World Journal of Pharmaceutical Science and Research

www.wjpsronline.com

Research Article

ISSN: 2583-6579
SJIF Impact Factor: 6.916
Year - 2026
Volume: 5; Issue: 4
Page: 488-493

DEVELOPMENT OF ANALYTICAL METHOD FOR ESTIMATION OF TADALAFIL AND MACITENTAN BY HPLC

Dr. Sachin H. Rohane*

Faculty of Pharmacy, Yashoda Technical Campus, Satara, Maharashtra, India.

Article Received: 16 February 2026 | | ***Article Revised: 8 March 2026*** | | ***Article Accepted: 29 March 2026***

***Corresponding Author: Dr. Sachin H. Rohane**
Faculty of Pharmacy, Yashoda Technical Campus, Satara, Maharashtra, India.
DOI: https://doi.org/10.5281/zenodo.19413646

How to cite this Article: Dr. Sachin H. Rohane (2026) DEVELOPMENT OF ANALYTICAL METHOD FOR ESTIMATION OF TADALAFIL AND MACITENTAN BY HPLC. World Journal of Pharmaceutical Science and Research, 5(4), 488-493.

ABSTRACT

The primary goal of this suggested RP-HPLC approach was to develop and validate analytical procedures for the simultaneous quantitative and qualitative determination of Tadalafil and Macitentan in synthetic mixtures. The HPLC procedure was carried out on a C18 column (particle size 250 mm x 4.6 mm, 5 m). The chromatographic separation was accomplished in gradiant mode with a 92:08% v/v, methanol, and water mobile phase. The flow rate was held constant at 1 ml/min, and the wavelength chosen for estimation was 249nm. The suggested analytical method had a retention time of 3.625 min for Tadalafil and 4.675 min for Macitentan. The linearity range for Tadalafil was 2–12 g/mL. The percentage recoveries for Tadalafil and Macitentan were 100.03% and 100.35%, respectively.To summarize, the proposed endeavor might be used successfully for routine quality control analysis of both medications.

KEYWORDS: HPLC, Validation, Degradation, Tadalafil, Macitentan.

INTRODUCTION

Macitentan (MACI) is used to treat pulmonary arterial hypertension (PAH) in the long term. It also reduces morbidity in patients with PAH, which can be idiopathic or heritable, as well as related to connective tissue disease or congenital heart disease. When used alone or in combination with phosphodiesterase-5 inhibitors, the medication is effective.

Tadalafil (TADA) is approved for the treatment of idiopathic ("primary") pulmonary arterial hypertension (PAH), as well as PAH caused by connective tissue disease, congenital heart disease, or anorexigen usage, in individuals who have not responded to conventional therapy.

Tadalafil (TADA), chemically (6R-trans) -6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12ahexahydro-2-methyl-pyrazino (1',2':1,6.) pyrido (3,4-b) indole 1,4-dione belongs to the phospho di esterase-5 inhibitor class of drugs.

While Macitentan (MACI), N- [5- (4-Bromophenyl)-6- [2- [(5-bromo-2 pyrimidinyl) oxy] ethoxy] pyrimidinyl-4-pyrimidinyl]-N′propyl-sulfamide is a medication that belongs to the endothelin-receptor antagonist class.[1]

A review of the literature revealed that spectrophotometric (2-5), chromatographic techniques including bio analytical methods (6-21) were available individually to estimate these drugs, but no single analytical method that included spectrophotometry and chromatographic methods for the simultaneous estimation of TADA and MACI has been reported.

As a result, the goal was to create and validate a chromatographic method in accordance with the ICH guidelines.[22] The suggested study provides a dependable and sensitive RP-HPLC method for measuring TADA and MACI in synthetic mixture form. The proposed method was validated for accuracy, precision, robustness, and sensitivity.

EXPERIMENTAL

Materials and Instruments

TADA and MAC reference standards provided by Sun Pharma (Vadodara, Gujarat, India). Methanol, triple distilled water, sodium hydroxide, and hydrochloric acid were all obtained from Finar chemicals Pvt Ltd in Ahmedabad, India. It was of HPLC quality. The Quaternary gradient LC 2030 plus HPLC apparatus was made by Shimadzu Corporation in Japan. All of the information was recorded using lab-solution software. The weighing balance belonged to Sartorius.

Chromatographic condition

The following chromatographic conditions were used to estimate pharmaceuticals. The column is at the center of separation in HPLC. The Shimpack solar C18 column was employed, with dimensions of 250 mm x 4.6 mm and particle size of 5 µm. The temperature of the column oven was set to 25 °C. Several attempts for mobile phase selection were conducted before settling on Methanol: Water (92:08% v/v) to provide the optimum chromatographic parameters such as resolution, tailing factor, theoretical plates, and asymmetric factor. The flow rate and isobestic wavelength were both set to 1 ml/min and 249 nm.

Preparation of Mobile Phase: The mobile phase was made by combining methanol and water in a 92:08% v/v ratio. The prepared mobile phase was degassed and filtered using a nylon membrane filter with a 0.45 µm diameter.

Preparation of Solutions: 10 mg of MACI and TADA were precisely weighed and then added to a 10 ml volumetric flask. The drugs were dissolved in mobile phase to make a solution with a concentration of 100 µg/ml (stock solution).

METHOD VALIDATION

The proposed method was validated with several experimental parameters such as linearity, precision, accuracy, specificity, LOQ and LOD, and robustness.[22]

Linearity: MACI and TADA solutions were produced in 10 ml volumetric flasks at concentrations ranging from 2 to 12 µg/ml. Aliquots of 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 ml were transferred to a 10 ml volumetric flask and diluted with mobile phase to the mark.

Triplicate analysis were performed on the samples. Figure 1 depicts the overlay chromatogram of linearity. The relationship between concentrations and peak areas was illustrated using calibration curves for TADA and MACI, respectively. The correlation coefficients and regression line equations were computed.

System Sutability (Repeatability): To attain repeatability, a solution containing both medications at a concentration of 6 µg/ml was injected six times onto the HPLC system. Each time, the area under the curve and tailing factor were recorded and used to determine the relative standard deviation.

Accuracy: This parameter was calculated using the standard addition approach. TADA and MACI (2, 4 and 6 µg/ml) were spiked at three different doses (50,100, and 150%) in a sample solution of 4 µg/ml of both medications for recovery analysis. The mean recovery, as well as the SD and RSD, were computed.

Precision: (Reproducibility): Interday and intraday studies were carried out in order to express within-laboratory variances on different days of analysis. The precision of the proposed investigation was assessed at MACI and TADA concentrations ranging from 2 to 12 µg/ml. All samples were collected in duplicate.

Limit of detection and Limit of quantification

The LOD and LOQ of the drugs were calculated using following equations as per ICH guideline.

LOD = 3.3 X N/S and

LOQ = 10 X N/S.

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

Robustness: It was designed by deliberately adjusting key parameters such as mobile phase ratio, wavelength, flow rate, and % RSD.

Specificity: The specificity of an analytical method shows that the analytical method can measure the analyte of interest accurately and precisely without interference from blank and placebo. A specificity research was carried out to demonstrate that the presence of excipients had no effect on the procedure.

RESULTS AND DISCUSSION

Linearity: Peak area and concentrations of TADA and MACI in the range of 2 to 12 µg/ml were found to have a linear relationship. The calibration curve revealed that the correlation coefficient for TADA was 0.9966 and 0.9975 for MACI. (Figure:1)

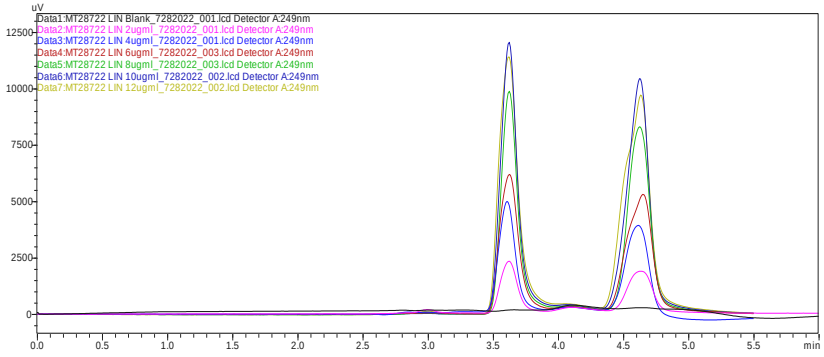

Figure 1: Chromatogram of TADA and MACI: (TADA: RT: 3.625 min. and MACI: RT: 4.675 min.)

System suitability: The relative standard deviation of the resulting area under curve was calculated and found to be 0.12% for TADA and 0.06% for MACI, respectively. All of the results were within an acceptable range, less than 2%, indicating that the approach was reproducible.

Accuracy: The recovery experiment was carried out by standard addition method. Values of % mean recovery of TADA and MACI was found to be 99 – 101 %.

Precision: The intra-day and inter-day precision % RSD was obtained between 0.02 – 1.0 for both the drugs.

Limit of detection and Limit of quantification: The limits of detection for TADA and MACI were 0.018 µg/ml and 0.0194 µg/ml, respectively, while the limits of quantification were 0.055 µg/ml and 0.058 µg/ml, respectively. All data was calculated using a formula.

Specificity: Specificity was determined by injecting blank and combined standard solution. HPLC chromatograms were compared to check the interference.

Robustness: On deliberate, minor changes of chromatographic parameters include wavelength, mobile phase and flow rate. % RSD was between 0.05 to 0.91 for both the drugs.

Assay: The prepared solutions from synthetic mixture were injected into the HPLC apparatus. The result was within an acceptable range. There was no evidence of excipient interference with peaks of interest. The proposed approach would be used to conduct quantitative and qualitative analyses of the medications mentioned.

Summary of validation parameters has been mentioned in Table 1.

Table I: Summary of validation parameters.

Parameter	Observation	
	TADA	**MAC**
Linearity (Range: 2-10 µg/ml)	$R^2 = 0.9966$	$R^2 = 0.9975$
Accuracy (% Recovery)	99 – 101 %	99 – 101 %
Precision (% RSD) (n=3)	0.02 – 1.0	0.02 – 1.0
System Suitability (% RSD) (n=6)	0.05 – 0.14	0.05 – 0.14
LOD (µg/ml)	0.018	0.019
LOQ (µg/ml)	0.055 µg/ml	0.058 µg/ml
Robustness	Robust	Robust
Assay	100.09 %	98.92 %
Specificity	No Interaction of diluent was observed	

CONCLUSION

The LC-MS/MS method available for identification of Macitentan in biological fluids is highly superior and sensitive. It is also applied to determine metabolites produced in human body during clinical trials study which is mandatory requirement for drug molecule registration with FDA.[9] Here, the drugs TADA and MACI were estimated by RP LC method quantitatively and qualitatively. The developed method was validated and found accurate, precise, sensitive and robust. The method also useful in determination of degradation pattern and bioanalytical study of proposed drugs.

REFERENCES

1. Indian Pharmacopoeia; Government of Indian ministry of health and family welfare, 2019; 1037.
2. Khan ZG, Patil AS, Shirkhedkar AA.; Estimation of tadalafil using derivative spectrophotometry in bulk material and in pharmaceutical formulation; International Journal of Spectroscopy, 2014); 1-6.
3. Yunoos M, Sankar DG, Kumar BP, Hameed S.; UV spectrophotometric method for the estimation of tadalafil in bulk and tablet dosage form; E-journal of chemistry, 2010; 7(3): 833-6.
4. Magdy MA, Anwar BH, Naguib IA, Abdelhamid NS.; Quantitative determination of dapoxetine hydrochloride and tadalafil using different validated spectrophotometric methods; Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2020; 226.
5. Vyas AJ, Gol DA, Patel AI, Patel AB, Patel NK, Chudasama A.; A stress degradation kinetic study of tadalafil bulk and tablet dosage form by UV spectrophotometry; Asian Journal of Pharmaceutical Analysis, 2020; 10(4): 177-81.
6. Bojanapu A, Subramaniam AT, Munusamy J, Dhanapal K, Chennakesavalu J, Sellappan M, Jayaprakash V.; Validation and method development of tadalafil in bulk and tablet dosage form by RP-HPLC; Drug research, 2015; 65(02): 82-5.
7. Cheng CL, Chou CH.; Determination of tadalafil in small volumes of plasma by high-performance liquid chromatography with UV detection; Journal of Chromatography B, 2005; 822(1-2): 278-84.
8. Rajeshwari M, Chenthilnathan A, Rama K.; Validated RP-HPLC method for simultaneous estimation of tadalafil and dapoxetine hydrochloride in combined pharmaceutical dosage forms; Int. J. Pharm. Biol. Sci., 2014; 4: 72-82.
9. Patel, C., Patel, B., Gutikar, S.; Development and Validation of bioanalytical method for Macitentan in human plasma using LC-MS; International Journal of Pharmaceutical, chemical and biological Sciences, 2018; 8(1): 43-52.
10. Aboul-Enein HY, Ali I.; Determination of tadalafil in pharmaceutical preparation by HPLC using monolithic silica column; Talanta, 2005; 65(1): 276-80.
11. Farthing CA, Farthing DE, Koka S, Larus T, Fakhry I, Xi L, Kukreja RC, Sica D, Gehr TW; A simple and sensitive HPLC fluorescence method for determination of tadalafil in mouse plasma; Journal of Chromatography B, 2010; 878(28): 2891-2895.
12. Zhu X, Xiao S, Chen B, Zhang F, Yao S, Wan Z, Yang D, Han H.; Simultaneous determination of sildenafil, vardenafil and tadalafil as forbidden components in natural dietary supplements for male sexual potency by high-performance liquid chromatography–electrospray ionization mass spectrometry.; Journal of Chromatography A, 2005; 1066(1-2): 89-95.
13. Satheesh B, Sree Ganesh KK, Saravanan D.; Simultaneous determination of tadalafil and its related compounds in pharmaceutical dosage forms by UPLC; Journal of Liquid Chromatography & Related Technologies, 2013; 36(10): 1451-65.
14. Shimpi PS, Mahajan H.; Bioanalytical Method Development and Validation of Tadalafil in Human Plasma Using RP-HPLC; Doi: 10.20959/Wjpr20208-18195
15. Naguib IA, Magdy MA, Anwar BH, Abdelhamid NS.; A validated green HPTLC method for quantitative determination of dapoxetine hydrochloride and tadalafil in bulk and pharmaceutical formulations; Journal of Chromatographic Science, 2020; 58(4): 303-8.

16. Rezk MR, Abdel-Moety EM, Wadie M, Tantawy MA.; Stability assessment of tamsulosin and tadalafil co-formulated in capsules by two validated chromatographic methods; Journal of Separation Science, 2021; 44(2): 530-8.
17. Nikolaou P, Papoutsis I, Athanaselis S, Alevisopoulos G, Khraiwesh A, Pistos C, Spiliopoulou C.; Development and validation of a GC/MS method for the determination of tadalafil in whole blood; Journal of pharmaceutical and biomedical analysis, 2011; 56(3): 577-81.
18. Purushothaman M, Subramanian R.; Bioanalytical method validation for determination of macitentan in K2EDTA human plasma by LC–MS/MS; International Journal of ChemTech Research, 2017; 10: 752-61.
19. Yerra NV, Pallerla P, Pandeti S, Tabet JC, Thota JR. ;Characterization of degradation products of macitentan under various stress conditions using liquid chromatography/mass spectrometry; Rapid Communications in Mass Spectrometry, 2018; 32(13): 1075-84.
20. Yu L, Zhou Y, He X, Li H, Chen H, Li W.; Simultaneous determination of Macitentan and its active metabolite in human plasma by liquid chromatography-tandem mass spectrometry; Journal of Chromatography B, 2015; 1002: 358-63.
21. Vyas AJ, Gol DA, Patel AI, Patel AB, Patel NK, Chavda JR, Lumbhani A, Chudasama A.; Implementing Analytical Quality by Design (AQbD) Approach for Simultaneous Estimation of Tadalafil and Macitentan by RP-HPLC Method; Analytical Chemistry Letters, 2021; 11(4): 539-52.
22. Panchal HJ, Suhagia BN, Patel M M, Patel B H, Estimation of Pitavastatin Calcium in Tablet Dosage Forms by Column Liquid Chromatography and Ultraviolet Spectrophotometry, 2009; 92(01): 158-164.