ejbps, 2025, Volume 12, Issue 3, 165-172. **Research Article** **SJIF Impact Factor 7.482**

EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 12
Issue: 3
Year: 2025

A VALIDATED RP-HPLC METHOD FOR QUANTITATIVE DETERMINATION OF MACITENTANENTAN IN BULK AND ITS PHARMACEUTICAL DOSAGE FORMS

Dr. M. Lakshmi Surekha[1]*, K. Prasanth Raju[2], D. Vasanthi[3], G.Swapna[4], M. Dinesh[5] and U. Vani[6]

[1,2,3,4,5,6]Department of Pharmaceutical Analysis, A.M. Reddy Memorial College of Pharmacy, Narasaraopet-522601, Guntur (Dt)., A.P. 2023.

***Corresponding Author: Dr. M. Lakshmi Surekha**
Department of Pharmaceutical Analysis, A.M. Reddy Memorial College of Pharmacy, Narasaraopet-522601, Guntur (Dt)., A.P. 2023. **Email id:** drlakshmipuvvula@gmail.com

Article Received on 20/01/2025 **Article Revised on 10/02/2025** **Article Accepted on 01/03/2025**

ABSTRACT

The objective of the current study was to develop a simple, accurate, precise and rapid RP-HPLC method with subsequently validate as per ICH guidelines for the determination of Macitentanentan using mobile phase [mixture of Phosphate buffer pH-3.9 and methanol in the ratio of 45:55] as the solvent. The proposed method involves the measurement of Retention time at selected analytical wavelength. 220.0 nm was selected as the analytical wavelength. The retention time of Macitentanentan was found to be 3.81. The linearity of the proposed method was investigated in the range of 5-600 µg/ml (r = 0.9996). The method was statistically validated for its linearity, accuracy and precision. Both inter-day and intra-day variation was found to be showing less % RSD (Relative Standard Deviation) value indicating high grade of precision of the method.

KEYWORDS: Macitentanentan; Tablet dosage forms; RP-HPLC; ICH Guidelines.

INTRODUCTION

Macitentanentan[1] is chemically N-[5-(4-Bromophenyl)-6-[2-[(5-bromo-2- pyrimidinyl) oxy]ethoxy]- 4-pyrimidinyl]-N'-propylsulfamide. It is freely soluble in DMSO and methanol. The molecular formula is C19 H20 N6 O4 S Br2 and Molecular weight is 588.273. Macitentanentan is an orphan drug for the treatment of pulmonary arterial hypertension. It acts as a dual endothelin receptor antagonist. Macitentanentan for the treatment of idiopathic pulmonary fibrosis[2] Idiopathic pulmonary fibrosis is a progressive, fatal disease. This prospective, randomised, double-blind, multicentre, parallel-group, placebo-controlled phase II trial investigated the efficacy and safety of the endothelin receptor antagonist Macitentanentan in idiopathic pulmonary fibrosis. The therapeutic importance of this compound justifies that, necessary to establish analytical methods for its determination in bulk and pharmaceutical formulation Literature survey reveals that no analytical methods have been reported for the quantitative estimation of Macitentan In the present work attempt has been made to develop a simple, accurate, sensitive, rapid and economic a RP-HPLC method for the quantitative estimation of Macitentanentan in bulk and pharmaceutical formulation. As chromatographic methods of analysis is a pre-requisit for the marketing of most of formulations.

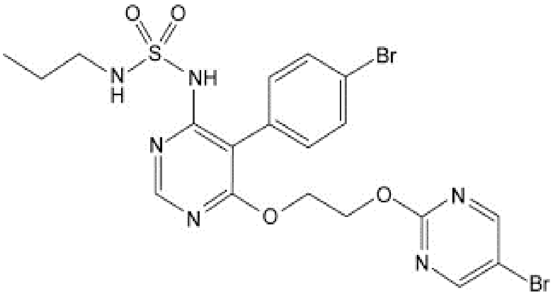

Fig. 1: Chemical structure of Macitentan.

Aim and Objective

The aim of the present work is to develop a RP-HPLC method for the tablet formulation of Macitentan Review of literature for Macitentanentan gave information regarding the various studies conducted and analytical methods established for the drug in tablet dosage form.

There are few methods reported in literature for analysis of Macitentanentan drug in the pure form, pharmaceutical formulations by UV-Spectrophotometer, RP-HPLC.

According to detailed survey of analytical literature none of the reported analytical procedure describes a simple and satisfactory RP-HPLC method for determination of Macitentanentan in tablet dosage form. So the objective of this work is to develop a simple, precise, accurate and cost effective analytical method for drug product containing Macitentan

The aim of the proposed work is:

- To develop a suitable HPLC method for determination of Macitentanentan in Tablet dosage form.
- Validate the developed method as per ICH guidelines.

Experimental 2.1

Chemicals and Reagents

The working standard of Macitentan was gifted by Mr. Jone Beijing Tiankai Pharma Co.,Ltd (China). The tablet formulation of Macitentan (Label claim: Macitentanen 10mg) Opsumit tablet container was purchased from the Canadian local market. Distilled water is obtained from local market for analytical work and rinsing purpose. 2.2 Instrument Used A Shimadzu class HPLC unit accomplished with SPD-20AD UV-Visible detector; Enable C18 (250*4.6*5) Column (Shimadzu); LC-20 AD Pump; Quantitative HPLC was performed on a isocratic mode with 20 µl injection of sample loop (manual). The output signal was monitored and integrated using software class LAB SOLUTIONS (Shimadzu). 2.3 Preparation of Mobile Phase The HPLC grade methanol was filtered through 0.4 µm membrane filter paper. Buffer (0.585 gm. of anhydrous disodium hydrogen phosphate and 0.843gm. citric acid monohydrate in 650 ml distilled water) was filtered through 0.4 µm membrane filter paper. Mobile phase was prepared by mixing 350 ml of buffer with 650 ml of methanol and sonicated for 15 min. 2.4 Preparation of Standard Stock Solution 100 mg of standard Macitentan was weighed accurately and transferred to 100 ml volumetric flask. Both the drugs were dissolved in 50 ml of mobile phase with sonication for 15 min and then volume was made up to the mark with mobile phase (solution–A). Further the stock.

solutions were diluted to get 100 µg/ml final concentration of standard stock solution of drug (solution–B). This stock solution was filtered through 0.4 µ membrane filter paper. 2.5 Preparation of Calibration Curves Appropriate dilutions were prepared separately and 20 µl of each was injected into the HPLC system and the chromatograms were recorded under the same chromatographic conditions as described below. Peak areas were recorded for all the peaks and a standard calibration curve of AUC against concentration was plotted. 2.6 Chromatographic Condition The mobile phase containing both Buffer and methanol in the ratio of 35:65 was selected as the optimum composition of mobile phase, because it was found that this solvent system resolved both the components ideally. The flow rate was set to 1.0 ml/min and UV detection was carried out at 268.0 nm. The mobile phase and samples were degassed by sonication for 15 min and filtered through 0.4 µm membrane filter paper. All determinations were performed at constant column temperature (250C). 2.7 Selection of Analytical Concentration Range Appropriate aliquots were pipetted out from the standard stock solution (solution B- 100 µg/ml) in to a series of 10 ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 20 100 µg/ml of Macitentan. Triplicate dilutions of each of the above-mentioned concentrations was prepared separately and from these triplicate solutions, 20 µl of each concentration of the drug were injected into the HPLC system two times separately and their chromatograms were recorded under the same chromatographic conditions as described above. Peak areas were recorded for all the peaks and a standard calibration curve of AUC against concentration was plotted. 2.8 Analysis of Tablet Formulation Twenty tablets each containing 10 mg of Macitentan weighed accurately and powdered. A quantity equivalent to 100 mg of Macitentan was weighed accurately and transferred to 100 ml volumetric flask containing approximately 50 ml of mobile phase. The contents were sonicated for 15 min and volume was made upto the mark with the mobile phase. The resulting solution was filtered through a membrane filter. The solution obtained was then diluted with the mobile phase so as to obtain a concentration of 1000 µg/ml. Sample solution was injected under the same chromatographic conditions and the chromatogram was recorded in triplicate. The amount of Macitentanentan present in tablet formulation was determined by comparing the peak area from the standard. The results are furnished in Table 2. 2.9 Method validation.[3-5] The developed analytical method was subjected to validation with respect to various parameters such as linearity, limit of quantification (LOQ), limit of detection (LOD), accuracy, precision, recovery studies and reproducibility as per the ICH guidelines. Linearity The standard curve was obtained in the concentration range of 20-100 µg/mL. The linearity was evaluated by linear regression analysis using the least square method. It was found that correlation coefficient and regression analysis are within the limits. Precision The precision was assesed in terms of intra-day and inter-day variation. The intra-day and inter-day variation in the peak area of drug solution was calculated in terms of coefficient of variation (C.V.). The results are furnished in Table 4. Limit of Detection (LOD) and Limit of Quantitation (LOQ) The LOD and LOQ for Macitentanentan were predicted basing on the parameters of standard error of estimate and slope, calculated from linearity of the response data of Macitentan Robustness The robustness was checked by changing the flow rate to 0.9 and 1.1 ml/min. Accuracy The accuracy of the HPLC method was assessed by adding known amount of standard drug solution to a pre-analyzed tablet formulation. The recovery studies were carried out in triplicate. The accuracy was expressed in terms of recovery at three levels 80%, 100% and 120%. The results are furnished in Table 5.

Method Validation and Results

Validation

"Establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes."Method validation was performed as per ICH guidelines. The developed method was validated for the following parameters.

LINEARITY

- **Procedure:** Linearity of the method was demonstrated over the concentration range of 5-600µg/ml Macitentan. Each concentration was prepared in triplicate. 20µl of each standard solution were injected at the optimized chromatographic conditions and the chromatograms were recorded. The retention time, average peak areas were recorded. Calibration curves were constructed by plotting peak area on Y-axis against concentration on X-axis and regression equation was calculated by the method of least squares. The correlation coefficient, y-intercept, slope of the regression line were submitted.
- **Preparation of standard stock solution:** 25mg of was accurately weighed and transferred in to 50ml volumetric flask.25ml of methanol was added, sonicated for 5minutes and then volume was made up to the mark with the methanol to give a standard stock solution containing 1000µg/ml.
- **Diluent:** Methanol: sodium dihydrogen Ortho phosphate (40:60)
- **Preparation of working standard solution:** Working standard solutions were prepared by transferring 0.05, 0.1, 0.5 1.0, 1.5 2.0, 3.0, 4.0, 5.0, 6.0.ml of the standard stock solution in to 10ml volumetric flasks separately and volume was made up to mark with diluent. Then, the working standard solutions were filtered and sonicated.

Table 1: Preparation of working standard solutions for Linearity.

S. NO	Working standard solutions Concentration(µg/ml)	Stock solution taken in (ml)	Diluted to volume (ml) with diluents
1	5	0.05	10
2	10	0.1	10
3	50	0.5	10
4	100	1.0	10
5	150	1.5	10
6	200	2.0	10
7	300	3.0	10
8	400	4.0	10
9	500	5.0	10
10	600	6.0	10

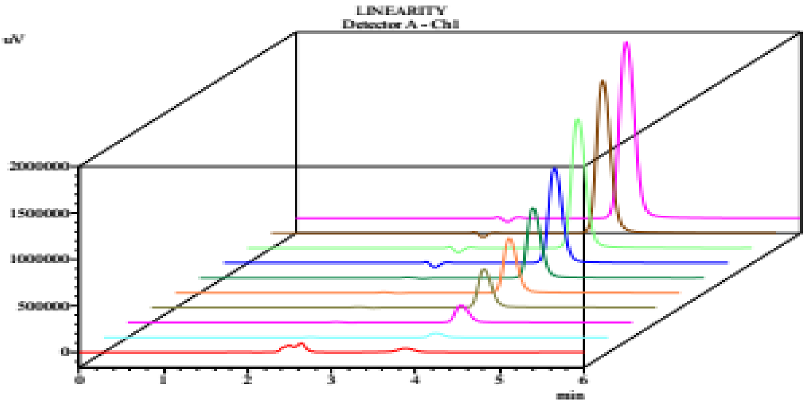

Fig. 2: Overlain Chromatogram for Linearity.

Table 2: Linearity data for Macitentan.

S. NO	Macitentan	
	Concentration (µg/ml)	Peak Area
1	5	513502
2	10	624796
3	50	2269149
4	100	4804079
5	150	6880420

6	200	9213686
7	300	14579904
8	400	19319253
9	500	22939032
10	600	27011819
Statistical analysis	**Slope** 45634 **y-intercept** ±25131 **Correlation Coefficient(R2)** 0.998	

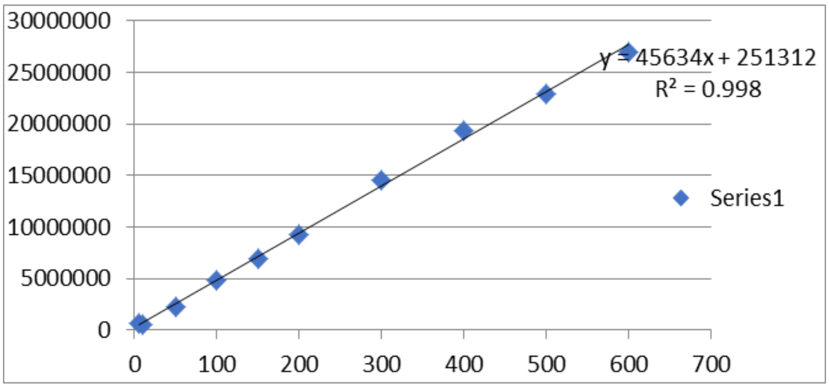

Fig. 3: Linearity Graph of Macitentan.

Report: Linearity was established over the concentration range of 5-600µg/ml, Correlation coefficient was found to be 0.998.

RANGE

The range of analytical method is the interval between the upper and lower levels of analytical that had been demonstrated to be determined within a suitable accuracy, precision and linearity.

The upper and lower levels of analyte showing accuracy, linearity and precision and found to be in the concentrations range 5 and 600µg/ml.

- **Preparation of standard stock solution:** 25mg of was accurately weighed and transferred in to 50ml volumetric flask.25ml of methanol was added, sonicated for 5minutes and then volume was made up to the mark with the methanol to give a standard stock solution containing 1000µg/ml.
- **Diluent:** Mobile phase
- **Preparation of working standard solution:** Working standard solutions were prepared by transferring 0.05, 6.0.ml of the standard stock solution in to 10ml volumetric flasks separately and volume was made up to mark with diluent. Then, the working standard solutions were filtered and sonicated.

Table 3: Range Peak for lowest concentration (5µg/ml).

S. NO	Peak	R_t	Peak area	Theoretical plates	Tailing factor
1	Macitentan	3.83	424883	2639	1.28

Table 4: Range Peak for Highest concentration (600µg/ml).

S.NO	Peak	R_t	Peak area	Theoretical plates	Tailing factor
1	Macitentan	3.96	23090912	2464	1.19

Table 4: Range data for Macitentan.

S. NO	Macitentan	
	Peak Area Lowest concentration (5 µg/ml)	Peak Area Highest concentration (600µg/ml)
1	420036	22634858
2	422564	22765034
3	423685	22634892
4	420965	22863401
5	424883	23090912
6	429650	23036580
Mean	423630.5	22837613
SD	3434.20	195901.19
%RSD	0.81	0.85

C. PRECISION

System Precision: It expresses the precision of the system. It includes analysis on system.

Procedure: It was performed by injecting six replicate injections of standard solution at 200µg/ml concentration under same experimental conditions. The mean value, standard deviation and % RSD was calculated for all six replicate injections.

Table 5: Results of System precision.

Injections	Peak area
1	246012
2	248564
3	248002
4	251654
5	251793
6	245412
Mean	248572.8
SD	2710.63
%RSD	1.0

Table 6: Peak Results of Method precision.

Injections	Peak area
1	201295
2	202563
3	203195
4	204569
5	205037
6	201169
Mean	202971.3
SD	1618.31
%RSD	0.79

Table 7: Inter Day Precision Results.

S. NO	Day I	Day II
Injections	**Peak area**	**Peak area**
1	3245666	3705836
2	3288621	3725832
3	3279705	3759238
4	3295431	3763584
5	3316820	3789019
6	3305182	3805863
Mean	3288571	3758229
SD	24659.43	37526.81
%RSD	0.74	0.99

D.ACCURACY

Procedure

Accuracy of the method was established by performing recovery studies according to the ICH guidelines. It was ascertained on the basis of recovery studies by standard addition method. Recovery studies were carried out at three different levels (80%, 100%, and 120%) by the addition of standard drug to pre-analyzed sample solution each in triplicate. Mean percentage recovery values at three different concentrations of drug were calculated.

$$\%\ \text{Recovery} = \frac{\text{Spiked} - \text{Test}}{\text{Standard}} \times 100$$

Table 8: Preparation of standard solutions for Accuracy.

Standard solution (%)	Stock solution taken in (ml)	Diluted to volume (ml) with diluents	Concentration of Macitentan (µg/ml)
80%	1.0	10	40
100%	2.0	10	50
120%	3.0	10	60

Preparation of Test solution

Three tablets were weighed accurately and crushed to form a fine powder. Accurately weighed a quantity of powder equivalent to 10 mg Macitentan, and transferred in to a 25 ml of volumetric flask, 25ml of methanol was added. The flask was then sonicated for 5 minutes and then made up to the mark with methanol. This gives the test stock solution having concentration of 1000µg/ml.

Table 9: Accuracy.

Level of spiking	**Test solution**		**Standard solution**		**Test and Std solutions Diluted to volume (ml) with diluents(mobile phase)**
	Concentration of test stock solution (µg/ml)	Stock solution taken in (ml)	Concentration of standard stock solution (µg/ml)	Stock solution taken in (ml)	
80%	1000	0.4	1000	0.4	10
100%		0.4		0.5	10
120%		0.4		0.6	10

Table 10: Accuracy Study data.

Spiked level	**Standard**		**Mean area**	**Spiked**		**Mean area**	**Recovery**
	Conc. (µg/ml)	**Peak area**		**Conc. (µg/ml)**	**Peak area**		
80%	40	1384962	1384962	40	3386924	3369637	99.60
		1379852			3047941		
		1395239			3342568		
100%	50	1466062	1444879	50	3425638	3428468	99.52
		1443470			3428561		
		1425106			3431205		
120%	60	1775891	1763372	60	3725462	3765537	100.6
		1744417			3789019		
		1769808			3782130		

ROBUSTNESS

Robustness of the method was demonstrated by making deliberate changes in the optimized conditions of the developed method.

Procedure: From the standard stock solution, a sextet of 150µg/ml solutions were prepared by appropriate dilutions and were treated in the similar manner as that of working standards. Six replicate injections were given and the effects of variations were observed in the respectively recorded chromatograms and the %RSD of the peak areas were calculated for the drug at each of the following conditions.

a. At different Flow rate (±0.1ml/min)
b. At different concentration of mobile phase ratio (±2mL)
c. At different wavelength(±3nm)

a. Effect of variation in flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution of 150µg/ml concentration was prepared and injected in to HPLC system by keeping flow rates 0.9ml/min and 1.1ml/min. The effect of variation of flow rate was evaluated.

b. Effect of variation in Mobile phase composition:

A study was conducted to determine the effect of variation in Mobile phase ratio by changing the ratio of organic phase i.e. Buffer: Methanol by ±2ml.standard solution of 150µg/ml concentration was prepared and injected in to HPLC system and the chromatograms were recorded. %RSD of the peak areas and retention times were calculated for the drug.

Effect of variation of detection wavelength

Standard solution of 150µg/ml concentration was prepared and injected in to HPLC system by keeping the wavelength at 224nm and 230nm. The effect of variation of detection wavelength was evaluated.

Table 11: Robustness Study (Change in Flow rate) Data.

Flow rate	**Parameters**	**Replicate injections**						**Statistical analysis**		
		1	**2**	**3**	**4**	**5**	**6**	**Mean**	**S.D**	**%RSD**
0.9mL/min	**Peak area**	4690255	4719205	4764420	4781632	4612854	4610352	4696453	73257.38	1.55
	RT	4.36	4.34	4.32	4.38	4.31	3.99	4.33	0.76	0.76
1mL/min	**Peak area**	3318350	3325634	3345632	3375632	3386924	3371235	3350735	33604.38	0.84
	RT	3.81	3.84	3.87	3.82	3.80	3.88	3.83	0.03	0.85
1.1mL/min	**Peak area**	2193582	2186368	2209974	2233562	2255230	2262359	2223513	31858.7	1.43
	RT	3.56	3.55	3.57	3.56	3.53	3.54	3.55	0.014	0.41

Table 12: Robustness Study (Change in mobile phase ratio) Data.

Mobile phase	Parameters	Replicate injections						Statistical analysis		
		1	2	3	4	5	6	Mean	S.D	%RSD
A:B:38:62	Peak area	8676596	8726367	8757502	8791481	8781833	8780806	8752431	43932.94	0.50
	RT	4.52	4.56	4.53	4.51	4.59	4.53	4.54	0.02	0.65
A:B:40:60	Peak area	3439635	3440236	3443689	3447483	3449483	3481107	3450272	15597.44	0.45
	RT	3.86	3.86	3.84	3.84	3.87	3.83	3.85	0.01	0.40
A:B:42:58	Peak area	7706380	770253	7835609	7890380	7875809	7896350	7817870	9037.03	1.15
	RT	3.53	3.55	3.50	3.58	3.52	3.53	3.53	0.02	0.77

F. RUGGEDNESS

This is to prove the lack of influence of operational and environmental variables of the test results by using the method. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst. All the system suitability parameters shall be met as per the method.

Variability due to Analyst

Accurately 322.5mg of Macitentan tablet powder was weighed and transferred into 100ml volumetric flask. 70 ml of diluent was added and sonicated to dissolve for 15min.Volume was made with diluent, further dilute 1ml to 10ml. filter through 0.45µ filter and injected into HPLC system in 6 replicate injections.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions were carried out with various combinations of buffer and methanol and by observing the peak parameters, the run time of the method was set at 10 min, Macitentanentan.appeared on the typical chromatogram at 3.83 min, which indicates a good base line. When the same drug solution was injected 3 times, the retention time of the drug was same. Linearity range was observed in the concentration range of 20 100 µg/ml. The regression equation of Macitentanentan concentration over its peak area ratio was found to be Y = 421237x - 27303(r=0.999) where Y is the peak area ratio and x is the concentration of Macitentanentan (Fig. 2). The proposed HPLC method was also validated for intra-day and inter-day variation. The coefficient of variation in the peak area of the drug for 3 replicate injections was found to be less than 2%. The tailing factor was found to be 1.114, which indicates good shape of peak. The number of theoretical plates was found to be 10829, which indicates efficient performance of the column. The limit of detection and limit of quantitation was found to be 5.3 x 10-5 µg/ml and 1.8 x 10 4 µg/ml which indicates the sensitivity of the method. The use of buffer and methanol in the ratio of 45:55 v/v resulted in peak with good shape and resolution. The high percentage of recovery of Macitentanentan ranging from 99.33-99.79 indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by proposed HPLC method.

CONCLUSION

The proposed HPLC method was found to be simple, sensitive, accurate and precise for determination of Macitentan The method utilizes easily available and cheap solvent for analysis of Macitentanentan hence the method was also economic for estimation of Macitentanentan

ACKNOWLEDGEMENT

The authors are express their gratitude to the AM Reddy college of Pharmacy,Narsaropet,A.P. for providing all the facilities and for providing gift sample of Macitentan.

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