EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 11
Year: 2024

REVIEW ON ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF BEMPEDOIC ACID AND EZETIMIBE IN PURE AND PHARMACEUTICAL DOSAGE FORM

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Article Received on 28/08/2024 **Article Revised on 18/09/2024** **Article Accepted on 08/10/2024**

ABSTARCT

Bempedoic acid, a novel adenosine triphosphate citrate lyase (ACL) inhibitor, and ezetimibe, a cholesterol absorption inhibitor, are both utilized for managing hyperlipidemia. Bempedoic acid is specifically indicated for reducing LDL cholesterol in patients who are resistant to statins, while ezetimibe is employed either as a monotherapy or in conjunction with other cholesterol-lowering agents to control hyperlipidemia. On February 21, 2020, bempedoic acid received FDA approval. Shortly after, on February 26, 2020, NEXLIZET—a combination of bempedoic acid and ezetimibe—was also approved. This combination is prescribed to treat hypercholesterolemia and elevated triglycerides, alongside dietary modifications. A comprehensive literature review has been conducted, examining various methods for determining these drugs as both single agents and in combination forms within bulk drugs, pharmaceutical formulations, and biological fluids. The review highlights several analytical techniques, including spectrophotometry, chromatographic methods such as HPLC, RP-HPLC, and HPTLC, as well as liquid chromatography-tandem mass spectrometry (LC-MS/MS).

KEYWORDS: Bempedoic acid, Ezetimibe, Hyperlipidemia, HPLC, UV.

INTRODUCTION

Bempedoic Acid is a first-in-class adenosine triphosphate citrate lyase (ACL) inhibitor that is taken once daily to lower LDL cholesterol levels in statin-resistant patients[1,2], Espersion therapeutics inc. [3,4] Structurally Bempedoic acid is also known as 8-hydroxy-2,2,14,14-tetramethyl penta decanedioic acid. It is a prodrug that needs to be activated in the liver.[5] The very-long-chain acyl-CoA synthetase-1 (ACSVL1) enzyme is responsible for its conversion to the pharmacologically active metabolite ETC-1002-CoA. The enzyme ATP lyase (also known as ATP synthase) is essential for cholesterol synthesis. After the parent drug is activated in the liver by coenzyme A (CoA), ETC-1002-CoA directly inhibits this enzyme[6,7], Ezetimibe is a lipid lowering compound that inhibits intestinal cholesterol and phytosterol absorption.[10,11] The discovery and research of this drug began early 1990s, Ezetimibe structure consists of (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3- hydroxypropyl]-4-(4-hydroxyphenyl) azetidin-2-one[12], Ezetimibe is used as an adjunctive therapy to a healthy diet to lower cholesterol levels in primary Hyperlipidemia, mixed Hyperlipidemia, Homozygous familial hypercholesterolemia and phytosterolemia. Ezetimibe mediates its blood cholesterol-lowering effect via selectively inhibiting the absorption of cholesterol and phytosterol by the small intestine without altering the absorption of fat-soluble vitamins and nutrients.[13,14,15] There are some other RP-HPLC methods published.[17,18,19]

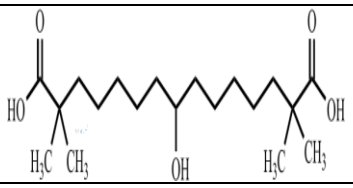
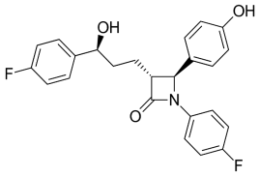
Physical and Chemical properties[8] [9]

Bempedoic acid is a white to off-white crystalline substance with the IUPAC designation of 8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid. It has the molecular formula C19H36O5 and a molecular weight of 344.5 g/mol. Its melting point ranges between 87ºC and 92ºC. Bempedoic acid is highly soluble in solvents such as ethanol, isopropanol, and phosphate buffer at pH 8, but it is insoluble in water and aqueous solutions with a pH below 5. Ezetimibe, on the other hand, is a white solid. Its IUPAC name is (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one. Ezetimibe's molecular formula is C24H21F2NO3, and it has a molecular weight of 409.4 g/mol. It has a melting point of 163ºC. Due to its insolubility in aqueous media suitable for injection, its absolute bioavailability cannot be accurately determined.

Analytical Method Development[20]

Analytical method development and validation play a crucial role in drug discovery, development, and the manufacturing of pharmaceutical products. This process is essential for identifying the purity and potential toxicity of a drug substance. Analytical method development involves selecting an accurate assay procedure to determine the composition of a formulation, ensuring the method is suitable for measuring the concentration of samples in the laboratory. This development must adhere to the protocols and acceptance criteria outlined in the ICH guidelines Q2(R1). Both analytical method development and validation are integral to the entire lifecycle of pharmaceutical products, from discovery to manufacturing. A literature review indicates that no single method has been reported for the combination of Bempedoic acid and Ezetimibe. However, techniques such as UV Spectrophotometry, RP-HPLC, HPTLC, Stability-indicating RP-HPLC, and UFLC have been employed for Bempedoic acid and Ezetimibe in combination with other drugs.

Drug profile[8, 9]

	Bempedoic acid	**Ezetimibe**
CAS number	738606-46-7	163222-33-1
Molecular formula	$C_{19}H_{36}O_5$	$C_{24}H_{21}F_2NO_3$
Drug category	Adenosine triphosphate-citrate lyase (ACL) inhibitors	Cholesterol absorption inhibitors
Chemical name	8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid	(3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidin-2-one
Structure		
Characteristics	A white to off-white crystalline powder	A white, crystalline powder.
Solubility	Highly soluble in Ethanol, Isopropanol and pH 8 phosphate buffer.	Freely soluble in ethanol, methanol and acetone.
Molecular weight	344.5 g/mol	409.4 g/mol
Melting point	87ºC to 92ºC	164 to 166 °C
Boiling point	506.5±35.0 °C at 760 mmHg	654.9±55.0 °C at 760 mmHg
Protein binding	99.3%	More than 90%
Elimination half life	21±11 hrs	Approx. 22 hrs
Pka	4.88	9.48
LogP	3.65	4.14
LogS	-4.2	-4.7
Λmax	265 nm	232 nm
Uses	Hypercholesterolemia; Dyslipidemias	Anticholesteremic drug, Antilipemic drug and Antimetabolite

Mechanism of action of Bempedoic acid

LDL cholesterol is typically synthesized in the liver and circulates in the bloodstream. However, when the blood becomes oversaturated with LDL, the excess can accumulate in blood vessels, including the coronary arteries, elevating the risk of cardiovascular events. Bempedoic acid, a prodrug, must be activated in the liver to exert its effects. This activation is facilitated by the very-long-chain acyl-CoA synthetase-1 (ACSVL1) enzyme, converting the drug into its active form, ETC-1002-CoA. The active metabolite then inhibits ATP citrate lyase, a key enzyme in cholesterol biosynthesis, by interfering with its activity after activation through coenzyme A (CoA).[21- 25]

Mechanism of action of Ezetimibe

Ezetimibe lowers blood cholesterol by selectively inhibiting the absorption of cholesterol and phytosterols in the small intestine, without affecting the absorption of fat-soluble vitamins and other essential nutrients. Its primary action is directed at the Niemann-Pick C1-Like 1 (NPC1L1) protein, which is involved in cholesterol transport. NPC1L1 is found on the apical surface of intestinal enterocytes as well as at the hepatobiliary canalicular interface. It facilitates the uptake of free cholesterol into enterocytes through interaction with adaptor protein 2 (AP2) and clathrin. Once cholesterol from the gut or bile is incorporated into the enterocyte membrane, it binds to NPC1L1's sterol-sensing domain, forming an NPC1L1-cholesterol complex. This complex is then internalized by clathrin-mediated endocytosis, forming a vesicle that is transported to the endocytic recycling compartment for storage.[26-27]

Literature Review

Sr. No.	Title/ Method	Descriptions	Ref. No.
1.	RP-HPLC Method Development and Validation for the Simultaneous Estimation of Bempedoic Acid and Ezetimibe in Pharmaceutical Dosage Form	Column: Agilent 150mm x 4.6 mm, 5µm Mobile phase: 55% Acetonitrile: 45% KH_2 Flow rate: 1.0 mL/min Wavelength: 230 nm Retention time: 6 mins	28
2.	Development and Validation of a RP-HPLC Method for the Simultaneous Determination of Bempedoic Acid & Ezetimibe in Pure and Pharmaceutical Dosage Form	Mobile phase: 30% HSA: 70% Acetonitrile Flow rate: 1.0 mL/min Wavelength: 225 nm Retention time: 3.246 and 3.865	29
3.	Development and Validation of Novel RP-HPLC Method for the Simultaneous Estimation of Ezetimibe and Bempedoic Acid in a Tablet dosage Form	Column: Prontosil C18 (250 x 4.6 mm, 5 µm) Mobile phase: 60% Acetonitrile: 40% Water Flow rate: 1.0 mL/min Wavelength: 225 nm Retention time: 4.7 and 5.7 mins	30
4.	Validated method for the simultaneous estimation of Bempedoic acid and Ezetimibe in bulk and tablet formulation by RP-HPLC method	Column: Kromosil C18 150 x 4.6 mm, 5µm Mobile phase: 55% KH_2PO_4: 45% Acetonitrile Flow rate: 0.9 mL/min Wavelength: 246 nm Retention time: 2.240 and 2.956 mins	31
5.	Stability indicating RP-UPLC method for simultaneous quantification of Bempedoic acid and Ezetimibe in bulk and pharmaceutical formulations	Column: Waters Acquity C18 [50×2.1 mm, 1.7 µm] Mobile phase: 50% Methanol: 30% Acetonitrile: 20% Water Flow rate: 0.5 mL/min Wavelength: 260 nm Retention time: 1.827 and 3.577 mins	32
6.	Characterization of novel stress degradation products of Bempedoic acid and Ezetimibe using UPLC–MS/MS: development and validation of stability-indicating UPLC method	Column: C18 (150 mm×4.6 mm, 3.5 µm). Mobile phase: 50% Orthophosphoric acid: 50% Acetonitrile Flow rate: 1 mL/min Wavelength: 230 nm	33
7.	Synchronized analysis of bempedoic acid and ezetimibe in pure binary mixture and their combined tablets by a new stability indicating RP-UPLC method	Column: Phenyl XBD (100 x 2.1mm, 1.7 µm) Mobile phase: 60% TFA: 40% Water Flow rate:0.4 mL/min Wavelength: 236 nm Retention time: 0.43 and 0.86 mins	34
8.	RP HPLC Method Development and Validation for Simultaneous Estimation of Bempedoic Acid, Ezetimibe and Atorvastatin in Synthetic Mixture	Column: C 18 (250 mm x 4.6 mm),5 µm Mobile phase: 30% Potassium Dihydrogen Phosphate: 60% Methanol: 10% Acetonitrile Flow rate:1 mL/min Wavelength: 262 nm Retention time: 3.76, 5.49 and 6.85 mins	35
9.	Development and validation of a reversed-phase HPLC method for the determination of Ezetimibe in pharmaceutical dosage forms	Column: Kromasil 100 (250 x 4mm, 5 µm) Mobile phase: 30% Water: 60% Acetonitrile Flow rate:0.5 mL/min Wavelength: 232 nm Retention time: 2.6 mins	36
10.	High performance liquid chromatographic method for determination of Ezetimibe in pharmaceutical formulation tablets	Column: C18 analytical column (250mm x 4.6mm, particle size 5µm Mobile phase: 75% Acetonitrile: 25% Ammonium Acetate Flow rate:1 mL/min Wavelength: 240 nm Retention time: 5.083 mins	37
11.	A stability indicating RP-HPLC method development for determination of	Column: Zorbax SB C18 (250mm x 4.6mm), 5 µm	38

	ezetimibe in tablet dosage form	Mobile phase: 20% Orthophosphoric acid: 80% Acetonitrile Flow rate: 1 mL/min Wavelength: 232 nm Retention time: 3.5 mins	
12.	RP-HPLC Method Development and Validation for the Simultaneous Estimation of Atorvastatin and Ezetimibe in Pharmaceutical Dosage Form	Column: C18 (250 x 4.6 mm, 5 mm) Mobile phase: 35% Phosphate Buffer: 65% Acetonitrile Flow rate: 1 mL/min Wavelength: 228 nm Retention time: 2.36 and 3.43 mins	39
13.	LC–MS–MS Simultaneous Determination of Atorvastatin and Ezetimibe in Human Plasma	Column: C18 (100 x 4.6 mm, 3.5 µm) Mobile phase: 30% Formic acid: 70% Acetonitrile Flow rate:0.6 mL/min Retention time: 3.0 mins	40
14.	Development and Validation of a Method for Simulataneous Densitometric Estimation of Atorvastatin Calcium and Ezetimibe as the Bulk Drug and in Tablet Dosage Forms	Stationary phase: - Precoated Silica gels F254 aluminium Mobile phase: - Toluene: methanol (8:2; v/v) Detection wavelength: - 240nm	41
15.	HPTLC Method Development, Validation and Stress Degradation Studies for Atorvastatin and Ezetimibe in Multicomponent Tablet Dosage Form	Stationary phase: - Precoated Silica gels F254 aluminium Mobile phase: - Toluene: ethyl acetate: methanol (12:5:3; v/v/v) wavelength: - 254nm	42
16.	High Performance Liquid Chromatographic Method for Estimation of Ezetimibe in Pharmaceutical Formulation Tablets	Column: C18 (250 x 4.6 mm, 5 µm) Mobile phase: Acetonitrile: ammonium acetate (75:25, v/v), pH = 3.0 Wavelength: 240 nm Flow rate: 1.0 mL/min Retention time: 3.6 mins	43
17.	Validated RP-HPLC Method for Estimation of Ezetimibe in Different Tablet Dosage Form	Column: C18 (250 x 4.6 mm, 5 µm) Mobile phase: 50% Acetonitrile: 50% Methanol Wavelength: 245 nm Flow rate: 1.0 mL/min Retention time: 4.959 mins	44
18.	A Stability Indicating RP-HPLC Method Development for Determination of Ezetimibe in Tablet Dosage Form	Column: Zorbax SB C18 (250 x 4.6 mm, 5 µm) Mobile phase: 0.02N ortho phosphoric acid: acetonitrile (20:80, v/v), pH = 3.0 Wavelength: 232 nm Flow rate: 1.0 mL/min Retention time: 3.5 mins	45
19.	Development and Validation of a Reversed Phase HPLC Method for the Determination of Ezetimibe in Pharmaceutical Dosage Forms	Column: Kromasil 100 C18 (250 x 4.6 mm, 5 µm) Mobile phase: Water: Acetonitrile: ammonium acetate (30:70 v/v) Wavelength: 232 nm Flow rate: 0.5 mL/min Retention time: 6 mins	46
20.	HPLC Analysis for Simultaneous Determination of Atorvastatin and Ezetimibe in Pharmaceutical Formulations	Column: Inertsil ODS- 3V (250 x 4.6 mm, 5 µm) Mobile phase: - 0.01M ammonium acetate buffer: acetonitrile (50:50, v/v) Wavelength: 254 nm Flow rate: 1.0 mL/min Retention time: 15.5 and 19.3 mins	47
21.	A RP-HPLC Method for Simulataneous Estimation of Atorvastatin and Ezetimibe in Pharmaceutical Formulations	Column: Phenomenex C18 (250 x 4.6 mm, 5 µm) Mobile phase: Water and 0.4%(v/v) TEA: acetonitrile (50:50, v/v); pH = 6.5, adjusted suing orthophosphoric acid Wavelength: 248 nm Flow rate: 1.0 mL/min	48

		Retention time: 3.42 and 6.90 mins	
22.	A Validated Stability Indicating RP-HPLC Method for the Simultaneous determination of Atorvastatin Calcium and Ezetimibe Hydrochloride in Bulk and Tablet Dosage Form	Column: X- terra C8 (150 x 4.6 mm, 3.5 µm) Mobile phase: Phosphate buffer: acetonitrile (pH = 3.5) pH adjusted with orthophosphoric acid; (55:45, v/v) Wavelength: 232 nm Flow rate: 1.2 mL/min Retention time: 6.81 and 4.96 mins	49
23.	Application of a Stability Indicating HPTLC Method for the Quantitative determination of Ezetimibe in Pharmaceutical Dosage Form	Stationary phase: - Precoated Silica gels F254 aluminium Mobile phase: - Toluene: ethyl acetate (7:3; v/v) Detection wavelength: - 254 nm	50
24.	Development and Validation of a Liquid Chromatography-Tandem Mass Spectrometry Method for the determination of Ezetimibe in Human Plasma and Pharmaceutical Formulations	Ion transition for atorvastatin (m/z): - 392/161 Ion transition for fluvastatin (m/z): - 359.3/280 Column: - Phenomenex (Torrance, USA) Luna C18 column (150 x4.5mm, 4µm) Mobile phase: - 0.02M phosphate buffer (pH=7): acetonitrile: methanol (40:55:5, v/v/v) Flow rate: - 1.0ml/min	51
25.	LC-MS/MS Simultaneous Determination of Atorvastatin and Ezetimibe in Human Plasma	Ion transition for atorvastatin (m/z): - 422.0/290.0 Ion transition for fluvastatin (m/z): - 408.0/271.0 Column: - Zorbax Eclipse plus (USA) C18 column (4.6*100mm, 3.5µm) Mobile phase: - 0.2% formic acid in water: acetonitrile (30:70, v/v) Flow rate: - 0.6ml/min Retention time: - 2.680 & 3.361 min	52

CONCLUSION

The comprehensive review of the study emphasizes the various analytical techniques employed for determining Bempedoic acid, Ezetimibe, and their combination in bulk drugs, pharmaceutical formulations, and biological samples. Among these, High-Performance Liquid Chromatography (HPLC) and spectrophotometry emerged as the most commonly utilized methods. HPLC, in particular, is preferred due to its superior sensitivity, specificity, and enhanced separation capabilities for both qualitative and quantitative analysis. Additionally, other techniques such as High-Performance Thin-Layer Chromatography (HPTLC) and LC-MS/MS have been applied in the assessment of Bempedoic acid, Ezetimibe, and their combinations in serum, pharmaceutical preparations, and stability testing.

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