Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

A Specific and sensitive analytical method development and validation for Atorvastatin for pharmaceutical preparation with a green chemistry perspective by high performance liquid chromatography

Md Nazmus Sakib Chowdhury

nscphr@gmail.com

Gopalganj Scienec and Technology University https://orcid.org/0000-0001-8307-2808

Research Article

Keywords: Atorvastatin, HPLC, Bioequivalence, Green chemistry, Method development, Method Validation

Posted Date: July 27th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10465451/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: The authors declare no competing interests.

Abstract

Atorvastatin is a widely prescribed lipid-lowering agent used for the management of hyperlipidemia and prevention of cardiovascular diseases. Reliable, sensitive, and environmentally sustainable analytical methods are essential for ensuring the quality, safety, and efficacy of atorvastatin pharmaceutical products. The objective of this study was to develop, optimize, and validate a rapid, sensitive, and green reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantitative determination of atorvastatin in pharmaceutical formulations.

Chromatographic method development was performed by comparing a previously reported control method with two newly developed chromatographic conditions. The optimized method was achieved using a GL Sciences ODS-3V C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of 0.5% (v/v) aqueous acetic acid and ethanol (42.5:57.5, v/v) at a flow rate of 1.0 mL/min and UV detection at 240 nm. Method optimization demonstrated that the proposed method significantly improved chromatographic efficiency by reducing retention time compared with the control method ($p < 0.0001$) while maintaining excellent peak characteristics. The optimized method exhibited enhanced sensitivity, with experimentally confirmed limits of detection and quantification of 5 ppm and 8 ppm, respectively, compared with a 10 ppm quantification limit for the control method.

The developed method was validated according to analytical validation requirements for specificity, linearity, accuracy, precision, limit of detection, and limit of quantification. The method demonstrated excellent linearity over the concentration range of 0.004–0.800 mg/mL with a correlation coefficient (R^2) of 1.000. Accuracy studies showed recoveries ranging from 96.88% to 103.13%, with an average recovery of 100.39%. The method showed good repeatability and intermediate precision with %CV values of 1.83% and 1.79%, respectively. Specificity evaluation confirmed the absence of interference from blank and placebo components, and peak purity analysis verified analyte specificity.

The proposed RP-HPLC-UV method provides a rapid, sensitive, accurate, precise, and environmentally favorable approach for atorvastatin quantification. The utilization of ethanol as the major organic solvent reduces environmental impact and supports green analytical chemistry principles. The validated method is suitable for routine pharmaceutical quality control, manufacturing process monitoring, regulatory analysis, and potential application in bioequivalence studies.

Introduction

Atorvastatin is a widely prescribed drug for managing hyperlipidemia and preventing cardiovascular diseases, necessitating robust and reliable analytical methods for its quantification in pharmaceutical formulations (Lee et al., 2017). Development of a specific and sensitive analytical method, followed by thorough validation, is crucial in the pharmaceutical industry to ensure the quality, efficacy, and safety of drug products (Li et al., 2012; Lokhande, 2019). Analytical method development involves establishing the suitability of the method for its intended purpose, particularly the quantitative estimation of the target analyte in pharmaceutical drugs, playing a vital role in both development and manufacturing (Lakka & Kuppan, 2019)

Most of the industrial companies couldn't keep a clean atmosphere or make decent products since they didn't have a proven method for measuring that was based on green chemistry. To avoid marketing faulty products and make sure that green chemistry is in line with good quality, a precise and sensitive method for analysis must be developed and validated.

Atorvastatin, a synthetic lipid-lowering agent, belongs to the statin class of drugs, acting as an inhibitor of the enzyme HMG-CoA reductase. The permitted daily exposure for atorvastatin was established at 200 µg/day based on the most sensitive toxicological endpoint which is liver toxicity (Arca & Pigna, 2011). The plasma half-life of atorvastatin is approximately 14 hours, but the HMG-CoA reductase inhibitory activity last for 20–30 hours because of the contribution from active metabolites. Atorvastatin has multidose of 10 mg, 20 mg, 40 mg, and 80 mg with a specific way of using it. It has many pharmaceutical dosages form for its intended uses (Lins et al., 2003). Due to the intricate nature of pharmaceutical matrices, including excipients, degradation products, and potential impurities, the analytical determination of atorvastatin presents several challenges (Szücs et al., 2017). Atorvastatin is one of the highest manufacturing medicines across the globe that has several health benefits (P´erez-Grijalba et al., 2016). So, a specific and sensitive method is required for Atorvastatin quantification from pharmaceutical formulation with green chemistry perspectives.

According to the previous study (Kokilambigai, K. S., & Lakshmi, K. S. (2022) the retention time of Atorvastatin peak was eluted at 6.9 min and developed by HPLC. But for long time analysis this method is time consuming and expensive. In order to develop and validate a new sensitive HPLC-UV method 240 nm of wavelength; column length of 250 mm, 4.6 mm of inner diameter, 5 µm of particle size, C18 GL science of ODS-3V; mobile phase comprised of 0.5% v/v aqueous acetic acid: ethanol were used.

The previously reported chromatographic method and the newly developed method were comparatively evaluated during the method optimization process. The proposed method demonstrated improved chromatographic performance for atorvastatin analysis, with a statistically significant difference observed in the main analyte peak elution characteristics ($p < 0.0001$). Furthermore, the developed method utilizes ethanol as the primary organic solvent, providing a cost-effective and environmentally sustainable alternative that is consistent with the principles of green analytical chemistry.

The primary objective of this study was to develop and validate a specific, sensitive, and environmentally friendly RP-HPLC method for the quantitative determination of atorvastatin in pharmaceutical formulations. The validated method is suitable for routine pharmaceutical quality control and may provide potential applications in bioequivalence assessment and other analytical investigations involving atorvastatin quantification.

Materials

Acetic acid, absolute ethanol, and ultrapure-grade water were used as reagents in this study. All chemicals were of analytical reagent grade and were within their specified expiry periods at the time of use. Ultrapure water was used for the preparation of mobile phase and sample solutions.

Instrument

Chromatographic analysis was performed using a high-performance liquid chromatography (HPLC) system equipped with ultraviolet (UV) and photodiode array (PDA) detectors. An ODS-3V C18 column (GL Sciences, 250 mm × 4.6 mm internal diameter, 5 µm particle size) was used for chromatographic separation. Analytical and semi-micro balances were used for accurate weighing of standards and samples. The pH measurements were performed using a Mettler Toledo pH meter. Sample preparation and mobile phase degassing were carried out using an ultrasonic water bath (Hwashin Tech Co.).

Method development and optimization

Before method validation, chromatographic method development and optimization were performed by comparing the previously reported method (control method) with two newly developed chromatographic conditions (Method 1 and Method 2). The chromatographic parameters, including mobile phase composition, flow rate, detection wavelength, and column specifications, were evaluated to identify the most suitable analytical conditions for atorvastatin determination. **Table 1** summarizes the chromatographic conditions investigated for the control method, Method 1, and Method 2. Method 1 was used foe method validation.

Method Validation

Method validation is essential to confirm that the analytical procedure employed for a specific test is reliable and appropriate for its intended application, as it provides documented evidence that the method consistently yields accurate and precise results (P´erez-Grijalba et al., 2016). In order to confirm it specificity, accuracy, precision, linearity, range, limit of detection, limit of quantitation should be validated.

Linearity and Range

Linearity was assessed by analyzing a series of standard solutions of atorvastatin at different concentration levels. The response was plotted against the concentrations, and the data was subjected to regression analysis to calculate the slope, intercept, and correlation coefficient. The range of the method was determined by verifying that it provides acceptable precision, accuracy, and linearity when applied to samples containing atorvastatin concentrations within the established limits (Nawrocki et al., 1995). The correlation coefficient, R2 should be 0.999 for acceptable range (P´erez-Grijalba et al., 2016).

The Regression equation of linearity is y = mx +c; Where, y = detector response, m = slope, x = concentration of analyte and c = intercept

The range can be calculated based on this formula: Range = Highest concentration – Lowest concentration where they are producing till a linear graph. (Mittal et al., 2015)

In order to conduct this, 11 different concentrations of linearity solution were prepared from a stock solution. The stock was prepared by weighing accurately 2000 mg of Atorvastatin standard and dissolved in 10 ml of ethanol and sonicate for 5 minutes and diluted to 100 ml of mobile phase in 100 ml volumetric flask (stock solution). Subsequently, serial dilutions were prepared by taking (0.2, 1, 2 & 1.2, 1.6, 2, 3.2, 4, 4.8, 6, 8 ml) from the stock solution (2.0 mg/ml) and completed to individual 100 ml & individual 20 ml with mobile phase, then injected in single injection for each concentration 0.004, 0.02, 0.04, 0.12, 0.16, 0.2, 0.32, 0.4, 0.48, 0.6, 0.8 mg/ml into HPLC system.

Limit of detection and limit of quantitation

The LOD and LOQ are crucial parameters for assessing the sensitivity of the analytical method. Limit of detection is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. limit of quantitation is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy (P´erez-Grijalba et al., 2016). Sensitivity is calculated as blank signal plus two standard deviations from multiple assays (Mehta et al., 2020). The signal to noise ratio of limit of detection should be not less than 3:1 (S/N ≥ 3). and for limit of quantitation is not less than 10:1 (S/N ≥ 10). LOD and LOQ can be determined using various approaches, including visual evaluation, signal-to-noise ratio, and calibration curve method.

The limit of detection and limit of quantitation were determined based on the signal-to-noise ratio method, using the formulas: LOD = 3.3 × σ/S and LOQ = 10 × σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve. LOD and LOQ can be experimentally verified by analyzing a series of samples with known concentrations of the analyte near the estimated LOD and LOQ values (Tang et al., 2025). This study was performed after getting the slope and standard deviation from calibration curve of linearity and range study (Vial & Jardy, 1999).

From graph I, regression equation was, y = 301588x – 10693 where slope = 301588, intercept = -10693 and calibration curve was 115649.3 (calculated on MS Excel by using “STEYX” for calibration curve).

So, Estimated LOD should be = 3.3 x 115649.3/301588 = 1.27% = 1.27 x 0.4/100 = 0.00508 mg/ml = 5 ppm

Estimated LOQ should be = 10 x 115649.3/301588 = 3.83% = 3.83 x 0.4/100 = 0.01532 mg/ml = 15 ppm

Based on this information, 4 ppm, 5 ppm and 6 ppm for LOD and 8 ppm, 15 ppm and 20 ppm was injected into HPLC system for the confirmation of LOD and LOQ.

For superiority confirmation LOQ was conducted for control method, method 1 and method 2.

Accuracy

Accuracy is the closeness of agreement between a test result and the accepted reference value. It can be expressed as the percentage of recovery by the assay of known amounts of analyte added to the sample (P´erez-Grijalba et al., 2016). Accuracy can be assessed by performing recovery studies by spiking known amounts of atorvastatin standard to the placebo and calculating the percentage recovery. Calculation of accuracy

% Recovery = (Amount found after spiking – Amount before spiking / Amount added) * 100

The accuracy limit should be 95% to 105%.

To confirm accuracy study triplicate concentration three sample solution for each concentration total nine concentrations were prepared. Prepared concentrations were 0.32 mg/ml, 0.4 mg/ml and 0.48 mg/ml and injected into HPLC system.

Moreover, the comparability study was conducted for 100% sample for the control, and the new method (method 1 and method 2).

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as excipients, impurities, and degradation products. A method should be specific to measure the analyte response without interference of placebo. The specificity can be performed by injecting the blank, placebo and sample and check for the interference of the peaks. The peak purity of main peak should pass to demonstrate that there is no interference of peak. The purity angle should be less than purity threshold.

To confirm specificity blank, placebo, sample and standard solution injected into HPLC system.

Precision

Precision is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. Precision is usually expressed as the standard deviation or coefficient of variation of a series of measurements.

calculation formula for precision for instance, precision is often expressed as the coefficient of variation, with lower values indicating higher precision (P´erez-Grijalba et al., 2016). The difference between repeatability and intermediate precision for assay method should be not more than 2% (P´erez-Grijalba et al., 2016).

In order to do the repeatability parameter, 6 independent samples of Atorvastatin 0.4 mg/ml prepared and on the 2nd day another fresh 6 independent sample of Atorvastatin 0.4 mg/ml prepared on different HPLC.

Results

Method development and optimization

Both control and the new method was satisfactory results for good peak elution however method 1 was the best for rapid peak elution. To evaluate chromatographic efficiency, retention times (RT) were compared among the Control method, Method 1, and Method 2. The Control method yielded an average RT of approximately $7.0\,\mathrm{min}$. Method 1 significantly reduced the retention time to approximately $4.0\,\mathrm{min}$, representing a substantial increase in analysis throughput ($p < 0.0001$, indicated by ****). In contrast, Method 2 resulted in a statistically significant increase in

retention time to approximately $7.7\,\mathrm{min}$ compared to the Control method ($p < 0.01$, indicated by **). One-way ANOVA test confirmed that both test methods exhibited statistically significant shifts in retention time relative to the Control method (Figure II, A).

Linearity and Range

The linear regression equation was y = 301588x – 10693 over the concentration 1% to 200% considering 0.4 mg/ml as 100% concentration. The correlation coefficient value, R^2 was 1.000 (table III).

Figure I represent the linearity graph where the linearity regression equation is displayed was y = 301588x – 10693. Here, slope presents 301588; intercept presents – 10693. The graph represents the entire method character. It defined the method that the method will show linear result over the concentration of 0.004 mg/ml to 0.8 mg/ml.

Accuracy

The method showed a good degree of accuracy over the concentration of 80% to 120% and it showed accurate results with the predefined specification limit 95% to 105% (table IV). All the nine samples % recovery results were within the specification limit. The calculation of the results was done by using Microsoft excel software. The method shows accurate results within 80% to 120% of target concentration 0.4 mg/ml.

The measured % recovery at 0.4 mg/ml for for **Method 1** and **Method 2** and compared against the **Control method**. Both test methods demonstrated excellent accuracy, yielding approximately 100% recovery ($100.0 \pm \mathrm{SEM}$). One-way ANOVA revealed no statistically significant differences in percent recovery between the control method and either Method 1 or Method 2 ($p > 0.05$). These findings confirm that both test methods perform with high fidelity and equivalent accuracy to the control standard at this concentration (Figure II,C).

Limit of detection and limit of quantitation

The signal to noise ratio for 4 ppm, 5 ppm and 6 ppm was 1:1, 5:1 and 6:1 and for 8 ppm, 15 ppm and 20 ppm the S/N was 11:1, 14:1 and 23:1.

So, the limit of detection and limit of quantitation of Atorvastatin for this method was found 5 and 8 ppm. The LOD & LOQ value was observed based on signal to noise ratio.

LOQ of control method was 10 ppm. So the method sensitivity was evaluated at the limit of quantification concentration ($10\,\mathrm{ppm}$) by comparing peak area responses across all three analytical approaches (Figure II, B). Method 1 demonstrated a statistically significant increase in signal intensity (~ 3200 area units) compared to the Control method (~ 2300 area units, $*p < 0.05$), indicating enhanced analytical sensitivity at lower concentration thresholds. Conversely, Method 2 yielded a lower average peak area response (~ 1800 area units); however, this decrease was not statistically significant relative to the Control method ($\mathrm{ns},\ p > 0.05$). Overall, Method 1 provided superior sensitivity at $10\,\mathrm{ppm}$.

Specificity

No interreference observed for mobile phase and peak purity passed for standard and sample. Purity angle was 0.032 & purity threshold was 0.128 in standard and the purity angle was 0.141 & purity threshold was 0.679 in sample for Atorvastatin. HPLC with PDA detector was used for the purity test.

Precision

The table V shows repeatable and reproducible precision results with low % CV. Six different samples % CV as 1.83% for the methods repeatability test and on the second day different HPLC instrument were used for 6 individual fresh samples % CV was for 1.79% for the intermediate test. % difference of these two parameters mean result was 1.48% which ensured the method reproducibility

Discussion

Linearity and Range

The square of correlation coefficient of the current study was R2 = 1.000 which was greater than previous findings of R2 0.999 (Alruwaili N. K. (2021)), R2 0.993 (Li, W., 2023), R2 0.9971 (Silva, J., 2019), R2 0.990 (Li, W., 2022), R2 0.998 (Al-Hashimi, 2021), R2 0.9939 (Kasem, H., 2025), R2 0.998 (Londhe, S. V., 2011), R2 0.992 (Bhinge, S. D., 2012). The previous findings of R2 0.999 (Alruwaili, N. K. (2021)), R2 0.999 (Alhazmi, H. A., 2018), R2 0.9995 (Kokilambigai, K. S., & Lakshmi, K. S. (2022)), R2 0.9993 (Shulyak, N., 2021), R2 0.9999 (Mostafa, E. A., 2023), R2 0.9994 (Spiridon, A. M., 2015), R2 0.9999 (Sithole, M. N., 2021), R2 1 (Maree, S., 2020), R2 0.999 (Gite, S., & Patravale, V. (2015)), R2 0.999 (Ibesh, S., 2023), R2 0.9998 (Maged, K., 2023), R2 0.999 (Alruwaili N. K. (2021)) were identical to the present findings of R2 1.000.

Moreover, the current findings show the range linear graph (Figure I) between 0.004 to 0.8 mg/ml which was better than 0.04 mg/ml to 1.25 mg/ml (Spiridon, A. M., 2015). The previous finding shows higher sensitivity method with 1.00 to 250 ng/mL (Le, J., 2021). The higher sensitivity of the previous findings might be due to the use of LC-MS/MS method. However, the current findings are comparable with the previous findings of same condition.

Accuracy

The current findings of this method show good recovery results and the minimum to maximum recovery of the nine samples are 96.88% to 103.13% (table IV) and the average of them was 100.39%. This result was better than the previous findings observed was within 96.3 to 102.8% (Abady, M. M., 2023), average recovery 92.8% (Talele, G. S., 2015), 91.4 to 106.7% (Kasem, H., 2025), 92.7-108.5% (Turner, 2018), 95.0 to 104.5 (Turner, R. M., 2018), 90 to 99% (Croitoru, O., 2015), 91.19 ± 1.75 (Porwal, P. K., & Talele, G. S. (2017)). The previous findings were within limit of 90% to 109%. The high variation of results might be due to the lower sensitivity of the method and lower concentration of the sample.

The current findings 96.88% to 103.13% and average recovery was 100.39% which was identical of previous findings 98.66 to 100.26 (Alruwaili N. K. (2021)), 98.175 to 101.06% (Ibesh, S., 2023), 98.93 to 101.16% (Sangshetti, J. N., 2016), 99.33 to 99.83% (Sharma, R., 2012), average recovery 101.76% (Jain, N., 2008). All of the findings were within 95% to 105% and all of the results are comparable.

Limit of detection and limit of quantitation

The current findings of the LOD and LOQ was 5 ppm and 10 ppm. The previous study shows 0.254 *µ*g/ml for LOD and 0.838 *µ*g/ml for LOQ (Alruwaili N. K. (2021)), 0.48 µg/ml for LOD and 1.43 µg/ml for LOQ (Maged, K., 2023), 0.13 for LOD and 0.44 µg/mL for LOQ (Abdallah, N. A. 2023), 0.030 µg/ml for LOD 0.093 µg/ml for LOQ (Attaa Bakr, N., 2020), 0.013 for LOD and 0.035 µg/ml for LOQ (Maree, S., 2020), 0.05µg/ ml for LOD and 0.17 µg/ml for LOQ (Spiridon, A. M., 2015), 0.010 for LOD and 0.032 µg/mL for LOQ (Mostafa, E. A., 2023), 0.189 for LOD and 0.603 µg/mL for LOQ (Alhazmi, H. A., 2018). The sensitivity of the previous findings were high than the current findings. This low sensitivity of the current findings might be due to the instrumental condition, environment temperature and humidity of that time, mixing of mobile phase, mixing of diluent problematic occurrence.

which has the higher sensitivity than the previous studies of 0.0005 mg/mL LOD (Al-Rimawi, F., & Kharoaf, M. (2010)), 3.0 g/ml LOQ (Yang, Z. Y., Wang, L., & Tang, X. (2009)), 28.7 µg/ml and 86.9 µg/ml (Al-Hakkani, M. F. (2019)), 0.0005 and 0.0008 mg/ml (Al-Rimaw)i, F., & Kharoaf, M. (2010)).

Precision

The % RSD of the previous findings of repeatability study was between 1.70 to 2.79 and for intermediate precision study 1.25 to 2.71 (Alruwaili N. K. (2021)), RSD < 6% (Abady, M. M., 2023), RSD < 7.7% (Kasem, H., 2025). On the contrary the % CV of the current study for repeatability test was 1.83% and for intermediate precision 1.79%. The absolute difference between the two precision was 1.48% which was lower than 2% (table V). The previous findings variation was more than 5% (Abady, M. M., 2023) (Kasem, H., 2025) and this might be use of very low concentration as sample solution. Low concentration sample might trigger the fluctuation of analytical results.

Data Analysis: All the data analysis were done as means ± SD using GraphPad prism V:11

Conclusion

The comparative evaluation of the control method and the two newly developed chromatographic methods demonstrated that all three methods produced acceptable chromatographic performance with satisfactory peak shape and analyte elution. Method 1 exhibited the most favorable chromatographic characteristics, providing a significantly shorter retention time than the control method ($p < 0.0001$), thereby substantially improving analytical throughput without compromising chromatographic performance. In contrast, Method 2 produced a significantly longer retention time ($p < 0.01$), making it less suitable for rapid routine analysis.

Method 1 also demonstrated superior analytical sensitivity, achieving lower LOD and LOQ values (5 ppm and 8 ppm, respectively) compared with the control method (LOQ = 10 ppm). At the LOQ concentration, Method 1 generated significantly higher peak responses than the control method ($p < 0.05$), confirming improved detector response at low analyte concentrations. Furthermore, accuracy studies showed no significant differences among the three methods ($p > 0.05$), with all methods achieving recoveries close to 100%, indicating equivalent analytical accuracy.

Overall, Method 1 was selected as the optimized chromatographic method because it combined rapid analysis, enhanced sensitivity, and excellent accuracy while maintaining the principles of green analytical chemistry. These findings support its suitability for subsequent method validation and routine quantitative determination of atorvastatin in pharmaceutical formulations.

Declarations

Figure II. Comparative evaluation of chromatographic performance, sensitivity, and accuracy of the developed methods.

(A) Comparison of retention time (RT) values among the control method, Method 1, and Method 2 during chromatographic method optimization; (B) Evaluation of limit of quantification (LOQ) sensitivity based on peak area response at 0.8 ppm concentration of atorvastatin; (C) Comparative accuracy assessment of the control method, Method 1, and Method 2 at 0.4 mg/mL atorvastatin concentration. Statistical analysis was performed using one-way analysis of variance (ANOVA). Data are presented as mean ± SEM. Statistical significance is indicated as follows: ****$p < 0.0001$, **$p < 0.01$, *$p < 0.05$, and ns, not significant ($p > 0.05$), ($n = 3$).

References

1. Lee JH, Kim S, Choi D, Tahk S, Yoon J, Choi SW, Hong T, Kim H-S (2017) Efficacy and tolerability of two different formulations of atorvastatin in Korean patients with hypercholesterolemia: a multicenter, prospective, randomized clinical trial. Drug Des Dev Therapy 2277. https://doi.org/10.2147/dddt.s112241
2. Li D, Martini N, Wu Z, Wen J (2012) Development of an isocratic HPLC method for catechin quantification and its application to formulation studies. Fitoterapia 83(7):1267. https://doi.org/10.1016/j.fitote.2012.06.006
3. Lokhande P (2019) Analytical Method Development and Validation of Teneligliptin by using RP-HPLC with ICH Guidelines. Int J Trend Sci Res Dev 259. https://doi.org/10.31142/ijtsrd21735
4. Lakka NS, Kuppan C (2019) Principles of Chromatography Method Development. In IntechOpen eBooks. IntechOpen. https://doi.org/10.5772/intechopen.89501
5. Szücs R, Brunelli C, Lestremau F, Hanna-Brown M (2017) Liquid chromatography in the pharmaceutical industry. In Elsevier eBooks. Elsevier BV 515. https://doi.org/10.1016/b978-0-12-805392-8.00017-7
6. ´erez-Grijalba P, Fandos V, Canudas N,´us J, Insua D, Casabona D, Lacosta AM, Monta ̃n´es,´ıa M, Pesini P, Sarasa M (2016) Validation of Immunoassay-Based Tools for the Comprehensive Quantification of A40 and A42 Peptides in Plasma
7. Lins R, Matthys KE, Verpooten GA, Peeters PC, Dratwa M, Stolear J-C, Lameire N (2003) Pharmacokinetics of atorvastatin and its metabolites after single and multiple dosing in hypercholesterolaemic haemodialysis patients. Nephrol Dialysis Transplantation 18(5):967. https://doi.org/10.1093/ndt/gfg048
8. Arca M, Pigna (2011) Treating statin-intolerant patients. Diabetes Metabolic Syndrome Obes 155. https://doi.org/10.2147/dmso.s11244
9. ´erez-Grijalba P, Fandos V, Canudas N,´us J, Insua D, Casabona D, Lacosta AM, Monta ̃n´es,´ıa M, Pesini P, Sarasa M (2016) Validation of Immunoassay-Based Tools for the Comprehensive Quantification of A40 and A42 Peptides in Plasma
10. Nawrocki JW, Weiss S, Davidson MH, Sprecher DL, Schwartz S, Lupien P-J, Jones PH, Haber H, Black DM (1995) Reduction of LDL Cholesterol by 25% to 60% in Patients With Primary Hypercholesterolemia by Atorvastatin, a New HMG-CoA Reductase Inhibitor. Arterioscler Thromb Vascular Biology 15(5):678. https://doi.org/10.1161/01.atv.15.5.678
11. Mittal A, Parmar S, Imam SS, Gilani SJ, Aqil M, FOR SIMULTANEOUS ESTIMATION OF FINASTERIDE AND TAMSULOSIN IN TABLET FORMULATIONS (2015) INDONESIAN J Pharm 26(1):45. https://doi.org/10.14499/indonesianjpharm26iss1pp45. RP-HPLC METHOD
12. Mehta PD, Patrick BA, Miller DL, Coyle PK, Wisniewski T (2020) A Sensitive and Cost-Effective Chemiluminescence ELISA for Measurement of Amyloid- 1–42 Peptide in Human Plasma.
13. Tang J, Li Y, Yao Y, Hu R (2025) Colorimetric/electrochemical dual-mode aptasensor with phosphatase-like ceria nanozyme for fast and ultrasensitive detection of Ochratoxin A. Sens Actuators B Chem 137482. https://doi.org/10.1016/j.snb.2025.137482
14. Vial J, Jardy A (1999) Experimental Comparison of the Different Approaches To Estimate LOD and LOQ of an HPLC Method. Anal Chem 71(14):2672. https://doi.org/10.1021/ac981179n
15. Darwısh IA, Khalil NY, Darwish HW, Alzoman NZ, Al-Hossaini AM (2021) Spectrophotometric and computational investigations of charge transfer complexes of chloranilic acid with tyrosine kinase inhibitors and application to development of novel universal 96-microwell assay for their determination in pharmaceutical formulations. Spectrochimica Acta Part Mol Biomol Spectrosc 252:119482. https://doi.org/10.1016/j.saa.2021.119482
16. Alruwaili NK (2021) Analytical Quality by Design Approach of Reverse-Phase High-Performance Liquid Chromatography of Atorvastatin: Method Development, Optimization, Validation, and the Stability-Indicated Method. *International journal of analytical chemistry*, *2021*, 8833900. https://doi.org/10.1155/2021/8833900
17. Li W, Xu X, Wang S, Li Y, Zhang Y, Zhang T (2023) Development of a UPLC-MS/MS method for the simultaneous determination of atorvastatin, 2-hydroxy atorvastatin, and naringenin in rat plasma and its application to pharmacokinetic interaction studies. Biomedical chromatography: BMC 37(1):e5515. https://doi.org/10.1002/bmc.5515
18. Maged K, El-Henawee MM, Abd El-Hay SS (2023) Development and validation of an eco-friendly HPLC-UV method for determination of atorvastatin and vitamin D_3 in pure form and pharmaceutical formulation. BMC Chem 17(1):62. https://doi.org/10.1186/s13065-023-00975-6
19. Chowdhury MNS (2024) Comparative In vitro Assessment of Quality Control Parameters of Omeprazole Enteric-Coated Pellets in Capsules from Local Pharmacies in Bangladesh
20. Abady MM, Jeong JS, Kwon HJ (2023) Development and validation of an analytical method using liquid chromatography-tandem mass spectrometry for the therapeutic drug monitoring of seven cardiovascular drugs in clinical usage. J Chromatogr B Analyt Technol Biomed Life Sci 1214:123552. https://doi.org/10.1016/j.jchromb.2022.123552
21. Talele GS, Porwal PK (2015) Development of Validated Bioanalytical HPLC-UV Method for Simultaneous Estimation of Amlodipine and Atorvastatin in Rat Plasma. Indian J Pharm Sci 77(6):742–750. https://doi.org/10.4103/0250-474x.174969
22. Silva J, Basso J, Sousa J, Fortuna A, Vitorino C (2019) Development and full validation of an HPLC methodology to quantify atorvastatin and curcumin after their intranasal co-delivery to mice. Biomedical chromatography: BMC 33(10):e4621. https://doi.org/10.1002/bmc.4621

23. Chowdhury NS (2023) A Review on Specific and Sensitive Analytical Method Development and Validation. Int J Pharm Chem Anal 10:28–30
24. Li W, Wang S, Wang W, Gong L, Ni D, Li Y, Wu W, Zhang Y, Xu X, Jiang Q, Zhang J, Zhang T (2022) Ultra-high performance supercritical fluid chromatography-tandem mass spectrometry method for simultaneous determination of atorvastatin, 2-hydroxy atorvastatin, and tangeretin in rat plasma and its application to the pharmacokinetic study. J Sep Sci 45(21):3985–3994. https://doi.org/10.1002/jssc.202200185
25. Ibesh S, Bitar Y, Trefi S (2023) A New method for simultaneous qualitative and quantitative determination of amlodipine besylate and atorvastatin calcium in bulk and pharmaceutical formulations using transmission FT-IR spectroscopy. Heliyon 9(3):e14189. https://doi.org/10.1016/j.heliyon.2023.e14189
26. Al-Hashimi NN, Shtaiwi MH, Hamed SH, El-Sheikh AH, Shahin RO, Banisakher LS, Tahboub DM (2021) Development of Dioctyl Phthalate@Fe_3O_4 Nanocomposite Reinforced Hollow Fiber Solid/ Liquid Phase Microextraction Followed by HPLC-DAD for the Determination of Atorvastatin and Gemfibrozil in Human Urine. Pharm Nanatechnol 9(5):372–382. https://doi.org/10.2174/2211738510666211222113358
27. Kasem H, Ntorkou M, Tzanavaras PD, Zacharis CK (2025) Application of AQbD principles on the development of a stability-indicating HPLC method for the determination of acetylsalicylic acid, ramipril and atorvastatin in their fixed-dose polypills. Anal methods: Adv methods Appl 17(17):3459–3470. https://doi.org/10.1039/d5ay00021a
28. Turner RM, Fontana V, Bayliss M, Whalley S, Santoyo Castelazo A, Pirmohamed M (2018) Development, validation and application of a novel HPLC-MS/MS method for the quantification of atorvastatin, bisoprolol and clopidogrel in a large cardiovascular patient cohort. J Pharm Biomed Anal 159:272–281. https://doi.org/10.1016/j.jpba.2018.06.062
29. Le J, Liao Y, Li S, Chen X, Hong Z (2021) High-throughput LC-MS/MS Method for Simultaneous Determination of Pantoprazole and Atorvastatin in Rat Plasma: Application to a Pharmacokinetic Interaction Study. Curr Drug Metab 22(6):481–490. https://doi.org/10.2174/1389200222666210520090632
30. Gite S, Patravale V (2015) Validation of RP-HPLC method and stress degradation for the combination of metformin HCl, atorvastatin calcium and glimepiride: application to nanoparticles. J Chromatogr Sci 53(10):1654–1662. https://doi.org/10.1093/chromsci/bmv068
31. Abdallah NA, El-Brashy AM, Ibrahim FA, El-Awady MI (2023) Micellar eco-friendly HPLC method for simultaneous analysis of ternary combination of aspirin, atorvastatin and ramipril: application to content uniformity testing. BMC Chem 17(1):19. https://doi.org/10.1186/s13065-023-00929-y
32. Rezk MR, Badr KA (2018) Quantification of amlodipine and atorvastatin in human plasma by UPLC-MS/MS method and its application to a bioequivalence study. Biomedical chromatography: BMC 32(7):e4224. https://doi.org/10.1002/bmc.4224
33. Attaa Bakr N, Saad S, Elshabrawy Y, Eid M (2020) First-derivative synchronous spectrofluorimetric method for estimation of losartan potassium and atorvastatin in their pure forms and in tablets. Luminescence: J Biol Chem luminescence 35(4):561–571. https://doi.org/10.1002/bio.3755
34. Attia KAM, Abdel-Monem AH, Abdel-Raoof AM, Eissa AS (2025) Development and validation of a highly sensitive HPLC method for quantifying cardiovascular drugs in human plasma using dual detection. Sci Rep 15(1):12229. https://doi.org/10.1038/s41598-025-94907-0
35. Maree S, du Preez JL, Plessis du, Plessis LH, J., Gerber M (2020) A novel HPLC method developed and validated for the detection and quantification of atorvastatin, fluvastatin, pitavastatin and pravastatin during transdermal delivery studies. Pharmazie 75(5):164–166. https://doi.org/10.1691/ph.2020.0007
36. Sithole MN, Oosthuysen E, Jaarsveld JV, Preez JLD, Plessis JD, Gerber M (2021) Development and validation of an HPLC method to be used for simultaneous detection and quantification of different statins after *ex vivo* skin diffusion studies. Pharmazie 76(12):583–587. https://doi.org/10.1691/ph.2021.1164
37. Jain N, Raghuwanshi R, Jain D (2008) Development and Validation of RP-HPLC Method for Simultaneous Estimation of Atorvastatin Calcium and Fenofibrate in Tablet Dosage Forms. Indian J Pharm Sci 70(2):263–265. https://doi.org/10.4103/0250-474X.41473
38. Londhe SV, Deshmukh RS, Mulgund SV, Jain KS (2011) Development and Validation of a Reversed-phase HPLC Method for Simultaneous Determination of Aspirin, Atorvastatin Calcium and Clopidogrel Bisulphate in Capsules. Indian J Pharm Sci 73(1):23–29. https://doi.org/10.4103/0250-474X.89753
39. Spiridon AM, Neamtu J, Belu I, Turcu-Stiolica TS, Croitoru O (2015) Simultaneous Analysis of Clopidogrel Bisulfate, Acetylsalicylic Acid and Atorvastatin calcium in Tablets by HPLC Method. Curr health Sci J 41(2):172–178. https://doi.org/10.12865/CHSJ.41.02.13
40. Mostafa EA, El-Ashrey MK, Mahmoud ST (2023) An innovative combination of Box-Behnken design and ecofriendly approaches for the simultaneous determination of aspirin, clopidogrel, atorvastatin and rosuvastatin in their fixed-dose combination tablets. BMC Chem 17(1):164. https://doi.org/10.1186/s13065-023-01079-x
41. Shulyak, N., Piponski, M., Kovalenko, S., Bakovska Stoimenova, T., Balkanov, T., El-Subbagh,H. I., … Logoyda, L. (2021). Development of a novel, fast, simple HPLC method for determination of Atorvastatin and its impurities in tablets. Scientia Pharmaceutica, 89(2), 16.
42. Kokilambigai KS, Lakshmi KS (2022) Analytical quality by design assisted RP-HPLC method for quantifying atorvastatin with green analytical chemistry perspective. J Chromatogr Open 2:100052
43. Alhazmi, H. A., Alnami, A. M., Arishi, M. A., Alameer, R. K., Al Bratty, M., Rehman,Z. U., … Arbab, I. A. (2018). A fast and validated reversed-phase HPLC method for simultaneous determination of simvastatin, atorvastatin, telmisartan and irbesartan in bulk drugs and tablet formulations. Scientia Pharmaceutica, 86(1), 1.

44. Sharma R, Khanna S, Mishra GP (2012) Development and Validation of RP-HPLC Method for Simultaneous Estimation of Ramipril, Aspirin and Atorvastatin in Pharmaceutical Preparations. J Chem 9(4):2177–2184
45. Bhatia NM, Gurav SB, Jadhav SD, Bhatia MS (2012) RP-HPLC method for simultaneous estimation of atorvastatin calcium, losartan potassium, atenolol, and aspirin from tablet dosage form and plasma. J Liq Chromatogr Relat Technol 35(3):428–443
46. Chowdhury NS, Dalal SN, Islam A, Hossain MA, Das PK, Hossain S, Das P (2024) Robustness Study and Superior Method Development and Validation for Analytical Assay Method of Atropine Sulfate in Pharmaceutical Ophthalmic Solution. Am J Anal Chem, *10*
47. Alruwaili NK (2021) Analytical Quality by Design Approach of Reverse-Phase High-Performance Liquid Chromatography of Atorvastatin: Method Development, Optimization, Validation, and the Stability-Indicated Method. Int J Anal Chem 2021(1):8833900
48. Bhinge SD, Malipatil SM, Jondhale A, Hirave R, Savali AS (2012) A new approach to the RP-HPLC method for simultaneous estimation of atorvastatin calcium and fenofibrate in pharmaceutical dosage forms. J Chem 9(3):1223–1229
49. Sangshetti JN, Aqeel M, Zaheer Z, Ahmed RZ, Dehghan MHG, Gonjari I (2016) Development and validation of RP-HPLC method for determination of Atorvastatin calcium and Nicotinic acid in combined tablet dosage form. J Saudi Chem Soc 20:S328–S333
50. Chowdhury MNS (2023) A stability indicating RP-HPLC method development and validation for simultaneous quantification for assay of rifampicin in pharmaceutical solid dosage form. *International Journal of Pharmaceutical Chemistry and Analysis*
51. Turner RM, Fontana V, Bayliss M, Whalley S, Castelazo AS, Pirmohamed M (2018) Development, validation and application of a novel HPLC-MS/MS method for the quantification of atorvastatin, bisoprolol and clopidogrel in a large cardiovascular patient cohort. J Pharm Biomed Anal 159:272–281
52. Croitoru O, Spiridon AM, Belu I, Turcu-Ştiolică A, Neamţu J (2015) Development and validation of an HPLC method for simultaneous quantification of clopidogrel bisulfate, its carboxylic acid metabolite, and atorvastatin in human plasma: application to a pharmacokinetic study. J Anal Methods Chem 2015(1):892470
53. Porwal PK, Talele GS (2017) Development of validated HPLC-UV method for simultaneous determination of metformin, amlodipine, glibenclamide and atorvastatin in human plasma and application to protein binding studies. Bull Fac Pharm Cairo Univ 55(1):129–139

Tables

Table I: Control, method 1 and method 2 chromatographic HPLC method

	Control method	Method 1	Method 2
	0.5% v/v aqueous acetic acid: ethanol (42.5: 57.5 v/v)	0.5% v/v aqueous acetic acid: ethanol (20: 80 v/v)	0.5% v/v aqueous acetic acid: ethanol (50: 50 v/v)
Flow (ml/min)	1	1	1
Wavelength	240	240	240
Column	ODS, 3V; C18, 250 x 4.6 mm; 5µm	ODS, 3V; C18, 250 x 4.6 mm; 5µm	ODS, 3V; C18, 250 x 4.6 mm; 5µm

Table II: Outcome of Control, method 1 and method 2 chromatographic HPLC method

	Control method	Method 1	Method 2
USP Tailing factor	1.2, 1.3, 1.1	1.4, 1.5, 1.4	1.2, 1.1, 1.0
USP Theoretical plate	5580, 5570, 5420	4580, 4614, 4815	6479, 6523, 6524
Retention time min	6.9, 6.8, 7.0	4.0, 3.9, 4.1	7.7, 7.6, 7.9

Table III: Linearity table

Concentration, mg/ml	% Concentration	Area	Correlation coefficient, R	Correlation coefficient, R2
0.004	1	290512	0.99998	1.000
0.02	5	1502345		
0.04	10	3002145		
0.12	30	9005432		
0.16	40	12034554		
0.2	50	15057843		
0.32	80	24234567		
0.4	100	30265092		
0.48	120	35902345		
0.6	150	45345567		
0.8	200	60290345		

Table IV: Accuracy

% of Accuracy level	Sample no	Actual concentration, mg/ml	Measured concentration mg/ml	% Recovery
80	1	0.32	0.318	99.38
	2	0.32	0.330	103.13
	3	0.32	0.310	96.88
100	1	0.4	0.390	97.50
	2	0.4	0.410	102.50
	3	0.4	0.405	101.25
120	1	0.48	0.485	101.04
	2	0.48	0.479	99.79
	3	0.48	0.490	102.08

Table V: Precision table

1^{st} day (Repeatability)				2^{nd} day (Intermediate precision)		
Sample no	Standard concentration	Measured sample concentration	% Assay	Standard concentration	Measured sample concentration	% Assay
1	0.4	0.3824	95.6	1	0.369	92.25
2	0.4	0.39	97.5	2	0.374	93.5
3	0.4	0.377	94.25	3	0.378	94.5
4	0.4	0.37	92.5	4	0.381	95.25
5	0.4	0.385	96.25	5	0.384	96.0
6	0.4	0.384	96.0	6	0.367	91.75
		Mean	95.35		Mean	93.88
		standard deviation	1.75		standard deviation	1.68
		% CV	1.83		% CV	1.79

Table VI: LOQ Sensitivity

Trial	Concentration	Control method	Method 1 Area response	Method 2 Area response
1	10 ppm	2112	3250	1820
2		2520	3140	1560
3		2310	3213	1945

Note: LOQ of control method was 10 ppm and method 1 was 8 ppm, method 2 was 12 ppm

Table VII: 100% Accuracy study

Trial	Concentration	Control method	Method 1	Method 2
1	0.4 mg/ml	97.50	98.8	99.9
2		102.50	100.5	101.5
3		101.25	101.9	101.2

Figures

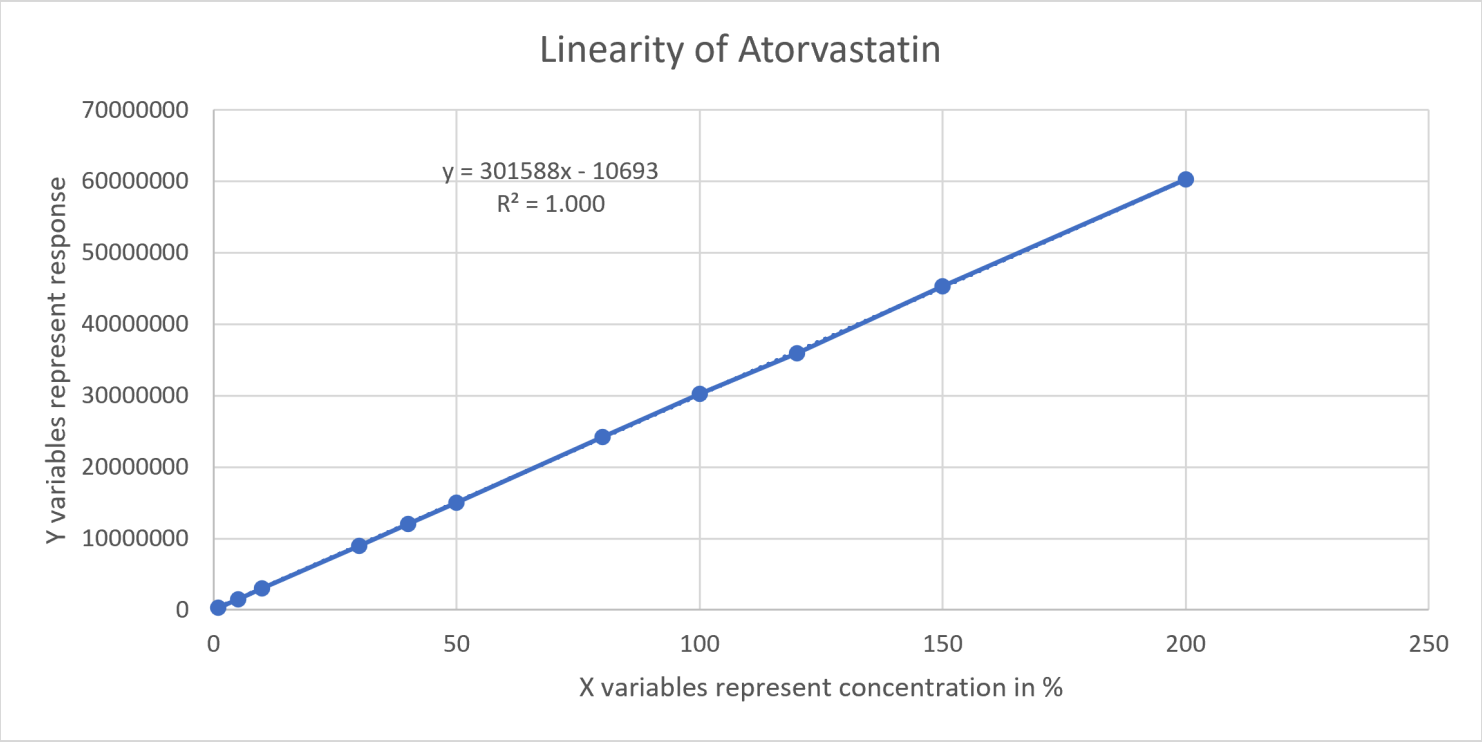


Figure 1

Linearity curve of Atorvastatin

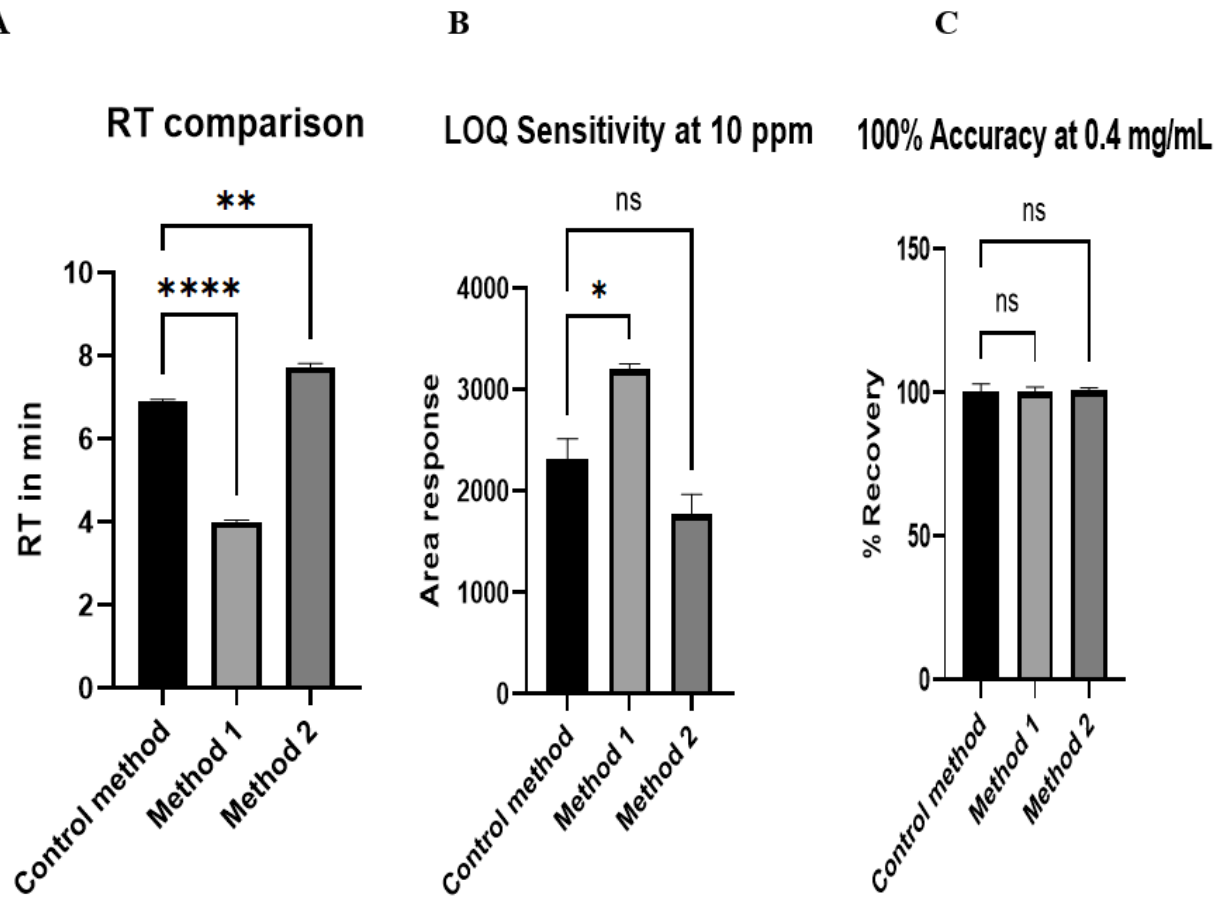


Figure 2

Comparative evaluation of chromatographic performance, sensitivity, and accuracy of the developed methods.
(A) Comparison of retention time (RT) values among the control method, Method 1, and Method 2 during chromatographic method optimization; (B) Evaluation of limit of quantification (LOQ) sensitivity based on peak area response at 0.8 ppm concentration of atorvastatin; (C) Comparative accuracy assessment of the control method, Method 1, and Method 2 at 0.4 mg/mL atorvastatin concentration. Statistical analysis was performed using one-way analysis of variance (ANOVA). Data are presented as mean ± SEM. Statistical significance is indicated as follows: ****$p < 0.0001$, **$p < 0.01$, *$p < 0.05$, and ns, not significant ($p > 0.05$), ($n = 3$).