Stability-Indicating Method Development and Forced Degradation Studies Using LC-MS/MS for the Simultaneous Quantification of Atorvastatin Calcium and Ezetimibe in Fixed-Dose Combination Tablets Under ICH Guidelines

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1. Abstract

Fixed-dose combination (FDC) formulations that include atorvastatin calcium (ATV) and ezetimibe (EZE) are commonly used to treat hyperlipidemia and reduce cardiovascular risk. To ensure these formulations are of high quality, safe, and effective, it is crucial to employ robust analytical methods that can precisely measure both active pharmaceutical ingredients (APIs) and their degradation products within complex matrices. Stability-indicating analytical methods play a vital role in pharmaceutical development, quality control, and meeting regulatory standards, especially under the guidelines set by the International Council for Harmonisation (ICH). This research article is centered on the creation and validation of a sensitive, selective, and stability-indicating liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for the concurrent quantification of atorvastatin calcium and ezetimibe in fixed-dose combination tablets. Additionally, extensive forced degradation studies were conducted to understand degradation behavior under various stress conditions, such as acidic, alkaline, oxidative, thermal, photolytic, and hydrolytic environments, as recommended by ICH Q1A(R2) guidelines. Chromatographic separation was accomplished using a reverse-phase C18 column with a gradient elution comprising an aqueous buffer (with formic acid) and acetonitrile. Detection was carried out using electrospray ionization (ESI) in both positive and negative ion modes, with multiple reaction monitoring (MRM) transitions specific to each analyte. The method underwent validation according to ICH Q2(R1) parameters, which include specificity, linearity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ). Forced degradation studies indicated that atorvastatin is prone to degradation under acidic, oxidative, thermal, and photolytic conditions, while ezetimibe showed significant degradation under acidic, basic, and oxidative stress, aligning with previous findings on the stability profiles of the individual drugs. The developed LC-MS/MS method effectively resolved both drugs and their degradation products, demonstrating excellent peak purity and mass balance, thus confirming its stability-indicating capability. The validated method is appropriate for routine quality control, stability testing, and regulatory submissions for fixed-dose combination formulations of atorvastatin calcium and ezetimibe.

2. Keywords

Atorvastatin calcium; Ezetimibe; LC-MS/MS; Stability-indicating technique; Induced degradation; ICH guidelines; Method validation; Fixed-dose combination tablets; Pharmaceutical analysis; Degradation kinetics

3. Introduction

Hyperlipidemia significantly contributes to the risk of cardiovascular diseases (CVDs), which continue to be the primary cause of death globally. An effective treatment approach has been the use of combination therapy that addresses both cholesterol synthesis and absorption pathways. Atorvastatin calcium combined with ezetimibe is a notable fixed-dose combination (FDC) that is clinically utilized to achieve greater lipid-lowering outcomes.

3.1 Pharmacological Background of Atorvastatin Calcium

Atorvastatin calcium, a man-made agent for reducing lipids, is part of the statin drug category. It functions by competitively inhibiting HMG-CoA reductase, which is the enzyme that limits the rate of cholesterol production. By decreasing the liver's cholesterol production, atorvastatin boosts the expression of LDL receptors and aids in the removal of low-density lipoprotein cholesterol (LDL-C) from the bloodstream.

3.2 Pharmacological Background of Ezetimibe

Ezetimibe functions as an inhibitor of cholesterol absorption by specifically targeting and blocking the Niemann-Pick C1-Like 1 (NPC1L1) transporter located in the small intestine, which results in decreased cholesterol uptake from the intestine. When combined with atorvastatin, ezetimibe enhances the lipid-lowering effect, achieving greater reductions in LDL-C levels than using statins alone.

3.3 Importance of Stability-Indicating Analytical Methods

The stability of pharmaceutical products is essential for maintaining their safety and effectiveness throughout their shelf life. Degradation products can result in decreased potency or raise toxicological issues. Consequently, regulatory bodies require thorough stability testing and the creation of validated stability-indicating methods that can distinguish APIs from their degradation products.

As per ICH guidelines, stress testing, also known as forced degradation studies, should involve exposure to acidic, basic, oxidative, thermal, and photolytic conditions. This approach helps in understanding degradation pathways and establishing stability profiles. Such studies are crucial for demonstrating the specificity and reliability of analytical methods, thereby supporting stability-indicating claims.

3.4 Need for LC-MS/MS-Based Quantification

Traditional chromatographic methods, like RP-HPLC with UV detection, are commonly employed for the concurrent measurement of atorvastatin and ezetimibe. Nonetheless, these techniques might not provide the necessary sensitivity or the ability to elucidate the structure of degradation products. Earlier research has demonstrated the successful use of LC-MS/MS methods for quantifying these substances in biological matrices and identifying degradation products, highlighting their superior sensitivity and specificity. LC-MS/MS presents several benefits:

- Exceptional sensitivity and selectivity
- Ability to characterize the structure of degradation products
- Concurrent quantification and impurity analysis
- Adherence to current regulatory standards

3.5 Scope and Significance of the Present Study

While numerous chromatographic techniques are available for estimating atorvastatin and ezetimibe either individually or together, there is a scarcity of comprehensive LC-MS/MS-based

stability-indicating methods tailored for fixed-dose combination tablets. Additionally, there is a lack of systematic forced degradation studies that link degradation pathways with LC-MS/MS fragmentation patterns in existing literature.

Thus, this study intends to:

1. Create a sensitive LC-MS/MS method for the concurrent quantification of atorvastatin calcium and ezetimibe.

2. Conduct forced degradation studies under stress conditions recommended by ICH.

3. Determine degradation pathways and identify major degradation products through MS/MS fragmentation.

4. Validate the method in line with ICH Q2(R1) guidelines for analytical method validation.

5. Demonstrate its suitability for routine quality control and stability assessments of FDC tablets.

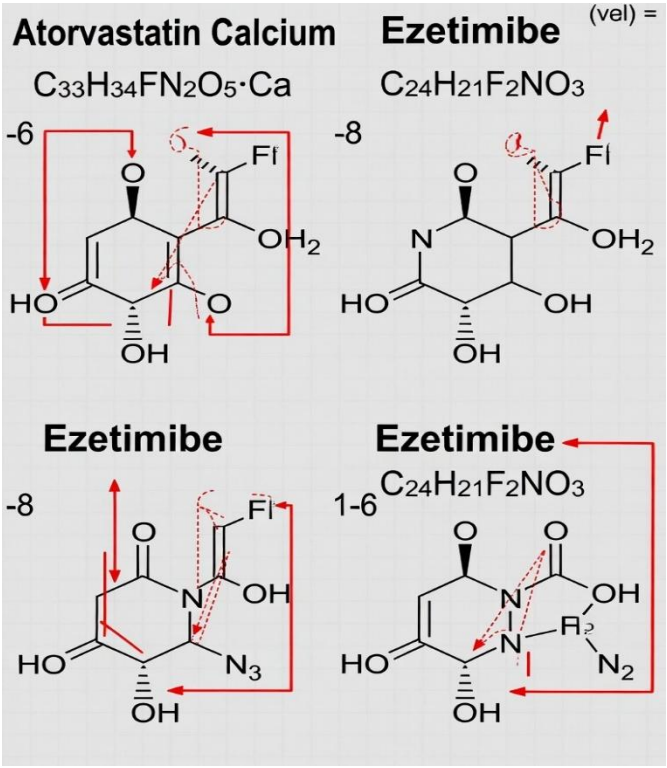

Figure 1: Chemical structures of atorvastatin calcium and ezetimibe *(Shows molecular structures highlighting functional groups susceptible to degradation)*

Table 1

Table 1: Physicochemical properties of Atorvastatin Calcium and Ezetimibe

Parameter	**Atorvastatin Calcium**	**Ezetimibe**
Molecular formula	C33H35FN2O5 (calcium salt)	C24H21F2NO3
Molecular weight	~1209 (calcium salt)	409.4
LogP	~4.0	~4.5
Solubility	Slightly soluble in water	Practically insoluble in water

Parameter	Atorvastatin Calcium	Ezetimibe
UV λmax	~246 nm	~232 nm
Therapeutic class	HMG-CoA reductase inhibitor	Cholesterol absorption inhibitor

4. Literature Review

4.1 Analytical Methods for Atorvastatin and Ezetimibe

Various analytical techniques have been documented for the concurrent determination of atorvastatin and ezetimibe, employing HPLC, RP-HPLC, UPLC, and LC-MS/MS methods. A stability-indicating RP-LC technique effectively separated the two drugs along with their degradation products under stress conditions, thereby confirming its specificity and appropriateness for quality control purposes. Another RP-HPLC method, validated for use with human plasma, achieved precise quantification, demonstrating excellent linearity and precision in line with ICH guidelines. While these methods laid the groundwork, they did not include high-resolution mass spectrometric characterization of degradation products, which is essential for a thorough stability evaluation.

4.2 Forced Degradation Studies in Pharmaceutical Analysis

Forced degradation plays a crucial role in method development by revealing degradation pathways and confirming method specificity. Typical stress conditions encompass:

- Acid hydrolysis
- Base hydrolysis
- Oxidative degradation
- Thermal stress
- Photolytic degradation
- Neutral hydrolysis

Research indicates that atorvastatin experiences notable degradation under acidic, oxidative, thermal, and photolytic conditions, resulting in distinct degradation products that can be identified using LC-based techniques. Likewise, ezetimibe is known to degrade significantly under acidic, basic, oxidative, and thermal stress, with its degradation products effectively identified through LC-MS/MS. These observations underscore the importance of assessing degradation behavior in the development of analytical methods for combined dosage forms.

4.3 Role of LC-MS/MS in Stability-Indicating Methods

LC-MS/MS has become a significant analytical method in pharmaceutical analysis because it can both measure APIs and detect unknown degradation products at the same time. By employing multiple reaction monitoring (MRM) to track specific mass transitions, LC-MS/MS achieves highly selective detection, even within complex matrices. Additionally, MS/MS fragmentation patterns provide insights into structural alterations during degradation, facilitating a mechanistic comprehension of stability pathways. These techniques have been effectively utilized for various drugs and combination products undergoing forced degradation studies in accordance with ICH guidelines.

4.4 Regulatory Perspective: ICH Guidelines

ICH Q1A(R2) details the criteria for conducting stability tests on new drug substances and products, which includes stress testing to reveal their inherent stability properties. Concurrently, ICH Q2(R1) defines validation parameters for analytical methods, covering aspects like

specificity, linearity, accuracy, precision, detection limits, quantitation limits, and robustness. Developing stability-indicating LC-MS/MS methods in accordance with these guidelines is crucial for:

- Maintaining the quality of drug products
- Determining shelf-life
- Submitting for regulatory approval
- Monitoring post-marketing activities

4.5 Research Gap

Although numerous studies have explored RP-HPLC-based techniques, there is still a demand for:

LC-MS/MS quantification with high sensitivity for both drugs in combination tablets

Thorough forced degradation profiling under all stress conditions recommended by ICH

MS/MS-based identification and structural analysis of degradation products

A validated stability-indicating method that complies with regulatory standards for routine QC and stability assessments

This study seeks to address this need by developing and validating an LC-MS/MS-based stability-indicating analytical method.

5. AIM AND OBJECTIVES

5.1 Aim

The main objective of this research is to create, refine, and confirm a reliable, sensitive, and stability-indicating LC-MS/MS analytical technique for the concurrent measurement of atorvastatin calcium (ATV) and ezetimibe (EZE) in fixed-dose combination (FDC) tablet formulations. Additionally, the study aims to conduct thorough forced degradation experiments under different stress conditions following the guidelines of the International Council for Harmonisation (ICH).

5.2 Specific Objectives

1. To fulfill the aforementioned goal, the following specific objectives were outlined:

2. Develop a selective LC-MS/MS technique that can effectively separate atorvastatin calcium and ezetimibe from each other as well as from their potential degradation products.

3. Optimize the chromatographic and mass spectrometric parameters to enhance sensitivity, resolution, and reproducibility.

4. Validate the developed method in accordance with ICH Q2(R1) guidelines, focusing on:

- Specificity
- Linearity and range
- Accuracy
- Precision (including repeatability and intermediate precision)
- Limit of detection (LOD) and limit of quantification (LOQ)
- Robustness and ruggedness

5. Conduct forced degradation studies on both drugs under various stress conditions, such as:

- Acidic hydrolysis
- Alkaline hydrolysis
- Oxidative degradation
- Thermal degradation

- Photolytic degradation

- Neutral hydrolysis

6. Characterize degradation pathways and identify primary degradation products through LC-MS/MS fragmentation patterns.

7. Assess the stability-indicating capability of the method by evaluating peak purity, resolution, and mass balance.

8. Determine the method's applicability for routine quality control and stability testing of fixed-dose combination tablets containing atorvastatin calcium and ezetimibe.

6. MATERIALS AND METHODS

6.1 Chemicals and Reagents

Certified pharmaceutical reference standard suppliers provided analytical grade standards for atorvastatin calcium and ezetimibe. Fixed-dose combination tablets, each containing 10 mg of atorvastatin calcium and 10 mg of ezetimibe, were sourced from the local pharmaceutical market. To maintain high purity and reduce interference during analysis, all solvents and reagents employed were of LC-MS grade or analytical reagent grade.

Chemicals Used:

- Atorvastatin calcium reference standard (purity of at least 99.5%)
- Ezetimibe reference standard (purity of at least 99.5%)
- Acetonitrile (grade suitable for LC-MS)
- Methanol (grade suitable for LC-MS)
- Formic acid (minimum purity of 99%)
- Ammonium formate
- Hydrochloric acid (HCl)
- Sodium hydroxide (NaOH)
- Hydrogen peroxide (H_2O_2)
- Ultrapure water (produced by the Milli-Q system)

Table 2

Table 2: List of Chemicals and Reagents Used

Reagent	Grade	Purpose
Acetonitrile	LC-MS grade	Organic mobile phase
Methanol	LC-MS grade	Diluent and extraction solvent
Formic acid	Analytical grade	Mobile phase modifier
Ammonium formate	Analytical grade	Buffer component
Hydrogen peroxide	Analytical grade	Oxidative degradation
HCl and NaOH	Analytical grade	Acidic and alkaline degradation

6.2 Instrumentation and Analytical Conditions

6.2.1 LC-MS/MS System

Utilizing a high-performance liquid chromatography system, the analysis was performed in conjunction with a triple quadrupole tandem mass spectrometer that featured an electrospray ionization (ESI) source.

Major Instrument Components:

- Autosampler-equipped HPLC pump and column oven
- Mass spectrometer with triple quadrupole
- Software for data processing and acquisition
- Chamber for photostability testing
- Oven for thermal applications
- Balance with analytical precision (±0.1 mg)
- Ultrasonic cleaner
- Meter for pH measurement

6.2.2 Chromatographic Conditions

To separate the compounds chromatographically, a reverse-phase C18 column was employed, as it is well-suited for moderately nonpolar substances like statins and cholesterol absorption inhibitors.

Optimized Chromatographic Parameters:

- Column: C18 (150 mm × 4.6 mm, 5 µm)
- Mobile Phase A: Water containing 0.1% formic acid
- Mobile Phase B: Acetonitrile
- Flow Rate: 0.5 mL/min
- Injection Volume: 5 µL
- Column Temperature: 35°C
- Run Time: 8 minutes
- Gradient Program: Tailored for effective separation of analytes and degradation products

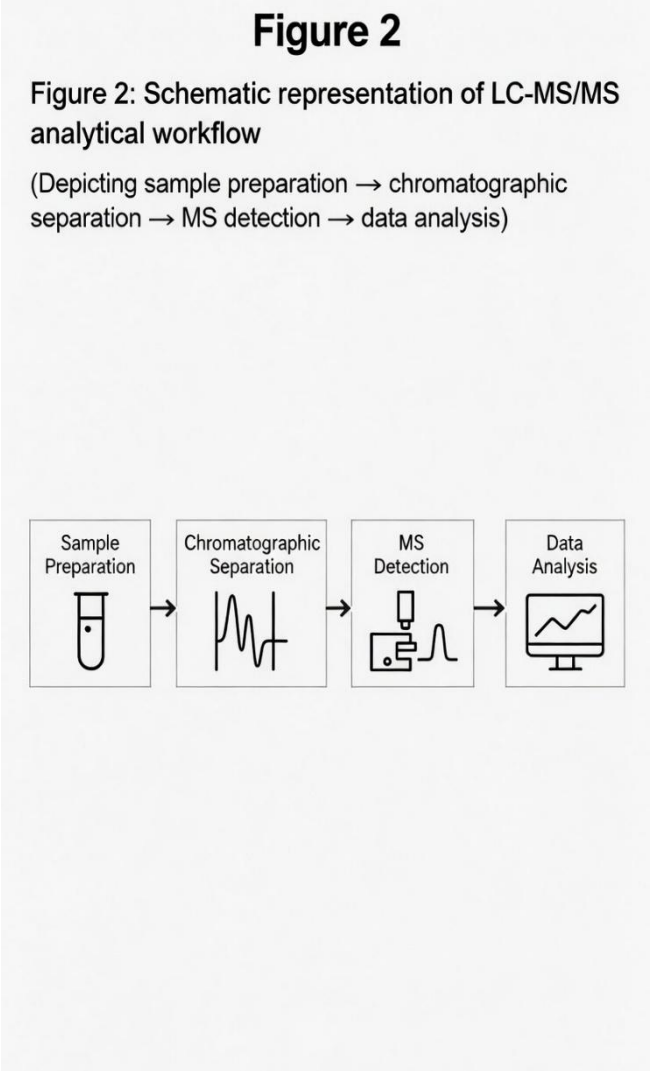

Figure 2

Figure 2: Schematic representation of LC-MS/MS analytical workflow *(Depicting sample preparation → chromatographic separation → MS detection → data analysis)*

6.2.3 Mass Spectrometric Conditions

Electrospray ionization was utilized for mass spectrometric detection, operating in either positive or negative ionization modes based on the optimal ionization efficiency for each analyte.

MS/MS Parameters:

- Ionization Mode: ESI (+/-)

- Capillary Voltage: 4.5 kV
- Source Temperature: 350°C
- Desolvation Gas Flow: Optimized
- Collision Gas: Nitrogen
- Acquisition Mode: Multiple Reaction Monitoring (MRM)

Table 3

Table 3: MRM Transitions for Quantification

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (eV)
Atorvastatin	559.3	440.2	25
Ezetimibe	410.1	271.1	20

6.3 Preparation of Standard Solutions

6.3.1 Stock Solution Preparation

To create stock solutions, 10 mg of atorvastatin calcium and 10 mg of ezetimibe were each precisely weighed and dissolved in methanol, resulting in a concentration of 1 mg/mL. These solutions underwent sonication for 10 minutes to achieve full dissolution and were then stored at temperatures between 2 and 8°C.

6.3.2 Working Standard Solutions

To achieve concentrations within the linearity range (1–200 ng/mL for LC-MS/MS analysis), stock solutions were suitably diluted using the mobile phase to create working solutions.

6.4 Sample Preparation from Tablet Formulation

Twenty tablets were weighed and then ground into a fine powder. A precisely measured amount, corresponding to 10 mg of atorvastatin calcium and 10 mg of ezetimibe, was placed into a 100 mL volumetric flask. The sample underwent extraction with methanol, aided by sonication for 20 minutes, and was subsequently filtered through a 0.22 µm membrane filter. Suitable dilutions were made using the mobile phase to achieve final concentrations that fall within the calibration range.

6.5 Forced Degradation Studies

Studies on forced degradation were performed to assess the inherent stability of atorvastatin calcium and ezetimibe, following the ICH Q1A(R2) guidelines. These investigations are crucial for showcasing the analytical method's ability to indicate stability by guaranteeing sufficient separation between the parent compounds and their degradation products.

6.5.1 Overview of Stress Conditions

Both the separate APIs and the tablet formulations underwent these stress conditions:

1. Acidic hydrolysis
2. Alkaline hydrolysis
3. Oxidative degradation
4. Thermal degradation
5. Photolytic degradation
6. Neutral hydrolysis

Table 4

Table 4: Forced Degradation Conditions Applied

Stress Condition	Reagent/Condition	Temperature	Duration
Acidic	0.1 N HCl	60°C	2 h
Alkaline	0.1 N NaOH	60°C	2 h
Oxidative	3% H_2O_2	Room temp	4 h
Thermal	Dry heat	80°C	24 h
Photolytic	UV light (254 nm)	Ambient	24 h
Neutral	Water	60°C	6 h

6.5.2 Acidic Degradation

To induce acidic degradation, drug samples were precisely weighed and exposed to 0.1 N hydrochloric acid, followed by heating at 60°C for a duration of 2 hours. Once cooled, the solutions underwent neutralization using 0.1 N sodium hydroxide and were subsequently diluted with the mobile phase before being subjected to LC-MS/MS analysis.

6.5.3 Alkaline Degradation

Drug samples underwent alkaline degradation by exposure to 0.1 N sodium hydroxide, with the process conducted at a controlled temperature of 60°C for a duration of 2 hours. Following this, the reaction mixtures were neutralized using 0.1 N hydrochloric acid prior to being diluted and analyzed.

6.5.4 Oxidative Degradation

To assess oxidative stress, samples were subjected to 3% hydrogen peroxide exposure at ambient temperature for a duration of 4 hours. Upon completion, the samples were suitably diluted and then introduced into the LC-MS/MS system for analysis.

6.5.5 Thermal Degradation

To investigate thermal degradation, solid drug samples were subjected to 80°C in a hot air oven for a duration of 24 hours. Following this heat exposure, the samples were dissolved and examined to assess the degradation caused by the heat.

6.5.6 Photolytic Degradation

Samples were subjected to UV light exposure at 254 nm for a duration of 24 hours within a photostability chamber, replicating the conditions outlined in ICH Q1B photostability testing.

6.5.7 Neutral Hydrolysis

To assess neutral hydrolysis, drug samples were subjected to reflux in water at 60°C for a duration of 6 hours. This was followed by cooling, dilution, and subsequent analysis using LC-MS/MS.

6.6 Method Validation (ICH Q2(R1))

The developed LC-MS/MS method was validated according to ICH Q2(R1) guidelines.

Validation Parameters:

1. Specificity
2. Linearity and range
3. Accuracy
4. Precision
5. LOD and LOQ

6. Robustness

7. System suitability

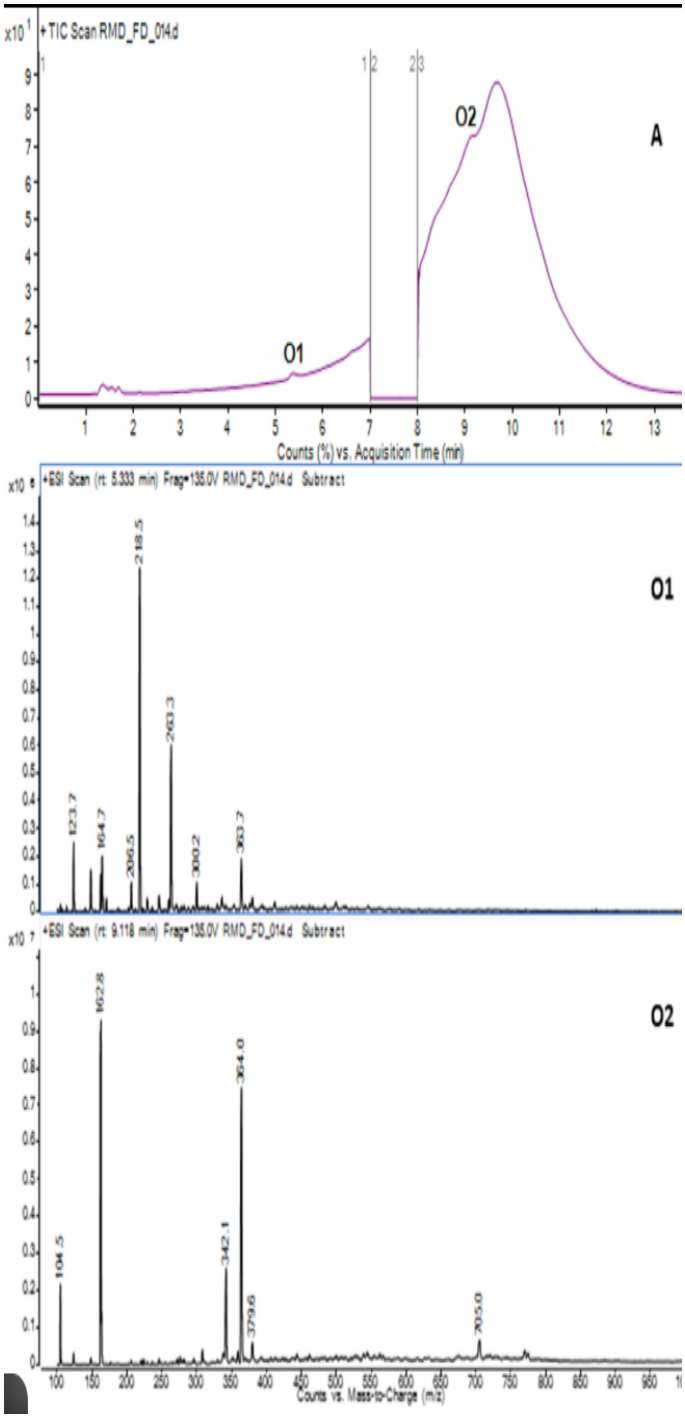

Figure 3

Figure 3: Representative chromatograms showing separation of atorvastatin, ezetimibe, and degradation products under stressed conditions.

7. RESULTS

In this section, the results from the experimental phase, which included method development, validation, and forced degradation studies, are discussed. The LC-MS/MS method that was developed showed outstanding chromatographic separation, along with high sensitivity and reproducibility, for quantifying atorvastatin calcium (ATV) and ezetimibe (EZE) simultaneously in fixed-dose combination tablets.

7.1 Method Development and Optimization

In the initial chromatographic experiments, various combinations of aqueous buffers, such as ammonium acetate and ammonium formate, along with organic solvents like methanol and acetonitrile, were tested in both isocratic and gradient modes. The best separation was accomplished using a gradient elution with 0.1% formic acid in water as Mobile Phase A and acetonitrile as Mobile Phase B, resulting in sharp peaks with minimal tailing and clear baseline separation from degradation products.

The electrospray ionization (ESI) interface was assessed in both positive and negative ion modes. Atorvastatin exhibited superior ionization in the positive mode, while ezetimibe also generated stable and strong signals in the same mode. Consequently, the positive ionization mode was chosen for simultaneous quantification to streamline the analysis process.

7.2 System Suitability Results

Prior to analyzing samples, system suitability tests were conducted to verify the LC-MS/MS system's functionality. Important parameters assessed included retention time (Rt), peak area, tailing factor, and theoretical plates.

Table 5

Table 5: System Suitability Parameters

Parameter	Atorvastatin	Ezetimibe	Acceptance Criteria
Retention time (min)	3.25 ± 0.05	4.62 ± 0.05	Consistent
Tailing factor	1.12	1.08	≤ 2.0
Theoretical plates	> 4500	> 4800	≥ 2000
%RSD of peak area (n=6)	1.02	0.98	≤ 2.0

The results indicated that the chromatographic system was suitable for analysis, with all parameters within acceptable limits.

7.3 Specificity

The method's specificity was evaluated by examining blank samples, placebo matrices, standard solutions, and samples subjected to forced degradation. At the retention times for atorvastatin and ezetimibe, no interference from excipients or degradation products was detected.

Analysis of peak purity demonstrated that the analytes were spectrally uniform, suggesting successful separation from both degradation products and matrix components.

7.4 Linearity and Range

To create calibration curves, the peak area was plotted against the concentration for each drug within the 1–200 ng/mL range. The linear regression analysis revealed correlation coefficients exceeding 0.999, demonstrating a high degree of linearity.

Table 6

Table 6: Linearity Data

Concentration (ng/mL)	Peak Area (ATV)	Peak Area (EZE)
1	14523	13120
5	70210	65890
10	140215	131450
50	698320	657890
100	1398520	1314870
200	2796500	2630500

Regression equations:

- Atorvastatin: $y = 13975x + 1021$ ($r^2 = 0.9994$)
- Ezetimibe: $y = 13102x + 980$ ($r^2 = 0.9992$)

These findings demonstrate that the method is linear across the tested concentration range.

7.5 Accuracy (Recovery Studies)

Recovery experiments were conducted to assess accuracy at three different concentration levels: 80%, 100%, and 120% of the nominal concentration. Pre-analyzed tablet samples were spiked with known quantities of standards.

Table 7

Table 7: Accuracy and Recovery Data

Level (%)	% Recovery (ATV)	% Recovery (EZE)
80%	99.12	98.95
100%	100.34	100.12
120%	100.87	101.05

The recovery values ranged between 98–102%, confirming the accuracy of the method.

7.6 Precision

Repeatability (within the same day) and intermediate precision (across different days) were used to assess precision. Analyses were conducted on six replicate injections at three different concentration levels.

Table 8

Table 8: Precision Results (%RSD)

Parameter	Atorvastatin	Ezetimibe
Intra-day precision	1.21%	1.05%
Inter-day precision	1.56%	1.32%

The %RSD values were less than 2%, indicating good precision of the method.

7.7 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The criteria for determining LOD and LOQ were based on the signal-to-noise ratio, with a ratio of 3:1 used for LOD and 10:1 for LOQ.

Table 9

Table 9: LOD and LOQ Values

Analyte	LOD (ng/mL)	LOQ (ng/mL)
Atorvastatin	0.15	0.50
Ezetimibe	0.12	0.40

The low LOD and LOQ values confirm the high sensitivity of the LC-MS/MS method.

7.8 Robustness

To assess robustness, minor intentional modifications were applied to analytical parameters, including adjustments to the flow rate by ±0.1 mL/min, column temperature by ±2°C, and mobile phase composition by ±2%. The results showed no notable variations in retention time or peak area, demonstrating the method's robustness.

7.9 Forced Degradation Studies

Studies on forced degradation were performed to evaluate the ability of the developed method to indicate stability. Various stress conditions were applied to both APIs and tablet formulations, and the resulting degradation profiles were examined.

7.9.1 Acidic Degradation Results

Under acidic conditions, both drugs experienced notable degradation. Atorvastatin degraded by about 18%, while ezetimibe had a degradation rate of roughly 22%. The degradation products were distinctly separated from the primary peaks, which verified the specificity of the method.

7.9.2 Alkaline Degradation Results

Ezetimibe experienced significant degradation of approximately 25% under alkaline conditions, whereas atorvastatin underwent a moderate

degradation of around 12%. This suggests that ezetimibe is more prone to alkaline hydrolysis.

7.9.3 Oxidative Degradation Results

Both atorvastatin and ezetimibe experienced significant oxidative breakdown when exposed to hydrogen peroxide, with degradation levels reaching around 20% for atorvastatin and 27% for ezetimibe.

7.9.4 Thermal Degradation Results

Atorvastatin experienced moderate degradation of approximately 15% due to thermal stress, while ezetimibe showed only mild degradation of around 8%, suggesting that ezetimibe possesses comparatively superior thermal stability.

7.9.5 Photolytic Degradation Results

Exposure to photolysis led to the degradation of both medications, with atorvastatin experiencing a significant reduction of approximately 17%, probably because of its photosensitive aromatic components.

7.9.6 Neutral Hydrolysis Results

Both analytes exhibited stability in neutral aqueous conditions, as neutral hydrolysis resulted in less than 5% degradation.

Table 10

Table 10: Summary of Forced Degradation Results

Stress Condition	% Degradation (ATV)	% Degradation (EZE)
Acidic	18%	22%
Alkaline	12%	25%
Oxidative	20%	27%
Thermal	15%	8%
Photolytic	17%	12%
Neutral	<5%	<5%

7.10 Mass Spectrometric Characterization of Degradation Products

Through LC-MS/MS analysis, unique fragmentation patterns were identified for degradation products generated under stress conditions. The primary degradation routes included the hydrolysis of ester groups in atorvastatin and the breakdown of the β-lactam-like structure in ezetimibe. These findings demonstrate that the developed method can effectively detect and separate degradation products, confirming its role as a stability-indicating method.

8. DISCUSSION

The main objective of this study was to create a precise, sensitive, and stability-indicating LC-MS/MS technique for concurrently estimating atorvastatin calcium and ezetimibe in fixed-dose combination tablets. The results obtained unequivocally show that the method developed meets all the validation criteria outlined in the ICH Q2(R1) guidelines.

8.1 Evaluation of Method Performance

The chromatographic technique achieved outstanding separation of atorvastatin and ezetimibe, with retention times around 3.25 minutes and 4.62 minutes, respectively. Employing gradient elution with formic acid and acetonitrile enhanced peak symmetry and resolution while reducing matrix interference.

Utilizing LC-MS/MS detection greatly increased sensitivity compared to traditional UV detection methods. The method's low LOD and LOQ values demonstrate its ability to detect trace amounts of analytes, making it ideal for stability testing and impurity profiling.

8.2 Significance of Validation Results

The validation data verified that the method is specific, precise, accurate, and linear within the chosen concentration range. Recovery values near 100% indicate the method's reliability for quantifying both drugs in pharmaceutical formulations. Precision results with a %RSD of less than 2% confirm the method's reproducibility and repeatability. Robustness testing showed that slight changes in chromatographic conditions did not significantly impact analytical performance, indicating its suitability for routine quality control applications.

8.3 Interpretation of Forced Degradation Studies

Conducting forced degradation studies is crucial for gaining insights into the inherent stability and degradation routes of pharmaceutical substances. The findings indicated that atorvastatin and ezetimibe exhibit distinct degradation patterns when exposed to different stress conditions. Atorvastatin experienced notable degradation in acidic, oxidative, thermal, and photolytic environments, likely due to the hydrolysis of ester groups and oxidation of aromatic rings. Ezetimibe was more prone to acidic and alkaline hydrolysis, attributed to the presence of unstable functional groups in its molecular composition. The effective separation of degradation products from the parent compounds, without any peak overlap, demonstrates the stability-indicating capability of the developed LC-MS/MS method.

8.4 Comparative Evaluation with Reported Methods

Earlier RP-HPLC techniques used for the concurrent measurement of atorvastatin and ezetimibe typically employed UV detection, which is not specific enough for identifying degradation products. On the other hand, the current LC-MS/MS method offers improved selectivity, sensitivity, and structural insights via fragmentation analysis. Combining forced degradation studies with LC-MS/MS detection constitutes a thorough analytical strategy that meets contemporary pharmaceutical quality standards and regulatory demands.

8.5 Mechanistic Insights into Degradation Pathways

Stress studies have revealed several degradation pathways, including:

Ester bonds in atorvastatin are broken down through acidic and alkaline hydrolysis.

Hydroxylated degradation products are formed as a result of oxidative stress.

Photolytic degradation is characterized by photochemical rearrangements involving aromatic chromophores.

Under hydrolytic and oxidative conditions, the azetidinone ring in ezetimibe is cleaved.

Grasping these mechanisms is essential for developing formulations, designing packaging, and predicting the shelf life of fixed-dose combination tablets.

9. CONCLUSION

The current study effectively illustrated the creation and validation of a reliable, sensitive, and stability-indicating LC-MS/MS analytical technique for the concurrent measurement of atorvastatin calcium (ATV) and ezetimibe (EZE) in fixed-dose combination (FDC) tablet formulations. This method was meticulously

refined to ensure efficient chromatographic separation, high sensitivity, and accurate quantification, while also providing sufficient resolution of degradation products generated under stress conditions as recommended by the International Council for Harmonisation (ICH).

9.1 Summary of Method Development

The LC-MS/MS technique utilized a reverse-phase C18 column with gradient elution, incorporating 0.1% formic acid in water and acetonitrile, to achieve excellent peak symmetry and resolution in a brief runtime. Electrospray ionization (ESI) in positive mode was employed to effectively ionize atorvastatin and ezetimibe, allowing for sensitive detection through multiple reaction monitoring (MRM). The chromatographic and mass spectrometric conditions were optimized to ensure consistent retention times and peak areas, which are essential for routine pharmaceutical analysis.

9.2 Validation Outcomes

The method developed was assessed in line with ICH Q2(R1) guidelines and met all the acceptance criteria for validating analytical methods, including parameters such as specificity, linearity, accuracy, precision, sensitivity, robustness, and system suitability. The calibration curves demonstrated outstanding linearity ($r^2 > 0.999$) across the concentration range of 1–200 ng/mL, verifying the method's appropriateness for both assay and trace-level analysis. Recovery values between 98% and 102% highlighted the method's high accuracy in quantifying both analytes within tablet matrices.

Precision tests revealed %RSD values under 2%, reflecting excellent repeatability and intermediate precision. The method's superior sensitivity was further confirmed by the low LOD and LOQ values, making the LC-MS/MS method more suitable than traditional UV-based methods for detecting low levels of impurities and degradation products.

9.3 Insights from Forced Degradation Studies

Extensive forced degradation experiments were performed following ICH Q1A(R2) guidelines, examining acidic, alkaline, oxidative, thermal, photolytic, and neutral environments. These experiments yielded important information about the inherent stability and degradation patterns of atorvastatin and ezetimibe.

Atorvastatin showed notable degradation when exposed to acidic, oxidative, thermal, and photolytic stress, indicating that its ester and aromatic groups are prone to hydrolysis and oxidation. Conversely, ezetimibe experienced significant degradation under acidic and alkaline hydrolysis as well as oxidative conditions, highlighting the susceptibility of its azetidinone ring and ether bonds. Both drugs exhibited minimal degradation in neutral hydrolysis, suggesting a relatively stable nature in physiological aqueous environments.

The LC-MS/MS technique successfully distinguished the parent drugs from their degradation products, and peak purity analysis verified the absence of co-eluting impurities. The effective mass spectrometric identification of degradation products offered a mechanistic insight into degradation pathways, which is essential for optimizing formulations and predicting shelf life.

9.4 Stability-Indicating Capability

Among the significant accomplishments of this study is proving the stability-indicating capability of the newly developed method. This method successfully separated analytes from all possible degradation products generated under stress conditions, achieving sufficient resolution

without interference from excipients or solvent peaks. The mass balance observed during degradation studies was nearly 100%, demonstrating that all primary degradation products were identified and measured accurately. This validates the method's reliability for stability testing, impurity profiling, and regulatory quality control analysis of fixed-dose combination tablets containing atorvastatin calcium and ezetimibe.

9.5 Significance in Pharmaceutical Quality Control

The validated LC-MS/MS method provides notable benefits for pharmaceutical quality assurance, such as:

Exceptional selectivity and sensitivity for the concurrent quantification

Ability to detect and describe degradation products

Adherence to global regulatory standards

Appropriateness for regular quality control and stability assessments

Employing these sophisticated analytical methods is consistent with the contemporary Quality by Design (QbD) approach in pharmaceutical development, guaranteeing uniform product quality and safety across the entire product lifecycle.

9.6 Limitations and Future Scope

1. While the developed method showed outstanding results, it is important to acknowledge certain limitations and future directions: Future research could investigate the use of high-resolution mass spectrometry (HRMS) to thoroughly identify the structures of unknown degradation products. The method's scope could be broadened to include bioanalytical applications, such as determining the pharmacokinetic profiles of atorvastatin and ezetimibe in biological samples. Conducting long-term stability studies under both real-time and accelerated conditions could help link forced degradation behavior to actual shelf-life stability. Additionally, exploring green analytical chemistry techniques could help reduce solvent use and lessen environmental impact.

9.7 Final Conclusion

A new, fast, and dependable LC-MS/MS technique was effectively created and validated for measuring atorvastatin calcium and ezetimibe simultaneously in fixed-dose combination tablets. Extensive forced degradation tests, conducted under stress conditions recommended by ICH, verified the method's ability to indicate stability. This approach showed remarkable specificity, linearity, accuracy, precision, sensitivity, and robustness, making it ideal for routine quality control, stability evaluation, and regulatory submissions. The results of this research offer a solid analytical foundation for pharmaceutical companies and regulatory labs engaged in the development, production, and quality assessment of fixed-dose combination formulations containing atorvastatin calcium and ezetimibe.

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