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Original Article

QBD ASSISTED STABILITY INDICATING RP-HPLC METHOD FOR ASSAY OF BEMPEDOIC ACID AND EZETIMIBE IN FORMULATION

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ABSTRACT

Objective: Bempedoic acid belongs to a class of drugs known as adenosine triphosphate citrate lyase inhibitors. Clinically, it's mainly prescribed for managing atherosclerotic cardiovascular disease, particularly in patients with hypercholesterolemia. Ezetimibe is a highly selective 2-azetidione derivative that works by blocking cholesterol absorption at the level of enterocytes, the cells lining the intestines. The study is to develop a Quality by Design based stability indicating RP-HPLC method for their simultaneous estimation in tablet dosage forms.

Methods: Chromatographic separation was achieved using a Zorbax SB C8 column (250 x 4.6 mm, 5 µm) under isocratic conditions. The mobile phase was a blend of two solutions: Solution A-a mixture of pH 2.5 buffer and methanol (80:20 v/v), and Solution B-a mix of acetonitrile, water, and methanol (70:20:10 v/v). These were combined in a 45:55 (v/v) ratio and mixed thoroughly. The flow rate was set at 1.2 ml/min, and detection was carried out at 215 nm for Bempedoic acid and 232 nm for Ezetimibe.

Results: The retention times were observed at 6.5 min for Bempedoic acid and 4.2 min for Ezetimibe. The Linearity was found to be 0.9999 and 0.9998 for Bempedoic acid and Ezetimibe respectively. The method is highly Precise and the Recovery was found to be 100.4%and 99.6%for Bempedoic acid and Ezetimibe respectively.

Conclusion: In conclusion, the RP-HPLC method developed using an Analytical QbD framework proved to be precise, sensitive, and highly reliable for the simultaneous estimation of Bempedoic acid and Ezetimibe in tablet formulations. The systematic evaluation of method parameters ensured optimal performance, while high recovery rates confirmed the absence of excipient interference. Validation outcomes demonstrated strong linearity, accuracy, precision, and robustness, establishing the method's suitability for routine quality control and pharmaceutical analysis.

Keywords: Bempedoic acid, Ezetimibe, Method validation, Quality by design, Validation, Central composite design

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INTRODUCTION

Heart disease remains a leading global cause of illness and death, especially among individuals with obesity, where abnormal cholesterol particularly elevated Low-density lipoprotein cholesterol (LDL-C) is a key modifiable risk factor. High Low-density Lipoprotein Cholesterol (LDL-C) levels drive atherosclerosis, the main cause of coronary artery disease. Extensive studies have consistently identified LDL-C as a major contributor to cardiovascular risk. This risk is even greater in individuals with type 2 diabetes and hypercholesterolemia [1, 2]. Statins are the primary treatment for lowering LDL-C, but they may not always achieve target levels or be well tolerated at high doses. Therefore, non-statin therapies are often needed to manage cholesterol effectively [3, 4]. Bempedoic acid (BDA) is a novel oral ATP-citrate lyase inhibitor that lowers LDL-C by blocking cholesterol synthesis upstream of HMG-CoA reductase and enhancing LDL receptor expression. Activated in the liver, it reduces muscle related side effects and offers a once-daily, first-in-class option for treating hypercholesterolemia [5-8]. Ezetimibe (EZE) is a lipid-lowering drug that reduces LDL-C by selectively blocking intestinal absorption of cholesterol and phytosterols, offering a unique alternative to statin therapy [9-11].

A critical analysis reveals significant gaps in current analytical methods for simultaneous estimation of BDA and EZE, which typically rely on traditional optimization approaches without comprehensive risk assessment or systematic evaluation of parameter interactions. Despite various published RP-HPLC and RP-UPLC methods, no studies have implemented a Quality by Design (QbD) approach or factorial design methodologies. Prior research, such as by Krishna (2022) [12], Suresh *et al.* (2024) [13], Vanga *et al.* (2024) [14], Yarra *et al.* (2021) [15], and Nilakh *et al.* (2024) [16], omitted structured analysis of critical factors, highlighting a clear methodological shortcoming. Koppisetty *et al.* (2023) [17], Dadi *et al.* (2024) [18], Panchamarthy *et al.* (2025) [19], Vasudha *et al.* (2024) [20], and Challa *et al.* (2025) [21] includes structured evaluation of critical factors, indicating methodological advancements through the application of QbD principles. The current study addresses this specific gap by developing a validated, QbD-assisted stability-indicating RP-HPLC method compliant with ICH guidelines [22-24] significantly enhancing robustness, analytical reliability, and overall pharmaceutical quality assurance for formulations containing these two important therapeutic agents.

MATERIALS AND METHODS

Materials

BDA was obtained from Lee Pharma Private Limited, Telangana, India; while EZE (fig. 1) was sourced from Lupin Limited, Maharashtra. All other Analytical grade chemicals were purchased from Merck Limited, Hyderabad. The formulation NEXLIZET (containing 180 mg of BDA and 10 mg of EZE), was acquired from a local pharmacy for the study. The buffer solution for the mobile phase was prepared with Milli-Q water.

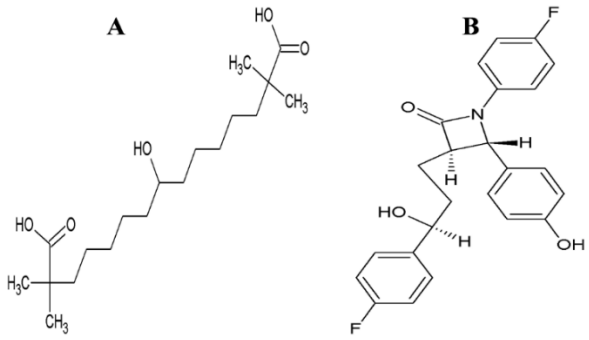

Fig. 1: Structure of BDA (A) EZE (B)

Statistical assessment

The statistical analysis was carried out using Microsoft Excel 2007, which was used to calculate the mean, coefficient of variation (CV), relative standard deviation (RSD), and perform linear regression. For more advanced modeling, Design Expert software (version 13.0.5.0) was used to run ANOVA, generate 3D response surface plots, and fine-tune the optimization of the response variables.

Instrumentation and chromatographic conditions of HPLC

The analysis was carried out using a HPLC system which came with a UV-PDA detector (Shimadzu 2030 series) having a wavelength of 215 nm and 232 nm for BDA and EZE respectively. It was operated through Lab Solutions Software. This setup included an auto-sampler, a degasser, and a temperature-controlled column compartment to ensure consistent performance. For chromatographic separation, a Zorbax SB C8 column (250×4.6 mm, 5 µm) was used with a mobile phase composed of Solution A and Solution B mixed in a 45:55 (v/v) ratio. This setup provided excellent peak resolution and sensitivity. The flow rate was maintained at 1.2 ml/min. Analytical balance (Mettler Toledo-ML303T), pH meter (Hanna), Sonicator (PCI Analytics Private Limited), 0.45µm Nylon filters (Axiva) were used. Each run involved a 20 µl** sample injection, and the entire HPLC system was operated under ambient laboratory conditions [25].

Preparation of buffer solution

Approximately 6.8 g of potassium dihydrogen orthophosphate was dissolved in 1000 ml of water, and the pH was adjusted to 2.50 using a diluted phosphoric acid solution.

Preparation of mobile phase

Solution A (a mixture of pH 2.5 buffer and methanol in an 80:20 v/v ratio) was combined with Solution B (a blend of ACN, water, and methanol in a 70:20:10 v/v ratio) in a 45:55 v/v proportion. The mixture was then thoroughly mixed.

Selection of diluent

During diluent selection, water, acetonitrile and glacial acetic acid were evaluated because of their solubility, polarity and pH-dependent solubility characteristics. Initial experiments with low proportions of acetonitrile produced asymmetric peak shapes and peak distortion for BDA. Higher acetonitrile reduced peak distortion for the relatively less polar BDA and lowering to injection volume to 20 µl** minimized overloading and improved peak symmetry, efficiency and optimum area response were achieved for both BDA and EZE. Based on these results, the diluent was finalized as water: acetonitrile: glacial acetic acid in a 40:60:1 (v/v/v) ratio.

Diluent

Mixture of water, acetonitrile, and glacial acetic acid in a 40:60:1 v/v ratio.

Preparation of standard stock solution of BDA

Approximately 54 mg of BDA working standard was accurately weighed and transferred into a 100 ml volumetric flask. Around 10 ml of diluent was added, and the solution was sonicated for about 3 min to ensure complete dissolution, then diluted to the mark with the same diluent.

Preparation of standard stock solution of EZE

Approximately 25 mg of EZE working standard was weighed and transferred into a 25 ml volumetric flask. About 10 ml of diluent was added, and the mixture was sonicated for roughly 3 min to achieve complete dissolution. The solution was then brought up to volume with the same diluent.

Preparation of standard solutions

Exactly 25 mg of pure BDA was carefully weighed and transferred into separate 25 ml volumetric flasks. About 18 ml of diluent was added to each, and the mixtures were sonicated for around 3 min to ensure complete dissolution. After that, the solutions were topped up to the mark with the same diluent, giving individual stock solutions. From these, a series of dilutions were made using the mobile phase to prepare working standard solutions containing 538.1 µg/ml of BDA and 38.7 µg/ml of EZE, respectively. Finally, the solutions were passed through 0.45µm Nylon filters and labeled as standard stock solutions.

Preparation of sample solution

To analyze NEXLIZET tablets (containing 180 mg of BDA and 10 mg of EZE) [26] ten tablets were accurately weighed and finely powdered using a glass mortar and pestle. A portion of this powder, equivalent to 25 mg each of BDA and EZE, was transferred into a 25 ml volumetric flask. About 18 ml of diluent was added, and the mixture was sonicated for 20 min to ensure thorough dissolution. The resulting solution was then diluted with the same diluent to achieve final concentrations of 538.1 µg/ml for BDA and 38.7 µg/ml for EZE. To prepare for recovery studies, this solution was spiked with additional BDA and EZE to cover a range from 50% to 200% of the specified concentration levels. The spiked samples were sonicated again for 15 min to ensure complete mixing. After filtration through a 0.45µm nylon membrane, the prepared solution was subjected to HPLC

analysis. The concentrations of BDA and EZE were quantified using a Central Composite Design (CCD) [27] approach to evaluate method performance and robustness.

Analytical quality by design-based method development

To fine-tune the chromatographic conditions, Response Surface Methodology using Central Composite Design (CCD) was applied. This approach helped evaluate not only the main effects but also the interactions and quadratic influences of the selected variables on key responses: the number of theoretical plates (N) for BDA (R-1), the tailing factor (Tf) for EZE (R-2), the number of N for EZE (R-3), and the resolution (RS) between BDA and EZE (R-4). Two independent variables organic phase solution B (F-1) and Flow Rate (F-2)—were selected based on prior scientific knowledge and insights from preliminary trials. Their nominal values were set at 55% and 1.2 ml/min, respectively. The experimental design included a total of 13 runs to explore the response space efficiently. The resulting models were used to generate 2D response surface and contour plots, which made it easier to visualize the behavior of each factor and identify optimal conditions. An ANOVA was performed to determine the statistical significance of the variables. To ensure unbiased results and avoid any systematic trends in the residuals, all experimental runs were carried out in a randomized order.

Method validation

In line with ICH guidelines, system suitability tests and method validation covering parameters like specificity, linearity, accuracy, precision, and robustness were performed following established standard procedures [28].

RESULTS AND DISCUSSION

Refining the method through statistical data analysis and validating the model, the method was fine-tuned using statistical analysis, followed by validation of the predictive model. Based on the CCD design, each experimental run involved injecting a spiked sample while varying two key factors: the concentration of Organic Phase B (F-1), ranging from 20% to 80% v/v, and the flow rate (F-2), which varied between 0.5 ml/min and 1.5 ml/min. A summary of the experimental results is presented in table 1. The model turned out to be statistically significant, with a sequential p-value below 0.05, indicating good predictive strength. Importantly, the lack-of-fit test was not significant ($p>0.05$), suggesting the model fits the data well. The coefficient of determination (R^2) was above 0.4, and the difference between the adjusted R^2 and predicted R^2 stayed within 0.2, further supporting the reliability of the model.

Table 1: Experimental design domain (CCD) for each run with responses

Run	F-1	F-2	R-1	R-2	R-3	R-4
	A: Solution B (organic)	B: Flow rate (ml/min)	BDA (*N*)	EZE (*Tf*)	EZE (*N*)	*Rs*
1	30	0.7	1587	1.94	1372	18.12
2	55	1.2	10958	1.096	10320	13.77
3	30	1.5	4824	1.54	4401	17.68
4	20	1.1	1233	1.98	1021	20.24
5	70	1.5	15468	0.987	14513	3.12
6	50	0.5	4785	1.65	4547	13.8
7	80	1.2	16892	1.03	15850	0.89
8	55	1.2	10814	1.093	10147	13.72
9	55	1.2	10423	1.094	10298	13.71
10	55	1.2	10723	1.095	10471	13.71
11	55	1.2	10677	1.092	10276	13.74
12	55	1.5	13574	1.05	13114	10.82
13	70	0.7	13957	1.24	13161	8.64

Overall, these metrics confirm a strong match between the predicted and observed outcomes, validating the accuracy of the model under the tested conditions. Statistical insights from table 2 back up the model's robustness and its suitability for identifying optimal chromatographic conditions. The equations explain how the coded factors influence the responses: BDA(N): 8,911.23+5,421.02 * A+1,937.42 * B; Tf of BDA: 1.19565+-0.311226 * A+-0.166433 * B+0.0366047 * AB+0.142295 * A^2+0.089127 * B^2; EZE (N): 8,451.76+5,169.05 * A+1,864.56 * B; Rs between BDA and EZE: 15.9595+-6.07276 * A+-1.43667 * B+-1.27025 * AB+-2.18442 * A^2+-1.88718 * B^2. It was observed that a reduction in the proportion of organic solvent B in the mobile phase led to decreases in responses R-1 and R-3, whereas a decrease in flow rate resulted in increases in responses R-2 and R-4. To better understand how the variables interact, both contour and 3D response surface plots were generated (fig. 2 and fig. 3). Fig. 2A (R-1), 2B (R-2), 2C (R-3) and 2D (R-4) demonstrated a quadratic relationship between the input factors and their respective responses.

Table 2: ANOVA for quadratic models

1	2	3	4	5	6	7	8	9	10
R-1	870.85	9686	8.99	0.9754	0.9705	0.9468	40.03	<0.0001	0.1137
R-2	0.005	1.30	0.3922	0.9999	0.9998	0.9992	289.93	<0.0001	0.1647
R-3	885.82	9192	9.64	0.9722	0.9667	0.9418	37.53	<0.0001	0.1368
R-4	0.1046	12.46	0.8393	0.9998	0.9996	0.9983	269.89	<0.0001	0.0978

1: Response; 2: Standard deviation; 3: Mean; 4: %C. V; 5: R^2; 6: Adjusted R^2; 7: Predicted R^2; 8: Adequate Precision; 9: Sequential p value; 10: Lack of fit p value

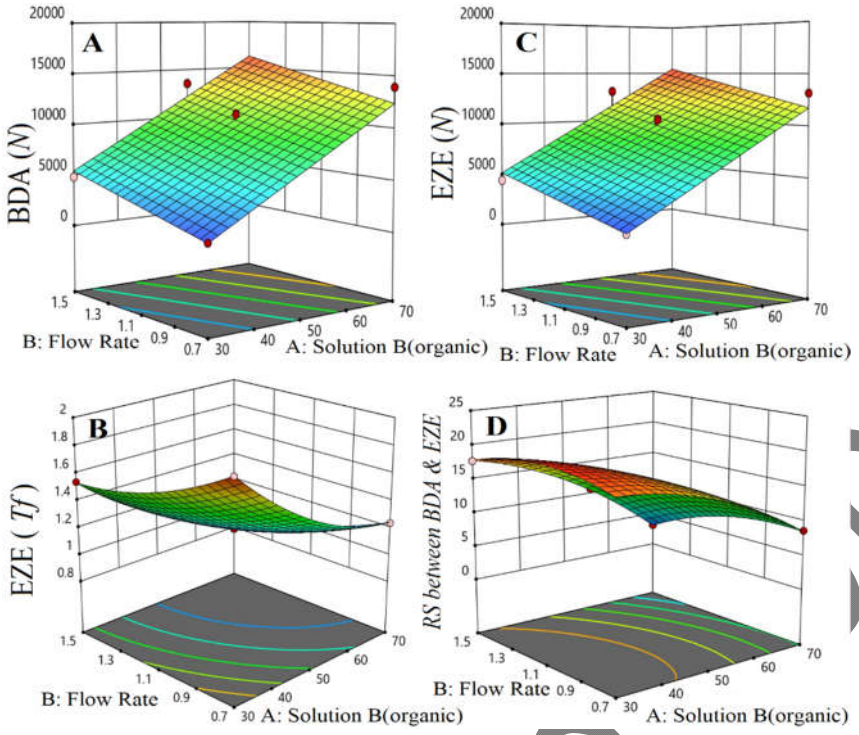

Fig. 2: 3D counter plots showing the effect of factors on responses A)*N* of BDA, B) *Tf* of EZE, C) *N* of EZE D) *Rs* between BDA and EZE

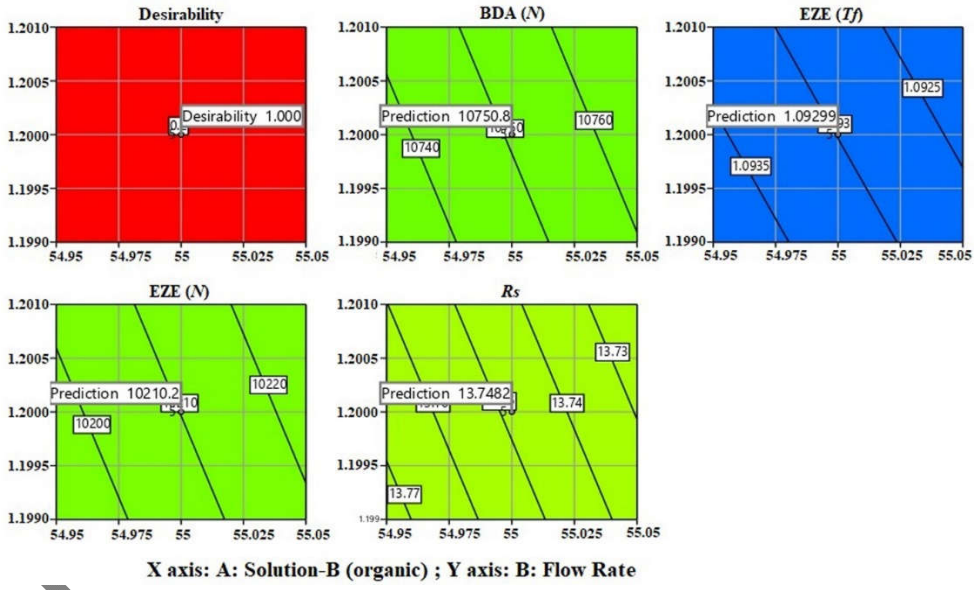

Fig. 3: 2D plots for desirability and responses

Design space

The main objective of method development is to define a reliable design space [22-24] under laboratory temperature conditions that ensures consistent performance. For this study, the target for Response-1 and Response-3 was to achieve an N of at least 7000 for both BDA and EZE. For Response-2, the ideal tailing factor (Tf) was set between 1.0 and 1.1, while for Response-4, the resolution (RS) between BDA and EZE needed to fall within the range of 13 to 15. Fig. 4 maps out the design space generated from the CCD experiments based on these criteria. The results clearly indicate that the desired responses can't be achieved when the concentration of organic phase solution-B drops below 45%. Similarly, using a flow rate under 0.8 ml/min leads to poor performance, signaling that the method isn't effective under those conditions.

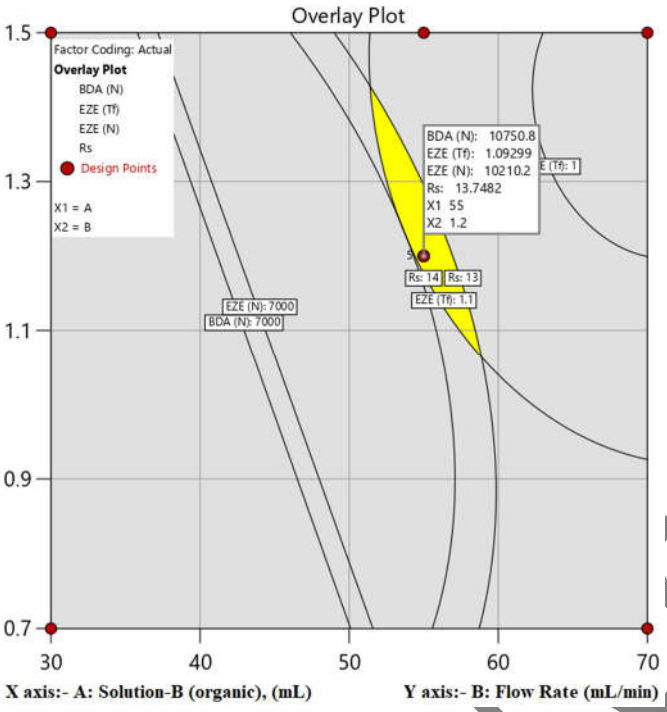

Fig. 4: Design space for a CCD experiment

Optimized method

After reviewing the DOE results, the optimal chromatographic conditions were found to include a mobile phase with 55% of Organic Solution B and a flow rate of 1.2 ml/min. The design space around these optimized conditions proved to be quite flexible, with a well-defined operable range between 51.5% and 59.0% for organic Solution-B and 1.0 to 1.4 ml/min for flow rate and theoretical plates greater than 7000. The responses remained stable regardless of the combined effect of the selected variables, indicating that the method is robust. This confirms that the optimized parameters fall comfortably within the Method Operable Design Region (MODR), supporting the reliability and quality of the developed method.

The optimized method utilized a mobile phase consisting of Solution A and Solution B in a 45:55 (v/v) ratio, prepared and analyzed under laboratory temperature conditions. This setup provided good peak resolution and sensitivity. The flow rate was maintained at 1.2 ml/min. The experimental BDA (N) exhibited a value of 10,958 with a Tailing factor of 1.096, while EZE (N) showed 10,320. The resolution between BDA and EZE was found to be 13.77. The comparison between the predicted means and actual experimental values, along with the corresponding error margins for each response, is provided in table 3.

Table 3: Predicted value versus experimental value

Response	Predicted mean	Experimental mean
R-1	9686	10958
R-2	1.30	1.096
R-3	9192	10320
R-4	12.46	13.77

System suitability study

Once the optimal chromatographic conditions were finalized, system suitability parameters were evaluated to ensure everything was within the acceptable limits. Each parameter met the required criteria, confirming that the method was well-suited for the intended analysis. The detailed results are presented in table 4.

Method validation

Based on the UV spectral analysis, both BDA and EZE showed strong absorbance at 215 nm and 232 nm, respectively. As a result, these wavelengths were selected for the analytical method. Fig. 5 displays the chromatograms for both individual standards and the formulation samples. The optimized method was then validated in accordance with ICH Q2 (R1) guidelines, covering key parameters such as linearity, precision, accuracy, and robustness. A summary of the validation results is presented in table 4.

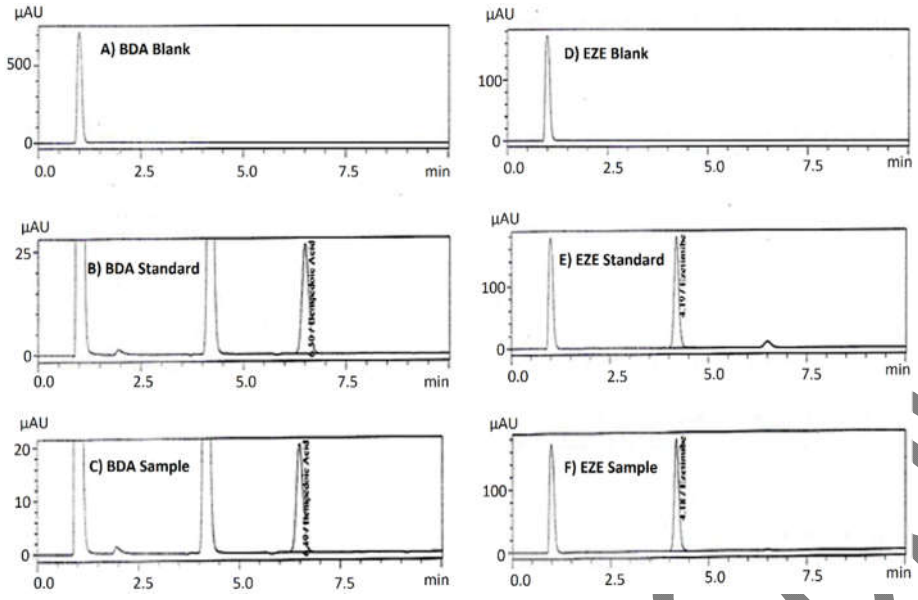

Fig. 5: Assay chromatograms A) BDA blank, B) BDA standard, C) BDA sample, D) EZE blank, E) EZE standard, F) EZE sample

Linearity

Calibration curves were established for BDA and EZE over concentration ranges of 53.8–1076.2 µg/ml and 3.17–63.42 µg/ml, respectively. For BDA, the regression equation was found to be 593.94x+5368.3, with an excellent correlation coefficient of 0.9999. Similarly, EZE showed a regression equation of 49937x+32704, with a correlation coefficient of 0.9998. The results from the linear regression analysis confirmed a strong, consistent linear relationship within the tested concentration ranges for both drugs. These calibration curves proved to be reliable for the accurate quantification of BDA and EZE in pharmaceutical formulations.

Precision

To assess the method's precision, both repeatability and intermediate precision were tested by analyzing three replicates at three concentration levels: 53.8, 538, and 1076 µg/ml for BDA, and 3.17, 31.7, and 63.4 µg/ml for EZE, spread across different days. For each set, the %RSD was calculated. In every case, the %RSD stayed well below 2%, confirming that the method is not only highly precise but also consistently reliable, even under slightly changing conditions.

Accuracy

Recovery studies were performed using the standard addition technique at five different concentration levels: 10%, 50%, 100%, 150%, and 200%. Each level was tested in triplicate to maintain consistency and rule out variability. The %recovery for both BDA and EZE turned out to be comfortably within the acceptable range. More specifically, recoveries fell between 99.6% and 101.9%, indicating that the method is not only accurate but also well suited for analytical use.

Sensitivity

The sensitivity of the proposed RP-HPLC method was assessed by calculating the LOD and LOQ using the standard deviation of the analytical response (σ) together with the slope of the corresponding calibration curve (S). The LOD and LOQ were calculated using the following ICH equations: LOD=(3.3×σ)/S and LOQ=(10×σ)/S. The results showed that the LOQ and LOD for BDA were 9.11 and 27.61µg/ml respectively, while for EZE, they were 0.82 and 2.50µg/ml.

Table 4: Results of validation studies

Parameters	**BDA**		**EZE**	
Tf	1.172±0.005		1.095±0.003	
Rs	-		13.96	
N	10958		10320	
%RSD for Peak Area (n=6)	0.0587%		0.0651%	
Linearity				
Range (µg/ml)	53.8 – 1076.2		3.17 – 63.4	
R^2	0.9999		0.9998	
Equation	y = 593.94x+5368.3		y = 49937x+32704	
Precision (%RSD)				
Repeatability (n=6)	0.81		0.94	
Inter. precision(n=6)	1.12		1.24	
Accuracy (%RSD)				
% Levels (n=3)	*% Recovery ±SD	% RSD	*% Recovery ±SD	% RSD
Level 10%	101.0±0.35	0.31	99.9±0.64	0.61
Level 50%	101.9± 0.12	0.16	100.3±0.62	0.64
Level 100%	101.6±0.29	0.32	101.4±0.25	0.23

Level 150%	101.8±0.15	0.24	101.6±0.21	0.34
Level 200%	100.5±1.31	1.32	100.9±1.12	1.14
LOD (µg/ml)	9.11		0.82	
LOQ (µg/ml)	27.61		2.50	

RSD-Relative standard deviation; All the values are presented as mean ±SD (n=6 for Precision, and n=3 for Accuracy)

Robustness

A robustness study was carried out through slight variations in method parameters. Specifically, the flow rate was adjusted by ±0.2 ml/min, the column temperature by ±5 °C, and the mobile phase composition by ±5%. The impact of these changes was assessed by evaluating the recovery of the analytes which was depicted in table 5. The results confirmed that the method remained robust despite the deliberate adjustments. To further assess the stability of both the sample formulation and standard solutions, analyses were performed at four different time intervals: 0, 12, 24, 36, and 48 h. No significant changes were observed in the formulation samples up to 30 h, and the standard solutions remained stable for at least 36 h. In all cases, the results were within the acceptable range, with %RSD values remaining below 2%.

Table 5: Robustness study

Variable parameter		**BDA**			**EZE**		
Original condition		***Tf* (<2.0)**	***N* (>2000)**	**%RSD (<2.0)**	***Tf*(<2.0)**	***N* (>2000)**	**%RSD (<2.0)**
		1.047	10958	0.18	1.055	10320	0.04
Change in mobile	1.3 ml/min	1.17	12247	0.06	1.05	12647	0.05
phase flow rate	1.1 ml/min	1.14	9547	0.15	1.05	9381	0.05
Change in column oven	30 °C	1.15	12467	0.03	1.05	11837	0.04
temperature 35± 5 °C	40 °C	1.15	13291	0.08	1.05	12193	0.07
Change in organic	-5%	1.15	9574	0.15	1.06	9836	0.09
phase –Solution A	+5%	1.15	11879	0.04	1.05	11024	0.07
Change in MeoH±5%	-5%	1.13	9879	0.08	1.06	9742	0.05
in Solution B	+5%	1.13	11367	0.08	1.06	11376	0.06

Assay of pharmaceutical formulation

The finalized method was successfully applied to analyze a tablet dosage form containing BDA and EZE as the active ingredients. The assay percentages were calculated by comparing the peak areas of the sample and standard solutions, considering their respective concentrations and the purity of the standard. The mean assay values showed excellent agreement with the label claims for both BDA and EZE. As shown in table 6, the consistency of results and low %RSD values further confirm that the method is reliable and well suited for quantifying BDA and EZE in commercially available tablet formulations.

Table 6: Analysis of BDA and EZE in commercial formulation

Formulation	**Label claim (mg)**		**Amount found(mg)**		*** %Recovery±%SD**	
	BDA	**EZE**	**BDA**	**EZE**	**BDA**	**EZE**
Nexlizet	180	10	180.87	9.96	100.41±0.34	99.6±0.41

*Each value represents the mean±SD (n=3)

Forced degradation study

The study explored how the formulation responded to different stress conditions by analyzing its degradation profile using LC. Under acidic conditions BDA and EZE showed degradation rates of 7.38% and 13.87%, respectively. When exposed to alkaline conditions, BDA degraded by 2.95%, while EZE showed a more pronounced degradation of 19.97%. In neutral conditions, degradation was minimal only 1.19% for BDA and 0.84% for EZE. Under oxidative stress, BDA showed 2.68% degradation, and EZE followed closely at 2.41%. Thermal conditions caused slight degradation of 0.68% in BDA and 4.48% in EZE. A complete breakdown of these results is presented in table 7 and table 8. With an acceptance criterion of 95–100%, the observed mass balance values for BDA and EZE were found to be within the acceptable range, indicating satisfactory mass balance. For a peak to be considered free from spectral interferences, the peak purity index must be greater than or equal to the single-peak threshold. In the present study, the evaluated peaks fulfilled this criterion, confirming acceptable peak purity. The degradation chromatograms of BDA and EZE are presented in fig. 6 and fig. 7 respectively.

Table 7: Forced degradation studies for BDA

BDA	**As such sample**	**Acid stressed sample**	**Base stressed sample**	**30% Peroxide stressed sample**	**Neutral stressed sample**	**Thermal sample 18 d**	**Humidity sample 18 d**
Total impurities	0.55	7.38	2.95	2.68	1.19	0.68	0.88
Assay	98.76	89.87	95.24	95.13	96.96	98.97	96.86
RS (Total impurities)+assay	99.31	97.25	98.19	97.81	98.15	99.65	97.74
% Mass balance	100.55	98.47	99.42	99.03	99.38	100.9	98.97

Table 8: Forced degradation studies for EZE

EZE	As such sample	Acid stressed sample	Base stressed sample	30% Peroxide stressed sample	Neutral stressed sample	Thermal sample 18 d	Humidity sample 18 d
Total impurities	0.4	13.87	19.97	2.41	0.85	4.48	7.34
Assay	98.23	83.2	78	96.2	97.5	93.15	89.52
RS (Total impurities)+Assay	98.27	97.07	97.97	98.61	98.35	97.63	96.86
% Mass balance	99.94	98.82	99.73	100.38	100.12	99.38	98.60

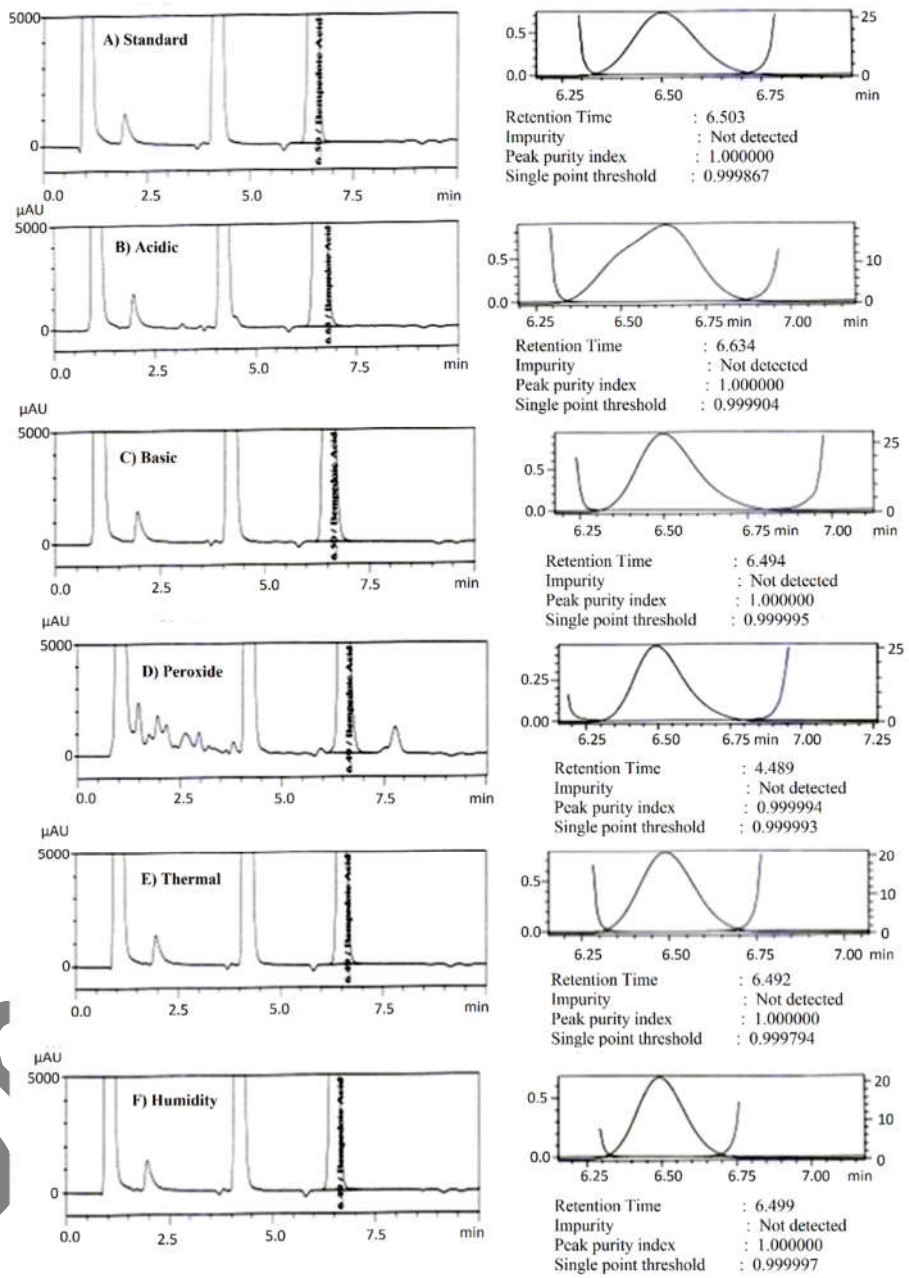

Fig. 6: Forced degradation studies for BDA

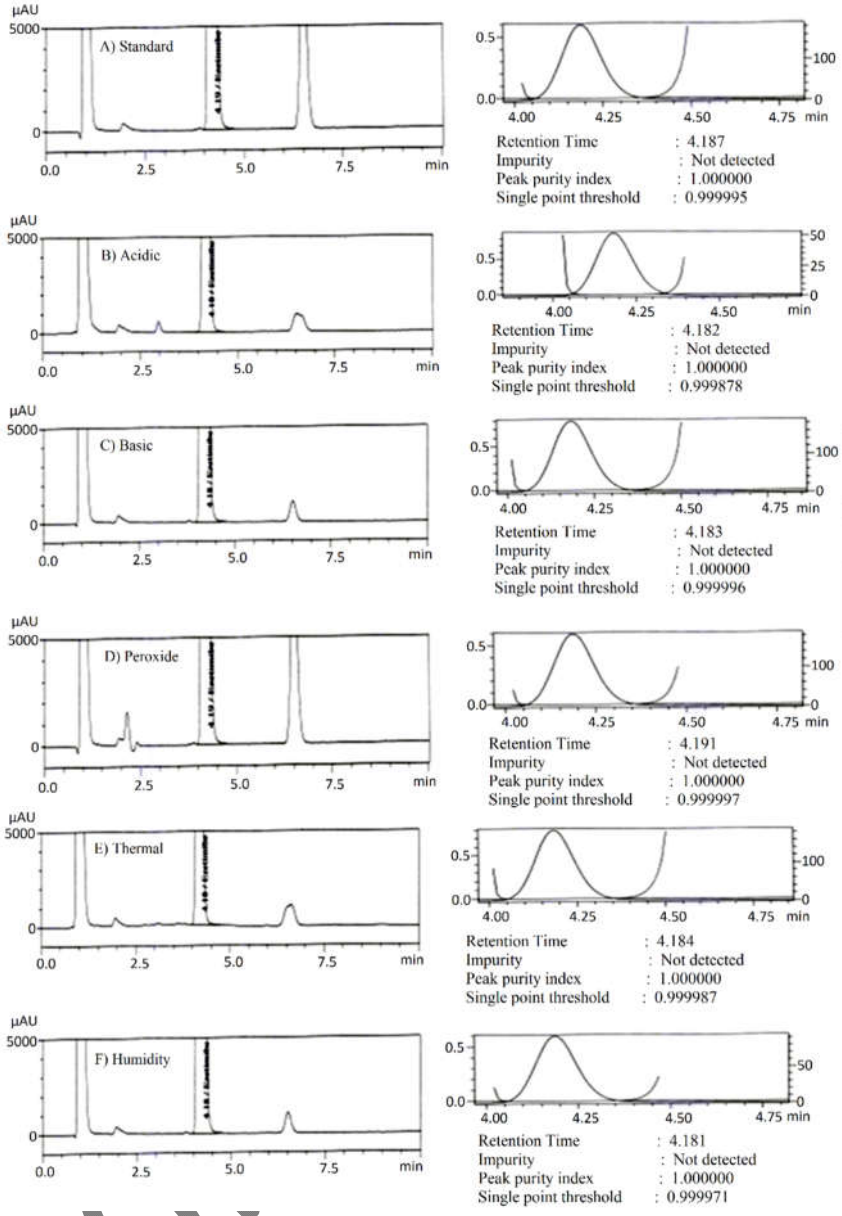

Fig. 7: Forced degradation studies for EZE

DISCUSSION

The present study successfully established and validated a robust, stability-indicating RP-HPLC method for the simultaneous quantification of Bempedoic Acid (BDA) and Ezetimibe (EZE) in a combined pharmaceutical formulation. This methodology enabled precise evaluation of critical method variables and their influence on chromatographic responses, which results in the construction of a detailed design space. The use of response surface methodology and three-dimensional contour plots provided the key analytical attributes, making the optimization process both predictive and highly reproducible.

The implementation of Design of Experiments (DoE) was instrumental in defining the optimal chromatographic conditions. Through this optimization, a mobile phase composition of Solution A and Solution B in a 45:55 (v/v) ratio, delivered at a flow rate of 1.2 ml/min, was identified as optimal which results in forming of Tailing factor of 1.096. And also, the bempedoic acid reports N value at 10,958 whereas Ezetimibe reports N value at 10,320 with a good resolution 13.77.

The final chromatographic conditions employed a Zorbax SB C8 column (250 × 4.6 mm, 5 µm) under isocratic elution. Dual-wavelength detection at 215 nm for BDA and 232 nm for EZE ensured optimal response for both molecules, considering their distinct chromophoric properties. The phosphate buffer–acetonitrile mobile phase (45:55 v/v) at ambient temperature facilitated rapid elution with a total run time of 10 min, contributing to solvent savings and increased sample throughput. Under these conditions, retention times were approximately 6.5 min for BDA and 4.2 min for EZE, allowing efficient separation within a relatively short analytical cycle. System suitability results further reinforced method reliability, with tailing factors of 1.047 (Bempedoic acid) and 1.055 (Ezetimibe), both comfortably below the threshold of 2.0. Linearity studies yielded excellent calibration characteristics, with regression equations of y = 593.94x+5368.3 for BDA and y = 49,937x+32,704 for EZE, and near-ideal correlation coefficients of 0.9999 and 0.9998, respectively. Precision evaluations showed %RSD values consistently below 2%, demonstrating high reproducibility. While earlier reported methods yielded recovery values around 101.2% for Bempedoic acid and 101.4% for Ezetimibe [28]. Additionally, low repeatability values (0.81% for BDA and 0.94% for EZE) reaffirmed method consistency [29].

Robustness testing, performed through systematic and deliberate variations in method parameters, yielded low %RSD values, confirming method ruggedness and compliance with ICH Q2(R2) guidelines. The A QbD approach not only improved peak characteristics such as retention time, area, and symmetry but also enhanced overall method reliability compared to previously reported chromatographic methods for these analytes.

Forced degradation studies revealed that both drugs were relatively stable under Acidic stressed, Base Stresses sample, Peroxide stressed, Neutral Stressed, Thermal Sample and Humidity Sample. The highest degradation was observed for Bempedoic acid under alkaline conditions (7.38%) and for Ezetimibe under Base stressed sample (19.97%) [30].

Peak purity values exceeding 0.990 indicated peak homogeneity, while mass balance values between 98.42% and 100.9% remained within acceptable ICH limits, demonstrating that the method successfully accounts for degradation pathways and maintains quantitative integrity.

Overall, the newly developed RP-HPLC method was rigorously validated for specificity, linearity, accuracy, precision, robustness, and system suitability. The incorporation of AQbD principles not only enhanced method performance but also provided statistical assurance and operational flexibility, distinguishing it from conventionally developed methods. Based on linearity studies, Bempedoic acid exhibited linearity across 10%–200% of the working concentration, whereas Ezetimibe showed linearity between 25%–200%. All recovery values fell within ICH-specified limits (80%–120%), with observed recoveries between 99.9% and 101.9%, confirming the method's reliability and suitability for routine pharmaceutical quality control.

CONCLUSION

A precise, sensitive, and dependable RP-HPLC method was successfully developed for the simultaneous estimation of BDA and EZE in tablet dosage forms, using an Analytical QbD approach. This strategy allowed for a more systematic and efficient method development process by evaluating key variables and their interactions with critical quality attributes. Out of all the parameters tested, the composition of the mobile phase and the flow rate turned out to be the key players influencing the Tf, N, and RS, as revealed by the CCD approach. One of the standout insights from the study was how effectively the DoE streamlined the process by cutting down the number of trials needed while still delivering solid, reliable data in less time and with less effort. The high recovery observed in the formulation confirmed that excipients didn't interfere with the quantification of the analytes. Validation results further reinforced the method's reliability indicating good linearity, accuracy, precision, and robustness under varied conditions.

ABBREVIATIONS

LDL-C: Low density lipoprotein cholesterol, RP-HPLC: Reverse phase high pressure liquid chromatography, BDA: Bempedoic acid, EZE: Ezetimibe, CCD: Central composite design, DOE-Design of experiments, QbD-Quality by design, ICH: International council for harmonisation, S/N: Signal to Noise, RP-UPLC: Reversed phase ultra-performance liquid chromatographic, CV: Coefficient of variation

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AUTHORS CONTRIBUTIONS

We declare that it's an original research work which was carried out by G. V Padmakar Rao and Bavisetti Lakshmi under the supervision of Dr. Meka Lingam. Dr. D. Suryakala proof read the manuscript, suggested the necessary corrections, and helped in writing the manuscript.

CONFLICTS OF INTERESTS

The authors have no competing interests to declare that are relevant to the content of this article.

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