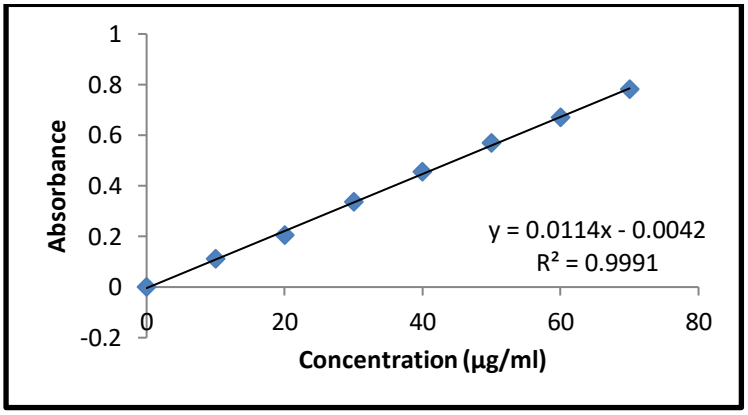

Figure No 3: Calibration Curve of Macitentan in 0.1N HCl

Method Validation

The developed method was validated as per ICH guidelines for the following parameters:

Linearity: The linearity for Macitentan was found to be linear in the range of 10-70 µg/ml. The regression equation was found to be y = 0.0114x - 0.0042, R^2 = 0.9991.

Range: The observed range of Macitentan in test solution was observed from 0.0981±0.0004 to 0.881± 0.001.

Accuracy: The accuracy of the analytical method for Macitentan was determined at 50%, 100% and 150% levels of standard solution. Absorbance was measured at 274 nm and results were expressed in terms of % recoveries in table 2.

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Table No 2: Results of Accuracy of Macitentan

Sr. No.	Level of % Recovery	Amount of tablet sample (ml)	Amount of standard drug added (µg/ml)	Amount added (µg)	Amount found (µg/ml)	% Recovery
1	0	1	0	0	0	0
2	50	1	0.5	15	14	93.83%
3	100	1	1	20	20	100%
4	150	1	1.5	25	26	104.2%

Precision: The Intra-day and Inter-day precision were carried out using same optimized conditions. The precision (measurement of inter-day, intra-day repeatability) results showed good reproducibility with the relative standard deviation (% RSD) below 2.0 % as shown in table 3 & 4 respectively. This indicated that the method was highly precise.

Table No 3: Results of Intra-Day Precision of Macitentan in Solvent

Sr. No.	Day Time	Concentration (µg/ml)	Absorbance	Mean Absorbance 274nm±SD (n=6)	%RSD
1	**Intraday morning precision**	4	0.606	0.607±0.001	0.14%
2			0.606		
3			0.606		
4			0.608		
5			0.607		
6			0.607		
1	**Intraday morning precision**	4	0.622	0.623±0.003	0.44%
2			0.625		
3			0.625		
4			0.627		
5			0.621		
6			0.620		
1	**Intraday morning precision**	4	0.613	0.616±0.005	0.59%
2			0.615		
3			0.619		
4			0.612		
5			0.620		
6			0.620		

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Table No 4: Results of Inter-Day Precision of Macitentan in Solvent

Sr. No.	Day Time	Concentration (µg/ml)	Absorbance	Mean Absorbance 274nm±SD (n=6)	%RSD
1	**Interday morning precision**	4	0.619	0.623±0.004	0.50%
2			0.621		
3			0.626		
4			0.627		
5			0.621		
6			0.622		
1	**Interday morning precision**	4	0.631	0.631±0.002	0.19%
2			0.632		
3			0.631		
4			0.630		
5			0.629		
6			0.632		
1	**Interday morning precision**	4	0.644	0.642±0.001	27%
2			0.645		
3			0.641		
4			0.643		
5			0.641		
6			0.641		

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ of method were determined to be 7.78 µg /ml and 21.25 µg /ml respectively. LOD and LOQ indicate that method was highly sensitive and fast.

Assay Marketed Formulation

The solution was then analyzed by developed UV-Visible spectroscopy method, and the results were indicated by % drug content. The % drug contents were found to be 101.6. As shown in table 5.

Table No 5: Assay Results of Formulation

Drug	Brand Name	Label Claim	Conc. Prepared	Amount Found (µg /ml)	%Assay	±SD	%RSD
Macitentan	Mecitent	10 mg	10 µg/ml	10.6	101.6	0.596	0.586

Conclusion

The method was validated and found to be simple, sensitive, accurate and precise as per ICH guidelines. The % RSD for the validation parameters was found to be less than 2%. Hence proposed method may be used for routine analysis of these drugs in pharmaceutical dosage forms. Accuracy of proposed method was confirmed by performing accuracy studies that showed the results within the range. Precision of proposed UV method was confirmed by performing intra-day and inter-day precision. Results were well within acceptance criteria that indicate excellent scope of the method for the determination of Macitentan in pharmaceutical dosage forms and bulk.

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