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**Research Article**

# Validated RP-HPLC Method for Estimation of Atorvastatin and Ezetimibe in Tablets

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## Abstract

A simple, sensitive and reproducible RP-HPLC method was developed and validated for the simultaneous estimation of Atorvastatin and Ezetimibe in combined tablet dosage form. Chromatographic separation was achieved on a Purospher C18 column (250 × 4.6 mm, 5 µm) using a mobile phase consisting of ammonium acetate buffer and acetonitrile in the ratio of 60:40 (v/v), delivered at a flow rate of 1.5 mL/min in isocratic mode. Detection was carried out at 248 nm with an injection volume of 20 µL, and the retention times of Atorvastatin and Ezetimibe were found to be approximately 16.0 and 10.0 minutes, respectively. The method was validated as per ICH guidelines. Linearity was observed in the range of 80–120 µg/mL for Atorvastatin and 40–60 µg/mL for Ezetimibe, with correlation coefficients (r) of 0.9999 for both drugs. System precision and method precision studies showed low %RSD values, indicating excellent repeatability. Accuracy, evaluated by recovery studies at 80%, 100% and 120% levels, was within acceptable limits for both drugs. The method demonstrated good specificity, with no interference from tablet excipients, and satisfactory system suitability parameters. The limits of detection and quantification were 2.150 and 6.51 µg/mL for Atorvastatin, and 0.0945 and 0.286 µg/mL for Ezetimibe, respectively, confirming the sensitivity of the method. The proposed RP-HPLC method is thus suitable for routine quality control analysis of Atorvastatin and Ezetimibe in combined tablet formulations.

**Keywords:** Method validation, Fixed-dose combination, Linearity, Accuracy, Precision, Specificity, Limit of detection, Limit of quantification, Tablet dosage form.

## INTRODUCTION

High-performance liquid chromatography (HPLC) has established itself as a cornerstone technique in pharmaceutical analysis due to its superior resolution, sensitivity, and reproducibility. Among its various modes, Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) is the most widely employed. This predominance is attributed to its robustness and versatility in separating a vast range of analytes, from non-polar to moderately polar compounds. The mechanism of separation in RP-HPLC is based on a non-polar stationary phase and a polar mobile phase, making it exceptionally suitable for the analysis of drug molecules, which are often hydrophobic in nature. The development and subsequent validation of an RP-HPLC method are critical to ensure that the analytical procedure is accurate, precise, specific, and robust, thereby fulfilling its intended purpose of reliable quantification in quality control and research settings.

The pharmaceutical landscape is increasingly witnessing the development and use of fixed-dose combination (FDC) products, which offer enhanced therapeutic efficacy, improved patient compliance, and synergistic effects compared to monotherapy. However, the analysis of such combinations presents a significant analytical challenge, as it requires the simultaneous quantification of multiple active pharmaceutical ingredients (APIs) that may possess diverse chemical properties. RP-HPLC emerges as an ideal technique for this purpose, as it can be meticulously optimized to achieve baseline separation of the components within a single run. This simultaneous analysis not only saves time and resources but also ensures that the excipients present in the formulation do not interfere, providing a specific and accurate assessment of each drug's content in the combined dosage form[1].

One such prominent combination is that of Atorvastatin and Ezetimibe, used extensively in the management of hypercholesterolemia. Atorvastatin[2] is a statin that acts by inhibiting HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis in the liver. Ezetimibe[3], on the other hand, works by selectively inhibiting the absorption of dietary and biliary cholesterol at the brush border of the small intestine. Their complementary mechanisms provide a potent dual inhibition of cholesterol synthesis and absorption. To ensure the safety, efficacy, and quality of this widely prescribed FDC in tablet form, it is imperative to have a reliable analytical method for its simultaneous estimation. While individual methods for these drugs exist, a dedicated, validated RP-HPLC method for their concurrent analysis is necessary to monitor the uniformity of content, stability, and dissolution profile of the formulated product, thereby guaranteeing that patients receive the correct therapeutic dose.

## MATERIALS AND METHODS

Ezetimibe, Atorvastatin Calcium are gift samples from Emcure Pvt Ltd. HPLC-grade ammonium acetate, acetonitrile, and distilled water were used, while glacial acetic acid was of AR grade.

### Analytical method development[4]

#### *Preparation of Mixed Standard Solution*

An accurately weighed quantity of 50 mg of ezetimibe and an amount equivalent to 100 mg of atorvastatin were transferred to a 100 mL volumetric flask, dissolved in a suitable volume of diluent, and diluted to volume with the same. The solution was filtered, and 10 mL of the filtrate was further diluted to 100 mL with diluent to obtain a mixed standard solution containing 500 µg/mL of ezetimibe and 1000 µg/mL of atorvastatin.

#### *Preparation of Mixed Sample Solution*

Twenty tablets were weighed, finely powdered, and a portion of the powder equivalent to 50 mg of ezetimibe was transferred to a 100 mL volumetric flask. The sample was dissolved by sonication with sufficient diluent and diluted to volume with the same, then filtered. A 10 mL aliquot of the filtrate was diluted to 100 mL with diluent to obtain a mixed sample solution containing 50 µg/mL of ezetimibe and 100 µg/mL of atorvastatin. A 20 µL volume of standard and sample solutions was injected into the HPLC system under the specified chromatographic conditions, and chromatograms were recorded. Each solution was injected six times at 10-minute intervals to ensure complete elution of the preceding injection.

The chromatographic analysis was carried out using a Water Alliance 2695 separation module (HPLC) equipped with a Purospher column having dimensions of 250 mm × 4.6 mm and a particle size of 5 µm. The detection wavelength was set at 248 nm, and the study was performed at ambient temperature. A flow rate of 1.5 mL/min was maintained throughout the run, with an injection volume of 20 µL. The mobile phase consisted of a mixture of buffer and acetonitrile in the ratio of 60:40 (v/v), and the chromatographic separation was achieved under isocratic mode of operation. The total run time for each analysis was 25 minutes.

**Analytical method validation**[5]

The analytical method validation of Atorvastatin calcium and Ezetimibe involved several preparatory steps. A buffer was prepared by accurately weighing 1.54 g of ammonium acetate, dissolving it in 1000 ml of HPLC-grade water, and adjusting the pH to 4.0 ± 0.5 with glacial acetic acid. The mobile phase was prepared by mixing this buffer and acetonitrile in a 60:40 ratio, followed by filtration through a 0.45 µm cellulose nitrate filter and degassing; the same procedure and ratio (60:40 of water and acetonitrile) were used for the preparation of the diluent. For the mixed standard solution, 50 mg of Ezetimibe and an amount equivalent to 100 mg of Atorvastatin were accurately weighed into a 100 ml volumetric flask, dissolved with sufficient diluent, and made up to volume, then filtered; 10 ml of this filtrate was further diluted to 100 ml with diluent to obtain final concentrations of 500 µg/ml of Ezetimibe and 1000 µg/ml of Atorvastatin. For the mixed sample solution, twenty tablets were weighed, powdered, and a quantity equivalent to 50 mg of Ezetimibe was transferred to a 100 ml standard flask, dissolved by sonication using diluent, made up to volume, and filtered; 10 ml of this filtrate was diluted to 100 ml with diluent to achieve 50 µg/ml of Ezetimibe and 100 µg/ml of Atorvastatin. Standard and sample solutions (20 µl each) were injected under the specified chromatographic conditions, with each solution injected six times at 10-minute intervals to ensure complete elution. The amounts of Ezetimibe and Atorvastatin present in each tablet formulation were calculated by comparing the peak areas with those of the standard.

**Precision**[6]

Weight accurately about 100mg of Atorvastatin and 50mg of Ezetimibe in-house reference standard and transferred it into a 100ml volumetric flaks. Added 10ml of diluent and sonicated to dissolve. Made up to the mark with diluent. 10ml of this solution was diluted to 100ml with diluent.

**Specificity**

Weighed accurately 230mg of placebo and transferred it into a 100ml volumetric flask, dissolved with sufficient diluent and made up to the volume with the same. Filtered the above solution by using Millipore filter paper. 20µl of this solution was injected and the chromatogram was recorded. 10ml of mixed standard stock solution was taken and transferred to 100ml a volumetric flask and added 230mg of placebo and made up to the mark. Filtered the above solution using Millipore filter paper. 20µl of this solution was injected and the chromatogram was recorded.

**Linearity and Range**[7]

To get a concentration of 80%, 90%, 100%, 110% & 120% of drug pipetted out. 8ml, 9ml, 10ml, 11ml and 12ml mixed standard stock solution into separate 100ml volumetric flasks labeled as linearity 80%, linearity 90%, linearity 100%, linearity 110% and linearity 120%,. The volume was made up with diluent. From these different solution 20µl was injected individually and the chromatograms were recorded. There exists a liner relationship in the two graphs for the two concentration ranges, which are prepared.

**Accuracy**

Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentrations / 3 replicates each of the total analytical procedure). Accuracy studies for drug substance and drug product are recommended to be performed at the 80, 100 and 120% levels of label claim as stated in the Guidance for submitting samples and analytical data for methods Validation.

**System suitability testing**[8]

System suitability tests are an integral part of gas and liquid chromatographic methods. They are used to variety that the resolution and reproducibility of the based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute and integral system that can be evaluated as such.

**Limit of detection**[9]

A specific calibration curve should be studied using samples containing an analyte in the range of detection limit. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may used as the standard deviation. The LOD was performed by the standard deviation of the response and the slope based on the calibration curve.

**Limit of quantitation**[10]

A specific calibration curve should be studied using samples containing an analyte in the range of quantitation limit. The residual standard deviation of a regression line or the standard deviation of y-intercepts of

regression lines may be used as the standard deviation. The LOQ was performed by the standard deviation of the response and the slope based on the calibration curve.

**RESULTS AND DISCUSSION**

For ezetimibe, the tablet label claim is 10 mg per tablet. The corresponding mean chromatographic peak area obtained for the sample is 1,379,498.3, which, when compared with the calibration curve (or standard solution response), corresponds to an amount present of 9.181 mg per tablet (reported as 91.81 mg/100 mg equivalent) and a percentage label claim of 98.15% w/w. This indicates that the actual content of ezetimibe in the tablet is very close to the nominal claim, with only a minor deviation well within typical pharmacopeial acceptance limits (usually 95–105%), demonstrating good assay accuracy for this drug.

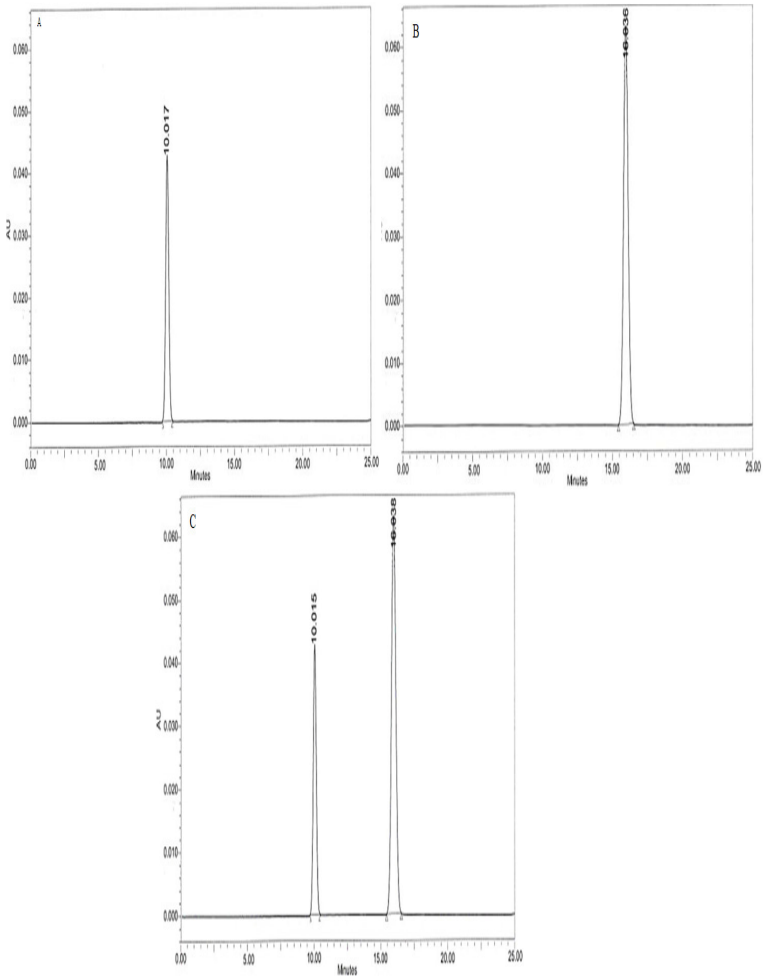


Figure 1: Chromatogram of A. Ezetimibe Standard, B. Atorvastatin Standard and C. Mixed standard.

For atorvastatin calcium, the tablet label claim is 20 mg per tablet. The mean peak area obtained is 2,891,748.8, corresponding to an amount present of 19.62 mg per tablet and a percentage label claim of 98.12% w/w. This again shows that the measured content is very close to the declared amount, with only a slight negative deviation that falls comfortably within acceptable specification limits.Overall, both ezetimibe and atorvastatin calcium are present in the tablets at about 98% of their label claim, indicating that the formulation meets the required assay specifications. The close agreement between the label claim and the experimentally determined amounts confirms that the developed HPLC method is suitable, accurate, and reliable for the routine quantitative estimation of these drugs in combined tablet dosage forms.

**Precision**

System precision was evaluated by six replicate injections of the standard solution containing Ezetimibe and Atorvastatin. The peak areas for Ezetimibe were 1,384,641; 1,381,611; 1,383,214; 1,382,845; 1,383,618; and 1,383,158, giving a mean area of 1,383,181.17 with a standard deviation (S.D) of 990.14 and a %R.S.D of 0.07. For Atorvastatin, the peak areas obtained were 2,924,652; 2,922,906; 2,924,189; 2,923,865; 2,921,489; and 2,924,158, resulting in a mean area of 2,923,543.17 with a standard deviation of 1,162.22 and a %R.S.D of 0.03. These low %R.S.D values indicate that the HPLC system shows good precision for the estimation of both Ezetimibe and Atorvastatin.

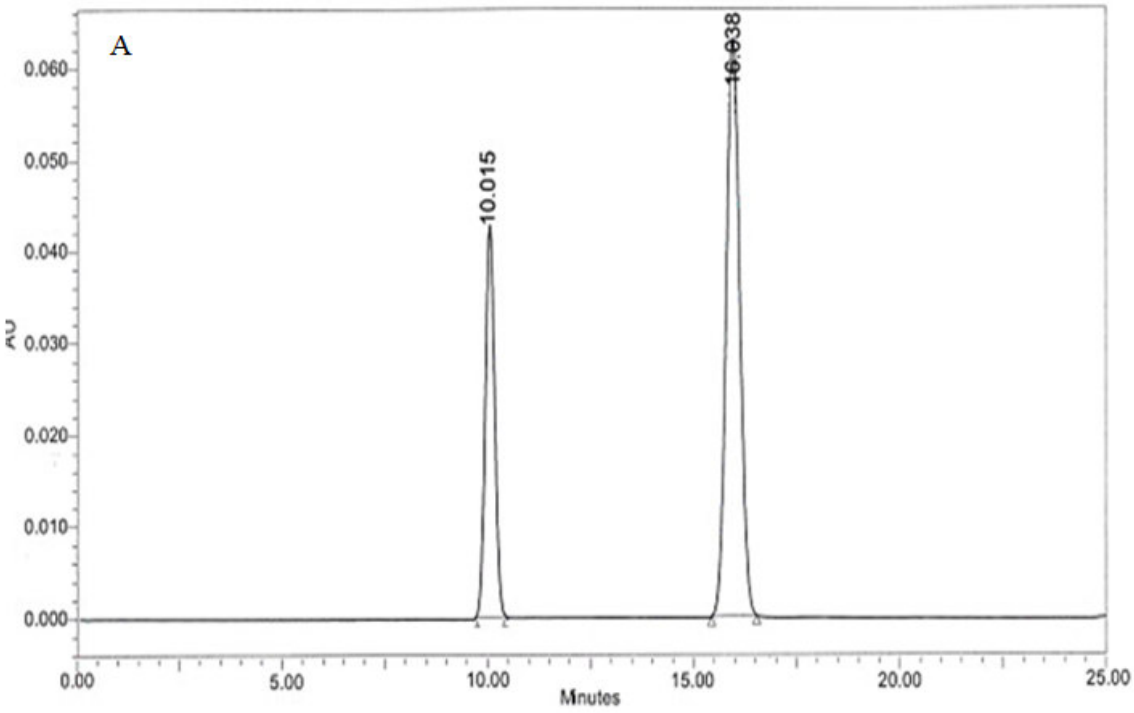


**Figure 2: Chromatogram of Method precision**

**Specificity**

The specificity of the method for Ezetimibe was evaluated using standard, standard with placebo, and placebo solutions. For Ezetimibe, the standard sample showed an area of 1,388,975 corresponding to 98.81% of the label claim, while the standard plus placebo sample gave an area of 1,381,458 corresponding to 98.93% of the label claim. The placebo alone showed no detectable peak, with an area of 0 and 0% label claim, confirming that the excipients did not interfere with the estimation of Ezetimibe.

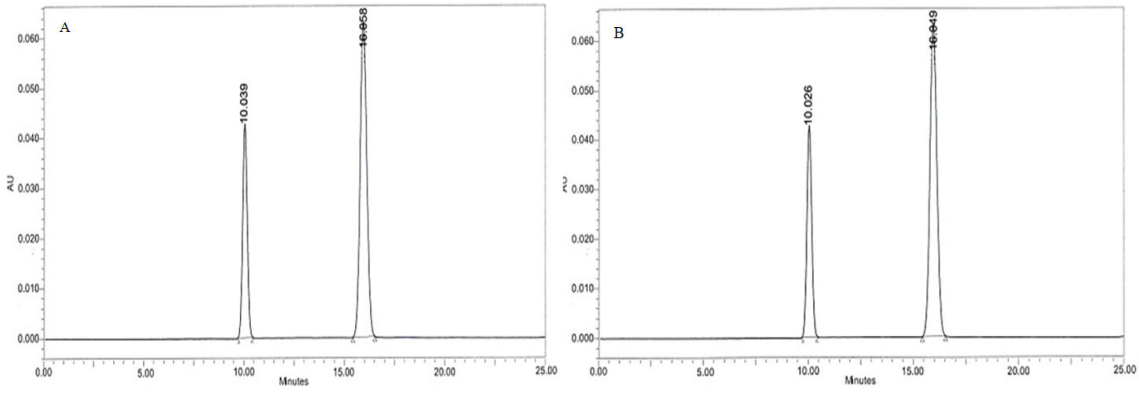


**Figure 3: Chromatogram of A. Specificity Standard and B. Specificity standard + placebo**

Similarly, the specificity for Atorvastatin was assessed using standard, standard plus placebo, and placebo. The standard sample of Atorvastatin showed an area of 2,928,581 with 98.21% of the label claim, whereas the standard plus placebo sample produced an area of 2,920,647 corresponding to 100.464% of the label claim. The placebo again showed an area of 0 and 0% label claim. These results indicate that there was no interference from the placebo components in the estimation of Atorvastatin, demonstrating the specificity of the analytical method for both drugs.

**Linearity and Range**

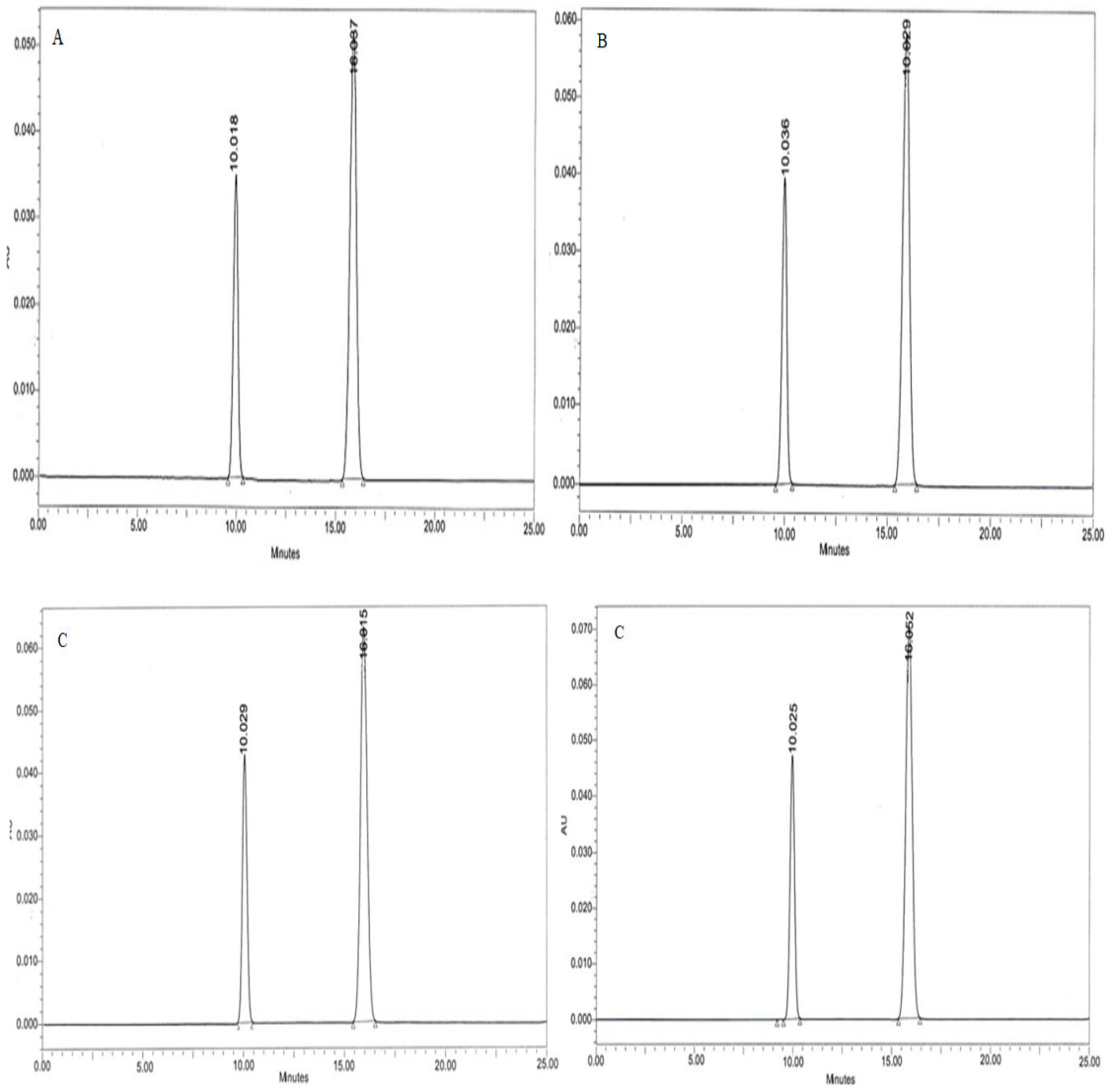


**Figure 4: Chromatogram of A. Linearity 80%, B. Linearity 90%, C. Linearity 100% and D. Linearity 110%.**

The linearity of Ezetimibe was evaluated over a concentration range of 40–60 µg/ml. The corresponding peak areas obtained were 11,09,482 at 40 µg/ml, 12,41,361 at 45 µg/ml, 13,83,840 at 50 µg/ml, 15,15,119 at 55 µg/ml, and 16,56,998 at 60 µg/ml. From these data, the analytical performance parameters were established, showing a linear dynamic range of 40–60 µg/ml with an excellent correlation coefficient (r) of 0.9999, indicating strong linearity between concentration and response. The calibration curve exhibited a slope

(m) of 13,800 and an intercept (c) of 1122.32, confirming the suitability of the method for the quantitative estimation of Ezetimibe within this range.

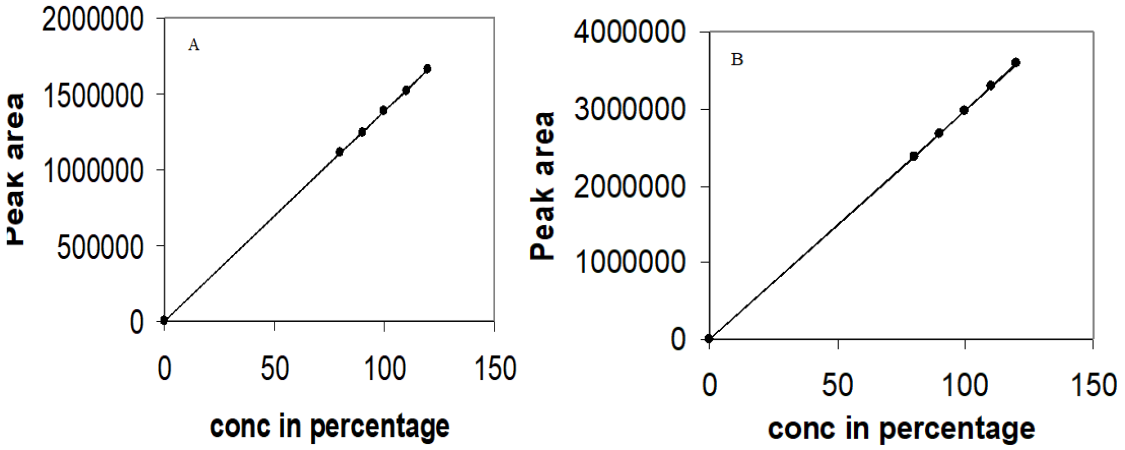


**Figure 5: Linearity Curve of A. Atorvastatin and B. Ezetimibe**

The linearity of Atorvastatin was assessed over the concentration range of 80–120 µg/ml. The corresponding peak areas obtained were 23,71,752 at 80 µg/ml, 26,81,517 at 90 µg/ml, 29,81,282 at 100 µg/ml, 32,91,047 at 110 µg/ml, and 35,91,812 at 120 µg/ml. Based on these results, the analytical performance parameters were determined, showing a linear dynamic range of 80–120 µg/ml with an excellent correlation coefficient (r) of 0.9999, indicating strong linearity between concentration and peak response. The calibration curve for Atorvastatin exhibited a slope (m) of 29,905.7 and an intercept (c) of –5907.86, confirming the reliability of the method for quantitative estimation of Atorvastatin within this range.

**Accuracy**

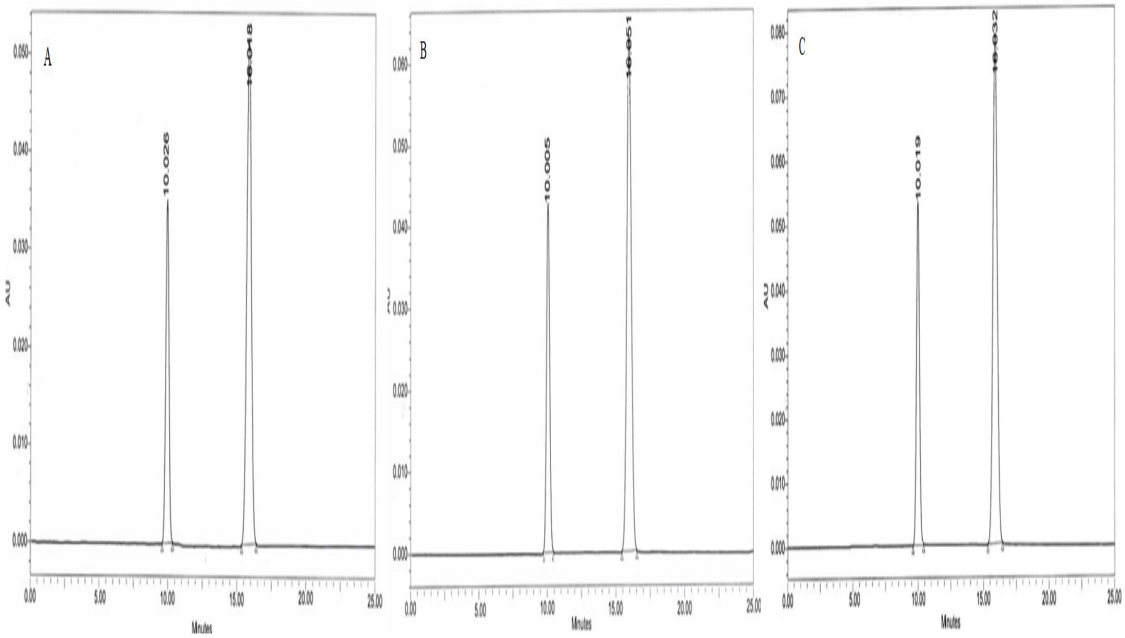


**Figure 6: Chromatogram of A. Accuracy 80%, B. Accuracy 100% and C. Accuracy 120%.**

**Table 1: Recovery study of Ezetimibe**

| S.No | Recovery | Area obtained | Average area | Amount recovered in mg | % Recovery |
|---|---|---|---|---|---|
| 1 | 80 | 1118106 | 1118110 | 8.033 | 80.33 |
| | | 1118021 | | | |
| | | 1118205 | | | |
| 2 | 100 | 1373458 | 1372555 | 9.86 | 98.61 |
| | | 1371351 | | | |
| | | 1372856 | | | |
| 3 | 120 | 1638202 | 1636094 | 11.75 | 117.5 |
| | | 1634823 | | | |
| | | 1635258 | | | |

**Table 2: Recovery of Atorvastatin**

| S.No | Recovery | Area obtained | Average area | Amount recovered in mg | % Recovery |
|---|---|---|---|---|---|
| 1 | 80 | 2382037 | 2388554 | 16.24 | 81.2 |
| | | 2399062 | | | |
| | | 2384563 | | | |
| 2 | 100 | 2994373 | 2993321 | 20.38 | 101.81 |
| | | 2992354 | | | |
| | | 2993236 | | | |
| 3 | 120 | 3599423 | 3599439 | 24.48 | 122.4 |
| | | 3599508 | | | |
| | | 3599388 | | | |

The recovery studies for Ezetimibe were performed at three concentration levels: 80%, 100%, and 120%. At the 80% level, the areas obtained were 11,18,106; 11,18,021; and 11,18,205, with an average area of 11,18,110, corresponding to an amount recovered of 8.033 mg and a percentage recovery of 80.33%. At the 100% level, the peak areas were 13,73,458; 13,71,351; and 13,72,856, giving an average area of 13,72,555 and an amount recovered of 9.86 mg with 98.61% recovery. At the 120% level, the areas recorded were 16,38,202; 16,34,823; and 16,35,258, with an average area of 16,36,094, corresponding to 11.75 mg recovered and 117.5% recovery.Similarly, recovery studies for Atorvastatin were conducted at 80%, 100%, and 120% levels. For 80% recovery, the areas obtained were 23,82,037; 23,99,062; and 23,84,563, with an average area of 23,88,554, corresponding to 16.24 mg recovered and 81.2% recovery. At the 100% level, the peak areas were 29,94,373; 29,92,354; and 29,93,236, with an average area of 29,93,321, giving an amount recovered of 20.38 mg and a percentage recovery of 101.81%. At the 120% level, the areas obtained were 35,99,423; 35,99,508; and 35,99,388, with an average area of 35,99,439, corresponding to 24.48 mg recovered and 122.4% recovery. These results collectively support the accuracy of the method for both Ezetimibe and Atorvastatin.

**System suitability testing**

System suitability for the developed method was evaluated using key chromatographic parameters for both Ezetimibe and Atorvastatin. The resolution between the two peaks was found to be 9.25, indicating good separation. The tailing factor was 1.1 for Ezetimibe and 1.2 for Atorvastatin, demonstrating symmetrical peak shapes within acceptable limits. The number of theoretical plates was 18,732.1 for Ezetimibe and 23,316 for Atorvastatin, confirming the high efficiency of the chromatographic column and the overall suitability of the system for simultaneous estimation of both drugs.

**Table 3: System suitability data**

| Parameter | Ezetimibe | Atorvastatin |
|---|---|---|
| Resolution | 9.25 | |
| Tailing Factor | 1.1 | 1.2 |
| Number of theoretical plates | 18732.1 | 23316 |

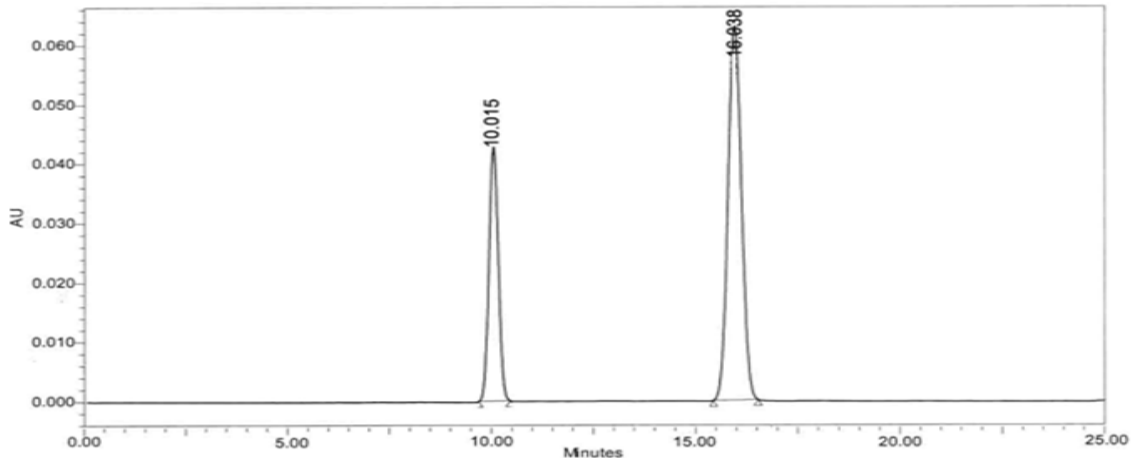


**Figure 7: Chromatogram of System Suitability Report.**

**Limit of detection**

The limit of detection (LOD) for the developed method was determined for both drugs.

Table 4: Limit of detection

| Drug | Limit of Detection (µg / ml) |
|---|---|
| EZETIMIBE | 0.0945 |
| ATORVASTATIN | 2.150 |

For Ezetimibe, the LOD was found to be 0.0945 µg/ml, indicating the minimum concentration at which the drug can be reliably detected but not necessarily quantified. For Atorvastatin, the LOD was 2.150 µg/ml. These values demonstrate the sensitivity of the analytical method for detecting low concentrations of both Ezetimibe and Atorvastatin.

**Limit of quantitation**

The limit of quantification (LOQ) for the developed method was established for both drugs. For Ezetimibe, the LOQ was found to be 0.286 µg/ml, representing the lowest concentration at which the drug can be quantitatively determined with acceptable precision and accuracy. For Atorvastatin, the LOQ was 6.51 µg/ml. These values indicate that the method is sufficiently sensitive for accurate quantitative estimation of both Ezetimibe and Atorvastatin at low concentration levels.

Table 5: Limit of quantification

| Drug | Limit of Quantification (µg / ml) |
|---|---|
| EZETIMIBE | 0.286 |
| ATORVASTATIN | 6.51 |

## CONCLUSION

A new HPLC method was developed for the simultaneous estimation of Atorvastatin and Ezetimibe in tablet dosage form. The analysis was carried out on an HPLC Water Alliance 2695 separation module equipped with a photodiode array detector (Waters 2996) using a Purospher column (250 mm × 4.6 mm, 5 µm). An injection volume of 20 µl was used, and the analytes were eluted with a mobile phase consisting of ammonium acetate buffer and acetonitrile in the ratio of 60:40 (v/v), delivered at a flow rate of 1.5 ml/min. Detection was performed at 248 nm using a UV detector. Under these conditions, the peaks of Atorvastatin and Ezetimibe were well resolved at retention times of 16.036 minutes and 10.017 minutes, respectively. The developed method was validated as per ICH guidelines for parameters such as accuracy, precision, linearity, specificity, system suitability, limit of detection, and limit of quantification. Hence, the proposed method is satisfactory and suitable for routine analysis of Atorvastatin and Ezetimibe in tablet dosage forms.

**REFERENCES**


1. Kumar SA, Debnath M, Rao JV, Sankar DG. New Validated Stability-Indicating RP-HPLC Method for Simultaneous Estimation of Atorvastatin and Ezetimibe in Human Plasma by Using PDA Detector. Adv Pharm Bull. 2015 Sep;5(3):385-91.
2. Neil M J. The Merck Index, The Encyclopedia of Chemicals, Drugs and Biologicals, 14th ed. Merck and Co Inc; 2006. P. 668-864.
3. Budavari S. The Merck Index. 12th ed. Whitehouse station (NJ): Merck and Co Inc; 1996. P.897.
4. Qutab SS, Razzaq SN, Khan IU, Ashfaq M, Shuja ZA. Simultaneous determination of atorvastatin calcium and ezetimibe in pharmaceutical formulations by liquid chromatography. J Food Drug Anal. 2007;15(2):139–44.
5. Dhaneshwar SS, Dhaneshwar SR, Deshpande P, Patil M. Development and validation of a method for simultaneous densitometric estimation of atorvastatin calcium and ezetimibe as the bulk drug and in tablet dosage forms. Acta Chromatogr. 2007;19:141–8.
6. Sama JR, Kalakuntla RR, Narayana VS, Reddanna RP. Simultaneous estimation of atorvastatin and ezetimibe in pharmaceutical formulations by RP-HPLC method. Der Pharm Lett. 2010;2(1):427–36.
7. Nagaraju P, Vishnu Vardhan Z. A validated reverse phase HPLC method for the simultaneous estimation of simvastatin and ezetimibe in pharmaceutical dosage forms. J Global Pharm Technol. 2010;2(4):113–7.
8. Kumar P, Ghosh A, Chaudhary M. Stability indicating method development for simultaneous estimation of ezetimibe and atorvastatin in pharmaceutical formulations by RP-HPLC. Pharm. Anal. Acta. 2012;3(6):1–6.
9. Tadikonda Rama Rao and Lode Madhuri (2024); validated RP - HPLC method for the simultaneous estimation of atorvastatin and ezetimibe in pure and combined pharmaceutical dosage forms, Int. J. of Adv. Res. (Sep). 31-41.
10. Nagavalli D, Srinivas B, Chakravarthi KC. Simultaneous estimation of atorvastatin calcium, ezetimibe and fenofibrate in pure and in combined dosage form by RP-HPLC and HPTLC Methods. J. Pharm Biomed Sci. 2010;4(4):1–6.