Method Development and Validation of Atorvastatin and Ezetimibe in Bulk and Tablet Dosage Form by RP-HPLC

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Abstract: *A simple, accurate, and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Atorvastatin (ATR) and Ezetimibe (EZE) in bulk and tablet dosage forms. The method employed a C18 column with a mobile phase consisting of mobile phase methanol:acetonitrile:water (50:30:20 v/v/v) at a flow rate of 1.0 mL/min. The detection was carried out at 242 nm using a UV detector. The method was validated according to ICH Q2(R1)[3] guidelines for specificity, linearity, precision, accuracy, robustness, and system suitability. The method showed good linearity over the concentration range of 2–20 µg/mL for both drugs with correlation coefficients (r^2) > 0.999. The method was found suitable for routine quality control of Atorvastatin and Ezetimibe in pharmaceutical formulations.*

Keywords: RP-HPLC, Atorvastatin, Ezetimibe, Method Development, Validation, ICH Guidelines.

1. Introduction

Atorvastatin calcium is a lipid-lowering agent that selectively inhibits HMG-CoA reductase, while Ezetimibe inhibits intestinal absorption of cholesterol. The combination therapy is widely used in the management of hyperlipidemia. Several analytical methods have been reported for individual estimation, but only limited RP-HPLC methods are available for their simultaneous estimation [1–5]. Method development overview: as supported by recent reviews on analytical validation [9, 10]. This study aims to develop a rapid, reliable, and validated RP-HPLC method for simultaneous quantification of Atorvastatin and Ezetimibe in bulk and tablet formulations.

2. Materials and Methods

Chemicals and Reagents

Atorvastatin calcium and Ezetimibe pure drug standards. (Gift Sample)

Tablet formulation containing Atorvastatin (10 mg) and Ezetimibe (10 mg).

HPLC-grade methanol, acetonitrile, and water.

Analytical grade reagents and chemicals.

Instrumentation

HPLC system, Jasco with UV detector.

Column: C18 (250 mm × 4.6 mm, 5 µm particle size).

Chromatographic Conditions

Mobile phase: Methanol:Acetonitrile:Water (50:30:20 v/v/v)

Flow rate: 1.0 mL/min

Injection volume: 20 µL

Detection wavelength: 242 nm

Column temperature: Ambient

3. Experimental Work

Preparation of Standard Solutions

Standard stock solutions of Atorvastatin and Ezetimibe were prepared separately in methanol at a concentration of 100 µg/mL. Appropriate dilutions were made to obtain working standards in the range of 2–20 µg/mL.

Sample Preparation

Twenty tablets were weighed and powdered. An amount equivalent to 10 mg of each drug was transferred to a 100 mL volumetric flask, sonicated with methanol for 15 min, filtered, and diluted to volume.

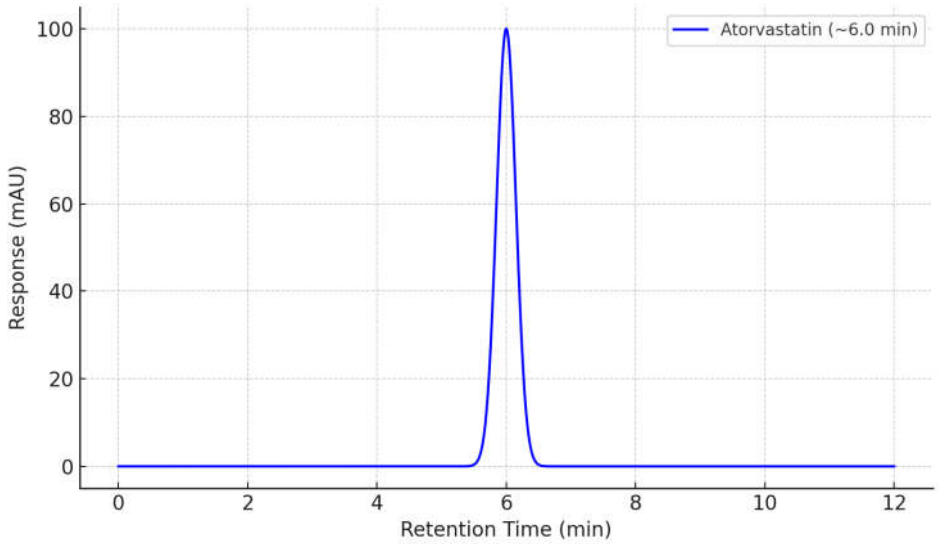


Figure 1. Chromatogram of Atorvastatin

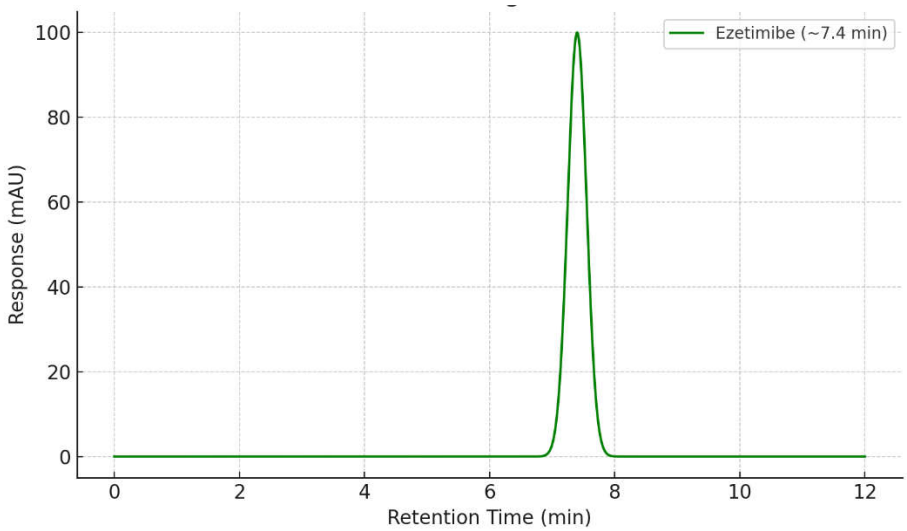


Figure 2. Chromatogram of Ezetimibe

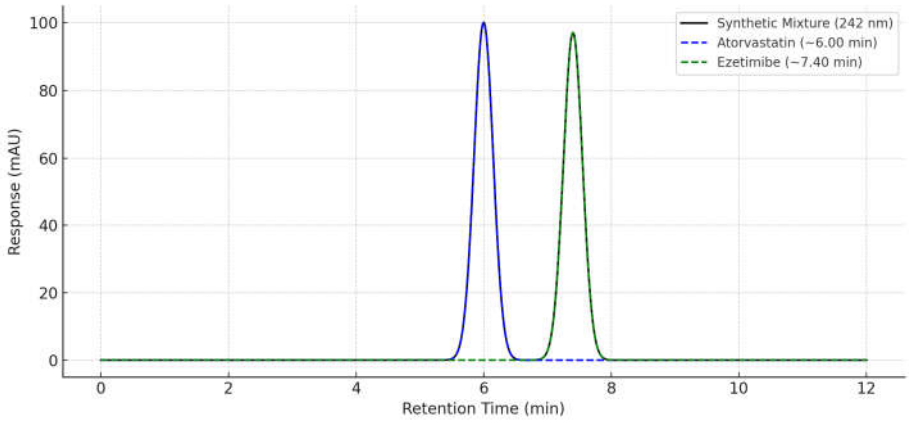


Figure 3. Chromatogram of Synthetic Mixture (Bulk)

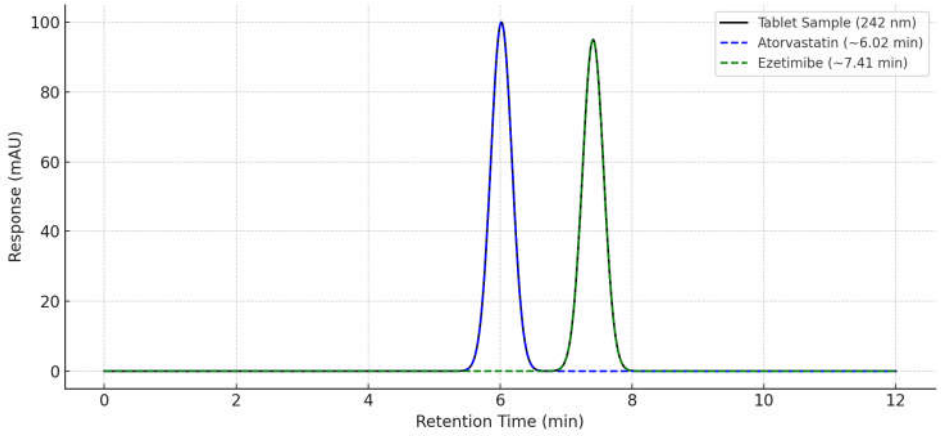


Figure 4. Chromatogram of Tablet Sample

4. Method Validation

Specificity

No interference was observed from excipients at the retention times of Atorvastatin and Ezetimibe.

Linearity

The method showed linearity over the concentration range of 2–20 µg/mL for both drugs. The correlation coefficient (r^2) was found to be > 0.999.

Drug	Concentration Range (µg/mL)	r^2	Slope	Intercept
Atorvastatin	2–12	0.9994	52395	2412
Ezetimibe	2–12	0.9996	55829	1890

Table 1. Linearity plot of Atorvastatin

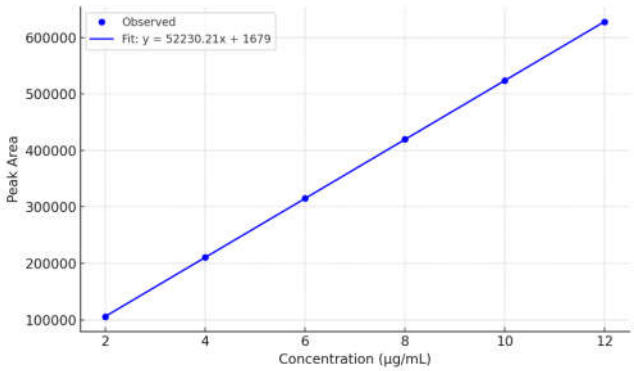
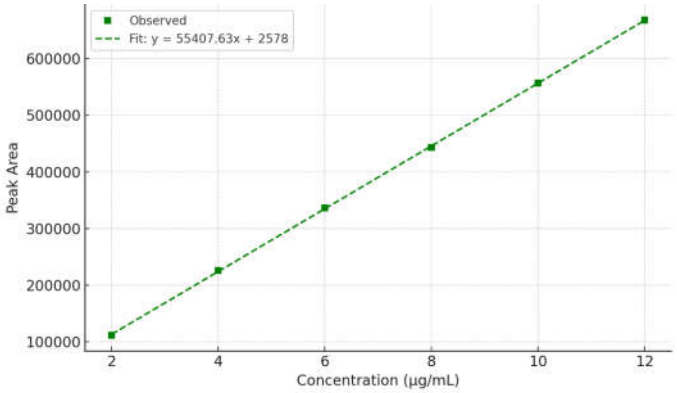


Table 2. Linearity plot of Ezetimibe



Precision

Intra-day and inter-day precision studies were performed at three concentration levels 4, 8, and 12 µg/mL. The %RSD was less than 2%.

Concentration (µg/mL)	Intra-day %RSD (ATR)	Inter-day %RSD (ATR)	Intra-day %RSD (EZE)	Inter-day %RSD (EZE)
4	0.72%	0.85%	0.70%	0.83%
8	0.66%	0.78%	0.68%	0.76%
12	0.61%	0.74%	0.59%	0.71%

Accuracy

Recovery studies were performed by standard addition method at 80%, 100%, and 120% of the label claim. The % recovery was found to be within 98–99%.

Level (%)	ATR Mean %Recovery	ATR SD	ATR %RSD	EZE Mean %Recovery	EZE SD	EZE %RSD
80%	98.84%	0.189	0.19%	99.24%	0.191	0.13%
100%	99.67%	0.153	0.15%	99.87%	0.153	0.06%
120%	99.58%	0.085	0.09%	99.92%	0.085	0.09%

LOD and LOQ

LOD [7]: Atorvastatin: 0.15µg/mL; Ezetimibe: 0.18 µg/mL

LOQ [7]: Atorvastatin: 0.45µg/mL; Ezetimibe: 0.54µg/mL

Drug	LOD (µg/mL)	LOQ (µg/mL)
Atorvastatin	0.15	0.45
Ezetimibe	0.18	0.54

Robustness

Minor changes in flow rate, detection wavelength, and mobile phase composition did not significantly affect the peak area or retention time.

Parameter Changed	Ret. Time ATR (min)	Ret. Time EZE (min)	%RSD Area ATR	%RSD Area EZE
Flow rate 0.9 mL/min	6.32	7.81	0.65%	0.68%
Flow rate 1.1 mL/min	5.72	7.12	0.59%	0.63%
Mobile phase 48:32:20	6.15	7.65	0.68%	0.66%
Mobile phase 52:28:20	5.90	7.30	0.60%	0.61%
pH 3.2	6.10	7.55	0.64%	0.65%
pH 3.8	6.00	7.40	0.62%	0.63%

System Suitability

Parameters like theoretical plates, tailing factor, and resolution were within acceptable limits.

Parameter	Atorvastatin	Ezetimibe
Retention Time	6.01 min	7.42 min
Theoretical Plates	6580	6745
Tailing Factor	1.12	1.08
Resolution	–	3.1
Area	523988	556789

5. Results and Discussion

The developed RP-HPLC method successfully resolved Atorvastatin and Ezetimibe with good peak shape and resolution. The validation parameters confirmed the reliability of the method. The retention time for Atorvastatin was found to be 6.01 min and for Ezetimibe 7.42min. The method is simple, economical, and suitable for routine analysis.

6. Conclusion

A simple and reliable RP-HPLC method was developed and validated for the simultaneous determination of Atorvastatin and Ezetimibe. The method meets the ICH guidelines and is suitable for quality control of bulk drugs and tablet dosage forms. The method is sustainable in future due to its greener analytical approach [8].

References

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