© 2017 IJSRST | Volume 3 | Issue 10 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
National Conference on Research and Developments in Synthetic Organic Chemistry

Development and Validation of Stability Indicating RP-HPLC Method on Core Shell Columnfor Determination of Degradation and Process Related Impurities of Macitentan- Anti-hypertension Drug

Shashikant B. Landge[1], Sanjay A. Jadhav[1], Sunil B. Dahale[1] ,Rajendra S. Shinde[1], Kunal M. Jagtap[1], Saroj R. Bembalkar[2], Vijayavitthal T. Mathad[1*]

[1]Department of Process Research and Development, Megafine Pharma Lakhmapur, Dindori, Nashik, Maharashtra, India
[2]Department of Chemistry, Deogiri College, Aurangabad, Maharashtra, India

ABSTRACT

A core shell chromatographic column was used to separate the nine process related and degradation related impurities(Imp-1 to Imp-9) of Macitentanis described in this article. The chromatographic separation was achieved on a Sigma-Aldrich's 'Ascentis Express ® C18 (4.6 mm x 100 mm, 2.7 µ)' HPLC column with a runtime of 35 min. Forced degradation study was carried out under acidic, alkaline, oxidative, photolytic, and thermal degradation conditionsto demonstrate the stability-indicating nature of developed RP-HPLC method.The methodology consists of mobile phase-A as aphosphate buffer and mobile phase-B as a mixture of acetonitrile andmethanol. The column oven temperature was set at 45°C, injection thermostat was set at 5°C, and photodiode array detector (PDA) was set at 215 nm.Adeveloped method was validated as per ICH guideline and found rapid, specific,precise, sensitive,androbust. The proposed RP-HPLC method was successfully applied to the analysis of drug substance and drug product of Macitentan.

Keywords: Macitentan, Core-Shell HPLC Column, RSD and Validation, Stability Indicating.

I. INTRODUCTION

Macitentan is an antagonist drug chemically known as N-[5-(4-Bromophenyl)-6-[2-[(5-bromo-2-pyrimidinyl)oxy]ethoxy]-4-pyrimidinyl]-N'propylsulfamide and sold under the brand name "Opsumit" to treat the people with pulmonary arterial hypertension (PAH), a chronic, life-threatening disorder which severely compromises the function of the lungs and heart. It is an endothelin receptor antagonist (ERA) approved for the treatment of pulmonary arterial hypertension (PAH). Macitentan is a dual ERA, meaning that it acts as an antagonist of two endothelin (ET) receptor subtypes, ETA and ETB[1].However, Macitentan has a 50-fold increased selectivity for the ETA subtype compared to the ETBsubtype [2].The drug received approval from the U.S. Food and Drug Administration (FDA) on October 13, 2013[3].

An analytical method development to determine the quality of product is a critical task during the synthesis of product in generic companies because there is continuous improvement in the process by usingnew reagents, intermediates or by changing the route of synthesis. To develop the robust and selective analytical method nine process related impurities have been discovered during the synthesis of product by process development laboratory (Imp-1 to Imp-9)(**Figure1**).

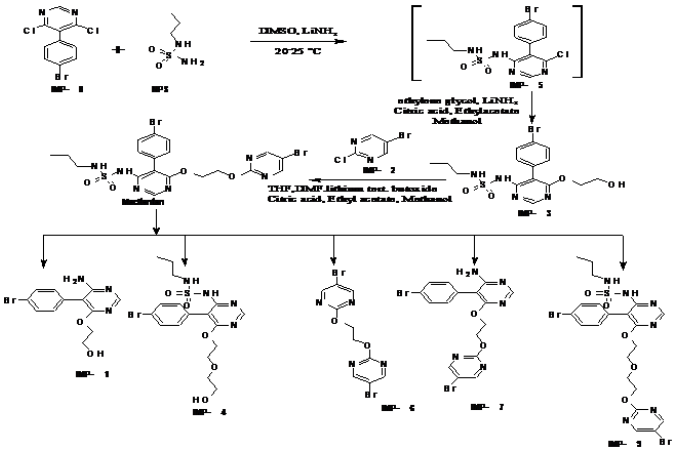


Figure 1. Process Related Impurities of Macitentan

Literature survey revealed that the Macitentan and/ or its metabolites were studied in human plasma by liquid chromatography-mass spectrometry method for theirpharmacokinetics[**4-5**].However, these papers were restricted to the determination of Macitentan and the details of process-related impurities and degradation related impurities formed under the stress conditions are not discussed.Few more analytical papers were also reported describing the method for determination of Macitentan content and not for the process-related impurities and degradation related impurities formed under the stress conditions employed[**6-8**].One of the articles reported on Quality by Design (QbD) HPLC method development of Macitentan lacks the forced degradation study and covers the limited number of impurities as compared to this article.Moreover, the column used in this article is porous and 3µ which often gives the high backpressure during the analysis[**8**].Further, Macitentan is not yet official in any of the pharmacopoeia andas per the requirements of various regulatory authorities, the impurity profile study of drug substance and drug product must be carried out using a suitable analytical method in the final drug product. Hence, we felt the need for the development of a selective, fast, and stability-indicating HPLC method on core shell column.

To the best of our knowledge no method on core shell column has been reported for the determination of Macitentan and its potential process related impurities in the drug substance and drug product for regular and stability study analysis in quality control laboratory.In present article the core shell chromatography column has been used to separate the nine process related impurities. The silica particle used in UPLC and HPLC column are porous in nature andsub-2µ and sub-5µ respectively. These columns give good resolution, speed, and sensitivity but same time it gives high backpressure[**9-11**].Hence core shell columns have been preferred to overcome these limitations. The present article describes the method development and method validation for determination of Macitentan and its process related impurities in bulk and dosage form using the core shellHPLC column [**12-13**].The core-objective of this research work was to develop a specific, precise, sensitive, and rapid stability-indicating RP-HPLC method for the determination of process and degradation related impurities of Macitentan. Thedeveloped method was successfully validated according to the USP<1225>Validation of Compendial procedures and ICH Q2 (R1) guideline[**14-15**].

II. EXPERIMENTAL

2.1. Materials and Reagents

The reagents like, ammonium dihydrogen orthophosphate, hydrochloric acid, sodium hydroxide, and hydrogen peroxide, triethylamine were all of AR grade, procured from Merck (India). The gradient grade acetonitrile and methanol was procured from J.T. Baker, Mumbai, India. HPLC grade water obtained from Millipore system (Millipore Inc., USA). The test sample of Macitentanand its potential process related impurities (Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7, Imp-8, and Imp-9) were received from synthetic laboratory of Megafine Pharma (P) Ltd, Nashik, India.

2.2. Instrumentation and Chromatographic Conditions

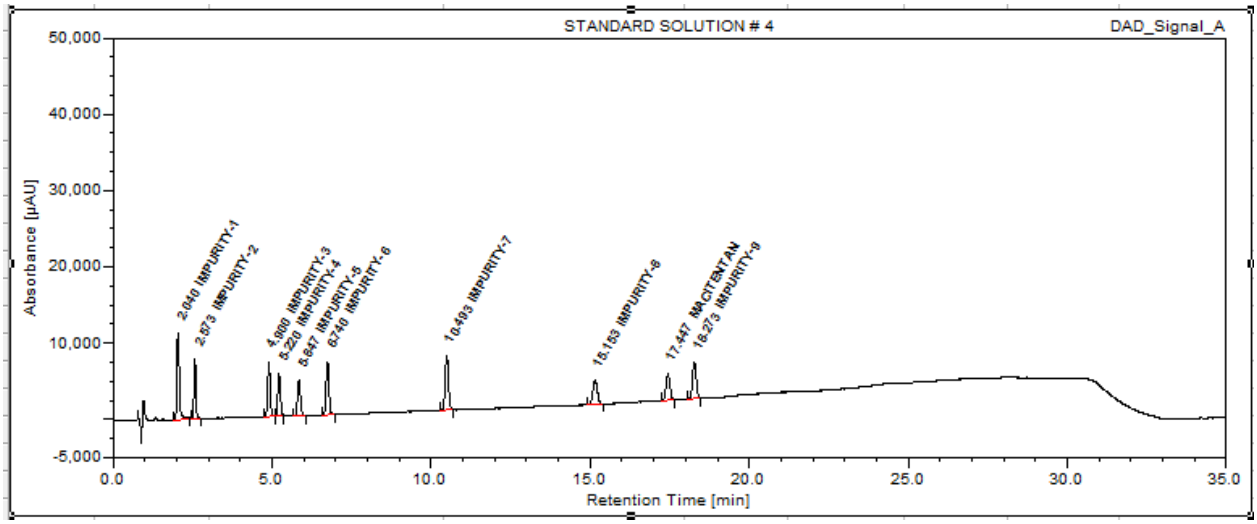
High Performance Liquid Chromatography (HPLC) equipped with photodiode array detector (1260, Agilent Technologies, Germany) was used for analytical method development and analytical method validation. 0.05M ammonium dihydrogen orthophosphate buffer was prepared bydissolving 5.75±0.10g ammonium dihydrogen orthophosphate in 1000 mL of waterand by adjusting pH 5.5 with triethylamine, further this buffer was filtered through 0.45µm membrane filter (0.45µ, Millipore) and degassed in ultrasonic bath prior to use as mobile phase A. 70:30 v/v,Acetonitrile and methanol was used as mobile phase B. Ascentis Express ® C_{18} (4.6 mm x 100 mm, 2.7µ) HPLC column thermostated at 45°C was used for the separation.The flow rate and injection volumes were 1.0 mL min^{-1} and 15µl respectively and injection thermostat was set at 5°C.The analysis was carried out under the gradient condition as time $_{(min)}$/A (v/v): B (v/v);$T_{0.01}$.60/40, $T_{25.0}$.35/65,$T_{29.0}$/35/65,$T_{31.0}$/60/40and $T_{35.0}$/60/40. The data was acquired at 215 nm for 35 min and processed by using Chromeleon software Ver. 6.80.

2.3. Preparation of Solutions and Analytical Procedure

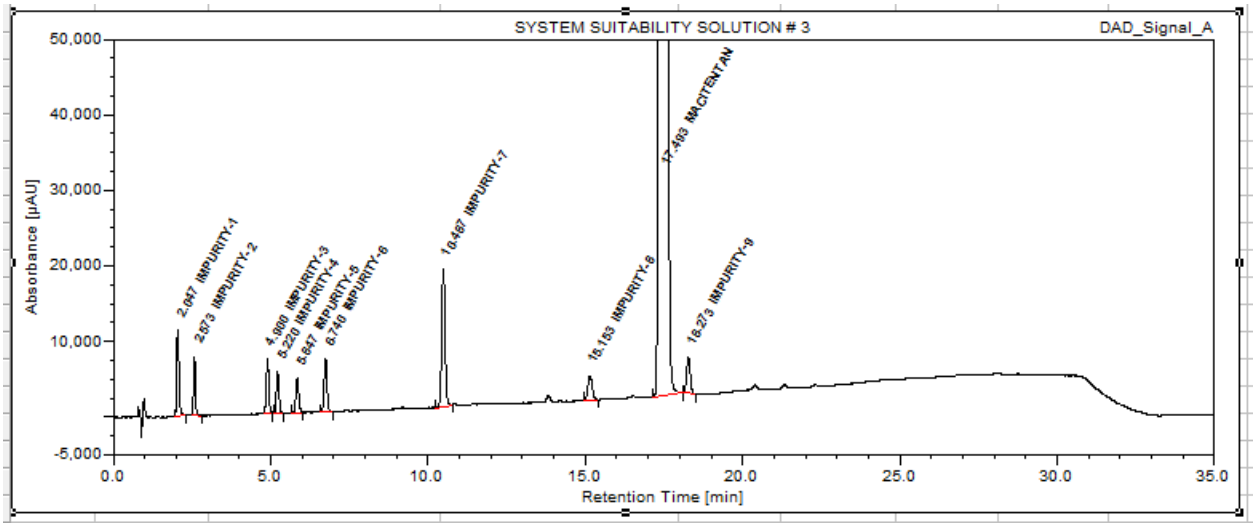
The diluent for analysis was prepared by mixing water and acetonitrile in the ratio of 50:50 (v/v).The stock

solutions of each impurity (Imp-1 to Imp-9) at concentration about75 µg $mL^{-1}$was prepared in diluent and further diluted to prepare the standard solution for quantification of impurities(**Figure2a**). The specification limits used for study was 0.15% for the related substances. Macitentan standard solution (500 µg mL^{-1}) spiked with all impurities at a specification level (w/w) was used as system suitability test (SST)(**Figure 2b**).The test sample solution having concentration of 500 µg mL^{-1} was prepared for the determination of related substances. The blank, system suitability test, 6 replicates of standard and test solution were injected separately. The resolution NLT 2.0, between Macitentan and Imp-9 and %RSD, NMT 5.0% for areas of six replicate injections of standard solution were set as system suitability criteria.

a) HPLC Chromatogram of Standard Solution:



b) HPLC Chromatogram of System Suitability Test Solution (SST):



c) HPLCChromatogram of Test Solution Spiked with Impurities at 1.0%w/w:

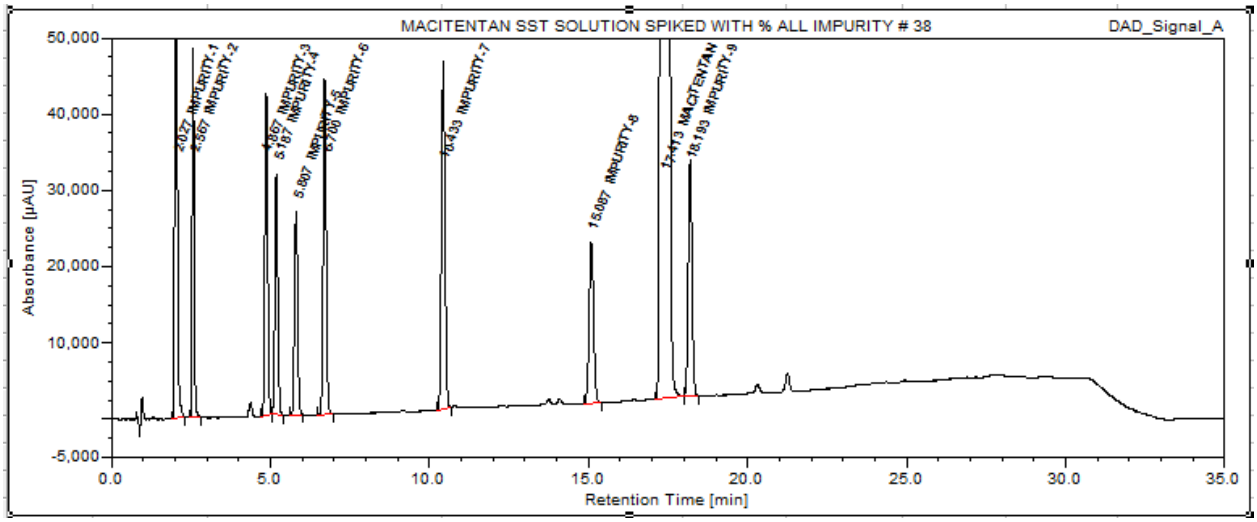


Figure 2. The chromatograms of Core shell column (a) standard solution (b) SSTand (c) test solution spiked with Impurities at 1.0%w/w

2.4. Characterization of Impurities

2.4.1. Fourier Transform Infrared Spectroscopy (FT-IR).FT-IR spectra were recorded for all the nine process-related and degradation impurities on Perkin Elmer model-spectrum-100 (California, USA) instrument using KBr pellet method.

2.4.2. ^{1}H NMR Spectroscopy.The ^{1}H NMR spectra were recorded on Bruker AV400 (400MHz) spectrometer using suitable solvent and tetramethylsilane (TMS) as internal standard.

2.4.3. Mass Spectrometry (MS).Mass spectra were recorded by using ESI source mass spectrometer equipped with a single quadrupole mass analyzer (Shimadzu LCMS-2020coupled with a Shimadzu UFLC Nexera, Japan). Ions were detected in electron spray ionization with positive/or negative ion mode (Event). Spectra were acquired from m/z 80 to 800 in scan mode.

All impurities (Imp-1 to Imp-9) were characterized using MS, FT-IR, and NMR spectroscopic techniques. The mass, FT-IR spectral data and ^{1}H NMR chemical shift values of these impurities are presented in **Table 1.**

Table 1. Mass, FTIR Spectral Data and 1H NMR Chemical Shift Value

Name of Impurity	**Mass value (m/z)** $(M+H)^+$	**FT-IR (KBr) absorption bands (cm^{-1})**	**^{1}H NMR Chemical shift values, δ in ppm, (multiplicity, integration)**
1) Imp-1	312.0	3463.65, 3443.03, 3341.27, 3136.68, 1640.12, 1578.71, 1432.65	4.44 (s, 1H), 4.23-4.24 (t, 2H), 3.56-3.57 (t, 2H), 6.40 (s, 2H)7.59-7.62 (d, 2H), 7.27-7.29 (d, 2H), 8.15 (s, 1H)
2) Imp-2	195.0	2935.40, 2965.51, 1597.08, 1752.30, 1193.71.	8.67 (s, 2H)

3) Imp-3	433.0	3331.18, 3502.85, 2983.98, 1572.04, 1334.78, 1163.11, 1064.74.	0.91-0.94 (t, 3H), 1.52-1.61 (m, 2H), 2.56-2.59 (t, 1H, OH), 2.92-2.97 (q, 2H), 3.81-3.84 (q, 2H), 4.46-4.48 (t, 2H), 5.59-5.62 (t, 1H, NH), 6.91 (bs, 1H, NH), 7.17-7.20 (dd, 2H), 7.62-7.65 (dd, 2H), 8.45-8.46 (s, 1H),
4) Imp-4	477.0	3310.0, 1570.0, 1430.0, 1340.0, 1170.0, 1080.0, 836.0.	0.77-0.82 (t, 3H), 1.36-1.48 (h, 2H), 2.78 (t, 2H), 3.35-3.42 (m, 4H), 3.60-3.63 (t, 2H), 4.39-4.42 (t, 2H) 4.58 (bs, 1H), 7.24-727 (d, 3H), 7.61-7.64 (d, 2H), 8.49 (s, 1H), 9.83 (s, 1H).
5) Imp-5	407.0	3265.59, 2966.62, 1543.10, 1340.57, 1165.04.	0.91-0.95 (t, 3H), 1.53-1.62 (m, 2H), 2.94-2.99 (q, 2H), 5.50-5.53 (NH) (t, 1H), 6.92 (NH), (bs. 1H), 7.16-7.19 (dd, 2H), 7.69-7.72 (dd, 2H), 8.65 (s, 1H).
6) Imp-6	377.0	3049.72, 1568.27, 1433.06, 1320.57, 1057.12.	4.73 (t, 4H), 8.52 (s, 4H)
7) Imp-7	468	3391.74, 3306.44, 3167.83, 1643.85, 1549.38, 1575.72, 1453.43, 1307.16, 1148.64, 1066.20, 791.58.	4.59-4.68 (m, 4H), 4.81(s, 2H), 7.19-7.22 (dd, 2H), 7.50-7.53 (dd, 2H), 8.22 (s, 1H) 8.48(s, 2H).
8) Imp-8	305.0	1546.93, 1508.27, 1371.69, 1224.95, 1070.70, 804.63	7.12-7.20 (m, 2H), 7.63-7.66 (m, 2H), 8.78 (s, 1H).
9) Imp-9	633.0	3292.38, 2972.84, 1569.47, 1556.17, 1431.43, 1312.95, 1172.29, 1087.81, 835.08	0.91-0.963 (t, 3H), 1.52-1.64 (h, 2H), 2.92-2.99 (q, 2H), 3.74-3.78 (q, 4H), 4.39-4.52 (m, 4H), 5.63-5.67 (t, 1H), 6.96 (s, 1H), 7.21-7.24 (dd, 2H), 7.60-7.63 (d, 2H), 8.46 (s, 1H), 8.53 (s, 2H).

III. RESULTS AND DISCUSSION

3.1 Development of Chromatographic Conditions

3.1.1 Optimization of Chromatographic Conditions by Using Core Shell Column

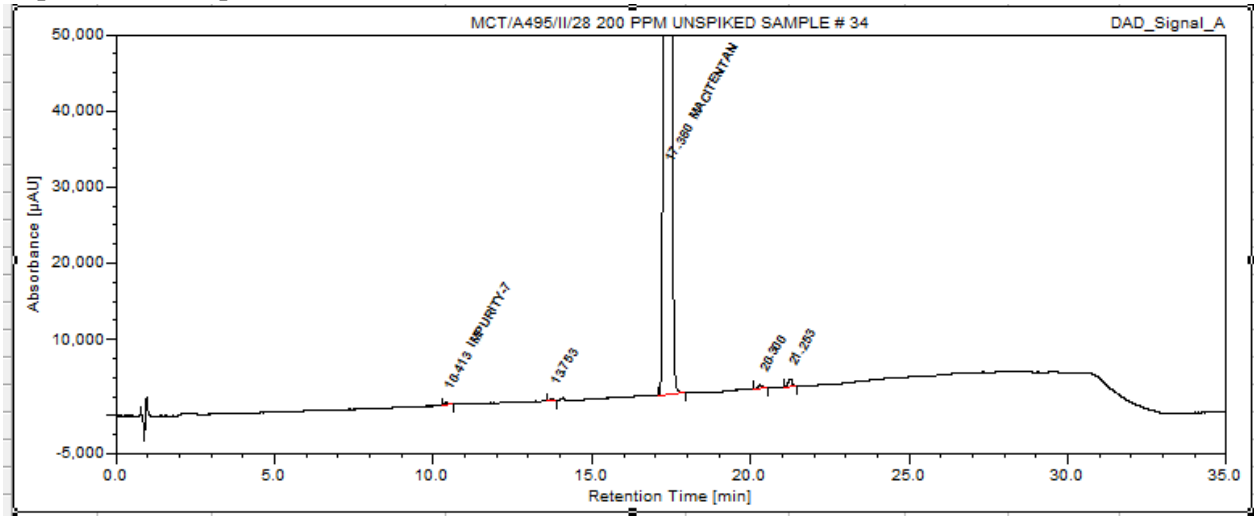
The objective of method development was to separate Macitentan and its process and degradation related impurities (Imp-1 to Imp-9) in a short run time with good resolution and good peak shape. Theresolution between Macitentan and Imp-9 was critical during the method development andhence selection of stationary phase was an important criterion during method development. The silica particle present in conventional HPLC column are porous in nature which results in higher back pressure, low resolution and higher run time of method. Therefore to reduce the run

time and backpressure and to maintain the good peak shape and resolution of impurities,the preference was given to core shell HPLC column than the conventional HPLC column.In core shell columns the modified silica particle having particle size of 2.7µ are used. Out of 2.7µ, 1.7µ is a solid core and 1.0µ is diffusion core/path. In core shell columns mobile phase is passing through only 1.0µ diffusion path whereas in conventional HPLC column mobile phase is passing through 3µ/5µ diffusion path.The core shell columns gives good peak shape, good resolution and theoretical plates due to less diffusion path to mobile phase.

Initial method development trials were conducted on different stationary phases like C_8, C_{18}, Phenyl-hexyl, andBiphenyl along with the optimization of other chromatographic conditions like detection of wavelength, the type, and quantity of organic/inorganic buffer, pH of mobile phase, thermostat, and column oven temperature. Every time system suitability criteria were evaluated during the different trial runs of method development to ensure the strength of developed method. Gradient mode was preferred than the isocratic mode to achieve the good resolution between all the impurities. We explored different core shell columns such as Ascentis Express ® C_{18} (4.6 mm x 100 mm, 2.7µ), Kinetex ® C_{18} (4.6 mm x 100 mm, 2.7µ), Kinetex ® Phenyl-hexyl (4.6 mm x 150 mm, 5µ), Kinetex ® C_8 (4.6 mm x 150 mm, 5µ), and Kinetex ® Biphenyl (4.6 mm x 150 mm, 5µ) during the development[**16**].

Among these columns satisfactory peak shape and good resolution of Macitentan and its process and degradation related impurities were achieved on Ascentis Express ® C_{18} (4.6 mm x 100 mm, 2.7µ) column with 35min run time, column flow rate 1.0 mL min^{-1}, λ 215nm, column oven temperature 45°C, injection thermostat 5°C and mobile phase consisting of phosphate buffer and combination of acetonitrile and methanol as a solvents.It was like a UPLC performance on conventional HPLC by using core shell column/technique. The typical chromatograms obtained from the analytical method development on core shell HPLC column are depicted in (**Figure 2c**) and (**Figure 3**)**.**

a) Un-Spiked Test Preparation:



b) Spiked Test Preparation:

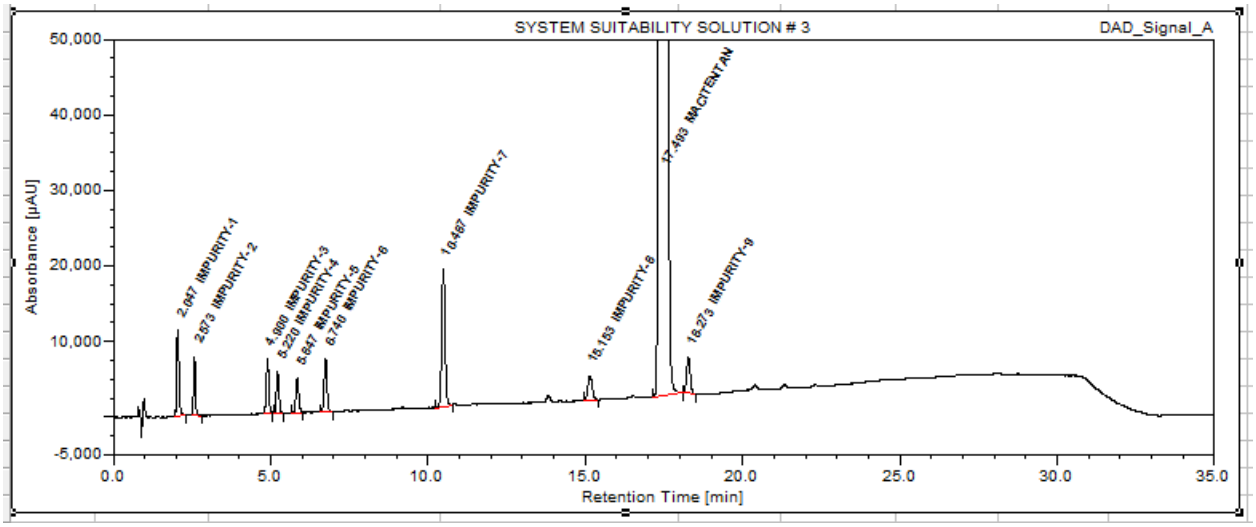


Figure 3. Typical HPLC chromatograms of; a) Macitentan, un-spiked test preparation, b) Macitentan spiked test preparation (0.15%w/w) with known impurities (Imp-1 to Imp-9)

3.1.2System Suitability Criteria

There was a critical resolution between Macitentan and Imp-9, hence a resolution criterion was set not less than 2.0.Other system suitability criteria were set as column efficiency/theoretical plates should not be less than 2,000, tailing factor should not be more than 2.0 and %RSD for six replicate injections of standard solution should not be more than 5.0%. The results of system suitability criterion are depicted in **Table 2**.

Table 2. System Suitability Test Results

Compound	Selectivity (α)	Resolution (R_s)	Tailing factor (T)	Theoretical Plates	RRT
Imp-1	0.533	-	1.33	3755	0.12
Imp-2	2.327	4.45	1.15	9733	0.15
Imp-3	0.32	18.42	1.09	17460	0.28
Imp-4	0.627	2.14	1.09	18870	0.30
Imp-5	0.893	3.96	1.04	19968	0.34
Imp-6	3.753	5.43	1.11	26860	0.39
Imp-7	4.66	20.95	1.05	47625	0.60
Imp-8	2.294	20.90	1.04	56501	0.87
Macitentan	0.826	9.51	1.05	102478	1.00
Imp-9	-	3.43	1.05	110051	1.05

RRT (Relative retention time)

3.2 Validation

3.2.1 Specificity (Selectivity)

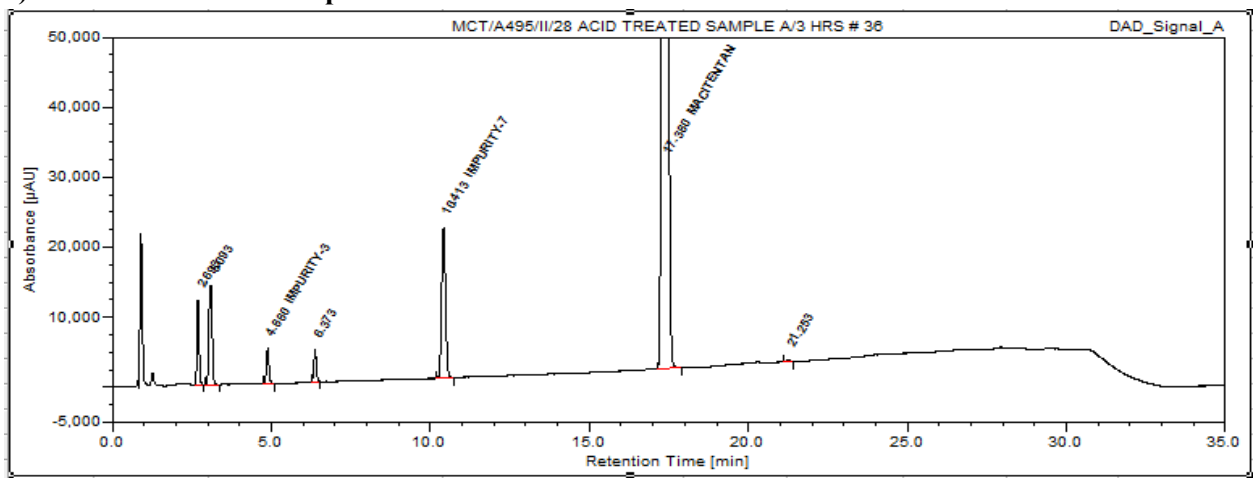
A forced degradation study was performed on Macitentanto provide an indication of the stability-indicating property and specificity of the proposed method.The specificity of developed RP-HPLC method for Macitentan was determined in presence of its impurities (Imp-1 to Imp-9) and degradation products. Aphotodiode array detector was employed to check and ensure the homogeneity and purity of Macitentan peak in all the stressed sample solutions. The stress conditions employed for the degradation study included light (1.2 million lux hours), heat (105°C), acid hydrolysis (5M HCl), base hydrolysis (1M NaOH) and oxidation (30%v/v H_2O_2). For heat sample was exposed for 4 days, for acid and base samples were treated for 3 hr and 40 minutes respectively at RT, whereas for oxidation sample was treated for 44 hr. The degradation was observed in acid, alkali, and peroxide degradation conditions. The mass balance was calculated for all the stressed samples. The mass balance is a process of adding together the assay value and the levels of degradation products to see how closely these add up to 100% of initial value with due consideration of the margin of analytical error[**17-19**].The results of forced degradation study are given in **Table 3** and**Figure 4.**

Table 3. Forced Degradation Results

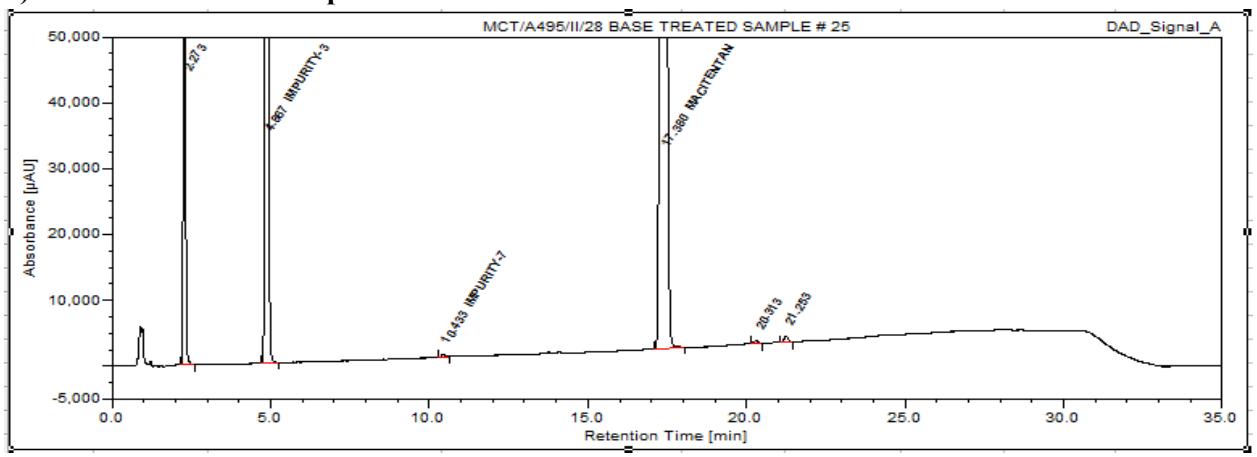
Stress condition	% of Macitentan	%of degredants	Observation and mass balance	Peak purity
Un-Treated	98.3	-	-	1.0000
Acid hydrolysis (5M HCl, 3 h at Room Temp.)	91.46	9.54	Major unknown degradation product (2.57%) formed (Mass balance: 97.69%)	1.0000
Base hydrolysis (1M NaOH, 40 min, at Room Temp.)	83.61	16.39	Major unknown degradation product (10.05%) formed (Mass balance: 97.88%)	1.0000
Oxidation (30% H_2O_2, 44 h. at Room Temp.)	96.50	3.50	Major unknown degradation product (3.13%) formed (Mass balance: 99.82%)	1.0000
Thermal (105°C, 4-days)	100.56	Nil	No any known and unknown degradation product formed (Mass balance: 100.71%)	1.0000
Photolytic as per ICH	99.85	Nil	No any known and unknown degradation product formed (Mass balance: 100.00%)	1.0000

Mass balance = % assay + % sum of all impurities + % sum of all degredants.

a) Acid Treated Test Sample



b) Base Treated Test Sample



C) Peroxide Treated Test Sample

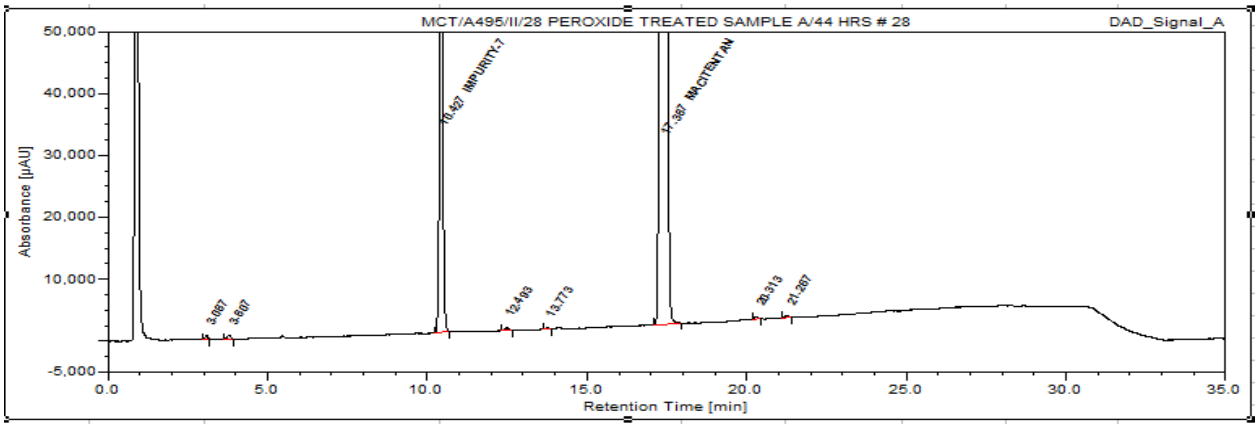


Figure 4. Typical HPLC chromatograms of forced degradation study; a) acid treated test sample, b) Base treated test sample, and c) Peroxide treated test sample.

3.2.2 Linearity

The linearity study of Macitentan and its related impurities was performed by using the six levels of linearity ranging from LOQ to 250% (LOQ, 0.187 µg mL^{-1}, 0.375 µg mL^{-1}, 0.75 µg mL^{-1}, 1.125 µg mL^{-1} and 1.50µg mL^{-1}) with respect to the specification level . The linearity plot was drawn for peak areas versus different concentrations of Macitentan and its related impurities. The linear regression data for all the components tested is presented in**Table 4**.

Table 4. Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ) data

Component	LOD /LOQ results		Linearity
	LOQ µg/ml, (% w.r.t.)[c]	LOD µg/ml, (% w.r.t.)[c]	
Imp-1	0.190 (0.038)	0.063 (0.013)	1.00000
Imp-2	0.188 (0.038)	0.063(0.013)	1.00000
Imp-3	0.187 (0.037)	0.062(0.012)	0.99998
Imp-4	0.178 (0.036)	0.059(0.012)	0.99999
Imp-5	0.188 (0.038)	0.063(0.013)	0.99995
Imp-6	0.189 (0.038)	0.063(0.013)	0.99989
Imp-7	0.186 (0.037)	0.062(0.012)	0.99994
Imp-8	0.189 (0.038)	0.063(0.013)	0.99994
Macitentan	0.129 (0.026)	0.043(0.009)	0.99982
Imp-9	0.188 (0.038)	0.063(0.013)	0.99992

[c] LOD LOQ values are in % with respect to test concentration of 500 µg/ml

3.2.3 Limits of Detection and Quantification(LOD and LOQ)

The limits of detection (LOD) and the limit of quantification (LOQ) of Macitentan and its process related impurities (Imp-1 to Imp-9) were estimated by calibration curve method [standard deviation of the response (σ) and the slope (S)], as per the ICH Q2 (R1) guideline. The values of LOD and LOQ for impurities and Macitentan were found in the range of 0.012%-0.013% and 0.036%-0.038% respectively. The precision was studied at the LOQ level by injecting six replicate injections of Macitentan and its related impurities, followed by the calculation of %RSD of the peaks areas. The %RSD of LOQ precision was found <10.0%. The results are depicted in **Table 4.**

3.2.4 Precision

A standard solution of Macitentan was injected for six times to determine the system precision of the method and %RSD was calculated for Macitentan and its all process related impurities. The %RSD of system precision was found in between 0.28%to 2.18%. For method precision six separate test sample solutions of Macitentan were prepared by spiking the related impurities (Imp-1 to Imp-9) at specification level. The %RSD (n = 6) for each related impurities was evaluated and found in between 0.72% to 2.44 %. For intermediate precision, similar procedure of method precision was carried out by a different analyst, on different instrument and on a different day with different lot of column. The %RSD of results for intermediate precision study was calculated and compared with the method precision results.

3.2.5 Accuracy (Recovery)

Macitentan sample solutions were spiked with all related substances at four different concentration levels, LOQ, 50,100, and 150% at specified limitin triplicate and theses spiked sample solutions were analyzed to determine the recovery of analytical method. The recovery of all these related substances were found to be in-between the predefined acceptance criterion, 80.0-120.0% and the data is given in **Table 5**.

Table 5. Accuracy Data of Related Substances

Component	Recovery results (Mean % Recovery[a]± %RSD)			
	LOQ level ; amount (%w/w)	50% of specification level[b]; amount (%w/w)	100% of specification level[b]; amount (%w/w)	150% of specification level[b]; amount (%w/w)
Imp-1	96.30±4.40	99.51±3.95	101.38±1.80	104.23±1.00
Imp-2	105.48±4.50	102.28±2.05	101.16±1.72	100.91±0.79
Imp-3	106.43±4.00	100.91±0.78	96.54±1.88	96.07±1.36
Imp-4	102.94±5.71	100.02±2.49	95.61±2.00	96.15±1.81
Imp-5	107.33±1.43	103.16±1.54	99.09±2.41	99.40±0.70
Imp-6	102.78±2.70	96.37±1.60	98.40±1.75	98.65±0.79
Imp-7	112.25±8.28	106.85±3.28	97.68±2.17	100.15±0.69
Imp-8	109.06±3.72	104.04±2.60	100.68±1.79	101.62±2.04
Imp-9	97.17±0.05	95.38±3.66	96.03±2.54	95.72±1.66

[a]% Recovery average of three determinations.
[b]0.15% of all related substances

3.2.6 Stability of Analytical Solution

To determine the stability of sample solution, Macitentan spiked with all related impurities at specified level were prepared and analyzed immediately and at after different time intervals up to 12 hrs. A sample cooler temperature was maintained at about 5°C. The result from these studies indicates that the sample solution is unstable and need to be injected freshly or within 8 hrs. at cooler temperature.

3.2.7 Robustness

The chromatographic conditions were deliberately altered to evaluate the robustness of developed method. The resolution between closely eluting peak pair i.e. Macitentanand Imp-9 was evaluated on altered chromatographic conditions.To study the effect of flow rate on the resolution the flow rate of mobile phase was altered by 0.1 units i.e. from 0.9 to 1.1 mL $min^{-1}$from 1.0 mL min^{-1}. The effect of column oven temperature on resolution was studied at 43°C and 48°Cinstead of 45°C whereas all other mobile phase components were held constant as described above. The tailing factor of Macitentanwas less than 2.0and the resolution between Macitentanand Imp-9 was greater than 2.0 in all the deliberately varied chromatographic conditions indicates that the robustness of the method.

3.2.8 Application of the Method

The analysis of bulk drug sample indicated that the method is specific and selective for determination of related substances in the bulk drug samples. The developed method is capable for quantitative analysis of Macitentanbulk drug and in a pharmaceutical dosage formand the data is given in **Table 6**.

Table 6. Results of Analysis of Bulk Drug Batches.

Component	Bulk drug sample batches		
	Batch No.1	Batch No.2	Batch No.3
Imp-1	ND	ND	ND
Imp-2	ND	ND	ND
Imp-3	ND	ND	ND
Imp-4	ND	ND	ND
Imp-5	ND	ND	ND
Imp-6	ND	ND	ND
Imp-7	0.02	0.02	0.02
Imp-8	ND	ND	ND
Macitentan	99.82	99.86	99.79
Imp-9	ND	ND	ND

ND: Not detected

IV. CONCLUSION

This is the first method reported in literature for the separation and quantification of Macitentanand its process related and degradation related impurities on core shell column. The RP-HPLC method is specific, linear, sensitive, accurate, precise, and robust. Moreover, the developed method was found to be more selective and rapid with respect to short runtime and low back pressure as compared to conventional HPLC column method. This method is validated as per ICH Q2 (R1) guideline. The developed method is stability indicating method which can be used for the analysis of routine and stability samples of Macitentan drug substance and drug products.

V. ACKNOWLEDGEMENTS

The authors wish to thank the management of MegafinePharma (P) Ltd., for supporting this work. The author is also thankful to colleagues in the division of Research and Development of MegafinePharma (P) Ltd for their cooperation in carrying out this work.

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