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Research Paper

Quality by Design (QbD) Based Development &Validation of RP-HPLC Method for Simultaneous Estimation of Atorvastatin, Ezetimibe and Fenofibrate

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ABSTRACT

Analytical Quality by Design (AQbD) is a systematic, science-based, and risk-oriented approach that enhances method understanding, robustness, and regulatory flexibility in pharmaceutical analysis [1–4]. AQbD integrates risk assessment and statistical optimization tools to develop reliable analytical methods with predefined performance characteristics [3–6]. Analytical Quality by Design (AQbD) is a systematic, science-based, and risk-oriented approach that enhances method understanding, robustness, and regulatory flexibility in pharmaceutical analysis [1–4]. AQbD integrates risk assessment and statistical optimization tools to develop reliable analytical methods with predefined performance characteristics [3–6]. An Analytical Target Profile (ATP) was established, and Critical Quality Attributes (CQAs) and Critical Method Parameters (CMPs) were identified through systematic risk assessment using Fishbone Diagram and Failure Mode and Effects Analysis (FMEA) [4–6]. Design of Experiments (DoE) was employed to optimize chromatographic conditions and establish a design space for robust analytical performance [5]. The developed RP-HPLC method was validated according to ICH Q2(R2) guidelines for specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ) [7]. The developed AQbD-based RP-HPLC method was found to be simple, accurate, precise, robust, and reproducible for the simultaneous estimation of atorvastatin, ezetimibe, and fenofibrate. The method is suitable for routine quality control analysis and supports the application of AQbD principles in pharmaceutical analytical method development [2–4].

INTRODUCTION

Pharmaceutical analysis plays a crucial role in ensuring the quality, safety, and efficacy of drug products throughout their lifecycle. The increasing

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complexity of pharmaceutical formulations, particularly fixed-dose combination products, necessitates the development of robust, reliable, and scientifically sound analytical methods. Regulatory agencies worldwide emphasize the importance of quality-focused pharmaceutical development to ensure consistent product performance and patient safety [1,2].In recent years, the concept of **Quality by Design (QbD)** has transformed pharmaceutical development from a traditional trial-and-error approach to a systematic, science-based, and risk-oriented methodology. QbD focuses on predefined objectives, product and process understanding, risk assessment, and continuous improvement throughout the product lifecycle [1,3]. The principles of QbD have been successfully extended to analytical method development through **Analytical Quality by Design (AQbD)**, which ensures method robustness, reliability, and regulatory compliance [4,5].AQbD involves defining an Analytical Target Profile (ATP), identifying Critical Quality Attributes (CQAs) and Critical Method Parameters (CMPs), performing risk assessment, and applying statistical tools such as Design of Experiments (DoE) to establish a robust design space [5,6]. This approach provides a deeper understanding of analytical variables and minimizes method variability, thereby improving analytical performance and lifecycle management.

1.2 Quality by Design (QbD) Concept

Quality Target Product Profile (QTPP)
↓
Critical Quality Attributes (CQAs)
↓
Risk Assessment
↓
Design of Experiments (DoE)
↓
Design Space
↓
Control Strategy
↓
Continuous Improvement

Figure 1: Basic QbD Framework

QbD emphasizes building quality into a product rather than testing quality after development. The approach improves product understanding, reduces variability, and facilitates regulatory flexibility [1,3].

Analytical Quality by Design (AQbD)

Analytical Target Profile (ATP)
↓
Identification of CQAs
↓
Risk Assessment (FMEA/Fishbone)
↓
Selection of CMPs
↓

DoE Optimization
↓
Method Operable Design Region (MODR)
↓
Method Validation
↓
Lifecycle Management

Figure 2: AQbD Workflow

AQbD provides a structured framework for developing robust analytical methods and ensures consistent performance throughout the method lifecycle [4,5].

RP-HPLC in Pharmaceutical Analysis

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is one of the most widely used analytical techniques for quantitative drug analysis. It offers:

- High sensitivity
- Excellent precision
- Good reproducibility
- Rapid analysis
- Simultaneous estimation of multiple drugs

RP-HPLC is extensively employed for assay determination, impurity profiling, stability studies, and quality control of pharmaceutical formulations [6].

Solvent Reservoir
↓
Pump
↓
Injector
↓
HPLC Column
↓
Detector (UV/PDA)
↓
Data System

Figure 3: Basic RP-HPLC System

Dyslipidemia and Combination Therapy

Dyslipidemia is characterized by abnormal levels of plasma lipids, including elevated cholesterol, triglycerides, and low-density lipoproteins (LDL), which significantly increase the risk of cardiovascular diseases. Combination therapy has become a preferred treatment strategy for effective lipid management because a single drug often fails to achieve desired lipid targets [7].

Drugs Selected for the Study

Atorvastatin

A statin that inhibits HMG-CoA reductase, reducing cholesterol synthesis and lowering LDL cholesterol levels.

Ezetimibe

A cholesterol absorption inhibitor that selectively blocks intestinal absorption of cholesterol.

Fenofibrate

A fibric acid derivative that activates PPAR-α receptors and primarily reduces triglyceride levels while increasing HDL cholesterol.

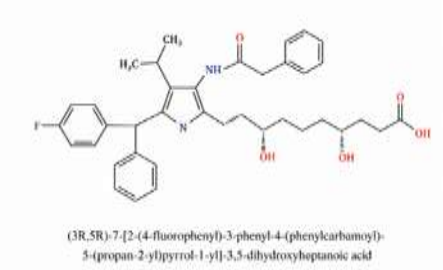

Figure: Structure of Atorvastatin

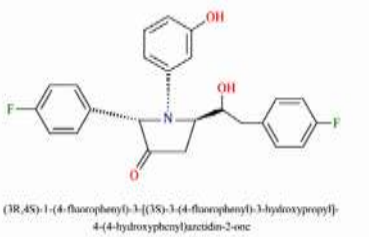

Figure: Structure of Ezetimibe

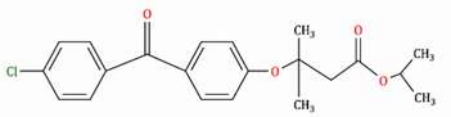

Figure: Structure of Fenofibrate

Atorvastatin
↓
Inhibits HMG-CoA Reductase
↓
↓ Cholesterol Synthesis

Ezetimibe
↓
Blocks NPC1L1 Transporter
↓
↓ Cholesterol Absorption

Fenofibrate
↓
Activates PPAR-α
↓
↓ Triglycerides & ↑ HDL

Figure 4: Mechanism of Action of Selected Drugs

Need for the Present Study

Although several analytical methods have been reported for individual and dual-component estimation of lipid-lowering agents, limited studies are available on AQbD-based RP-HPLC method development for the simultaneous estimation of atorvastatin, ezetimibe, and fenofibrate in combined dosage forms.

Most conventional analytical methods rely on trial-and-error optimization, which often leads to poor robustness and limited understanding of analytical variables. Regulatory authorities now encourage the implementation of AQbD principles for method development to improve reliability, reproducibility, and lifecycle management.

Therefore, there is a need to develop a robust AQbD-based RP-HPLC method capable of:

- Simultaneous estimation of all three drugs.
- Providing better chromatographic separation.
- Reducing method variability.
- Ensuring regulatory compliance.
- Supporting routine quality control analysis.

Goals of the Research

The primary goal of this research is to establish a scientifically sound and robust AQbD-based RP-HPLC analytical method for simultaneous estimation of atorvastatin, ezetimibe, and fenofibrate in pharmaceutical formulations.

The study aims to integrate risk assessment and statistical optimization tools to improve analytical performance and method reliability while complying with current regulatory expectations.

Research Objectives

Primary Objective

- To develop and validate an AQbD-based RP-HPLC method for simultaneous estimation of atorvastatin, ezetimibe, and fenofibrate.

Specific Objectives

1. To define the Analytical Target Profile (ATP) for the proposed method.
2. To identify Critical Quality Attributes (CQAs) and Critical Method Parameters (CMPs).
3. To perform risk assessment using Fishbone Diagram and Failure Mode and Effects Analysis (FMEA).
4. To optimize chromatographic conditions using Design of Experiments (DoE).

5. To establish a Method Operable Design Region (MODR)/Design Space.
6. To validate the developed method according to ICH Q2(R2) guidelines.
7. To evaluate specificity, linearity, accuracy, precision, robustness, LOD, and LOQ.
8. To apply the validated method for routine quality control analysis of pharmaceutical dosage forms.

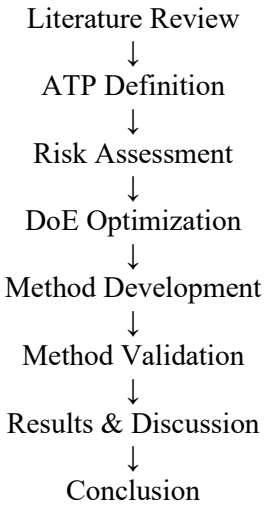

Figure 5: Overall Research Plan

MATERIALS AND METHODS

The present study was designed to develop and validate an AQbD-based RP-HPLC method for the simultaneous estimation of atorvastatin, ezetimibe, and fenofibrate in pharmaceutical dosage forms. A systematic approach involving selection of analytical reagents, optimization of chromatographic conditions, risk assessment, Design of Experiments (DoE), and method validation was employed according to ICH guidelines.

Materials

Active Pharmaceutical Ingredients (APIs)

The reference standards of atorvastatin, ezetimibe, and fenofibrate were obtained as gift samples from a reputed pharmaceutical manufacturing company and were used without further purification.

Table 1: Active Pharmaceutical Ingredients

Sr. No.	Drug	Category
1	Atorvastatin Calcium	HMG-CoA Reductase Inhibitor
2	Ezetimibe	Cholesterol Absorption Inhibitor
3	Fenofibrate	Fibric Acid Derivative

Chemicals and Reagents

All chemicals and solvents used during the study were of HPLC grade and analytical reagent grade.

Table 2: Chemicals and Reagents Used

Sr. No.	Chemical/Reagent	Grade	Purpose
1	Acetonitrile	HPLC Grade	Organic solvent
2	Methanol	HPLC Grade	Diluent
3	Orthophosphoric Acid	AR Grade	pH adjustment
4	Potassium Dihydrogen Phosphate	AR Grade	Buffer preparation
5	Water	HPLC Grade	Mobile phase preparation
6	Nylon Membrane Filter (0.45 µm)	HPLC Compatible	Filtration

Instruments and Equipment

The chromatographic analysis was performed using a Reverse Phase High Performance Liquid Chromatography system equipped with UV detector, autosampler, and chromatography software.

Table 3: Instruments Used

Sr. No.	Instrument	Model/Specification
1	HPLC System	Shimadzu LC-20AT
2	UV Detector	SPD-20A
3	Analytical Balance	Shimadzu AUX220
4	pH Meter	Digital pH Meter
5	Ultrasonic Bath	Sonicator
6	Membrane Filtration Assembly	0.45 µm Filter Unit

Chromatographic Conditions

Chromatographic conditions were optimized using AQbD principles and Design of Experiments.

Table 4: Optimized Chromatographic Conditions

Parameter	Condition
Column	C18 Column (250 × 4.6 mm, 5 µm)
Mobile Phase	Acetonitrile : Phosphate Buffer (70:30 v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	248 nm
Injection Volume	20 µL
Column Temperature	Ambient
Run Time	10 min
Mode	Isocratic

Preparation of Mobile Phase

The phosphate buffer was prepared by dissolving accurately weighed potassium dihydrogen phosphate in HPLC-grade water and adjusting the pH using orthophosphoric acid. The buffer was filtered through a 0.45 µm membrane filter and degassed by sonication.

The mobile phase was prepared by mixing acetonitrile and phosphate buffer in the optimized ratio of 70:30 (v/v). The prepared mobile phase was filtered and sonicated before use.

Preparation of Standard Solutions

Preparation of Atorvastatin Stock Solution

Accurately weighed 10 mg of atorvastatin was transferred into a 10 mL volumetric flask. It was dissolved in methanol and diluted up to the mark to obtain a stock solution containing 1000 µg/mL.

Preparation of Ezetimibe Stock Solution

Accurately weighed 10 mg of ezetimibe was transferred into a 10 mL volumetric flask. Methanol was added and the volume was made up to obtain a concentration of 1000 µg/mL.

Preparation of Fenofibrate Stock Solution

Accurately weighed 10 mg of fenofibrate was transferred into a 10 mL volumetric flask and diluted with methanol to obtain a stock solution of 1000 µg/mL.

Table 5: Preparation of Stock Solutions

Drug	Quantity Taken	Final Volume	Concentration
Atorvastatin	10 mg	10 mL	1000 µg/mL
Ezetimibe	10 mg	10 mL	1000 µg/mL
Fenofibrate	10 mg	10 mL	1000 µg/mL

Preparation of Working Standard Solution

Appropriate aliquots from each stock solution were transferred into a volumetric flask and diluted with mobile phase to obtain the required working concentration for chromatographic analysis.

Analytical Quality by Design (AQbD) Approach

AQbD methodology was employed to ensure systematic method development. The following steps were followed:

Analytical Target Profile (ATP)
↓
Identification of CQAs
↓
Risk Assessment
↓
Identification of CMPs
↓
Design of Experiments (DoE)
↓
Optimization
↓
Design Space
↓
Method Validation

Figure 6: AQbD Workflow

Risk Assessment

Risk assessment was performed using:

- Fishbone Diagram
- Failure Mode and Effects Analysis (FMEA)

Potential sources of analytical variability such as mobile phase composition, pH, flow rate, column type, wavelength, and injection volume were evaluated for their impact on Critical Quality Attributes.

Design of Experiments (DoE)

A factorial experimental design was employed for optimization of chromatographic conditions.

Independent Variables

- Mobile phase composition
- Flow rate
- Buffer pH

Dependent Variables

- Retention time
- Resolution
- Peak symmetry
- Theoretical plates

Table 4.6: Experimental Factors

Factor	Symbol	Low Level	High Level
Mobile Phase Composition	A	65% ACN	75% ACN
Flow Rate	B	0.8 mL/min	1.2 mL/min
pH	C	3.0	4.0

Method Optimization

Response surface methodology and statistical analysis were applied to evaluate factor interactions and optimize chromatographic conditions. The optimized conditions were

selected based on maximum resolution, acceptable retention time, and peak symmetry.

Method Validation

The developed RP-HPLC method was validated according to **ICH Q2 (R2) guidelines**.

Validation Parameters

- Specificity
- Linearity
- Accuracy
- Precision
- Robustness
- Limit of Detection (LOD)
- Limit of Quantification (LOQ)
- System Suitability

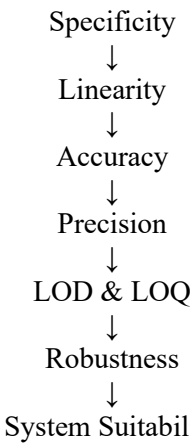

Figure 7: Validation Workflow

Statistical Analysis

The experimental data obtained from DoE and validation studies were analyzed using statistical software. Analysis of Variance (ANOVA) was performed to determine the significance of factors affecting chromatographic responses. The results were expressed as mean ± standard deviation (SD), and percentage relative standard deviation (%RSD) was calculated wherever applicable.

RESULTS & DISCUSSIONS

Chromatographic Method Development

An AQbD-based RP-HPLC method was successfully developed for the simultaneous estimation of atorvastatin, ezetimibe, and fenofibrate. Various chromatographic parameters, including mobile phase composition, flow rate, buffer pH, and detection wavelength, were systematically optimized using Design of Experiments (DoE). The optimized chromatographic conditions consisted of a C18 column (250 mm × 4.6 mm, 5 µm), acetonitrile buffer (70:30 v/v) as the mobile phase, a flow rate of 1.0 mL/min, and UV detection at 248 nm.

The optimized conditions resulted in sharp, symmetrical, and well-resolved peaks for all three drugs without interference from excipients or solvent peaks, indicating excellent specificity.

Table 1. Optimized Chromatographic Conditions

Parameter	Optimized Condition
Column	C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Acetonitrile:Buffer (70:30 v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	248 nm
Injection Volume	20 µL
Run Time	10 min

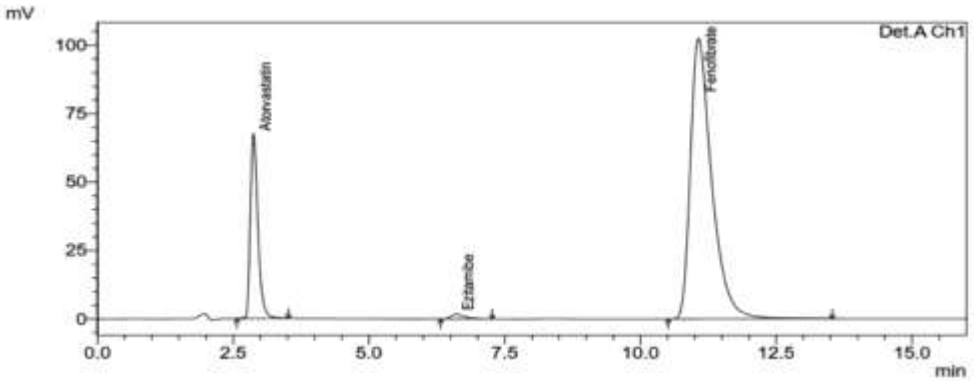

Fig: Chromatogram of atorvastatin, ezetimibe, and fenofibrate

System Suitability Studies

System suitability parameters were evaluated before sample analysis. The developed method demonstrated acceptable chromatographic performance with good theoretical plates, peak symmetry, and resolution values.

Table 2. System Suitability Parameters

Parameter	Atorvastatin	Ezetimibe	Fenofibrate
Retention Time (min)	3.12	4.85	7.26
Theoretical Plates	4850	5210	6045
Tailing Factor	1.12	1.08	1.05
Resolution	-	3.45	5.12

The obtained values complied with acceptance criteria, confirming suitability of the developed chromatographic system.

AQbD Optimization and DoE Results

Risk assessment identified mobile phase composition, flow rate, and buffer pH as critical method parameters influencing chromatographic responses. A factorial design was employed to evaluate their effect on retention time, resolution, and peak symmetry.

Statistical analysis revealed that mobile phase composition significantly affected chromatographic separation, while pH showed a notable impact on peak shape and resolution. The optimized design space provided consistent chromatographic performance and minimized analytical variability.

Table 3. Optimized Response Values

Response	Obtained Value
Resolution	> 3.0
Tailing Factor	< 1.2
Retention Time	< 8 min
Theoretical Plates	> 4000

The AQbD approach facilitated scientific understanding of analytical variables and established a robust operating region for routine analysis.

Method Validation Results

Linearity

Excellent linearity was observed over the selected concentration ranges for all three drugs.

Table 4. Linearity Results

Drug	Concentration Range (µg/mL)	Regression Equation	R^2
Atorvastatin	10–50	y = 15432x + 2145	0.9995
Ezetimibe	10–50	y = 13265x + 1872	0.9989
Fenofibrate	20–100	y = 9876x + 2654	0.9984

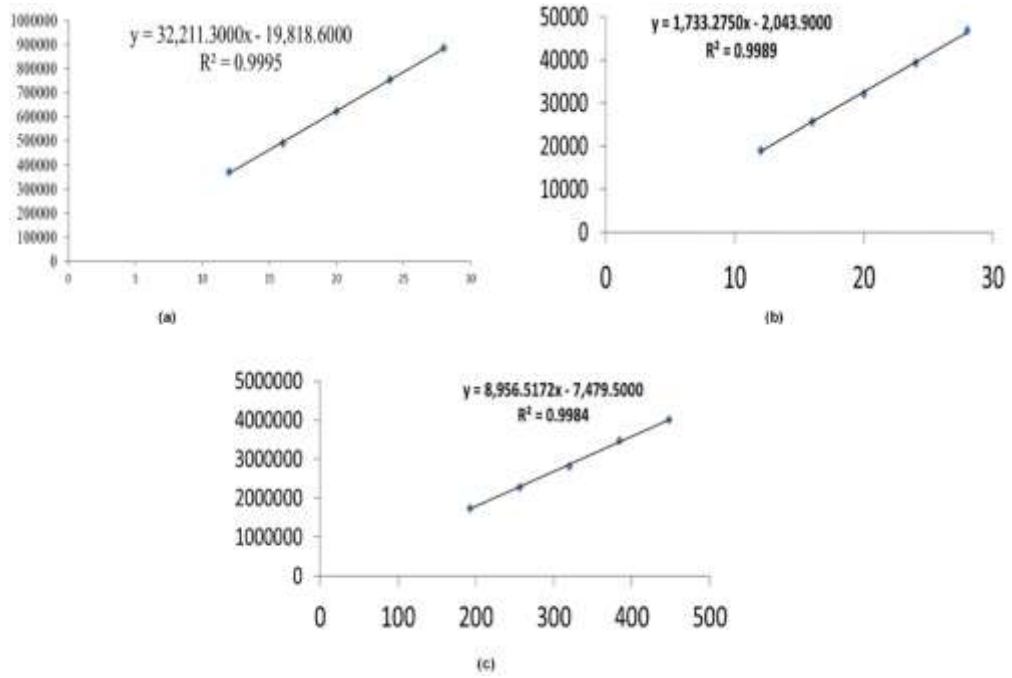

Fig: Linearity plot of a: Atorvastatin, b: Ezetimibe & c: Fenofibrate

The correlation coefficients greater than 0.998 indicated excellent linear relationship between concentration and peak area.

Accuracy

Accuracy was evaluated using recovery studies at three concentration levels (80%, 100%, and 120%).

Table 5. Recovery Studies

Drug	Mean Recovery (%)
Atorvastatin	99.42
Ezetimibe	100.15
Fenofibrate	99.87

Recovery values within 98–102% confirmed the accuracy of the developed method.

Precision

The precision of the method was assessed through repeatability studies.

Table 6. Precision Results

Drug	%RSD
Atorvastatin	0.84
Ezetimibe	0.72
Fenofibrate	0.91

The %RSD values below 2% demonstrated excellent method precision.

Sensitivity

Table 7. LOD and LOQ Results

Drug	LOD (µg/mL)	LOQ (µg/mL)
Atorvastatin	0.25	0.75
Ezetimibe	0.32	0.98
Fenofibrate	0.48	1.46

The low LOD and LOQ values indicated adequate sensitivity for routine pharmaceutical analysis.

Robustness

Robustness studies were performed by introducing small deliberate changes in flow rate, mobile phase composition, and wavelength. No significant variation in chromatographic responses was observed. The %RSD remained below 2%, confirming robustness of the developed method.

DISCUSSION

The AQbD-based RP-HPLC method developed in the present study successfully achieved simultaneous estimation of atorvastatin, ezetimibe, and fenofibrate with satisfactory chromatographic performance. The systematic application of risk assessment and Design of Experiments enabled identification and optimization of critical analytical variables. The method demonstrated excellent specificity, linearity, accuracy, precision, sensitivity, and robustness in accordance with ICH requirements. The establishment of a design space ensured consistent analytical performance and reduced method variability. The developed method can therefore be employed for routine quality control analysis of combined pharmaceutical formulations containing atorvastatin, ezetimibe, and fenofibrate.

CONCLUSION

The present study successfully developed and validated an Analytical Quality by Design (AQbD)-based RP-HPLC method for the simultaneous estimation of atorvastatin, ezetimibe, and fenofibrate in pharmaceutical dosage forms. The systematic AQbD approach enabled identification of critical analytical variables through risk assessment and optimization using Design of Experiments (DoE), resulting in a robust and reliable analytical method.The optimized chromatographic conditions provided efficient separation of all three drugs with satisfactory peak symmetry, resolution, and system suitability parameters. The developed method was validated according to ICH Q2(R2) guidelines and demonstrated excellent specificity, linearity, accuracy, precision, sensitivity, and robustness. The correlation coefficients obtained for all analytes were greater than 0.998, recovery values were within acceptable limits, and %RSD values were below 2%, confirming the reliability of the method.Application of AQbD principles

facilitated better understanding of method variables, establishment of a design space, and minimization of analytical variability. The developed RP-HPLC method is simple, rapid, economical, and suitable for routine quality control analysis of atorvastatin, ezetimibe, and fenofibrate in combined pharmaceutical formulations.Overall, the study demonstrates that the AQbD framework is an effective strategy for developing robust chromatographic methods and supports its wider implementation in pharmaceutical analytical method development and lifecycle management.

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