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RECENT DEVELOPMENTS IN ANALYTICAL TECHNIQUES FOR BEMPEDOIC ACID AND ATORVASTATIN: A SYSTEMIC REVIEW

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ABSTRACT

Bempedoic acid is a newer one for cardiovascular disease treatment. It generally alters the cholesterol synthesis pathway. This suppression affects fatty acid synthesis, lipogenesis and low-density level cholesterol. Previous trials of bempedoic acid showed fewer side effects as compared to other drugs used for hypercholesteremia. Fixed combinations of atorvastatin and bempedoic acid have sparked a rising interest in studying their probable synergistic effect when combined. During its studies, method development is one of the initial processes that includes optimizing analytical parameters such as chromatographic conditions, its settings, mobile phase selection and composition, and detection wavelength for efficient separation and quantification of both of these drugs in varied biological samples for analysis. This review article includes literature reports from past developments of these drugs, singly or in combination with others. Therefore, the finding reveals the potential to conduct further research and enhancements for treating cardiovascular diseases.

Keywords: Analytical method, Atorvastatin, Bempedoic Acid, HPLC, HPTLC, UV

1. INTRODUCTION

Cardiovascular diseases are considered one of the primary contributors to mortality that occur all over the world holding 17.9 million per year death. This disease is usually described by the term hypercholesteremia which is defined as a high level of cholesterol in blood stream. This causes myocardial infarction, heart failure, stroke, claudication and more **[1-3]**. Major factors that are being reported include environmental, metabolic, and behavioural factors. Elaborating these risk factors it reveals temperature variation, blood pressure elevation, greater body mass index (BMI), fluctuation in blood glucose level during fasting conditions, abnormal kidney function, alteration in eating habits, extreme use of alcoholic beverages, smoking and drugs, no or lack of physical activity **[4]**.

1.1 Bempedoic acid

Bempedoic acid (Nexletol) which is basically from the anti-lipidemic class is a white to pale white powder with molecular formula $C_{19}H_{36}O_5$ and molecular mass 344.492 g/mol carrying IUPAC name 8-hydroxy-2, 2, 14, 14-tetramethylpentanedecanedioicacid. It has a melting point of 87-92°C and is highly soluble in ethanol, isopropanol and pH8 phosphate buffer but insoluble in water and aqueous solution below pH 5 **[5]**.

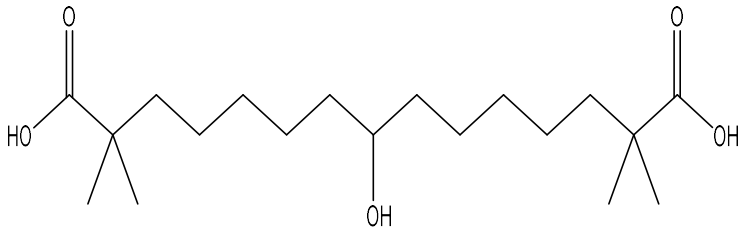

Figure 1: Structure of bempedoic acid

It is a newer drug that acts upon an enzyme that is involved in the synthesis of the cholesterol pathway. It is mainly a prodrug that gets converted into its active state called bempedoyl co-A, with the help of the long-chain acyl CoA synthetase enzyme. This enzyme usually exists in hepatocytes but not in skeletal muscle. This bempedoyl coA then binds with the ATP citrate lyase enzyme and blocks the step of converting citrate to acetyl coA. The citrate lyase enzyme is mainly responsible enzyme for generating acetyl co A and oxaloacetate from citric acid obtained from the TCA cycle occurring in mitochondria. Hence, reduction of these during the pathway leads to changes in fatty acid synthesis, cholesterol production, and gluconeogenesis as well **[6-7]**.

Table 1: Developed analytical method for determination of bempedoic acid and its combination in pharmaceutical dosage form

Sr. No.	Method	Description	Ref. No.
1.	Validated method for the simultaneous estimation of Ezetimibe and Bempedoic Acid in bulk and tablet formulations by RP-HPLC	Stationary phase: Kromosil C18 (150×4.6 mm, 5 µm) Mobile phase: Acetonitrile: KH_2PO_4 (45:55 v/v) Flow rate: 0.9 ml/min λmax: 246 nm Retention time: 2.240 min (Bempedoic Acid) 2.956 min (Ezetimibe)	[8]
2.	Stability indicating RP-UPLC method for simultaneous quantification of Bempedoic Acid and Ezetimibe in bulk and pharmaceutical formulations	Stationary phase: C18 (50×2.1 mm, 1.7 µm) Mobile phase: Methanol: Acetonitrile: Water (50: 30: 20 v/v/v) Flow rate: 0.5 ml/min λmax: 260 nm Retention time: 1.827 min (Bempedoic Acid) 3.577 min (Ezetimibe)	[9]
3.	RP-HPLC method development and validation of simultaneous estimation of Bempedoic Acid, Ezetimibe and Atorvastatin in a synthetic mixture	Stationary phase: C18 (250×4.6 mm, 5 µm) Mobile phase: Potassium Dihydrogen Phthalate: Methanol: Acetonitrile (30: 60: 10) 5.8 pH buffer Flow rate: 1 ml/min λmax: 262 nm Retention time: 3.76 min (Bempeodic Acid) 5.49 min (Ezetimibe) 6.85 min (Atorvastatin)	[10]
4.	Development and validation of novel RP-HPLC method for the simultaneous estimation of Ezetimibe and Bempedoic Acid in a tablet dosage form	Stationary phase: Prontosil C18 (150×4.6 mm. 5 µm) Mobile phase: Water: Acetonitrile (40: 60 v/v) Flow rate: 1 ml/min λmax: 225 nm Retention time: 4.7 min (Bempedoic Acid) 5.7 min (Ezetimibe)	[11]

1.2 Atorvastatin

Atorvastatin (Lipitor) belongs to a class statin, shows anticholestermic properties fall in BCS class II has an appearance of white to creamy white crystalline powder having molecular formula $C_{33}H_{35}FN_2O_5$, molecular weight of 558.64 g/mol and IUPAC name (3R,5R)-7-[2-(4fluorophenyl)-3-phenyl-4-(phenylcarbomoyl)-5-(propane-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoicacid. It has a melting point of 176°C and is very slightly soluble in distilled water, pH 7.4, phosphate buffer, acetonitrile, slightly soluble in ethanol and freely soluble in methanol **[12-13]**.

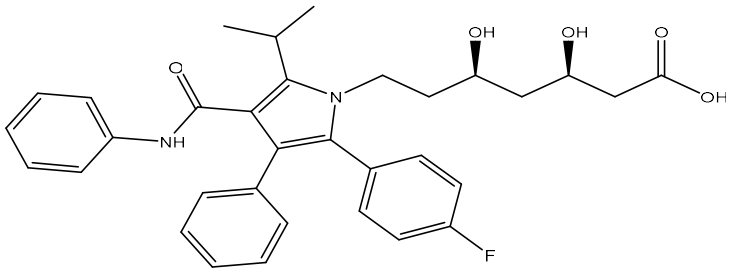

Figure 2: Structure of atorvastatin

Statin category atorvastatin is the most preferred one to lower LDL cholesterol. It shows its activity by blocking another enzyme other than the bempedoic acid inhibition site. Hence binds with HMG CO A reductase and competes for the active site of the enzyme in

the same way as native HMG CO A reductase substrates do. It is a rate-limiting enzyme in cholesterol synthesis that is competitively inhibited by statins, resulting in changes in the cholesterol synthesis pathway. These alterations benefit by lowering low-density level cholesterol; however repetitive muscle-related negative consequences, limit dosage and prevent dyslipidemic patients from achieving, maximum risk mitigation **[14-15]**.

Table 2: Developed analytical methods for determination of Atorvastatin and its combination in different pharmaceutical dosage forms

Sr. No.	Method	Description	Ref. No.
1.	A validated RP-HPLC method for the determination of Atorvastatin Calcium in tablets	Stationary phase: LiChroCART C18 (250×4 mm, 5 µm) Mobile phase: 0.1% Acetic Acid: Acetonitrile (45: 55 v/v) pH 3.8 Flow rate: 0.8 ml/min λmax: 246 nm Retention time: 6.312 min Linearity: 8.13-23.77 µg/ml	[16]
2.	Development of RP-HPLC method for simultaneous estimation of Atorvastatin Calcium and Clopidogrel Bisulphate in a pharmaceutical capsule dosage form	Stationary phase: Eurosphere C18 (250×4.6 mm, 5 µm) Mobile phase: Acetonitrile: 0.01M KH_2PO_4 (75: 25 v/v) pH 6.1 Flow rate: 1 ml/min λmax: 240 nm Retention time: 3.5394 min (Atorvastatin Calcium) 10.7578 min (Clopidogrel Bisulphate)	[17]
3.	Simultaneous estimation of Atorvastatin Calcium and Ezetimibe in combined formulation by RP-HPLC	Stationary phase: Phenomenex Gemini C18 (250×4.6 mm, 5 µm) Mobile phase: acetonitrile: ammonium acetate buffer (pH 3) (50: 50 v/v) Flow rate: 1.2 ml/min λmax: 247 nm Retention time: 3 min (Atorvastatin) 5.2 min (Ezetimibe)	[18]
4.	Development and validation of the RP-HPLC method for the simultaneous estimation of Irbesartan and Atorvastatin in a synthetic mixture	Stationary phase: Thermo C18 (250×4.6 mm, 5 µm) Mobile phase: Acetonitrile: Methanol: 0.1% formic acid (50: 10: 40 v/v/v) pH 3.5 with Triethylamine Flow rate: 1ml/min λmax: 262 nm Retention time: 4.01 min(Irbesartan) 7.03 min (Atorvastatin)	[19]
5.	Development and validation of RP-HPLC method for simultaneous estimation of Ramipril, Aspirin, and Atorvastatin in pharmaceutical preparations	Stationary phase: 150×4.6 mm, 5 µm) Mobile phase: (A) Acetonitrile: Methanol (65: 35) (B) 10 mM $NaH_2PO_4.H_2O$ Mixture of A & B (60: 40 v/v) pH 3 with 5% v/v o-phosphoric acid Flow rate: 1.5 ml/min λmax: 230 nm Retention time: 3.620 min (Ramipril) 4.920 min (Aspirin) 11.710 min (Atorvastatin)	[20]
6.	RP-HPLC method development and validation for simultaneous estimation of Atorvastatin Calcium and Pioglitazone Hydrochloride in pharmaceutical dosage form	Stationary phase: C8 (250×4.6 mm, 5 µm) Mobile phase: Acetonitrile: Water (45: 55 v/v) Ph 6.2 with o-phosphoric acid Flow rate: 1 ml/min λmax: 232 nm Retention time: 4.1 min (Atorvastatin Calcium) 8.1 min (Pioglitazone Hydrochloride)	[21]
7.	Development and validation of RP-HPLC method for determination of Atorvastatin Calcium and Nicotinic Acid in combined tablet dosage form	Stationary phase: Zorbax SB C18 (150×4.6 mm, 5 µm) Mobile phase: Acetonitrile: Water (85: 15) pH 4.5 with o-phosphoric acid Flow rate: 1 ml/min λmax: 261 nm Retention time: 6.092 min (Atorvastatin Calcium) 3.125 min (Nicotinic Acid)	[22]

8.	**Development and validation of an eco-friendly HPLC method for the determination of Atorvastatin and Vitamin D_3 in pure form and pharmaceutical formulation**	**Stationary phase: C18 (100×4.6 mm, 3.5 µm) Mobile phase: 0.1% o-phosphoric acid (pH 2.16): Ethanol Flow rate: 1 ml/min λmax: 246 nm (Atorvastatin); 264 nm (Vitamin D_3) Retention time: 6.129 min (Atorvastatin) 9.163 min (Vitamin D_3)**	**[23]**
9.	**Development and validation of RP-HPLC method for simultaneous estimation of Atorvastatin Calcium and Fenofibrate in tablet dosage forms**	**Stationary phase: Luna C18 (250×4.6 mm, 5 µm) Mobile phase: methanol: acetate buffer (pH 3.7) (82: 18 v/v) Flow rate: 1.5 ml/min λmax: 248 nm Retention time: 3.02 min (Atorvastatin Calcium) 9.05 min (Fenofibrate)**	**[24]**
10.	**Development and validation of an RP-HPLC method for the determination of Atorvastatin Calcium and Aspirin in a capsule dosage form**	**Stationary phase: C18 (250×4.6 mm) Mobile phase: 0.02M KH_2PO_4: Methanol (20: 80) pH 4 with o-phosphoric acid Flow rate: 1 ml/min λmax: 240 nm Retention time: 5.4 min (Atorvastatin Calcium) 3.4 min (Aspirin)**	**[25]**
11.	**Analytical method development and validation of simultaneous determination of Atorvastatin Calcium and Amlodipine Besylate in tablet dosage form by RP-HPLC**	**Stationary phase: C18 (250×4.6 mm, 5 µm) Mobile phase: 0.1% Phosphate buffer: Acetonitrile: Methanol (53: 43: 4 v/v/v) Flow rate: 1 ml/min λmax: 246 nm Retention time: 3.337 min (Amlodipine) 6.067 min (Atorvastatin)**	**[26]**
12.	**Development and validation of a new analytical method for the estimation of Atorvastatin Calcium Hydrate residue by using a UV spectrometer**	**Solvent: methanol: water (90: 10) λmax: 245 nm Linearity: 1-10 µg/ml**	**[27]**
13.	**HPLC-UV method for determination of Atorvastatin Calcium in pharmaceutical formulations**	**Stationary phase: Acclaim 120 C18 (250×4.6 mm, 5 µm) Mobile phase: Acetonitrile: Dichloromethane: Acetic Acid (68.6: 30.6: 0.8) Flow rate: 1 ml/min λmax: 246 nm Retention time: 2.68 min**	**[28]**
14.	**Simple spectrophotometric methods for the determination of Amlodipine and Atorvastatin in bulk and tablets**	**Solvent: 50% v/v aqueous Methanol λmax: 244 nm (max. Absorbance Amlodipine and Atorvastatin) 365 nm (Amlodipine)**	**[29]**
15.	**Validated UV-visible spectrophotometric method for estimation of Atorvastatin in pure and pharmaceutical dosage form using Methyl Orange reagent**	**Solvent: Methanol λmax: 410 nm Range: 50-300 µg/ml**	**[30]**
16.	**Development and validation of a UV-Spectrophotometric method for quantification of Atorvastatin in tablets**	**Solvent: Methanol: Water (50: 50) λmax: 248 nm Range: 5-15 µg/ml**	**[31]**
17.	**Simultaneous estimation of Atorvastatin Calcium and Aspirin in pure and capsule dosage form by using the UV-Spectrophotometric method**	**Solvent: Methanol λmax: 232 nm Range: 5-40 µg/ml (Atorvastatin Calcium) 5-30 µg/ml (Aspirin)**	**[32]**
18.	**Validated spectrophotometric methods for the quantitative determination of Atorvastatin Calcium and Metoprolol Succinate in capsules**	**Solvent: Methanol λmax: 246.5 nm (Atorvastatin) 276.5 nm (Metoprolol) Range: 4-24 µg/ml (Atorvastatin Calcium) 10-60 µg/ml (Metoprolol Succinate)**	**[33]**
19.	**Development and validation of an HPTLC method for simultaneous estimation of Atorvastatin Calcium and Ezetimibe**	**Stationary phase: precoated silica gel 60 F_{254} Mobile phase: Chloroform: Benzene: Methanol: Acetic Acid (6: 3: 1: 0.1 v/v/v/v) λmax: 250 nm Retardation factor: 0.3 (Atorvastatin) 0.53 (Ezetimibe)**	**[34]**
20.	**HPTLC method development, validation and stress degradation studies for Atorvastatin and Ezetimibe in multicomponent tablet dosage form**	**Stationary phase: precoated silica gel 60 F_{254} Mobile phase: Toluene: Ethyl Acetate: Methanol (12: 5: 3 v/v/v) λmax: 254 nm Retardation factor: 0.31 (Atorvastatin)**	**[35]**

		0.57 (Ezetimibe)	
21.	HPTLC method development and validation for the estimation of Atorvastatin Calcium and Pioglitazone Hydrochloride in pharmaceutical dosage form	Stationary phase: precoated silica gel 60 F_{254} Mobile phase: Chloroform: Methanol: Toluene (6: 3: 4 v/v/v) λmax: 259 nm Retardation factor: 0.45 (Atorvastatin) 0.30 (Pioglitazone)	[36]
22.	Validated HPTLC method for estimation of Atorvastatin Calcium and Fenofibrate in bulk drug and its tablets according to ICH guidelines	Stationary phase: precoated silica gel 60 F_{254} Mobile phase: Dichloromethane: Toluene: Methanol (2: 6: 2 v/v/v) λmax: 287 nm Retardation factor: 0.23±0.03 (Atorvastatin Calcium) 0.83±0.03 (Fenofibrate)	[37]
23.	Development and validation of novel HPTLC method for the simultaneous estimation of Atorvastatin Calcium and Telmisartan in tablet dosage form	Stationary phase: precoated silica gel 60 F_{254} Mobile phase: Dichloromethane: Methanol: Toluene: Ammonia (5: 2: 1: 0.2 v/v/v/v) λmax: 289 nm Linearity: 100-600 µg/ml Retardation factor: 0.17±0.02 (Atorvastatin Calcium) 0.49±0.02 (Telmisartan)	[38]

2. CONCLUSION

This describes the developed analytical methods like spectroscopy and chromatographic methods for atorvastatin and bempedoic acid alone or in combination with other medications. The method that was being created and deployed was easy, efficient, precise, affordable and reproducible. However, there is currently no way to combine atorvastatin and bempedoic acid altogether. Based on the literature survey, research and analysis may still be ongoing or technique development has not yet been finished.

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