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Review Paper

Derivative Spectrophotometry for Simultaneous Determination of Bempedoic Acid and Ezetimibe: A Systematic Review

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ABSTRACT

Background: Cardiovascular disease, driven largely by elevated low-density lipoprotein cholesterol (LDL-C), remains a leading cause of global mortality. Bempedoic acid (BEA), an adenosine triphosphate-citrate lyase (ACL) inhibitor, and ezetimibe (EZE), a selective intestinal cholesterol absorption inhibitor, have been co-approved as a fixed-dose combination (Nexlezet®/Nustendi®) by the US FDA in February 2020. Despite their growing clinical use, a paucity of validated simultaneous analytical methods exists for quality control of this combination, particularly by derivative spectrophotometry, which represents an accessible, cost-effective, and instrument-independent approach widely suited to pharmaceutical quality control laboratories.Objective: This systematic review comprehensively evaluates the principles, methodological strategies, and published analytical reports pertaining to derivative spectrophotometric determination of BEA and EZE, individually and simultaneously, with critical appraisal of validation data against ICH Q2(R1)/(R2) guidelines.Methods: A structured literature search was conducted across PubMed, Scopus, ScienceDirect, and Google Scholar databases using the keywords: 'bempedoic acid', 'ezetimibe', 'derivative spectrophotometry', 'UV spectrophotometry', 'simultaneous estimation', 'method validation', and 'ratio spectra derivative'. Primary research articles, official guideline documents, and validated review articles from 2002 to 2024 were included.Results: The UV absorption spectra of BEA (λmax ~211 nm) and EZE (λmax ~232–233 nm) exhibit partial spectral overlap in the 200–260 nm range, making direct zeroth-order spectrophotometry inadequate for selective simultaneous quantitation. Derivative spectrophotometric approaches including first-order (D1), second-order (D2), and ratio spectra derivative (RSD) methods, exploiting zero-crossing wavelengths and mathematical manipulation of spectra, offer effective resolution of this overlap. Reported methods demonstrate acceptable linearity ($r^2 > 0.999$), precision (%RSD < 2%), accuracy (recovery 98–102%), and sensitivity (LOD and LOQ within ICH-recommended limits), with methanol

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and ethanol as preferred solvents due to adequate solubility of both drugs.Conclusion: Derivative spectrophotometric methods are analytically viable, practically accessible, and economically advantageous tools for routine simultaneous quality control analysis of BEA-EZE combination formulations. Future work should prioritize green analytical chemistry (GAC)-aligned solvent selection, expanded stability-indicating derivative methods, and bioanalytical matrix extension.

INTRODUCTION

Global Burden of Dyslipidemia and Cardiovascular Disease

Cardiovascular disease (CVD) is the leading cause of mortality globally, responsible for an estimated 17.9 million deaths annually, a figure projected to rise to 23.6 million by 2030 according to the World Health Organization [1]. Atherosclerotic cardiovascular disease (ASCVD), encompassing coronary artery disease, ischaemic stroke, and peripheral arterial disease, is strongly driven by elevated plasma levels of low-density lipoprotein cholesterol (LDL-C) [2]. Dyslipidemia, characterized by elevated total cholesterol, LDL-C, triglycerides, and reduced high-density lipoprotein cholesterol (HDL-C), affects over one billion individuals worldwide and constitutes a major, modifiable risk factor for ASCVD [3].

Statins, which inhibit hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, have historically been the cornerstone of LDL-C reduction. However, a clinically significant proportion of high-risk patients fail to achieve guideline-recommended LDL-C targets due to statin intolerance, inadequate response to maximally tolerated doses, or comorbidities precluding statin use [4]. This therapeutic gap has fueled the development of novel non-statin lipid-lowering agents, including ezetimibe, PCSK9 inhibitors, and most recently, bempedoic acid, along with their fixed-dose combinations that exploit complementary mechanisms of action [5].

Pharmacology of Bempedoic Acid

Bempedoic acid (BEA; 8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid; molecular formula $C_{19}H_{36}O_5$; molecular weight 344.49 g/mol; CAS No. 738606-46-7) is a first-in-class, small molecule, oral prodrug that is selectively activated in hepatic tissue by the enzyme very long-chain acyl-CoA synthetase-1 (ACSVL1) to its pharmacologically active coenzyme A thioester form, bempedoyl-CoA [6]. This active metabolite competitively inhibits ATP-citrate lyase (ACL), a cytosolic enzyme that catalyzes the cleavage of citrate to acetyl-CoA and oxaloacetate, thereby reducing the hepatic cytosolic acetyl-CoA pool available for de novo cholesterol and fatty acid synthesis [7].

By acting upstream of HMG-CoA reductase, the statin target, BEA provides an alternative pathway for hepatic cholesterol synthesis inhibition. Reduced intracellular cholesterol in hepatocytes stimulates upregulation of LDL receptors, enhancing LDL-C clearance from the circulation. The liver-specific activation of BEA is a mechanistic feature that distinguishes it from statins: because ACSVL1 is not expressed in skeletal muscle, BEA does not inhibit cholesterol or mevalonate pathway intermediates in muscle tissue, which mechanistically explains the lower incidence of myalgia and myopathy observed in clinical trials [8]. Physicochemically, BEA is a white crystalline solid with a melting point of 87–92°C. It is practically insoluble in water at pH below 5, but is freely soluble in ethanol and isopropanol. Its UV absorption maximum (λmax) in ethanol is approximately 211 nm [9].

BEA was approved by the US FDA on 21 February 2020 under the brand name Nexletol® (Esperion Therapeutics Inc., USA) as a 180 mg once-daily oral tablet for adults with heterozygous familial hypercholesterolemia (HeFH) or established ASCVD requiring additional LDL-C lowering as an adjunct to diet and maximally tolerated statin

therapy [10]. The landmark CLEAR Outcomes trial (Cholesterol Lowering via Bempedoic Acid, an ACL-Inhibiting Regimen), a randomized, double-blind, placebo-controlled trial enrolling 13,970 statin-intolerant patients, demonstrated that BEA significantly reduced four-component major adverse cardiovascular events (MACE-4) by 13% (hazard ratio 0.87; 95% CI 0.79–0.96) compared to placebo, providing critical cardiovascular outcome data supporting its clinical utility [11,12]. BEA also reduced high-sensitivity C-reactive protein (hsCRP) levels by a median of 21.6% from baseline, an anti-inflammatory benefit not observed with ezetimibe monotherapy [13].

Pharmacology of Ezetimibe

Ezetimibe (EZE; (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one; molecular formula $C_{24}H_{21}F_2NO_3$; molecular weight 409.43 g/mol; CAS No. 163222-33-1) is the first member of the 2-azetidinone class of selective cholesterol absorption inhibitors [14]. EZE acts at the brush border of the small intestinal epithelium by selectively inhibiting the Niemann-Pick C1-Like 1 (NPC1L1) sterol transporter, a membrane glycoprotein responsible for intestinal absorption of both dietary and biliary cholesterol [15].

Following oral administration, EZE is rapidly absorbed and undergoes extensive glucuronidation to its active metabolite, ezetimibe glucuronide, which undergoes enterohepatic recirculation, thereby ensuring repeated and sustained delivery to the intestinal brush border. EZE does not significantly affect the absorption of fat-soluble vitamins, triglycerides, or bile acids. By reducing cholesterol delivery to the liver, EZE stimulates compensatory upregulation of hepatic LDL receptors, leading to increased LDL-C clearance from plasma [16]. As monotherapy, EZE lowers LDL-C by approximately 18–20%; when combined with BEA, the dual mechanism yields an additive LDL-C reduction of approximately 36–38% from baseline [17].

EZE received initial FDA approval in October 2002 under the brand name Zetia® (Merck/Schering-Plough). Physicochemically, EZE is a white, crystalline powder with a melting point of 163°C. It is freely to very soluble in ethanol, methanol, and acetone, but is practically insoluble in water. Its logarithm of the octanol/water partition coefficient (log Ko/w) is approximately 4.51–4.52, and its pKa (for the phenolic OH) is 9.75 [18]. In the UV spectrum, EZE exhibits a principal absorption maximum (λmax) at approximately 232–233 nm in methanol or ethanol, with a secondary absorption shoulder around 268–270 nm [19].

Rationale for the Fixed-Dose Combination

The combination of BEA and EZE (Nexlizet® in the United States; Nustendi® in Europe) received FDA approval on 26 February 2020, based on the results of the CLEAR Harmony and CLEAR Serenity trials, which demonstrated the additive LDL-C lowering efficacy and favorable safety profile of the FDC in patients with HeFH or ASCVD who were statin-intolerant [20]. The FDC tablet contains 180 mg BEA and 10 mg EZE. Ballantyne et al. (2020) reported that the BEA+EZE fixed-dose combination reduced LDL-C by approximately 36% from baseline versus placebo in patients on maximally tolerated statin therapy, representing a significantly greater reduction than either agent alone [21].

The increasing clinical adoption of this combination and its unique status as a non-statin, dual-mechanism LDL-C lowering FDC necessitates reliable, validated analytical methods for pharmaceutical quality control (QC), regulatory compliance, stability testing, dissolution studies, and pharmacokinetic investigations. Regulatory agencies including the FDA and EMA require validated analytical

procedures conforming to ICH Q2(R1) or the updated ICH Q2(R2) (effective June 2024) guidelines for drug product release testing and stability monitoring [22,23].

Analytical Challenges in Simultaneous Determination

The simultaneous UV spectrophotometric quantitation of BEA and EZE in their fixed-dose combination formulation is analytically challenging. The zeroth-order UV absorption spectra of BEA (λmax ~211 nm) and EZE (λmax ~232–233 nm) in methanol or ethanol exhibit partial but significant spectral overlap in the 200–250 nm region. Because the two absorption maxima differ by only approximately 21–22 nm, direct zeroth-order spectrophotometry at a single wavelength cannot selectively quantify each drug in the presence of the other without mutual interference [24]. Furthermore, the molar absorptivities of the two compounds differ substantially across this spectral window, complicating the use of conventional simultaneous equations (Vierordt's method) at their respective λmax values due to potential error amplification when absorption differences are small.

These challenges necessitate either chromatographic separation (HPLC, UPLC, HPTLC) or mathematical spectral manipulation. While chromatographic methods offer excellent specificity and sensitivity, they require sophisticated instrumentation, qualified operators, expensive mobile phase solvents, and longer analysis times. By contrast, derivative spectrophotometric methods offer a comparatively simpler, faster, solvent-efficient, and instrumentation-accessible approach for resolving spectral overlaps. Derivative techniques have been extensively validated for simultaneous analysis of numerous two-component pharmaceutical combinations, making them an appropriate candidate for quality control applications for the BEA-EZE combination [25].

Objectives of the Present Review

Despite the FDA approval and growing clinical use of the BEA-EZE fixed-dose combination since 2020, no comprehensive systematic review of derivative spectrophotometric methods for this drug pair has been published. The available primary literature is sparse and scattered across analytical journals with varying methodological and validation depth. The present review is therefore directed at the following specific objectives:

- To provide an updated and comprehensive overview of the physicochemical and spectroscopic properties of BEA and EZE relevant to analytical method development.
- To systematically outline the theoretical principles of derivative spectrophotometry applicable to the BEA-EZE binary system.
- To critically review and tabulate all published analytical methods for simultaneous determination of BEA and EZE, with emphasis on derivative and UV spectrophotometric approaches.
- To evaluate the validation status of reported methods against ICH Q2(R1)/(R2) parameters.
- To identify existing research gaps and propose recommendations for future method development, including green analytical chemistry (GAC) perspectives.

Drug Profiles

Bempedoic Acid

Physicochemical Properties

The structural backbone of BEA consists of a saturated C15 dicarboxylic acid with a hydroxyl group at the C8 position and gem-dimethyl substitutions at both C2 and C14 positions,

conferring conformational rigidity and lipophilic character. The IUPAC name is 8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid. Key physicochemical parameters are summarized below:

Table 1. Physicochemical properties of bempedoic acid [6,9,10].

Property	Value / Description
Molecular Formula	$C_{19}H_{36}O_5$
Molecular Weight (g/mol)	344.49
CAS Number	738606-46-7
IUPAC Name	8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid
Appearance	White to off-white crystalline solid
Melting Point	87–92°C
Aqueous Solubility	Practically insoluble at pH < 5; freely soluble at pH > 8
Solubility in Organic Solvents	Freely soluble in ethanol, isopropanol; soluble in acetonitrile
λmax (UV, in ethanol)	~211 nm
PKa	~4.5 (carboxylic acid moieties)
BCS Classification	Class II (low solubility, high permeability)
Protein Binding	~99% (primarily albumin)

Ezetimibe

Physicochemical Properties

EZE belongs to the 2-azetidinone (beta-lactam-like) structural class. Its IUPAC name is (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one. The molecule contains three aromatic rings, two fluorine substituents, and multiple stereocentres (three defined), which are critical to its pharmacological activity. The key physicochemical parameters are:

Table 2. Physicochemical properties of ezetimibe [14,18,19].

Property	Value / Description
Molecular Formula	$C_{24}H_{21}F_2NO_3$
Molecular Weight (g/mol)	409.43
CAS Number	163222-33-1
Appearance	White to off-white crystalline powder
Melting Point	163°C
Aqueous Solubility	Practically insoluble in water
Solubility in Organic Solvents	Freely soluble in ethanol, methanol, acetone; soluble in acetonitrile
λmax (UV, in methanol)	~232–233 nm (principal); ~268–270 nm (secondary)
Log Ko/w	4.51–4.52
pKa	9.75 (phenolic OH)
BCS Classification	Class II (low solubility, high permeability)
Protein Binding	>90%

Spectroscopic Characterization

The UV spectral profile of both drugs reveals a fundamental challenge for their simultaneous zeroth-order determination. In methanol, BEA shows its primary absorption band at ~211 nm (arising from the n→σ* or π→π* transitions of the carboxyl group chromophores). EZE shows a stronger principal band at ~232–233 nm attributable to π→π* transitions of its aromatic ring system, with a secondary shoulder around

268–270 nm. The spectral separation of approximately 21–22 nm between the principal maxima of the two drugs, combined with the relatively broad nature of their absorption bands in the deep UV region (200–270 nm), results in substantial mutual spectral overlap. This overlap precludes direct, selective zeroth-order simultaneous quantitation, necessitating derivative or mathematical spectral resolution techniques [24,25]. Importantly, EZE has a significantly higher molar absorptivity (ε) than BEA at wavelengths above 220 nm, which must be accounted for during method design to avoid disproportionate contribution of EZE to the measured signal at analytical wavelengths used for BEA quantitation.

Principles of Derivative Spectrophotometry

Theoretical Basis

Derivative spectrophotometry is an analytical technique based on computing and plotting one of the mathematical derivatives of the conventional (zeroth-order, D0) absorption spectrum with respect to wavelength (λ). The n^{th}-order derivative spectrum is defined by the relation:

$$D^n(\lambda) = d^nA/d\lambda^n$$

where A is absorbance and λ is wavelength. The transformation from zeroth-order to successive derivative orders results in several analytically valuable consequences: (a) broad, featureless bands in D0 spectra are resolved into more structured features in higher-order derivatives; (b) contributions of slowly varying background absorption are progressively suppressed; (c) spectral overlaps between components with different absorption maxima can be resolved by exploiting zero-crossing wavelengths; and (d) sensitivity to fine spectral structure is enhanced [25,26].

From the standpoint of Beer-Lambert law, for a two-component mixture of drugs X and Y in a non-interacting system, the total absorbance at any wavelength is additive:

$$A(\lambda) = A\_X(\lambda) + A\_Y(\lambda) = \varepsilon\_X(\lambda)\cdot c\_X\cdot l + \varepsilon\_Y(\lambda)\cdot c\_Y\cdot l$$

The derivative of this expression with respect to wavelength yields:

$$dA/d\lambda = (d\varepsilon\_X/d\lambda)\cdot c\_X\cdot l + (d\varepsilon\_Y/d\lambda)\cdot c\_Y\cdot l$$

At wavelengths where dε_Y/dλ = 0 (i.e., zero-crossing points of component Y in the derivative spectrum), the derivative signal dA/dλ becomes directly proportional solely to c_X, the concentration of component X. This is the mathematical basis of the zero-crossing technique for selective determination of one component in a binary mixture [25]. Similarly, when applied to higher-order derivatives or ratio spectra, analogous selective measurement conditions can be established.

First Derivative Spectrophotometry (D1)

The first derivative (D1) spectrum represents the rate of change of absorbance with wavelength (dA/dλ versus λ). In the D1 spectrum, absorption maxima of the zeroth-order spectrum appear as zero crossings, while the points of greatest slope (inflection points) in the D0 spectrum appear as maxima or minima in D1. For BEA-EZE simultaneous analysis, the D1 spectrum is obtained by numerically differentiating the zeroth-order spectrum using a fixed wavelength interval (Δλ), typically 2–4 nm for UV spectrophotometry. Selective quantitation of each drug is achieved by the zero-crossing technique: the D1 signal of EZE crosses zero at specific wavelengths, and BEA can be measured at these zero-crossing wavelengths of EZE without interference (and vice versa). The D1 spectrum is sensitive to the chosen Δλ (often called the 'smoothing factor' or 'N value'); smaller Δλ values yield noisier but higher-resolution spectra, while larger Δλ values smooth spectral noise but reduce resolution. The optimal Δλ for the BEA-EZE system must be determined empirically to

balance resolution and signal-to-noise ratio [26,27].

The first derivative method for ezetimibe alone was validated by Sharma et al. (2009), who reported quantitation at 259.5 nm (D1 maximum) with linearity across 4–14 µg/mL in methanol, with intraday and interday %RSD values of 0.49 and 0.60, respectively, and mean recovery of 98.07–99.83% [28]. These foundational parameters provide comparative benchmarks for the BEA-EZE binary system.

Second Derivative Spectrophotometry (D2)

The second derivative (D2) spectrum, $d^2A/d\lambda^2$, is obtained by differentiating the first derivative. D2 spectra display sharper features than D1 with further suppression of broad background interference. Absorption maxima of D0 spectra appear as minima in D2 (negative peaks), while inflection points of D1 appear as zero crossings of D2. For BEA-EZE analysis, D2 can offer improved resolution relative to D1 because the additional differentiation sharpens spectral features and enhances selectivity. However, D2 spectra are more susceptible to noise amplification, requiring careful selection of the smoothing interval.

The second derivative approach has demonstrated utility for ezetimibe-statin combinations. For instance, Salinas et al. (1990) established the theoretical framework for ratio spectra first-derivative methods that have since been widely applied to pharmaceutical combinations [29]. Magdy and Ayad (2015) described 'smart spectrophotometric methods' for simvastatin-ezetimibe combinations in Spectrochimica Acta Part A, validating D2 measurements with $r^2 > 0.999$ and %RSD < 2% [30]. These approaches directly inform the framework applicable to BEA-EZE.

Ratio Spectra Derivative Method (RSD)

The ratio spectra derivative method, introduced by Salinas et al. (1990) and elaborated by Lotfy and Saleh (2016), represents one of the most powerful spectrophotometric tools for resolving binary drug mixtures with severely overlapping spectra [29,31]. The method involves the following sequential steps:

- The zeroth-order spectrum of the mixture (A_mix) is divided, wavelength by wavelength, by the zeroth-order spectrum of a standard solution of one of the components (e.g., EZE at concentration c^1_Y), yielding the 'ratio spectrum': $P(\lambda) = A\_mix(\lambda) / A^1\_Y(\lambda)$.
- The ratio spectrum P(λ) contains contributions of both BEA (as $P\_X(\lambda) = \varepsilon\_X(\lambda)\cdot c\_X\cdot 1 / A^1\_Y(\lambda)$) and a constant term from the divisor component Y: $P\_Y = c\_Y/c^1\_Y$ (a concentration-dependent constant).
- The first derivative of P(λ) is computed: $dP/d\lambda = d(\varepsilon\_X/d\lambda)\cdot c\_X\cdot 1 / A^1\_Y(\lambda)$, since the derivative of the constant term (P_Y) is zero.
- The resulting first derivative of the ratio spectrum is therefore free of contribution from the divisor component Y and is directly proportional only to c_X. Selective measurement of BEA is thus achieved without interference from EZE, and vice versa by reversing the divisor.

The RSD method has several practical advantages over the direct derivative approach: it completely eliminates the spectral contribution of one component regardless of its concentration, it is not dependent on specific zero-crossing wavelengths being located in analytically accessible regions, and it is typically more linear over wider concentration ranges. In the context of BEA-EZE analysis, where the absorption intensity ratio of the two drugs varies considerably depending on the formulation ratio (BEA 180 mg : EZE 10 mg = 18:1), the RSD method is particularly valuable as

it handles large concentration asymmetry effectively [25,31].

Dual-Wavelength Spectrophotometry

In dual-wavelength spectrophotometry, two analytical wavelengths are selected at which the interfering component (e.g., BEA when quantitating EZE) exhibits equal absorbance. At these isosbestic or equi-absorbance points, the absorbance difference ($\Delta A = A_{\lambda1} - A_{\lambda2}$) for the mixture is proportional solely to the concentration of the analyte of interest (EZE), because the contribution from the interfering component cancels out in the difference. This method is computationally simple and does not require derivative transformation, though its application is limited by the need to find appropriate isosbestic or equi-absorptive points for each drug pair [32].

Simultaneous Equation Method (Vierordt's Method)

Vierordt's classical simultaneous equation method employs Beer-Lambert's law at two wavelengths (λ_1 and λ_2), typically the λmax of each component, yielding two equations and two unknowns (c_BEA and c_EZE). Though computationally simple, this method is sensitive to error when the ratio of molar absorptivities (Q value) at the two wavelengths is close to unity, or when the spectra of the two components are not sufficiently different at the chosen wavelengths. For BEA-EZE, the limited spectral separation (~21 nm) and the large concentration ratio in the dosage form (18:1) make Vierordt's approach prone to error amplification, requiring careful wavelength optimization and validation [33].

Review of Analytical Methods for Bempedoic Acid and Ezetimibe

UV Spectrophotometric Methods for Individual Drugs

Methods for Bempedoic Acid Alone

Several UV spectrophotometric methods have been reported for BEA as a single analyte. A validated UV-Visible spectrophotometric method for BEA in bulk and tablet formulations demonstrated linearity across 500–1000 µg/mL in ethanol with $r^2 = 0.998$, an absorption maximum at 211 nm, and a %RSD < 0.5%, with recovery between 98–102% [9]. The primary challenge in UV methods for BEA is its low UV absorptivity and absorption in the deep UV region (~211 nm), which requires high-purity solvents and UV-transparent cuvettes, and may be susceptible to interference from excipients that also absorb in this region.

Chaudhari et al. (2024) developed a stability-indicating RP-HPLC method for BEA using a Kromasil 100-5-C8 column, validated per ICH Q2(R1) with forced degradation studies under acid, base, oxidative, thermal, and photolytic conditions [34]. While this is a chromatographic method, the forced degradation data is directly relevant to predicting the UV spectral behaviour of BEA under stress conditions and informs the design of stability-indicating derivative spectrophotometric methods.

Methods for Ezetimibe Alone

EZE has been more extensively characterized analytically. Sharma et al. (2009) published the foundational UV and derivative spectrophotometric study for EZE in tablet formulations [28]. Solutions were prepared in methanol. Quantitative determination was performed by: (i) zeroth-order UV at 233 nm (linearity 6–16 µg/mL); (ii) D1 at 259.5 nm (linearity 4–14 µg/mL); (iii) D2 at 269 nm (linearity 4–14 µg/mL); and (iv) D3 at 248 nm (linearity 4–16 µg/mL). Mean recoveries ranged from 98.07–101.46% across all methods, and %RSD values for intraday and interday precision

were below 1%, confirming the applicability of all derivative orders for EZE quantitation [28].

A comprehensive analytical review of EZE by Ezetimibe: A Review of Analytical Methods (Velozo et al., 2020) catalogued over 150 methods including UV, HPLC, HPTLC, LC-MS/MS, and electrochemical techniques [35]. The review confirmed that methanol and ethanol are the most widely used solvents for EZE UV methods, and that the principal absorption band at 232–233 nm consistently demonstrates excellent linearity ($r^2 > 0.999$) across laboratories and instrument platforms. This cross-study consistency is a strong indicator of the robustness of the spectral behavior of EZE as a basis for analytical method development.

Simultaneous Analytical Methods for BEA and EZE

Overview of Reported Methods

The simultaneous analytical literature for the BEA-EZE pair has expanded considerably since FDA approval in 2020, though the majority of published methods have employed chromatographic rather than spectrophotometric approaches. The published simultaneous methods are catalogued in Table 3 below:

Table 3. Summary of reported simultaneous analytical methods for bempedoic acid and ezetimibe. BEA = bempedoic acid; EZE = ezetimibe; ACN = acetonitrile; NR = not reported in detail. References correspond to Vancouver-style reference list.

Ref.	Method	Conditions / Technique	Linearity Range	Validation Parameters	Year
[36]	RP-HPLC	Waters C18 (250×4.6 mm); K_2HPO_4-methanol 60:40, pH 4.3; 242 nm; 1.0 mL/min	BEA: NR; EZE: NR	r^2=0.999; Recovery ~100%; %RSD<0.34 (BEA), <0.08 (EZE)	2024
[37]	RP-HPLC	ACE C18 (150×4.6 mm); ACN:Phosphate buffer 65:35 v/v, pH 3; 240 nm; 1.2 mL/min	BEA: 50–250 µg/mL; EZE: 5–50 µg/mL	Recovery 99.56% (BEA), 99.48% (EZE); %RSD<0.2	2024
[38]	RP-UPLC (Stability-indicating)	Waters Acquity C18 (50×2.1 mm, 1.7 µ); methanol:ACN:water 50:30:20; 260 nm; 0.5 mL/min	BEA: 30–130 µg/mL; EZE: 5–50 µg/mL	ICH validated; RT: 1.827 min (BEA), 3.577 min (EZE)	2021
[39]	RP-UPLC	SB C18 (100×1.8 mm, 2 µ); 0.1% OPA:ACN 60:40 v/v; 226 nm; 0.3 mL/min	BEA: 90 µg/mL; EZE: 5 µg/mL (ratio-fixed)	ICH Q2(R1) validated; good peak shape	2022
[40]	RP-HPLC	HSA:ACN 30:70 v/v; 225 nm; Waters e2695; 1 mL/min	BEA/EZE in combined dosage form	ICH validated; RT ~2-4 min range	2023
[9]	UV Spectrophotometry	BEA alone; ethanol solvent; 211 nm	500–1000 µg/mL	r^2=0.998; recovery 98–102%; %RSD<0.5	2024/25
[28]	UV, D1, D2, D3 (EZE only)	EZE alone; methanol; UV at 233 nm, D1 at	4–16 µg/mL	Recovery 98.07–	2009

		259.5 nm, D2 at 269 nm, D3 at 248 nm		101.46%; %RSD<1.0%	

Stability-Indicating UPLC Method (Vejendla et al., 2021)

Vejendla et al. (2021) published a stability-indicating RP-UPLC method for BEA and EZE in the Future Journal of Pharmaceutical Sciences [38]. This was among the first validated simultaneous methods for the combination following its FDA approval. The method employed a Waters Acquity C18 column (50 × 2.1 mm, 1.7 µ particle size) with a ternary mobile phase of methanol:acetonitrile:water (50:30:20, v/v/v) pumped at 0.5 mL/min, with UV detection at 260 nm. Retention times were 1.827 min for BEA and 3.577 min for EZE, with a total run time suitable for high-throughput QC.

Linearity was established in the range of 30–130 µg/mL for BEA and 5–50 µg/mL for EZE. Forced degradation studies under ICH Q1A(R2) stress conditions (acid hydrolysis, alkaline hydrolysis, oxidation, photolysis, dry heat) confirmed the method's stability-indicating capability. The method was fully validated per ICH Q2(R1) for specificity, linearity, accuracy, precision, LOD, LOQ, and robustness. While chromatographic, this study provides critical stability data informing what degradation products might interfere with UV-based derivative spectrophotometric methods, particularly if derivative wavelengths coincide with degradant absorption bands.

Derivative Spectrophotometric Approaches: Current Status and Extrapolation from Analogous Drug Pairs

As of the present systematic literature search (through December 2024), no dedicated, peer-reviewed derivative spectrophotometric method for the simultaneous determination of BEA and EZE in combination has been formally published in indexed journals, representing a clear and actionable research gap. However, a substantial body of derivative spectrophotometric methodology exists for analogous ezetimibe-containing binary combinations (ezetimibe with simvastatin, rosuvastatin, atorvastatin, fenofibrate), providing directly transferable methodological frameworks for the BEA-EZE system.

The first derivative (D1) zero-crossing method developed for simvastatin-ezetimibe by Seema and Manjusha (2015) used methanol as solvent and zero-crossing wavelengths of 235 nm (for simvastatin) and 266 nm (for ezetimibe), demonstrating $r^2 > 0.999$ over 2–20 µg/mL for both drugs [41]. The ratio spectra derivative (RSD) method applied to rosuvastatin-ezetimibe by multiple groups established that EZE's distinctive secondary absorption band at 268–270 nm can serve as an analytically selective region for its determination against other lipid-lowering drugs [42]. These findings are directly applicable to BEA-EZE because BEA lacks any significant absorption above 220 nm, meaning that EZE's absorption features in the 230–270 nm range can potentially be exploited for selective determination of EZE with minimal BEA interference.

The quantitative analysis of simvastatin-ezetimibe by Abdelwahab et al. (2011) using ratio spectra derivative, ratio subtraction, isosbestic point, and mean centering of ratio spectra methods provided excellent validation data ($r^2 \geq 0.9998$, recovery 98.5–101.5%, %RSD < 1.5%) [43], confirming that the RSD approach is particularly robust for ezetimibe-lipid-lowering drug combinations with overlapping spectra. The chemometric-assisted PLS method of Lotfy et al. (2017) for EZE-simvastatin demonstrated linearity from 2–8 µg/mL and 4–16 µg/mL respectively with $r^2 > 0.99$ [44].

The eco-friendly second derivative synchronous fluorescence spectroscopic method for rosuvastatin-ezetimibe (ScienceDirect, 2024) demonstrates the expanding analytical landscape for ezetimibe combinations beyond conventional UV absorption, incorporating greenness assessment via GAPI metrics [16]. This contemporary approach indicates that the BEA-EZE system would be well-served by an eco-friendly derivative spectrophotometric method validated per ICH Q2(R2) with integrated greenness profiling.

ICH Q2(R1)/(R2) Validation Parameters for Derivative Spectrophotometric Methods

Overview of ICH Q2(R1) and the Updated Q2(R2)

The ICH Q2(R1) guideline (Step 4, 1994; revision 2005) has been the international standard for analytical method validation in pharmaceutical development for three decades, defining the tests and acceptance criteria for: specificity, linearity, range, accuracy, precision (repeatability and intermediate precision), detection limit (LOD), quantitation limit (LOQ), and robustness [22]. The updated ICH Q2(R2) guideline, adopted in November 2023 and legally effective from June 14, 2024, constitutes a comprehensive revision of Q2(R1), expanding its scope to include non-linear calibration models, multivariate/chemometric methods, and biotechnology products, while clarifying terminology (e.g., specificity versus selectivity) and strengthening guidance on method transfers and partial validations under change control [23]. For spectrophotometric methods applied to BEA-EZE, both Q2(R1) (for historical benchmarking) and Q2(R2) (for new method submissions) are relevant.

Specificity/Selectivity

Specificity (or selectivity under Q2(R2) terminology) is the ability of the method to measure the analyte of interest in the presence of other components in the sample matrix. For derivative spectrophotometric methods applied to BEA-EZE, specificity is demonstrated by: (i) confirming that the chosen analytical wavelength (zero-crossing or ratio derivative peak) gives zero signal from the non-analyte drug at the concentration encountered in the dosage form; (ii) showing that common tablet excipients (lactose, microcrystalline cellulose, magnesium stearate, polyvinylpyrrolidone, sodium stearyl fumarate) do not interfere with the spectral signal at the analytical wavelengths; and (iii) demonstrating acceptable recovery from synthetic mixtures prepared with and without excipients [22]. The absence of BEA absorption above ~220 nm in methanol or ethanol means that analytical wavelengths selected for EZE in the 230–270 nm region will inherently demonstrate high spectral selectivity for EZE over BEA, a significant advantage for this drug pair.

Linearity and Range

Linearity is established by analyzing a minimum of five concentration levels within the specified range and demonstrating that the derivative signal (amplitude or peak-to-peak value) bears a linear relationship to concentration. Acceptable criteria include a correlation coefficient (r or r^2) ≥ 0.999 and a y-intercept close to zero relative to the signal at the target concentration. For BEA in the FDC tablet (180 mg), the working concentration range for spectrophotometric analysis after appropriate dilution typically spans 50–250 µg/mL; for EZE (10 mg in the FDC), the working range is typically 2–50 µg/mL. The 10:1 to 18:1 concentration ratio of BEA to EZE must be accounted for during calibration and sample preparation to ensure both analytes fall within their respective validated linear ranges [22,38].

Accuracy

Accuracy is assessed by the recovery experiment (standard addition method), typically at three concentration levels corresponding to 50%, 100%, and 150% of the target concentration. For assay methods, ICH Q2(R1) recommends a minimum of nine determinations (three levels, triplicate each). Acceptance criteria for pharmaceutical assay methods are generally a recovery of 98.0–102.0%. Derivative spectrophotometric methods for ezetimibe-containing combinations in the literature consistently report recoveries within this range (98.07–101.46%) [28,41,42], confirming that derivative approaches are capable of meeting pharmacopoeial accuracy standards when properly optimized.

Precision

Precision is evaluated at two levels: (i) repeatability (intraday precision), performed by the same analyst on the same day using the same equipment, and (ii) intermediate precision (interday precision), performed across different days or by different analysts. ICH Q2(R1) specifies that %RSD should not exceed 2% for assay methods. Derivative spectrophotometric methods for EZE have demonstrated intraday %RSD values of 0.33–0.93% and interday values similarly below 1%, confirming adequate precision [28]. For BEA-EZE simultaneous methods, precision demonstration is required independently for each analyte.

Limits of Detection and Quantitation

The LOD and LOQ are calculated using the signal-to-noise (S/N) approach (for chromatographic methods) or the standard deviation and slope approach recommended by ICH: LOD = 3.3 × σ/S and LOQ = 10 × σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve. For the BEA-EZE system, published HPLC methods report LOD values of ~1.065 µg/mL (BEA) and ~0.203 µg/mL (EZE), and LOQ of ~3.55 and ~0.677 µg/mL respectively [36]. Derivative spectrophotometric methods typically demonstrate slightly higher LOD/LOQ compared to HPLC due to the inherent noise amplification associated with differentiation; however, given the high dosage strengths in the FDC (180 mg BEA / 10 mg EZE), these sensitivities are analytically sufficient for pharmaceutical QC purposes.

Robustness

Robustness testing evaluates the ability of the method to remain unaffected by small, deliberate variations in method parameters. For derivative spectrophotometric methods applied to BEA-EZE, critical robustness parameters include: the Δλ interval (smoothing factor) used for differentiation (typically varied by ±1–2 nm), the analytical wavelength (varied by ±1–2 nm), the solvent type (e.g., methanol versus ethanol), solvent pH (where applicable), sonication time for sample dissolution, and final solution concentration. The method should tolerate these deliberate variations with %RSD < 2% for the assay result. ICH Q2(R2) now elevates robustness to a core requirement rather than an optional consideration, with a risk-based approach to determining which parameters require evaluation [23].

Comparative Evaluation of Spectrophotometric Approaches for BEA-EZE

Comparison of Derivative Techniques

The choice among D1, D2, RSD, and dual-wavelength approaches for simultaneous BEA-EZE determination involves considerations of spectral resolution, sensitivity, linearity, ease of calibration, and equipment requirements. Table 4 presents a structured comparative evaluation:

Table 4. Comparative evaluation of derivative spectrophotometric approaches for simultaneous determination of bempedoic acid and ezetimibe.

Criterion	D1 (Zero-Crossing)	D2	D3	RSD Method	Dual-Wavelength
Spectral Resolution	Good	Better than D1	Best (noise risk)	Excellent	Good
Sensitivity (LOD)	Moderate	Good	Lower (noise)	Good	Moderate
Background Suppression	Moderate	Good	Excellent	Excellent	Moderate
Calibration Simplicity	Simple	Simple	Simple	Moderate	Simple
Noise Amplification	Moderate	Higher	Highest	Minimal	Low
Effect of Concentration Ratio	Moderate impact	Moderate	Moderate	Handles well	Moderate
Regulatory Acceptance	Established	Established	Established	Established	Established
Recommended for BEA-EZE	Yes (EZE at 232+ nm)	Yes	Cautiously	Strongly Recommended	Yes (at isosbestic pt.)

Based on the spectral characteristics of BEA and EZE and the large concentration ratio in the dosage form (18:1), the ratio spectra derivative (RSD) method is most strongly recommended as the primary derivative spectrophotometric approach for simultaneous quantitation of this drug pair. The complete elimination of spectral interference by the mathematical divisor operation makes RSD particularly suited to the BEA-EZE system, where BEA's absorption above 220 nm is substantially weaker than EZE's, ensuring that accurate quantitation of BEA would be most challenging by simple zero-crossing D1 approaches. The D1 zero-crossing approach, however, remains a simpler and viable complementary technique, particularly for EZE quantitation where zero-crossing wavelengths in the 260–270 nm range may offer good sensitivity and selectivity.

Comparison of Derivative Spectrophotometry with Chromatographic Methods

Chromatographic methods (RP-HPLC, UPLC) reported for BEA-EZE offer superior specificity, the ability to detect individual degradation products, and wider linear dynamic ranges compared to derivative UV spectrophotometry. HPLC methods also permit simultaneous stability-indicating capability, which is a regulatory expectation for drug product release testing. However, derivative spectrophotometric methods offer significant practical advantages:

- Instrumentation accessibility: A standard UV-Visible double-beam spectrophotometer is far more widely available in low- and middle-income country (LMIC) quality control laboratories than HPLC/UPLC systems.
- Lower operational cost: No HPLC-grade organic solvent consumption, no column replacement costs, no pump seal maintenance.
- Analysis speed: Derivative spectrophotometric analysis of a sample set can be completed in minutes without chromatographic run times.
- Simplified sample preparation: Direct dissolution in an appropriate UV-transparent solvent, without mobile phase compatibility requirements.

- Method transferability: Simpler to transfer between instruments and laboratories without chromatographic system suitability testing requirements.

The comparative analysis indicates that for primary pharmaceutical manufacturing QC of routine release testing in resource-limited settings, derivative spectrophotometric methods represent a rational, ICH-compliant alternative that should be further developed and validated for the BEA-EZE combination.

Green Analytical Chemistry Perspectives

Principles of Green Analytical Chemistry

Green analytical chemistry (GAC) is a subdiscipline of green chemistry directed at minimizing the environmental and health impact of analytical laboratory operations. The twelve principles of GAC, articulated in analogy to the twelve principles of green chemistry, include: minimizing sample and reagent use, avoiding toxic solvents, reducing energy consumption, using online or in-line analysis, and preventing rather than treating waste [45]. GAC metrics have been developed to objectively assess the 'greenness' of analytical methods, providing quantitative or semi-quantitative scores that allow comparison across method platforms.

GAC Metrics and Their Application to BEA-EZE Methods

The principal GAC assessment tools applicable to pharmaceutical spectrophotometric and chromatographic methods include: (i) the National Environmental Methods Index (NEMI), a binary pictogram tool assessing persistence, bioaccumulation, toxicity, and waste generation; (ii) the Green Analytical Procedure Index (GAPI), a five-category semi-quantitative traffic-light system covering health, environmental hazard, energy, waste, and safety; (iii) the Analytical Eco-Scale, a scoring system penalizing for hazardous solvents, waste, and instrument energy; and (iv) the Analytical Greenness Metric (AGREE), a holistic score from 0 to 1 integrating twelve GAC principles [46,47].

For derivative spectrophotometric methods applied to BEA-EZE, the solvent choice is the primary determinant of the GAC score. Methanol, the most common solvent used for EZE UV methods, is classified as a Class 2 solvent by ICH Q3C, with a permissible daily exposure (PDE) of 3.0 mg/day and established CNS toxicity at high exposures. Ethanol (ICH Q3C Class 3, PDE 50 mg/day) is a significantly greener alternative that may be substituted for methanol in BEA-EZE derivative spectrophotometric methods if solubility and spectral data are adequate. Both BEA (freely soluble in ethanol) and EZE (freely soluble in ethanol and methanol) are sufficiently soluble in ethanol for preparation of working standard solutions, supporting an ethanol-based, greener derivative spectrophotometric method [9,18]. Compared to HPLC/UPLC methods for BEA-EZE which consume 50–200 mL of acetonitrile:water or methanol:buffer mobile phase per analytical run, UV spectrophotometric methods generating < 5 mL of dilute organic solvent waste per determination are inherently greener across all GAC metrics [46].

DISCUSSION

Analytical Gap and Clinical Significance

The clinical importance of the BEA-EZE FDC (Nexlizet®/Nustendi®) has been firmly established by the CLEAR Outcomes trial (Nissen et al., 2023), which demonstrated a 13% reduction in MACE-4 with BEA versus placebo in 13,970 statin-intolerant patients [11]. The BEA+EZE combination has been shown to reduce LDL-C by approximately 36–38%, positioning it as a viable non-statin alternative for patients unable to tolerate

statins [17,24]. As global prescribing of this combination increases, the demand for validated quality control methods — particularly those accessible to a broad range of analytical laboratories in emerging economies — will grow correspondingly.

Yet, as this systematic review has established, there is currently no published, peer-reviewed, ICH-validated derivative spectrophotometric method for the simultaneous determination of BEA and EZE. The existing analytical literature for this combination is dominated by RP-HPLC and UPLC methods, all developed since 2021, reflecting the recency of the FDC's approval. This represents a clear and practically significant analytical gap. Derivative spectrophotometric methods offer an accessible, rapid, cost-effective, and sufficient-precision alternative that should be addressed in the research literature.

Key Methodological Considerations for Future Method Development

Based on the spectral properties of BEA and EZE and the extensive methodological literature for ezetimibe-containing combinations, the following considerations are proposed for future derivative spectrophotometric method development for BEA-EZE:

- Solvent selection: Methanol or ethanol are appropriate solvents; ethanol is preferred from a GAC perspective. The solvent must dissolve both drugs from the formulation matrix and show negligible UV absorption above 200 nm.
- Spectral scanning range: 200–350 nm provides coverage of all relevant UV absorption features of both drugs.
- Derivative order optimization: A D1 zero-crossing approach exploiting BEA's zero-crossing wavelength in the EZE spectral region (~230 nm) and EZE's zero-crossing wavelength in the BEA spectral region should be evaluated. Due to BEA's deep UV absorption, a RSD method using EZE as divisor may provide more reliable selective quantitation of BEA.
- Concentration ratio accommodation: Given the 18:1 weight ratio of BEA to EZE in the FDC tablet, calibration and working concentration ranges should be designed to accommodate this ratio while maintaining both analytes within their validated linear ranges.
- ICH Q2(R2) compliance: New methods should be validated per the updated ICH Q2(R2) guideline (effective June 2024), including robustness as a core requirement and explicit use of risk-based parameter evaluation.
- Stability-indicating capability: At minimum, specificity against major degradation products under acid, alkali, and oxidative conditions should be demonstrated to support potential use in stability testing.
- GAC assessment: Greenness profiling using AGREE and GAPI should be incorporated, with ethanol preferred over methanol as solvent.

Limitations of the Present Review

This review acknowledges several limitations. First, the absence of a dedicated peer-reviewed derivative spectrophotometric method for BEA-EZE in the indexed literature means that the spectral characterization, method optimization, and validation data discussed are substantially extrapolated from the analogous ezetimibe-containing combination literature and from individual drug UV data. Second, the database search may not have captured all grey literature, conference proceedings, or institutional thesis reports describing derivative spectrophotometric work on BEA-EZE. Third, as BEA is a relatively new drug (FDA approval 2020), its analytical

chemistry is still evolving, and new methods are being published at an accelerating pace; the present review covers literature through December 2024.

FUTURE DIRECTIONS

The following specific research directions are recommended based on the analytical gaps identified in this review:

- Development and ICH Q2(R2) validation of a ratio spectra first-derivative (RSD) spectrophotometric method for simultaneous determination of BEA and EZE in pharmaceutical tablets, using ethanol as a green solvent, with integrated AGREE/GAPI greenness profiling.
- Development of a stability-indicating derivative spectrophotometric method for BEA-EZE, with forced degradation studies under acid, alkaline, oxidative, photolytic, and thermal conditions per ICH Q1A(R2).
- Exploration of chemometric approaches (PLS, PCR) for BEA-EZE simultaneous UV spectrophotometric determination in complex sample matrices, including dissolution media and blended tablet powders.
- Development of a simple, equipment-minimal absorbance ratio (Q-analysis) or isoabsorptive point method for BEA-EZE that is suitable for resource-limited laboratory settings.
- Bioanalytical extension: Development of derivative spectrophotometric or chemometric-UV methods for BEA and EZE in spiked plasma or urine for pharmacokinetic screening, with appropriate sensitivity (LOQ suitable for anticipated plasma concentrations).
- Application of near-infrared (NIR) or attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy combined with multivariate calibration for non-destructive BEA-EZE tablet analysis, contributing to process analytical technology (PAT) frameworks.

CONCLUSION

Bempedoic acid and ezetimibe represent a clinically validated, FDA-approved non-statin fixed-dose combination for LDL-C reduction in statin-intolerant patients and those with established ASCVD. The CLEAR Outcomes trial has established their cardiovascular benefit, positioning the BEA-EZE combination as an important pharmacotherapeutic option. Correspondingly, validated analytical methods for their quality control are a regulatory and scientific necessity.

This systematic review has comprehensively examined the physicochemical and spectroscopic properties of BEA and EZE, the theoretical principles of derivative spectrophotometry relevant to their simultaneous determination, and the current state of the analytical method literature for this drug pair. The review confirms that the partial UV spectral overlap between BEA (λmax ~211 nm) and EZE (λmax ~232–233 nm) precludes direct zeroth-order simultaneous quantitation but is well-suited to resolution by derivative spectrophotometric approaches, particularly the ratio spectra first-derivative (RSD) method, which is recommended as the technique of choice for this combination.

The absence of a peer-reviewed, ICH-validated derivative spectrophotometric method for simultaneous BEA-EZE determination represents a clear and practically significant gap in the pharmaceutical analytical literature. Drawing on the rich methodological precedent from analogous ezetimibe-containing drug combinations and on the established UV characterization of BEA alone, a well-designed RSD or D1 zero-crossing derivative spectrophotometric method is entirely feasible and should be a priority for the

pharmaceutical analysis research community. Future methods should align with ICH Q2(R2) requirements, incorporate GAC principles preferably using ethanol as solvent, and address stability-indicating capability. Such methods will contribute meaningfully to the quality assurance infrastructure supporting the safe and effective use of this important lipid-lowering combination.

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