J. Pharm. Tech. Res. Management Vol. 11, No. 1 (2023), pp.71–76

CHITKARA

Journal of Pharmaceutical Technology Research and Management
Journal homepage: https://jptrm.chitkara.edu.in/

Simultaneous Quantification RP-HPLC Method Development and Validation for Atorvastatin and Tadalafil in Bulk Form

Ifroj[1], Baljeet Kaur[1] and Monika Gupta[1*]

[1]*Department of Pharmaceutical Chemistry, Amar Shaheed Baba Ajit Singh Jhujhar Singh Memorial College of Pharmacy, Bela, Ropar-140111, India*

[*]monikaguptaa@gmail.com (Corresponding Author)

ARTICLE INFORMATION

Received: February 10, 2023
Revised: February 20, 2023
Accepted: March 1, 2023
Published Online: April 23, 2023

Keywords:
Reverse Phase-HPLC, Atorvastatin, Tadalafil, Simultaneous estimation, Method validation

DOI: 10.15415/jprtm.2023.111007

ABSTRACT

Background: Tadalafil and Atorvastatin together have been shown to significantly enhance hemodynamics and sexual performance in middle-aged and older patients with hyperlipidemia. Despite of the number of techniques for estimating tadalafil and atorvastatin calcium, the Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) technique for simultaneous determination of Atorvastatin calcium and Tadalafil has not yet been disclosed.

Purpose: A sensitive, simple, rapid, reverse phase high pressure liquid chromatography (RP-HPLC) method has been developed and validated for the simultaneous estimation of Tadalafil and Atorvastatin in bulk form.

Methods: The method was carried out utilizing the C-18 column (Sapphirus, 5 x 250 mm) and mobile phase at 1 ml/min flow rate. 0.05M phosphate buffer (30:70 v/v gradient mode) and acetonitrile were the ideal mobile phase conditions. The pH was changed with glacial acetic acid to 4.0.

Results: Tadalafil and Atorvastatin had retention times of 3.517 and 4.117, respectively, according to data collected concurrently. The linearity range for Atorvastatin and Tadalafil (15 µg/ml to 90 µg/ml) was established using an external standard calibration method. It was calculated that the recovery rate ranges from 98.09 to 101.44 percent, and it was discovered that all values fall within this range. The limits of detection for Atorvastatin and Tadalafil are 0.318 µg/ml and 0.672 µg/ml, respectively.

Conclusion: The method was found to be robust, durable, accurate and linear for validated parameters. The % RSD observed for flow rate, wavelength, and analyst variation was found to be below 2, which was considered acceptable in the robustness investigation.

1. Introduction

Atorvastatin calcium is a lipid-lowering medicine from the statin class of drugs, Atorvastatin is also known by its brand name, Lipitor. Statins reduce aberrant lipid and cholesterol levels by preventing the liver's endogenous synthesis of cholesterol, ultimately leading to a lower risk of cardiovascular disease. The enzyme HMG-CoA reductase catalyses the conversion of HMG-CoA to mevalonic acid, and statins competitively block it (Chang *et al.,* 2013). The production of molecules like cholesterol, low-density lipoprotein (LDL), which is usually referred to as "bad cholesterol," and very low-density lipoprotein (VLDL) occurs in a number of metabolic processes. All of these molecules contribute to the transport and metabolism of lipids.

Tadalafil is used to treat benign prostatic hypertrophy (BPH), pulmonary arterial hypertension (PAH) and erectile dysfunction (ED). It is a selective phosphodiesterase-5 (PDE) inhibitor. Tadalafil improves erectile dysfunction by promoting sexual stimulation-dependent smooth muscle relaxation in the penis by resulting in the corpus cavernosum to fill with blood and causing an erection (Hatzimouratidis, 2014; Mónica & De Nucci, 2019). Relaxation of smooth muscles in the pulmonary arteries, which lowers the blood pressure in the pulmonary vasculature, aids in vasodilation in PAH (Henrie *et al.,* 2015). Tadalafil may aid in reducing smooth muscle cell proliferation in BPH, which could help shrink the prostate and lessen the structural blockage that causes BPH's urinary symptoms (Mónica & De Nucci, 2019). Tadalafil has a lesser affinity for PDE6 than other PDE5 inhibitors, which might be the reason why ocular side effects are less common.

Tadalafil and Atorvastatin together have been shown to significantly enhance hemodynamics and sexual

ISSN No.: 2321-2217(Print) ISSN No.: 2321-2225(Online); Registration No. : CHAENG/2013/50088

performance in middle-aged and older patients with hyperlipidemia worsened by ED (Du *et al.,* 2021).

Numerous techniques for liquid chromatography evaluation of Tadalafil and Atorvastatin calcium in aqueous samples and biological fluid were identified by the literature review. Additionally, a number of techniques for estimating Tadalafil and Atorvastatin calcium with other medications such as Telmisartan, Nicotinic acid, Amlodipine Besilate, Ambrisentan, Sildenafil Silodosin and its enantiomer and diastereomer, etc. were also reported.

However, the Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) technique for simultaneous determination of Atorvastatin calcium and Tadalafil has not yet been disclosed. Therefore, the goal of the current study is to create and validate an easy-to-use, highly accurate, and reasonably priced RP-HPLC method for figuring out the concentrations of Tadalafil and Atorvastatin calcium in both bulk and formulation. The accuracy, precision, linearity, specificity, system suitability, limit of detection and qualification of the established method has all been validated (Lal *et al.,* 2019; U.S. FDA Guidance for Industry, 2003; ICH Guidelines, 1996; United States Pharmacopeia, 2000; Bhagyasree *et al.,* 2014).

2. Experiments

2.1. Chemicals and Reagents

Pharmaceutical grade (>99%) drugs Atorvastatin and Tadalafil were obtained from Health Biotech (P) LTD (Chandigarh, India). Acetonitrile (HPLC grade) and sodium dihydrogen phosphate were obtained from Fisher Scientific India (P) LTD. The supplier of potassium dihydrogen orthophosphate was Merck from Mumbai. Unless otherwise noted, all other analytical-grade chemicals were purchased from local suppliers. Every dilution was carried out using conventional volumetric glassware.

2.2. Instrumentation

Shimazdu RP-HPLC Instrument equipped with PDA detector and Digital weighing balance (CY220) was used in method development; analysis and separation have been done on Sapphirus C18 Column (5x250 mm) at a flow rate of 1 ml min^{-1}. All HPLC systems are equipped with a column compartment with temperature control and an online degasser.

2.3. Selection of detection wavelength

The sensitivity of the UV-detection HPLC method is dependent on the proper wavelength selection. The wavelength that responds favourably to the drugs being detected is the optimum wavelength. After then, this solution's spectra were recorded when they were scanned in the UV spectrum between 200 and 400 nm.

2.4. Optimized Chromatographic Conditions

- Analytical Column: Sapphirus C_{18} Column (5 µm, 250 × 4.6 mm)
- Elution mode: Gradient mode (30:70 v/v)
- Mobile phase: 0.05M phosphate buffer (adjusted to pH 4.0 with Glacial acetic Acid) and Acetonitrile.
- Flow rate: 1 mL min^{-1}.
- Column Temperature: 30°C
- Injection volume: 10 µl
- Diluent: Mobile Phase
- Absorption maxima: 272 nm
- Run time: 10 minutes
- Detector: UV

2.5. Standard Stock Solution Preparation

The exact weights of 10.62 mg of Atorvastatin and 10.21 mg of Tadalafil were added to a 100 ml volumetric flask. To dissolve the drug, add sonicate with 30 ml of the diluent first. Then, add more diluent to the appropriate level.

3. Results and Discussion

The atorvastatin calibration curve was created using the 15 to 90 µg/ml solution. The area (Isosbestic point) was calculated to be 272. The calibration curve, as shown in the graph, indicated the regression equation Y= 20255x -819.7, with an R^2 value of 0.999, demonstrating good linearity. The tadalafil calibration curve was created using the 15 to 90 µg/ml solution. The area (isosbestic point) was calculated to be 272. The calibration curve, as shown in the graph, revealed the regression equation Y=17706 x + 40587, and the R^2 value was 0.999, showing good linearity.

According to the results of the pre formulation investigation (FT-IR spectra, HPLC, and melting point), atorvastatin and tadalafil were found to be pure and of good quality, and the estimate methodology was found to be quite dependable, accurate, and suitable for the method improvement. The best liquid chromatographic conditions for the separation of atorvastatin and tadalafil were achieved by performing multiple tests for the selection of column and mobile phase. The technique was developed using the C-18 Column (Sapphirus 5 x 250 mm) at 1 mL min^{-1} flow rate.

0.05M phosphate buffer (30:70 v/v gradient mode) and acetonitrile were the ideal mobile phase conditions. The pH was changed with glacial acetic acid to 4.0. Tadalafil and

atorvastatin had retention times of 3.517 and 4.117 (Figure 1), respectively, according to data collected concurrently. The method was found to be linear, accurate, durable, and robust for validated parameters. The linearity range for atorvastatin (15 µg/ml to 90 µg/ml) and tadalafil (15 µg/ml to 90 µg/ml) was established using an external standard calibration method. It was calculated that the recovery rate ranges from 98.09 to 101.44 percent, and it was discovered that all values fall within this range. The sample's repeatable analysis added to the method's accuracy. Low values of the % RSD were discovered, which suggested that the outcomes were precise. The limits of detection for atorvastatin and tadalafil are 0.318 µg/ml and 0.672 µg/ml, respectively. The % RSD reached for varying the flow rate, wavelength, and analyst was found to be below 2, which was considered acceptable in the robustness investigation.

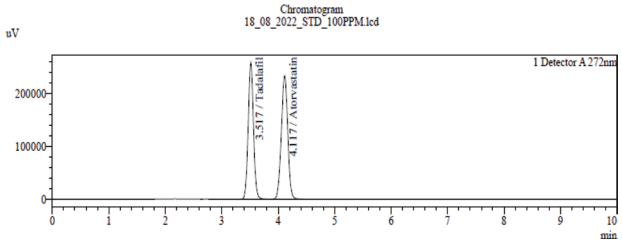

Figure 1: Simultaneous chromatogram of standard Atorvastatin & Tadalafil solution of 100 µg/ml and 100 µg/ml.

3.1. RP-HPLC Method Validation

3.1.1 Linearity and range

For atorvastatin and tadalafil, a calibration curve was plotted throughout the concentration range of 15 µg/ml to 90 µg/ml. Atorvastatin working stock solution was accurately measured at 15 µg/ml, 30 mg/ml, 45 µg/ml, 60 µg/ml, 75 µg/ml, and 90 µg/ml. All dilutions were filtered through a 0.22 filter before injection. Each solution's area was measured at the appropriate wavelength. By comparing concentration to the area where each reading was taken, the linearity was created (Table 1 and Figure 2-3).

Table 1: Linearity of Atorvastatin and Tadalafil

Conc. (µg/ml)	Atorvastatin	Tadalafil
15	310807	292198
30	623883	574858
45	925422	854432
60	1194980	1107270
75	1508004	1393604
90	1833474	1624874

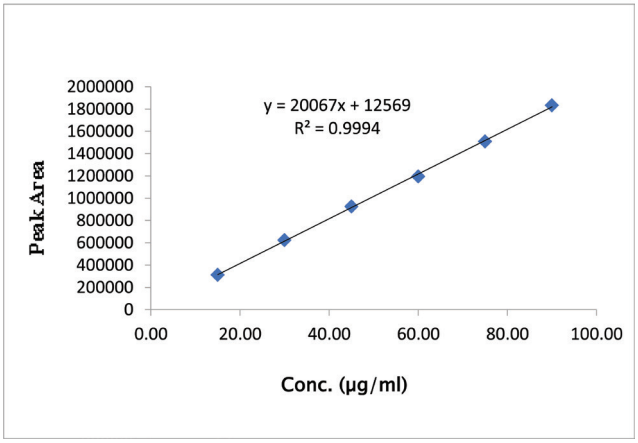

Figure 2: Linearity of Atorvastatin

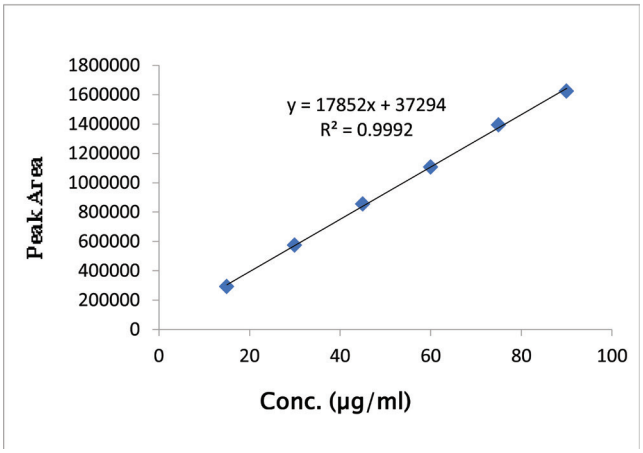

Figure 3: Linearity of Tadalafil

3.1.2. Accuracy

The accuracy of the approach was calculated as a percentage of standard recovery. By adding standard drug solution at the three concentration levels of 50%, 100%, and 150% to a pre-analyzed sample, recovery tests were conducted (Table 2-3). In this procedure, the assay sample was mixed with a standard medication at a known concentration.

Table 2: Summary of Accuracy Study of Atorvastatin

Level	Conc. Spiked (µg/ml)	Percentage recovered	% RSD
50%	30	100.73	0.331
100%	60	99.77	1.081
150%	90	98.20	0.705

Table 3: Summary of Accuracy Study of Tadalafil

Level	Conc. Spiked (µg/ml)	Percentage recovered	% RSD
50%	30	101.44	0.111
100%	60	101.15	1.128
150%	90	98.09	0.507

3.1.3. Precision

Standard solution of Atorvastatin and Tadalafil (Table 4) was prepared and analyzed as per the proposed method.

Table 4: Summary of Repeatability, Intermediate and Reproducibility precision study

Precision	Percentage recovery of Atorvastatin	% RSD	Percentage recovery of Tadalafil	% RSD
Repeatability	98.49	0.189	99.77	0.021
Intermediate	97.48	0.0185	98.62	0.004
Reproducibility	98.35	0.359	100.14	0.003

3.1.4. LOD and LOQ

The method's limit of detection (LOD) and limit of quantification (LOQ) (Table 5) were determined using the standard deviation of the response and the slope (s) of the calibration curve, respectively, at approximately the same values. The results fell within the expected range.

Table 5: LOD and LOQ data

Drug	LOD (µg/ml)	LOQ (µg/ml)
Atorvastatin	0.318	0.964
Tadalafil	0.672	1.037

3.1.5. Robustness

By examining the sample with purposeful variation in the procedure parameters, the resilience was investigated (Table 6-9). In terms of % RSD, the shift in drug reactions was detected. The robustness of the approach was investigated using wavelength and flow rate changes.

Table 6: Robustness data of Atorvastatin with deliberate change in wavelength

Conc.(µg/ml)	Wavelength 267nm (Area)	Wavelength 277nm (Area)
60	1279253	1110144
60	1275582	1108202
60	1267713	1097046
60	1268851	1102315
60	1271247	1092544
60	1262114	1124154
Mean	1270793	1105734
SD	6054.67	11189.12
%RSD	0.476	1.012

Table 7: Robustness data of Tadalafil with deliberate change in wavelength

Conc.(µg/ml)	Wavelength 267nm (Area)	Wavelength 277nm (Area)
60	936042	1220606
60	932949	1229618
60	924768	1233880
60	910187	1245844
60	937821	1224584
60	942157	1215485
Mean	930654	1228336
SD	11589.0	10750.70
%RSD	1.245	0.875

Table 8: Robustness data of Atorvastatin with deliberate change in flow rate (ml/min)

Conc.(µg/ml)	Flow rate 0.8ml/min (Area)	Flow rate 1.2ml/ min (Area)
60	1481723	987436
60	1483233	996436
60	1478993	1001682
60	1475248	992417
60	1482547	1001247
60	1475847	995214
Mean	1479599	995739
SD	3457.9	5411.0
%RSD	0.234	0.543

Table 9: Robustness data of Tadalafil with deliberate change in flow rate (ml/min)

Conc.(µg/ml)	Flow rate 0.8ml/min (Area)	Flow rate 1.2ml/ min (Area)
60	1373894	908356
60	1367086	918399
60	1369020	920634
60	1352471	928547
60	1365147	904548

60	1365147	913314
Mean	1365461	915633
SD	7146.47	8725.16
%RSD	0.523	0.953

There shouldn't be a % RSD of greater than 2. It was discovered that the % RSD achieved for the change in flow rate and wavelength was below 2, which was acceptable. Thus, the approach was reliable.

3.1.6. Ruggedness

By using different analysts to analyse the same samples of the same medicine, the ruggedness was evaluated (Table 10). Drug reactions were shown to change in terms of % RSD.

Table 10: Analyst variation data

S.No	Percentage recovery of Atorvastatin	%RDS	Percentage recovery of Tadalafil	%RDS
Analyst 1	99.87	0.292	99.62	0.467
Analyst 2	101.09	0.844	100.25	0.415

Conclusion

Using the new RP-HPLC approach, the quantitative determination of bulk Atorvastatin and Tadalafil was found to be fast, easy to use, sensitive, precise, and simple. The method was designed to outperform the majority of methods that have been reported for the simultaneous measurement of Atorvastatin and Tadalafil. The ICH Guidelines were used to grant approval for the procedure.

Acknowledgments

Authors duly acknowledge Management, Amar Shaheed Baba Ajit Singh Jhujhar Singh Memorial College of Pharmacy, Bela, Ropar, India for providing research facilities.

Authorship Contribution

IFROJ: Methodology, Investigation, Writing-Original draft, Baljeet Kaur: Writing-Review & Editing, Monika Gupta: Formal Analysis, Supervision, Project Administration.

Funding

There is no funding source for this article.

Conflict of Interest

There is no conflict of interest.

Declarations

It is an original data and has neither been sent elsewhere nor published anywhere.

References

Bhagyasree T., Injeti N., Azhakesan A., & Rao U.M.V., (2014). A review on analytical method development and validation, *Int. J. of Pharm. Res. & Anal., 8*, 444-448. https://www.ijpra.com/File_Folder/444-448(ijpra).pdf

Chang, C., Lee, J., Lin, J., Hung, Y., Liu, R., Shau, W., & Sheu, W.H. (2013). Effects of statins on lipid profile of hyperlipidemic patients in Taiwan. *Tzu-Chi Med. J.*, *25*, 168-174. https://doi.org/10.1016/j.tcmj.2013.06.001

Du L., Jia J. H., Xue W. Y., Qi J. C. (2021). Effect of Tadalafil combined with Atorvastatin on hemodynamics and sexual function in middle-aged and elderly patients with hyperlipidemia complicated with erectile dysfunction. *Pak. J. Med. Sci., 37*(7), 1965-1971. https://doi.org/10.12669/pjms.37.7.4257

Hatzimouratidis K. (2014). A review of the use of Tadalafil in the treatment of benign prostatic hyperplasia in men with and without erectile dysfunction. *Ther. Adv. Urol. 6*(4), 135-47. https://doi.org/10.1177/1756287214531639

Henrie A. M., Nawarskas J. J., & Anderson J. R. (2015). Clinical utility of Tadalafil in the treatment of pulmonary arterial hypertension: an evidence-based review. *Core Evid. 2*(10), 99-109. https://doi.org/10.2147/CE.S58457.

International Conference on Harmonization (ICH) Guidelines, (1996). *Impurities in new drug products*, IFPMA, Geneva.

Lal B., Kapoor D., & Jaimini M., (2019) A review on analytical method validation and its regulatory perspectives. *J. drug delive. Ther.*, *9*(2), https://doi.org/10.22270/jddt.v9i2.2403

Mónica F. Z., & De Nucci G. (2019). Tadalafil for the treatment of benign prostatic hyperplasia. *Expert Opin. Pharmacother. 20*(8), 929-937. https://doi.org/10.1080/14656566.2019.1589452.

The United States Pharmacopeia, (2000), 24th Revision, Asian Edition, United States, Pharmacopeial Convention, Inc., Rockville, MD.

U.S. Food and Drug Administration Guidance for Industry, (2003). ICH Q3A, *Impurities in New Drug Substances*.

Journal of Pharmaceutical Technology, Research and Management

Chitkara University, Saraswati Kendra, SCO 160-161, Sector 9-C, Chandigarh, 160009, India

Volume 11, Issue 1 **May 2023** **ISSN 2321-2217**