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Research Article

Simultaneous Estimation of Bempedoic Acid and Pitavastatin Calcium in Bulk Drug by RP-UPLC

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ABSTRACT

The present study focused on establishing and validating a reliable RP-UPLC method for the simultaneous quantification of Bempedoic acid and Pitavastatin calcium in bulk drug. Chromatograph analysis was performed on a C18 column (250 mm x 4.6 mm, 5 μ m) using a mobile phase comprising potassium dihydrogen phosphate buffer in acetone at pH 4.5, Adjusted with KOH, in a 50:50 (v/v). The mobile phase was pumped at a flow rate of 1 mL/min under ambient temperature conditions. UV detection was performed at 221 nm. The developed method provided satisfactory separation of both analytes, with retention times of 9.215 min for Pitavastatin calcium and 13.345 min for Bempedoic acid. The analytical procedure was evaluated in accordance with applicable ICH recommendations. The method exhibited a satisfactory linear response over five concentration levels and demonstrated good precision, with low percentage relative standard deviation (RSD%) values. Accuracy was assessed by recovery studies at 50%, 100% and 150% concentration levels, resulting in recoveries within the range of 98-102 % for both drugs. The robustness of the procedure was examined by intentionally modifying the flow rate by ± 0.1 mL/min. The method remained suitable under the investigated conditions. Forced degradation studies indicated appreciable degradation of the analytes under acidic and oxidative conditions. The developed procedure was capable of distinguishing the degradation products from the respective drug peaks, supporting its ability to monitor the stability of Bempedoic acid and Pitavastatin calcium. In conclusion, the developed RP-UPLC procedure provides a simple, rapid, accurate, precise, and economical analytical approach for the simultaneous determination of Bempedoic acid and Pitavastatin calcium in bulk drug. Owing to its satisfactory validation characteristics and ability to differentiate degradation products from the drug substances, the method can be considered appropriate for routine quality assessment and batch-release testing.

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INTRODUCTION

Ultra-Performance Liquid Chromatography (UPLC), commonly known as Ultra-High-Performance Liquid Chromatography (UHPLC), represents an advanced development of conventional HPLC. The technique utilizes very small stationary-phase particles, generally below 2 μm , together with high-pressure pumping systems to achieve enhanced chromatographic efficiency and rapid separation. First commercialized in 2004 (Waters ACQUITY UPLC), UPLC broke the traditional HPLC limits by enabling much higher plate counts and faster runs. In practice, UPLC achieves dramatically shorter analysis times and lower solvent use, often 5–10 $\times$ faster runs and 5–15 $\times$ less solvent use compared to a matched HPLC method [1]. Peaks are narrower and taller (higher efficiency, improved sensitivity), and thousands of theoretical plates per meter are common. These advances have led to broad industry uptake: by 2010 almost all LC instrument vendors offered UHPLC systems and modern pharmacopeial guidelines now permit using sub-2 μm columns in place of older HPLC columns under defined conditions [2].

UPLC is now standard in high-throughput pharmaceuticals, bioanalysis, environmental and food labs. This introduction reviews UPLC's history, physical principles (van Deemter, efficiency, sensitivity), instrumentation, column chemistries, method considerations, and comparison to conventional HPLC, concluding with applications and best practices [3].

Historical Background

Since the 1960s, liquid chromatography has undergone systematic evolution alongside process in chromatogram theory and instrumental design. The advantage of using finer particles for enhanced column efficiency was anticipated as

early as 1941 by Martin and Synge. This concept was put into practice in the mid-1960s, when packing materials with particle size $<1\ \mu\text{m}$ was tested by Piel (1966) and Bidlingmeyer (1969) using either gravity elution or extremely high operating pressures [4]. During 1970-1980, HPLC reached a mature stage with columns packed with 5-10 μm materials, and subsequently, 3 μm packings were introduced in the 1990s. A landmark development was reported in 2003 with Agilent's introduction of Zorbax Rapid Resolution HT columns containing 1.8 μm particles, which first crossed the 2 μm threshold. In 2004, Waters Corp. launched its ACQUITY UPLC system designed for sub-2 μm columns operating at 15,000 psi and trademarked the acronym UPLC. Around the same period, Jim Jorgenson introduced the designation UHPLC and demonstrated exceptionally high column efficiency, exceeding 200,000 theoretical plates, using capillary columns operated under pressures of approximately 4,000 bar.

Instruments capable of delivering pressures in the range of 15-19 kpsi were commercialized shortly thereafter, making sub-2 μm separations a routine practice. In 2010, a major transition was evident, as almost all modern LC systems were manufactured with UHPLC compatibility, low extra column dispersion, and high-pressure tolerant pumping modules. Regulatory guidelines have also been updated to reflect this shift; for example, the 2014 revision of USP <621> permits the replacement of conventional HPLC columns with sub-2 μm counterparts in numerous isocratic procedures [5].

1.1 Principles: Particles, Efficiency and Van Deemter

UPLC's gains stem from **particle size**. Smaller particles (e.g., 1.7 μm vs 5 μm) drastically the use of smaller stationary-phase particles improve chromatographically performance by increasing



the number of theoretical plates per unit length and minimizing the spreading of analyte bands.

According to the Van Deemter relationship, the plate height (H), which represents the height equivalent to a theoretical plate (HETP), is influenced by eddy diffusion (A), longitudinal diffusion (B/u), and mass transfer resistance (C*u). The A and C contributions are dependent on the particle size of the column packing material. The plate number for a given column length. Smaller particles also allow higher optimal linear velocities: van Deemter curves flatten and shift right, and UPLC can run much faster flows with minimal loss in efficiency. In practice, sub-2 μm columns routinely produce very narrow peaks (high N) and tall peak heights; efficiency (N) of 200,000–400,000 per meter is achievable. Resolution is improved ($R_s \propto \sqrt{N} \times \text{selectivity}$).

In simplified terms, narrower peaks (H smaller) increase sensitivity: peak height $\propto 1/\text{width}$ at equal area. Key trade-offs include much higher backpressure: pressure drop $\propto (\text{flow particle } \{-2\})$. So UPLC systems must handle $\sim 15,000\text{--}19,000$ psi (1000–1300 bar). In summary, UPLC fundamentally leverages sub-2 μm and core-shell particles to multiply column efficiency, raising throughput and sensitivity [6].

1.2 Instrumentation

A UPLC system is architecturally similar to HPLC but optimized for high pressure and low dispersion.

I) Pump: binary (or quaternary) high-pressure mixing pumps (>1000 bar rating) with minimal dwell volume is required [6-8].

II) Plumbing: stainless or PEEK tubing with minimized ID and length reduces extra-column volume (Ideally < 20 μL) [7-8].

III) Injector/Autosampler: low-volume injector loops or direct injection micro-syringes are used; zero-dead-volume valves and narrow-bore sample loops ($\sim 1\text{--}10$ μL) minimize dispersion.

IV) Column oven: Typical temperatures 30–50 $^{\circ}\text{C}$; temperature control can reduce viscosity and help offset viscous heating in long runs.

V) Columns: UPLC columns are shorter/smaller than typical HPLC. Common formats are 2.1 mm ID $\times$ 30–150 mm length, packed with 1.3–1.9 μm particles (fully porous or core-shell) [9].

VI) Detectors: standard UV/VIS, PDA or MS detectors are used. Microbore flow cells (e.g., 0.1–1 μL cell volume) improve sensitivity and reduce band spreading.

VII) Pressure limits: modern UPLC systems routinely handle 15,000–19,000 psi, though typical runs use lower pressures (e.g., 300–1200 bar). Notably, all components must withstand these pressures; many UHPLC systems use reinforced pistons, metal-free fluid paths, and specialized ferrules. Because system dispersion must be very low to reap the benefits of UPLC, system dwell volume, mixing volume and dead volumes are minimized by design [10].

1.3 Column Chemistry and Stationary Phases

UPLC columns cover similar chemistries as HPLC, but with fine particles or novel designs.

I) Fully porous particles: Traditional silica or hybrid silica packing with 1.3–1.9 μm diameters (e.g., Waters BEH columns, Agilent Infinity Lab) are common. These give maximal efficiency but at very high pressure.

II) Core-shell (superficially porous) particles: These have solid silica cores (e.g., 1.6–2.7 μm outer diameter) and thin porous shells.



Core-shell columns (e.g., 1.7 μm Halo®, 2.6 μm Kinetex®) deliver near-UPLC efficiencies at much lower pressure (often <600 bar for 2.6 μm core-shell). Thus, they performance features of HPLC AND UHPLC [12-14].

III) Monolithic and polymer columns exist but are less common for UPLC.

Available stationary phase chemistries include all reversed-phase and specialized phases: C18/C8 alkyls (broad utility), phenyl (π - π interactions), PFP/fluoro-phenyl, cyano, HILIC phases for polar analytes, strong cation/anion exchange, and biocompatible or polar-embedded phases. Pore size is chosen by analyte: 60–120 Å for small molecules; 150–300 Å for peptides; ≥ 300 Å for intact proteins. Very narrow-bore (1.0 mm) and larger-bore (3.0 mm) UPLC columns exist for trace analysis or increased load ability, respectively. All UPLC columns require ultra-pure, 0.2 μm -filtered mobile phases to avoid clogging their small frits [16].

1.4 Mobile Phase and Method Development

Mobile phases in UPLC are the same solvents/buffers as in HPLC, but with tighter control and often steeper gradients due to faster runs. Common LC-MS grade solvents include water, acetonitrile, and methanol, with additives such as dilute acids or low-molarity buffers for pH adjustment [17].

I) Solvent purity and degassing are critical: even tiny particulates or salt precipitates ($\geq 0.2 \mu\text{m}$) can block UPLC frits. Use high-grade solvents (0.22 μm filtration) and freshly prepared buffers.

II) Flow rates: are scaled to column dimensions. A rule of thumb for 2.1 mm ID, 1.7–1.9 μm columns are ~0.3–0.6 mL/min for reversed-phase. These flows maintain linear velocities near the optimum. Very short columns (e.g., 30 mm) permit higher flows (1–2 mL/min) for ultrafast peaks, whereas 50–150 mm columns might run at 0.2–0.8 mL/min depending on pressure constraints [18].

III) Temperature: Operating at moderately elevated temperature (e.g., 30–40 °C) can reduce viscosity (lower pressure) and sometimes improve peak shape. Buffer pH should respect column stability (commonly pH 2–8 for silica) [19].

IV) Method scaling: When translating an HPLC method to UPLC, one generally keeps stationary phase chemistry the same, then adjusts flow and gradient for the new column dimensions. Under USP guidelines, isocratic methods can often be transferred to UPLC with minimal revalidation by applying established scaling rules. However, gradient methods usually require re-optimization [20].

UPLC

Table 1.1: UPLC. (Sources: manufacturer data and reviews.)

Metric	UPLC/UHPLC (sub-2 μm)
Max Pressure	15,000–19,000 psi (1000–1300 bar)
Particle Size	Sub-2 μm (1.3–1.9 μm) fully porous; 1.3–2.6 μm core-shell
Efficiency (N)	~100,000–400,000 plates/m (dramatically higher)
Analysis Time	Rapid (often 1–10 $\times$ faster; sub-5 min gradients common)
Solvent Use	Much lower (≈ 20 –80% of HPLC volume)
Sensitivity (UV)	Higher: narrower peaks yield 3–10 $\times$ signal increase
Detection LOD	5–10 $\times$ lower LOD (due to concentration of analyte in smaller volume peak)
Precision	Often better: RT RSD 0.1–0.5%, area <0.1%RSD
Column Lifetime	Comparable in sample volumes, but more sensitive to particulates



Instrument Cost	Higher (specialized high-pressure systems)
Robustness	Requires careful plumbing, filtering; maintenance more critical (e.g., guard columns)

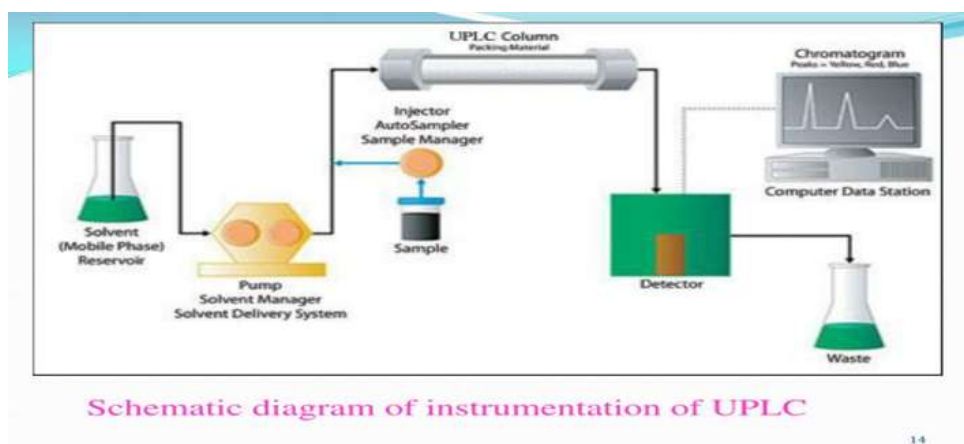


Fig.1.1 Instrumentation of UPLC Diagram

Table 1.2: Common validation Criteria for RP-UPLC Method

Validation Parameter	Acceptance Recommendation
Retention Factor (K')	The analyte peak must be clearly separated from other peaks and from the void. In general, $K' \geq 2$.
Repeatability (%RSD)	RSD should be $\leq 1\%$; it is preferable to have $N \geq 5$ injections.
Relative Retention Time	Not essential as the resolution is already reported.
Resolution (Rs)	The resolution between the main peak and the nearest interfering peak should be more than 2.
Tailing Factor (T)	It should be $T \leq 2$.
Column Efficiency (N)	The number of theoretical plates should generally be > 2000 .

Table 1.3: System Suitability Parameters with Acceptance Limits

Characteristics	Acceptance Limit
Accuracy/Trueness	Individual recovery within 98–102%
Precision	$RSD \leq 2\%$
Repeatability	$RSD \leq 2\%$
Intermediate Precision	$RSD \leq 2\%$
Limit of Detection (LOD)	Signal-to-noise ratio should be greater than 2 or 3
Limit of Quantification (LOQ)	$S/N > 10$
Linearity	Correlation coefficient $R^2 > 0.999$

2.DRUG PROFILE

2.1. BEMPEDOIC ACID:

Bempedoic acid is an oral lipid-lowering agent primarily used to reduce elevated LDL cholesterol levels. It is mainly used to manage hypercholesterolemia. This drug comes under the category of adenosine triphosphate (ATP) citrate lyase (ACL) inhibitors. It is indicated as an adjunct

to dietary therapy and maximally tolerated statin treatment for the reduction of LDC-C levels in adults with primary hypercholesterolemia or established cardiovascular disease who require additional LDL-C lowering⁽²¹⁾.

It is a prodrug in nature. After administration, it gets converted to its pharmacologically active form, Bempedoyl-CoA, with the help of the enzyme very long-chain acyl-CoA synthetase-1

(ACSVL1). This enzyme is present predominantly in the liver. Bempedoic acid inhibits ATP-citrate lyase, an enzyme involved in cholesterol synthesis before HMG-CoA reductase. This reduces cholesterol production in the liver and increases LDL receptors on liver cells, helping to remove LDL-C from the blood. This results in higher removal of LDL-cholesterol from the blood circulation ⁽²²⁾.

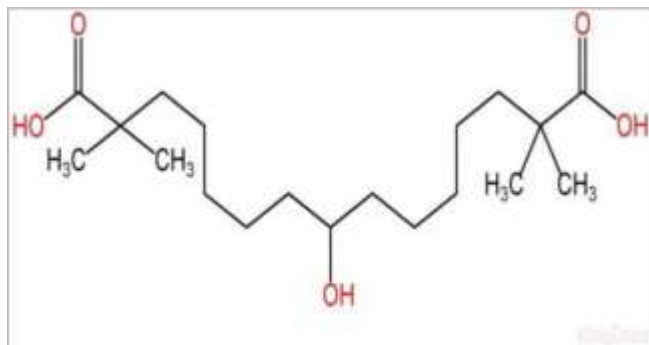


Fig 2.1: Structure of Bempedoic Acid

IUPAC Name: 8-Hydroxy-2,2,14,14-tetramethylpentadecanedioic acid.

Molecular Formula: $C_{19}H_{36}O_5$

Molecular Weight: 344.49 g/mol

CAS Number: 738606-46-7

Description: A white to off-White crystalline powder.

Solubility: Highly soluble in ethanol and soluble in methanol, acetonitrile, and potassium dihydrogen phosphate, but practically insoluble in water ^[23].

Mechanism of Action:

Bempedoic acid is a first-in-class ATP-citrate lyase (ACL) inhibitor used to reduce LDL-C levels. It is a prodrug that is converted in the liver to its active form, bempedoyl-CoA, by very long chain acyl-CoA synthetase-1 (ACSVL).

The active metabolite inhibits ATP-citrate lyase, an enzyme located upstream of HMG-CoA reductase in hepatic cholesterol synthesis. Inhibition of ACL decreases hepatic cholesterol synthesis, thereby increasing LDL receptor expression on hepatocytes. The increased number of LDL receptors enhances the removal of circulating LDL cholesterol from the bloodstream, thereby lowering plasma LDL-C levels. Consequently, it has a lower incidence of muscle related adverse effects compared with statins, making it a suitable option for statin-intolerant patients.

Table 2.1: Pharmacokinetics

Parameter	Description
Absorption	Bempedoic acid is rapidly absorbed following oral administration, reaching peak plasma concentration (T _{max}) at approximately 3.5 hours.
Bioavailability	Good oral absorption; food has no clinically significant effect.
Protein binding	Approximately 99%, primarily to plasma proteins.
Distribution	Volume of distribution is approximately 18 L, indicating limited tissue distribution.
Metabolism	Primarily metabolized in the liver by glucuronidation through UGT2B7 and converted to the active metabolite by ACSVL1. Minimal CYP450 involvement.
Elimination	It is eliminated predominantly in the urine as metabolites, with a minor fraction excreted through feces.
Elimination half-life	Approximately 21 hours. Steady-state achieved after approximately 7 days of once-daily dosing.

Adverse Reactions:

Common adverse reactions observed during clinical studies include: respiratory tract infection, Muscle spasms, Abdominal pain, Hyperuricemia (increased serum uric acid), Gout, Elevated liver



enzymes (ALT and Anaemia, Increased blood urea nitrogen, Tendon rupture (rare but serious), Fatigue, Headache.

Serious adverse effects:

Hyperuricemia leading to gout, Elevated hepatic transaminases, Hypersensitivity reactions (rare)

Drug Interactions: Bempedoic acid has relatively few clinically significant drugs. interactions because it is not extensively metabolized by cytochrome P450 enzymes.

Table 2.2: Dosage and Administration

Parameter	Recommendation
Recommended adult dose	180 mg orally once daily
Route of administration	Oral
Administration	May be taken with or without food. Swallow tablets whole with water.
Combination therapy	Can be used alone or in combination with statins and/or ezetimibe.
Missed dose	Take as soon as remembered on the same day. If it is almost time for the next dose, skip the missed dose and continue the regular schedule. Do not double the dose.
Renal impairment	No dose adjustment is generally required in mild or moderate renal impairment.
Hepatic impairment	No dose adjustment is generally required in mild or moderate hepatic impairment; use is not recommended in severe hepatic impairment due to limited clinical data.
Storage	Store at 20–25°C, protected from excessive moisture and light.

2.2 PITAVASTATIN CALCIUM:

Pitavastatin calcium is a synthetic lipid-modifying compound classified among the statin drugs. It exerts its therapeutic action by inhibition HMG-CoA reductase and is primarily indicated for the management of elevated cholesterol levels and mixed lipid abnormalities, thereby contributing to the prevention of cardiovascular complications.

Its therapeutic efficacy is attributed to its ability to substantially lower plasma concentrations of low-density lipoprotein cholesterol (LDL-C), total cholesterol, triglycerides, and apolipoprotein B, Accompanied by an elevation in high-density lipoprotein cholesterol (HDL-C).

Pitavastatin calcium reduces cholesterol levels by competitively inhibiting HMG-CoA reductase, thereby suppressing hepatic cholesterol synthesis and increasing LDL-receptor expression on hepatocytes. This enhances the hepatic clearance

of circulating LDL-C and lowers plasma LDL-C levels. Its limited metabolism through the cytochrome P450 system, particularly CYP2C9 with minimal CYP3A4 involvement, results in a relatively low potential for drug–drug interactions.

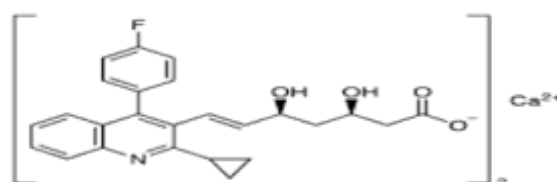


Fig 2.2: Structure of Pitavastatin calcium

- **Molecular Formula:** $(C_{22}H_{20}FNO_4)_2 \cdot Ca$
- **Molecular Weight:** 880.98 g/mol (as a calcium salt; the free base is 421.46 g/mol)
- **IUPAC Name:** Calcium bis[(3R,5S,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl) quinolin-3-yl]-3,5-dihydroxyhept-6-enoate].



- **CAS Number:** 147511-69-1
- **Description:** Pitavastatin calcium is a white to pale yellow crystalline powder.
- **Solubility:** It exhibits very low solubility in water, is readily Soluble in methanol, and shows slight solubility in acetonitrile.

Mechanism of Action

Pitavastatin calcium competitively inhibits HMG-CoA reductase, the rate-limiting enzyme involved in hepatic cholesterol biosynthesis. This inhibition decreases intracellular cholesterol production, resulting in compensatory upregulation of LDL receptors on the hepatocyte surface. The increased receptor activity enhances the uptake and clearance of circulating LDL particles from the

bloodstream, thereby reducing plasma LDL-Cholesterol levels. Consequently, Pitavastatin calcium produces a lipid-lowering effect and contributes to the management of hypercholesterolemia.

The enhanced receptor expression facilitates greater uptake and elimination of circulating low-density lipoprotein cholesterol (LDL-C) from the bloodstream.

Additionally, Pitavastatin decreases the hepatic synthesis of very-low-density lipoprotein (VLDL) and lowers plasma triglyceride concentrations, while producing a moderate elevation in high-density lipoprotein cholesterol (HDL-C) levels. Collectively, these lipid-modulating actions contribute to lowering the risk of atherosclerotic cardiovascular diseases ⁽²⁴⁾.

Table 2.3: Pharmacokinetic Properties

Parameter	Description
Absorption	Pitavastatin calcium is rapidly absorbed after oral administration and reaches its peak plasma concentration within 0.5–1.2 hours.
Bioavailability	Approximately 51% following oral administration.
Protein Binding	Greater than 99%, mainly to albumin and α 1-acid glycoprotein.
Distribution	It shows extensive distribution throughout the body, with an approximate volume of distribution of 148 L.
Metabolism	The drug undergoes limited metabolism mainly through CYP2C9 and, to a lesser extent, CYP2C8. Glucuronidation is the major metabolic process and results in the formation of an inactive lactone metabolite.
Elimination	Primarily excreted via bile and feces; a small proportion is eliminated in urine.
Elimination half-life	Approximately 12 hours.
Steady State	Achieved within 4–6 days of once-daily administration [25].

Adverse Reactions

Pitavastatin calcium is generally well tolerated. The adverse effects reported most frequently include: Myalgia (muscle pain), Arthralgia (joint pain), Back pain, Constipation, Diarrhea, Nausea, Headache, Elevated liver enzymes (ALT and AST), Increased creatine kinase (CK).

Serious Adverse Reactions: Myopathy, Rhabdomyolysis (rare but serious), Hepatotoxicity, Hypersensitivity reactions, New-onset diabetes mellitus (rare).

Drug Interactions: Pitavastatin has fewer drug interactions than many other statins because it undergoes minimal metabolism via the cytochrome P450 system.



Therapeutic Uses

Primary Hypercholesterolemia

- It is indicated for the management of primary hypercholesterolemia in adults by reducing elevated levels of low-density lipoprotein cholesterol (LDL-C), total cholesterol, and apolipoprotein B (ApoB).
- Mixed Dyslipidaemia - Indicated for the treatment of mixed dyslipidaemia by lowering LDL-C, total cholesterol, triglycerides, and increasing high-density lipoprotein cholesterol (HDL-C).
- Prevention of Atherosclerotic cardiovascular Disease (ASCVD): Pitavastatin calcium is used to reduce the risk of major cardiovascular events, including myocardial infarction, stroke, and the requirement for coronary revascularization in patients with an elevated cardiovascular risk ^[26].

Combination of Bempedoic Acid and Pitavastatin Calcium

The combination of Bempedoic acid and Pitavastatin calcium provide a complementary therapeutic approach for managing hypercholesterolemia and reducing the risk of atherosclerotic cardiovascular disease (ASCVD). These two agents possess complementary mechanisms of action, producing greater reductions in low-density lipoprotein cholesterol (LDL-C) than either drug alone.

Bempedoic acid suppresses cholesterol production by targeting ATP-citrate lyase (ACL), which functions at an earlier stage of the cholesterol synthesis pathway. Pitavastatin calcium acts at a later stage by inhibiting HMG-COA reductase, the principal regulatory enzyme of cholesterol biosynthesis.

Acting at different points in the same metabolic pathway results in additive inhibition of clearance of circulating LDL-C. reduced hepatic cholesterol synthesis, upregulation of LDL receptors, and enhanced clearance of circulating LDL-C.

Unlike many statins, Pitavastatin undergoes minimal metabolism through the cytochrome P450 enzyme system, and bempedoic acid is activated only in the liver. These characteristics reduce the likelihood of clinically significant pharmacokinetic interactions and may lower the lower likelihood of muscle-related adverse effects than regimens containing higher statin doses some other statins.^[27].

This combination is particularly beneficial in patients who:

- Have primary hypercholesterolaemia or mixed dyslipidaemia.
- Do not achieve target LDL-C levels with statin therapy alone.
- Require additional LDL-C reduction while minimizing statin dose.
- Are intolerant to high-intensity statin therapy because of muscle-related adverse effects ^[28].

Advantages of the Combination

- Dual inhibition of cholesterol synthesis through different molecular targets.
- Greater LDL-C reduction compared with Pitavastatin monotherapy.
- A reduced likelihood of muscle-related adverse effects may be associated with bempedoic acid because its activation occurs predominantly in the liver.



- Potential to reduce the required statin dose, improving tolerability in statin-intolerant patients.
- Suitable for patients at high cardiovascular risk requiring aggressive lipid management [29].

3. LITERATURE REVIEW

Table 3.1: Literature review

Sr. No.	Author(s) & Year	Drug(s)	Method Developed	Key Findings
1	Jain H.K. et al., [30] 2009	Pitavastatin Calcium	RP-HPLC and UV Spectrophotometric Method	Developed and validated accurate, precise, and economical methods for estimation of Pitavastatin calcium in tablet dosage forms.
2	Niranjani S. and Venkatachalam K., [31] 2019	Pitavastatin Calcium	UV Spectrophotometric Stability-Indicating Method	Evaluated degradation behaviour under acidic, alkaline, oxidative, thermal, and photolytic conditions. Method showed good accuracy and precision.
3	Kumar NS, Bhagya Lakshmi J., [32] 2018–2020	Pitavastatin Calcium	HPTLC Method	Developed simple and cost-effective HPTLC methods suitable for routine quality control analysis.
4	Di B, Su MX, Yu F, Qu LJ, Zhao LP, Cheng MC, et al., [33] 2020	Pitavastatin Calcium	LC-MS/MS Method	Established highly sensitive methods for pharmacokinetic and bioanalytical studies.
5	Bapatu P.K.R. and Sharma A.K., [34] 2023	Pitavastatin Calcium	Stability-Indicating RP-HPLC Method	Successfully separated Pitavastatin from degradation products and validated according to ICH guidelines.
6	Chakraborty G.S. et al., [35] 2024	Bempedoic Acid	Review of Analytical Methods	Summarized available HPLC, HPTLC, UV, and LC-MS methods and highlighted future analytical challenges.
7	Neelofar H. et al., [36] 2026	Bempedoic Acid + Rosuvastatin	Stability-Indicating UPLC Method	Simultaneously estimated both drugs with excellent separation and compliance with ICH validation requirements.
8	Kashid A.M. et al., [37] 2026	Bempedoic Acid	AQbD-Based Stability-Indicating HPTLC Method	Applied Analytical Quality by Design principles and obtained robust, accurate, and reproducible results.
9	Present Research Gap [34-37].	Bempedoic Acid + Pitavastatin Calcium	Simultaneous Stability-Indicating RP-HPLC Method	Limited literature available for simultaneous estimation of bempedoic acid and Pitavastatin calcium, indicating a need for novel method development and validation. development and validation of a stability-indicating RP-UPLC method for simultaneous estimation of bempedoic acid and Pitavastatin calcium is justified. [34-37].

4. AIM, OBJECTIVES & PLAN OF WORK 4.1 Aim



The present study focuses on the development and validation of a rapid and accurate analytical method. Stability-indicating RP-UPLC method for the simultaneous qualification of Bempedoic acid and Pitavastatin calcium in bulk form ⁽³⁸⁾.

4.2 Objective

The primary objectives of this study are:

- To establish a stability-indicating RP-UPLC analytical procedure for the concurrent determination of bempedoic acid and Pitavastatin calcium in bulk drug.
- To assess the developed RP-UPLC procedure for its suitability, reliability, and analytical performance in the simultaneous determination of both drugs in bulk material.
- To utilize the validated RP-UPLC procedure for the simultaneous quantitative analysis of bempedoic acid and Pitavastatin calcium in bulk drug ⁽³⁸⁾.

4.3 Plan of Work

The study was designed to establish an RP-UPLC method for the simultaneous determination of bempedoic acid and Pitavastatin calcium in bulk drug. The experimental work was carried out in two main phases.

Phase 1: Optimization of chromatographic condition

- Selection of analytical wavelength
- Selection of preliminary separation conditions
- Selection of mobile phase Parameters (pH, peak modifier, solvent strength, ratio, and flow rate)
- Nature of stationary phase

- Selection of separation method.

Phase 2: Validation of method

The developed method was proposed to be validated using the various validation parameters such as:

- Accuracy
- Precision, Interday, Intraday, repeatability
- Linearity
- Limit of detection (LOD)
- Limit of quantification (LOQ)
- Robustness.

5. MATERIALS AND METHODS

Table 5.1: CHEMICALS & REAGENTS:

Sr. No	Chemicals	Quality
1	Bempedoic acid, Pitavastatin calcium	Central Drugs Testing Laboratory
2	Water	HPLC Grade
3	Potassium dihydrogen phosphate	HPLC Grade
4	Acetonitrile	HPLC Grade
5	Methanol	HPLC Grade
6	Orthophosphoric Acid	AR Grade

Table 5.2: EQUIPMENT AND INSTRUMENTS:

Sr. No	Instruments	Model
1	UPLC	Ultimate 3000, Autosampler
2	UV Spectrometer	Shimadzu
3	PH meter	Lab India
4	Analytical Weighing balance	Sortorius, BAL-SA-06
5	Hot Air oven	Thermo fishcer scientific

5.3 Method Development of an RP-UPLC Method for the Simultaneous Estimation of Bempedoic acid and Pitavastatin Calcium:



The equipment and chemicals utilized during the study are presented in Tables 5.1 and 5.2. respectively.

5.3.1 Preparation of Buffer solution:

Weigh and dissolve 6.8 g of KH_2PO_4 in 1000 mL of HPLC grade water and adjust pH to 4.5 with KOH. The Prepared solution was passed through a 0.45 μm Millipore membrane filter paper.

5.3.2 Mobile phase Preparation (KH_2PO_4 in 50:50 ACN / H_2O)

Combine 500 mL of ACN and 500 mL of H_2O , add 85% of KH_2PO_4 , mix together, degas in an ultrasonic water shower for 10 minutes, and then separate using a 0.45 μm filter while being filtered via a vacuum.

5.3.3 Preparation of Diluent (65:35 ACN/ H_2O)

Combine 325 mL of Acetonitrile and 175 mL of Water and mix well.

5.3.4 Preparation of Primary Standard stock solution

Accurately weigh 180 mg of Bempedoic acid into 100 mL volumetric flask. The substance was dissolved using the selected diluent, and the resulting solution was diluted to the required volume.

5.3.5 Preparation of Standard stock solution 2

Accurately weigh 10 mg of Pitavastatin calcium into 100 mL volumetric flask. The contents were

dissolved completely in the diluent and the volume was adjusted to the calibration mark.

5.3.6 Preparation of Working standard solution

Aliquots of 5 mL each from the Bempedoic acid and Pitavastatin calcium stock solutions were accurately transferred into a 50 mL volumetric flask. The volume was then adjusted to the calibration mark using the selected diluent.

5.3.7 Determination of Isobestic point (λ max):

An accurately weighed quantity of 15 mg each of Bempedoic acid and Pitavastatin calcium reference standards was transferred separately into 10 mL volumetric flasks. Approximately three-fourths of the final volume was added with methanol, followed by sonication for 10 minutes to ensure complete dissolution. The volume was subsequently made up to the calibration mark with methanol. Methanol was used as the blank during UV spectrophotometric analysis. The maximum absorbance (λ max) of the drug mixture was observed at 221 nm.

For chromatographic method development, different mobile-phase compositions containing water, methanol, acetonitrile, and suitable buffer solutions were evaluated in various combinations and proportions. Based on the chromatographic response obtained during the trials, methanol was selected as the mobile phase component. A flow rate of 1.0 mL/min provided sharp and well-defined chromatographic peaks.

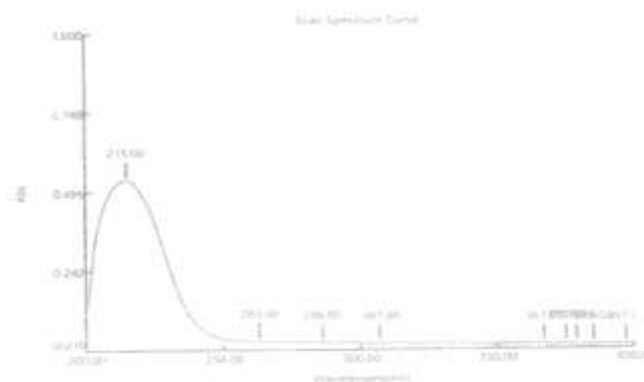


Fig 5.1: UV spectrum of standard Bempedoic acid

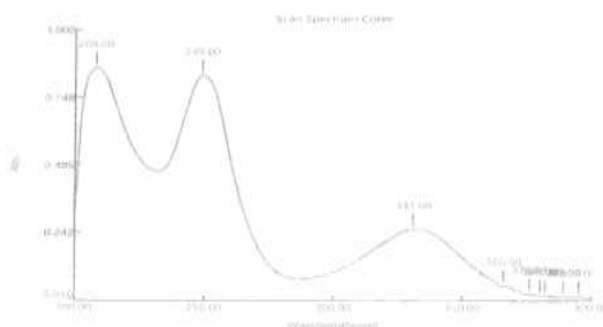


Fig 5.2: UV spectrum of standard Pitavastatin calcium

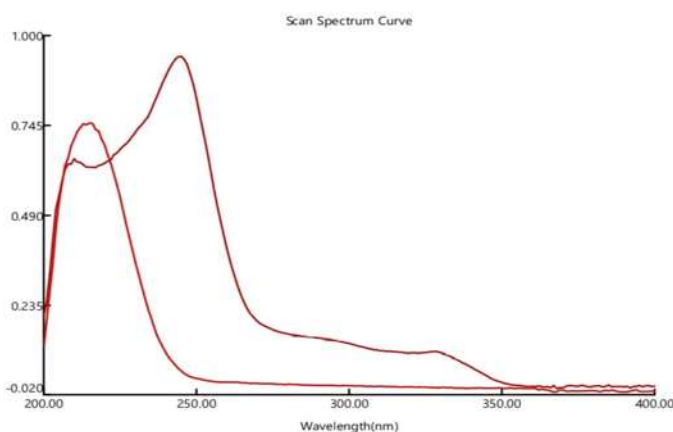


Fig 5.3: UV spectrum of Isobestic point of Bempedoic Acid & Pitavastatin calcium.

5.3.8 CHROMATOGRAPHIC CONDITIONS: Mobile phase: Water: Acetonitrile (50:50) (v/v)

Trail-1 Flow rate: 1.0 mL/min

Method development was carried out by varying the mobile-phase composition and buffer conditions. Column: C18 Column (250 mm x 4.6 mm, 5 μ m)
Detection wavelength: 245 nm

Chromatographic conditions: Column temperature: 30°C

Injection volume: 10 μ L

Run time: 30 min

Diluent: Water and Acetonitrile in the ratio of 50:50 (v/v)

Results: Bempedoic acid and Pitavastatin calcium peaks were obtained; however, the peak shapes were unsatisfactory, further trial was carried out.

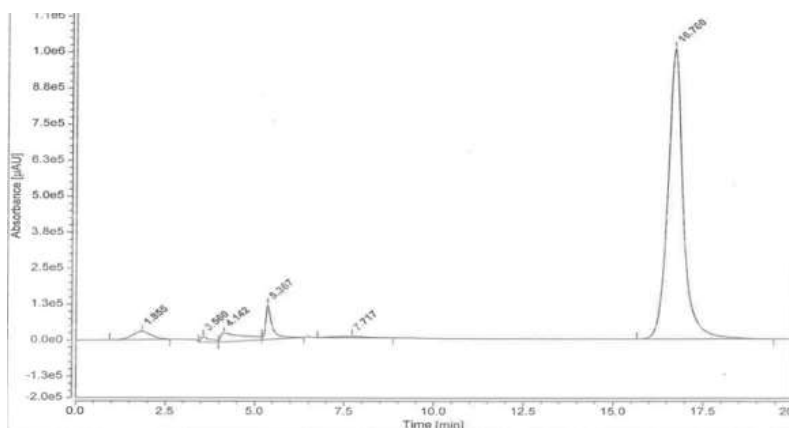


Fig 5.4: Trail-1 Chromatogram of Bempedoic acid and Pitavastatin calcium

Trail-2: Chromatographic conditions:

Injection volume: 10 μ L

Mobile phase: Methanol: Opa (30:70) (v/v)

Run time: 30 min

Flow rate: 1.0 mL/min

Diluent: Water: Acetonitrile in the ratio 50:50 (v/v)

Column: C18 column (250 mm x 4.6 mm, 5 μ m)

Detection wavelength: 247 nm

Results: Pitavastatin calcium was eluted successfully, whereas the peak shape of bempedoic acid was unsatisfactory. Hence, further optimization was performed.

Column temperature: 30°C

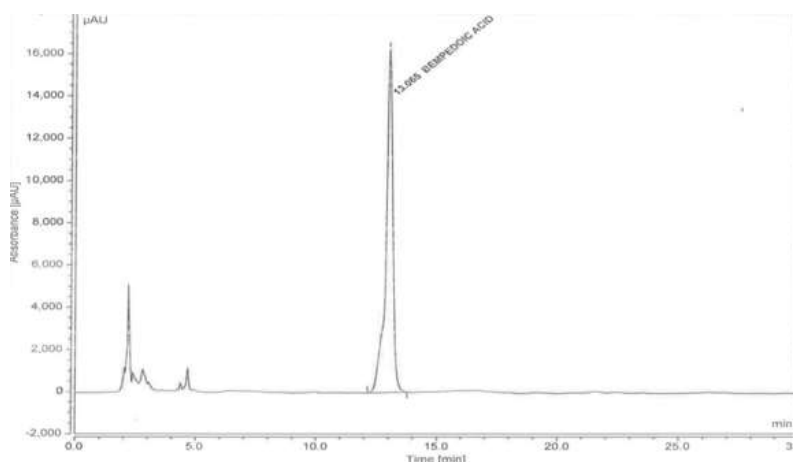


Fig 5.5: Trail-2 Chromatogram Bempedoic acid and Pitavastatin calcium

Trail-3: Chromatographic conditions:

Mobile phase: Buffer: acetonitrile (50:50) (v/v)



Flow rate: 1.0 mL/min

Run time: 30 min

Column: C18 column (250 mm x 4.6 mm, 5 μ m)

Diluent: Water: Acetonitrile (50:50) (v/v)

Detection wavelength: 230 nm

Result: In this trial Pitavastatin calcium and bempedoic acid peaks were eluted but satisfactory peak shape was not achieved. Therefore, further optimization was undertaken.

Column temperature: 30°C

Injection volume: 10 μ L

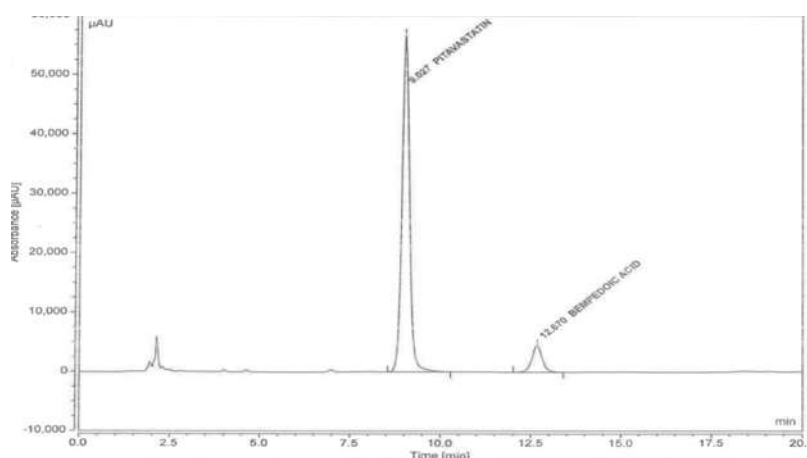


Fig 5.6: Trail-3 Chromatogram Bempedoic acid and Pitavastatin calcium

Trail-4 :5.3.9 Optimized Chromatographic conditions:

Run time: 30 min

Mobile phase: KH₂PO₄ in (50:50) ACN/H₂O

Diluent: Water and Acetonitrile in the ratio (35:65) (v/v)

Flow rate: 1.0 mL/min

Column: C18 Column (250 mm x 4.6 mm, 5 μ m)

Detector wave length: 221 nm

Column temperature: 30°C

Injection volume: 10 μ L

Observation: The developed conditions provided satisfactory separation, with retention times of approximately 13.32 and 30.0 min for the respectively. Obtained theoretical plate count and tailing factor indicated adequate column efficiency and peak symmetry. Hence, these chromatographic conditions were considered suitable and selected for further method development and validation.

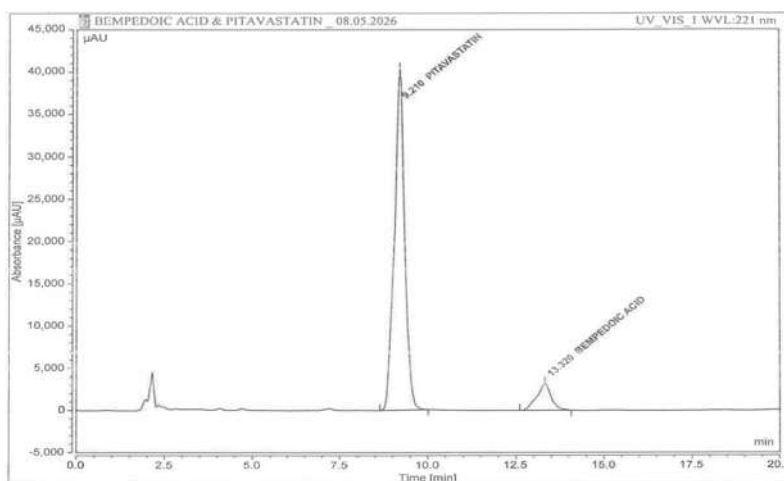


Fig 5.7: Trail-4 Chromatogram Bempedoic acid and Pitavastatin calcium

6. RESULTS AND DISCUSSION

6.1 validation of the Developed Analytical Method for Simultaneous Quantification of Bempedoic acid and Pitavastatin calcium:

A systematic process of documenting evidence that demonstrates with high certainty that a given analytical method consistently produces results aligned with predefined quality standards and specifications.

6.1.1 Linearity:

Linearity was evaluated using five standard concentrations within the range of 72 – 360 µg/mL for Bempedoic acid and 4-20 µg/mL for Pitavastatin calcium.

Calibration curves were constructed by plotting the corresponding peak area against the respective drug concentration. The obtained regression equations showed sloped of 9294.1 for bempedoic acid and 83399.35 for Pitavastatin calcium, with correlation coefficients (R^2) of 0.9994 and 1.0000, respectively. The results demonstrated an excellent relationship between concentration and peak response over the investigated ranges. Linearity plot was shown in Fig 6.10.

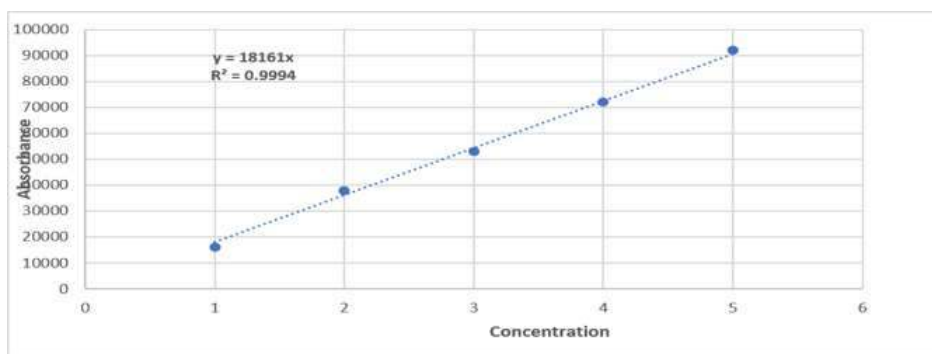


Fig 6.1: Linearity (calibration) curve of Bempedoic acid

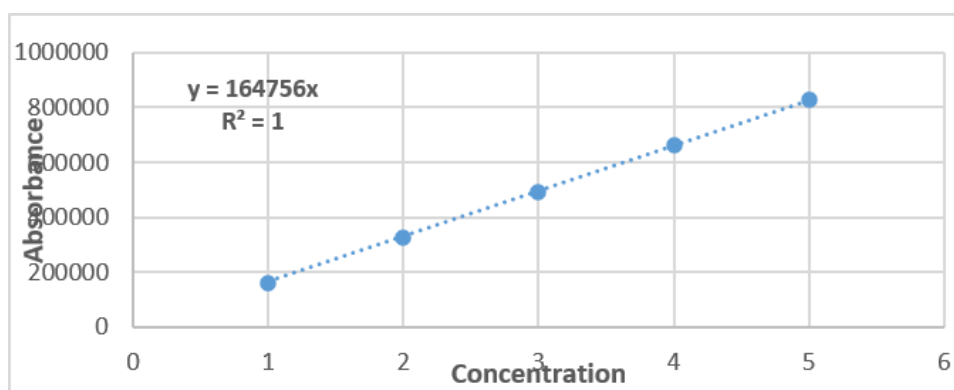
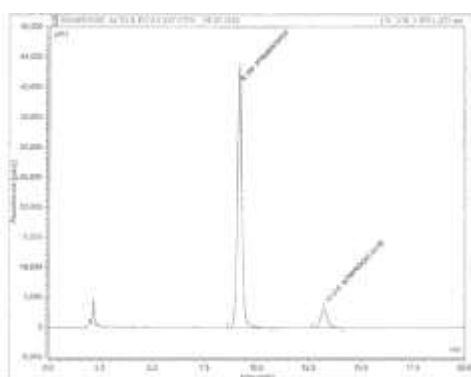
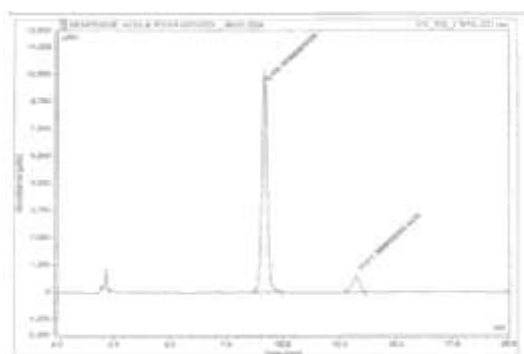


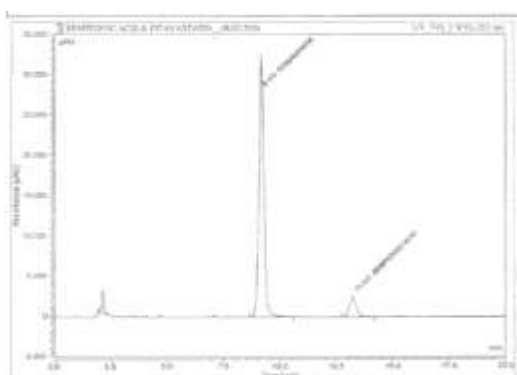
Fig 6.2: Linearity (calibration) curve of Pitavastatin Calcium.



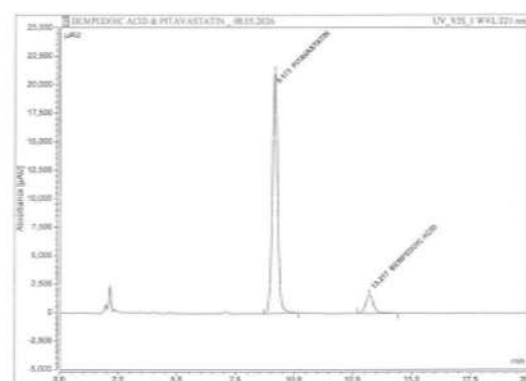
Linearity chromatogram-1



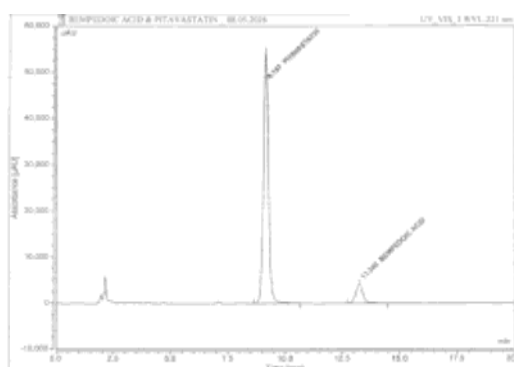
Linearity chromatogram-2



Linearity chromatogram-3



Linearity chromatogram-4



Linearity chromatogram-5

Fig 6.3: Linearity Chromatograms of Bempedoic acid and Pitavastatin calcium

Table 6.1: Linearity results for Bempedoic acid and Pitavastatin calcium

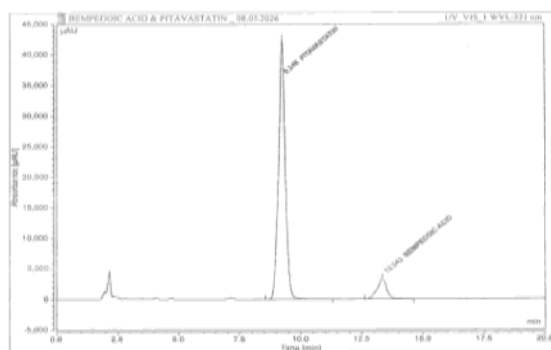
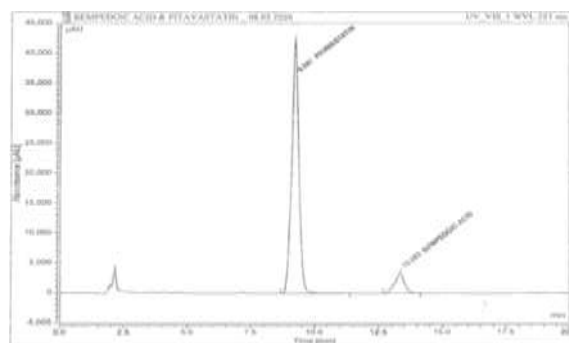
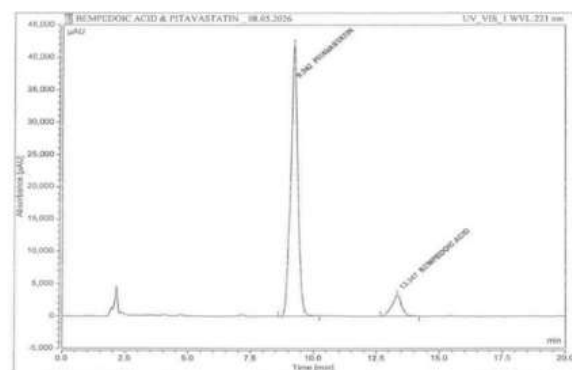
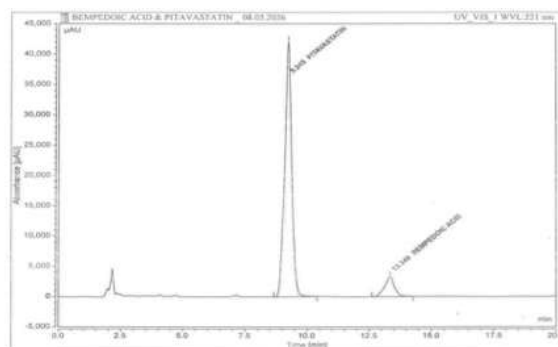
Sr. No	Concentration	Bempedoic acid		Pitavastatin calcium	
		Area	Ret Time	Area	Ret Time
1	2	16101	13.217	159015	9.172
2	4	37872	13.217	327474	9.173
3	6	53038	13.223	490998	9.172
4	8	71990	13.233	660581	9.165
5	10	91983	13.240	826455	9.152
Correlation Coefficient (r2)		$R^2 = 0.9994$		$R^2 = 1$	

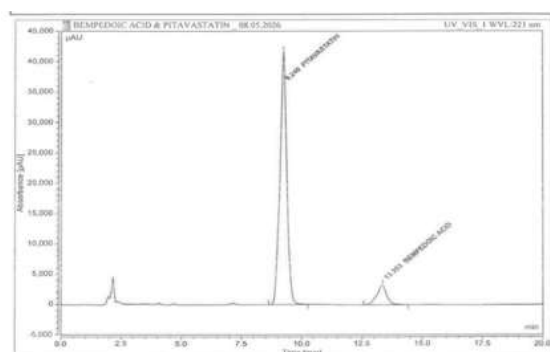
Result: Linearity was established over 50%-150% of the nominal concentration, corresponding to 72-360 µg/mL for bempedoic acid and 4-20 µg/mL for Pitavastatin calcium.

The correlation coefficients were 0.9994 and 1.0000, respectively, confirming good linearity.

6.1.2 Method precision (Repeatability):

Repeatability of the proposed method was assessed from five replicate preparations (n=5) at concentrations of 180 µg/mL for bempedoic acid and 10 µg/mL for Pitavastatin calcium. Each preparation was analysed using unchanged chromatographic conditions and the resulting peak areas and retention times were recorded.

**Precision Chromatogram-01****Precision Chromatogram-02****Precision Chromatogram-03****Precision Chromatogram-04**



Precision Chromatogram-05

Fig 6.4: Precision Chromatograms of Bempedoic acid and Pitavastatin calcium.

Table 6.2: Precision results for Bempedoic acid and Pitavastatin calcium

SR. NO	BEMPEDOIC ACID				PITAVASTATIN CALCIUM			
	RT (min)	Area	%Area	Asymmetry (EP/USP)	RT (min)	Area	% Area	Asymmetry (EP/USP)
Injection-1	13.343	91282	10.01	0.95	9.248	820925	89.99	0.97
Injection-2	13.353	88074	9.66	0.91	9.247	824105	90.34	0.98
Injection-3	13.348	90937	9.97	0.93	9.245	821048	90.03	0.98
Injection-4	13.347	89312	9.84	0.93	9.242	818125	90.16	0.98
Injection-5	13.353	91238	10.03	0.93	9.240	818235	89.97	0.98
Mean	13.3488	90168.6	9.902	0.93	9.2444	820487.6	90.098	0.978
%RSD	0.031959	1.577678	1.557011	1.52066	0.036363	0.300073	0.171119	0.457274

Result: The %RSD value was below 2.0%, confirming the satisfactory precision of the proposed method.

6.1.3 Repeatability Chromatogram

Intermediate precision was assessed by analysing six sample preparations at a concentration of 100 µg/mL on the subsequent day. The percentage assay was determined, and the %RSD was found to be 0.5%, indicating good intermediate precision. The corresponding chromatograms are shown in Fig 2.14.

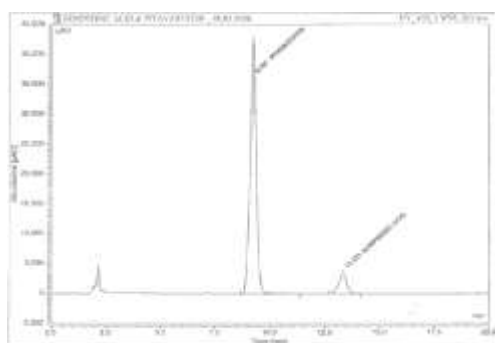


Fig 6.4.1: Intermediate Precision -01

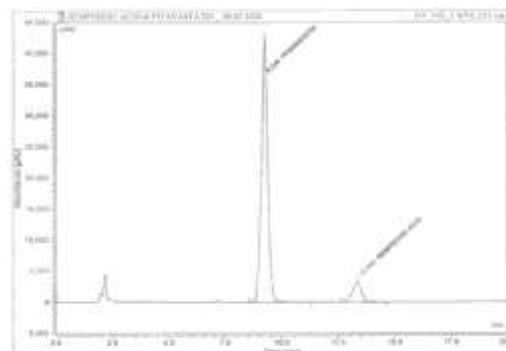


Fig 6.4.2: Intermediate Precision - 02



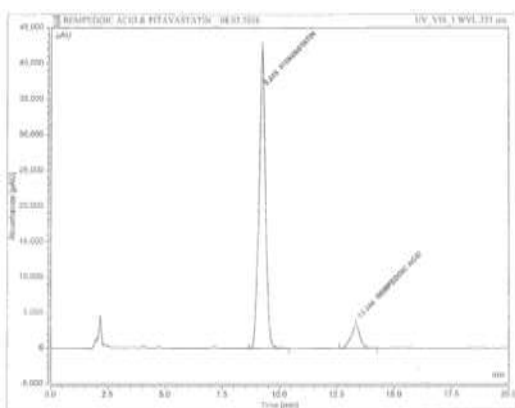


Fig 6.4.3: Intermediate Precision – 03

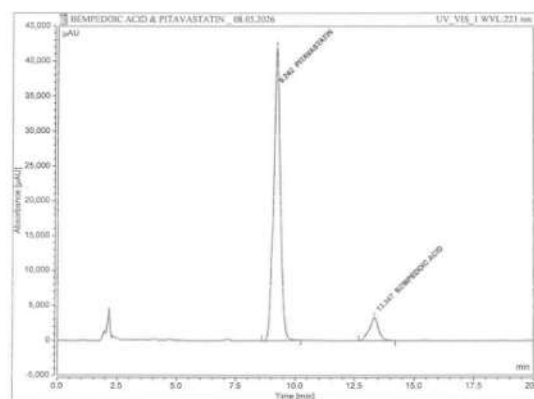


Fig 6.4.4: Intermediate Precision -04

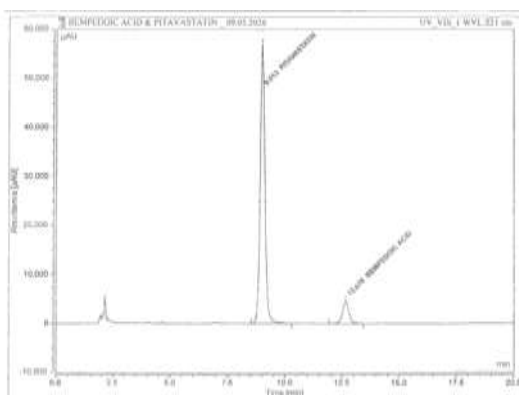


Fig 6.4.4: Intermediate Precision -05

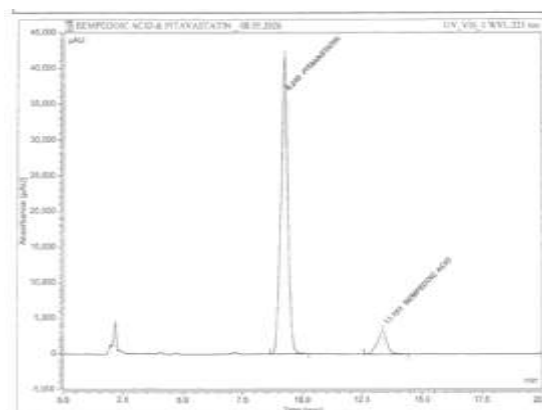


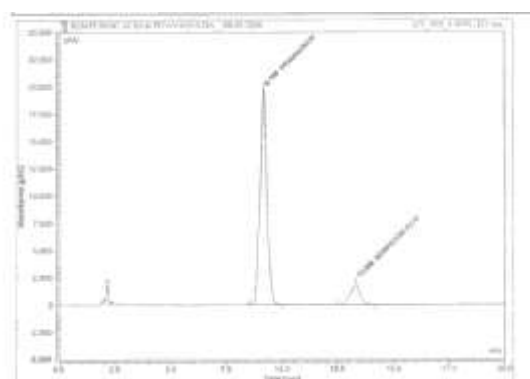
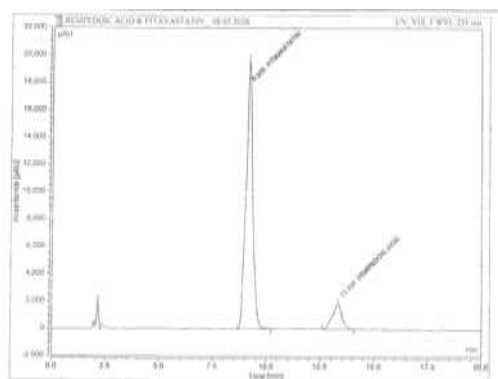
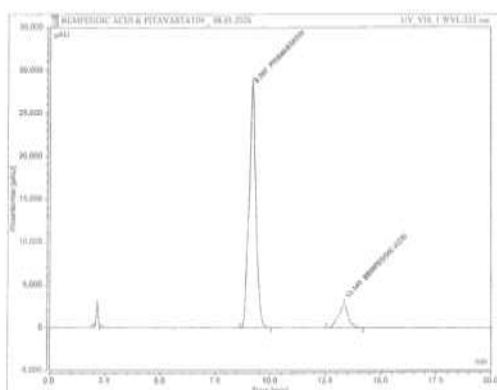
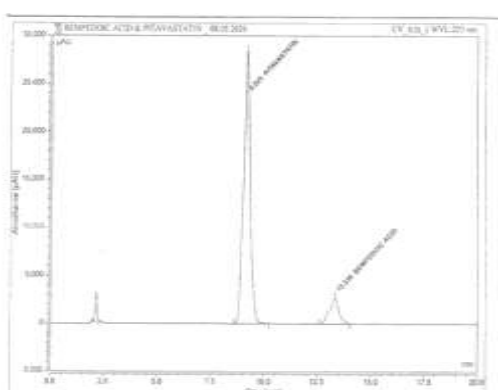
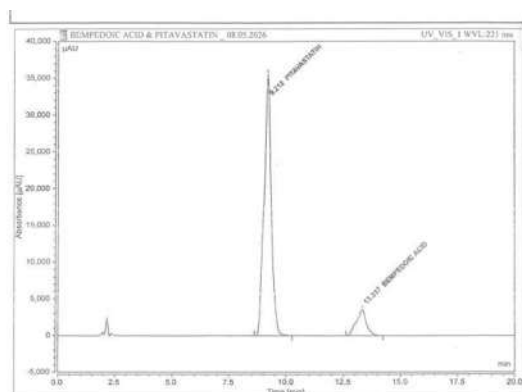
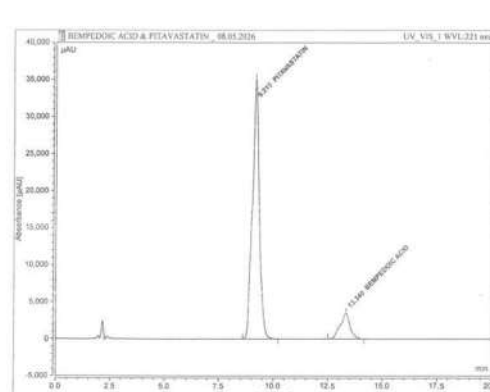
Fig 6.4.4: Intermediate Precision -06

Table 6.3: Results of Intermediate Precision for Pitavastatin calcium and Bempedoic acid.

Sr. No	Pitavastatin calcium			Bempedoic acid		
	Conc. (µg/mL)	Peak area	Retention Time (min)	Conc. (µg/mL)	Peak area	Retention Time (min)
1	2	824820	9.033	180	90637	12.683
2	2	823118	9.027	180	89894	12.670
3	2	822598	9.020	180	91348	12.667
4	2	827710	9.017	180	91114	12.663
5	2	828813	9.018	180	91132	12.672
6	2	826980	9.013	180	91490	12.668

6.1.4 Accuracy: The accuracy of the method was determined using samples fortified at 50%, 100%,

and 150% of the target concentration. Three replicate injections were performed at each level.

**Chromatogram of Accuracy at 50% -1****Chromatogram of Accuracy at 50% -2****Chromatogram of Accuracy at 50% -3****Chromatogram of Accuracy at 50% -4****Chromatogram of Accuracy at 50% -5****Chromatogram of Accuracy at 50% -6****Fig 6.5: Accuracy Chromatograms of Bempedoic acid and Pitavastatin calcium****Table 6.4: Accuracy results of Bempedoic acid and Pitavastatin calcium**

SR. NO	ACCURACY % Level	Amount Spiked		Amount Spiked	
		Area	BPA	Area	PTV
1	50%-1	57744	57744	421998	421998
2	50%-2	57022	57022	420718	420718
3	100%-1	84687	84687	617831	617831
4	100%-2	82758	82758	619281	61928
5	150%-1	107122	10712	780144	780144
6	150%-2	108139	108139	777545	777545

Result: The results indicate that the developed method exhibits satisfactory accuracy and conforms to the established acceptance criteria.

6.1.5 Limit of Detection (LOD) and Limit of Quantification (LOQ): The values were determined from the standard deviation (σ) of the y-intercept and the slope (S) of the calibration curve with the following equations:

$$\text{LOD} = 3.3 \times \sigma / S$$

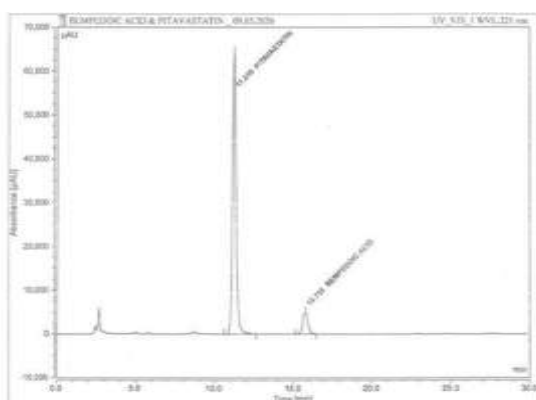
$$\text{LOQ} = 10 \times \sigma / S$$

Table 6.5: The LOD and LOQ values determined for Bempedoic acid and Pitavastatin calcium are presented.

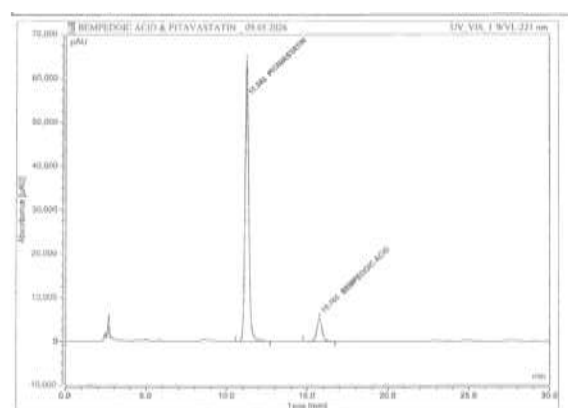
	Bempedoic acid	Pitavastatin calcium
LOD	10.44807	10.43564
LOQ	31.6608	31.62314

6.1.6 Robustness: The method robustness was evaluated by making minor deliberate changes in flow rate, mobile phase composition, and detection wavelength.

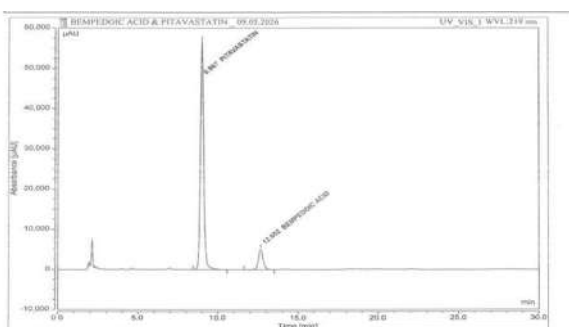
For this purpose, small changes were made in critical chromatographic conditions like flow rate, wavelength, proportion of mobile phase, and temperature. The %RSD was determined for all these varied conditions. The obtained results are presented in Table 5.8.



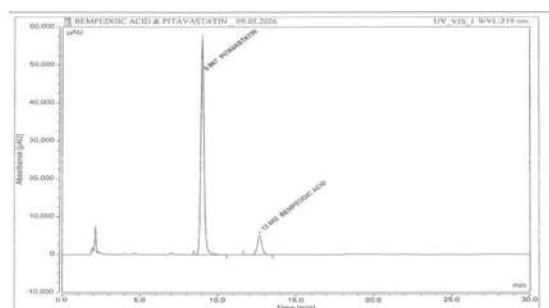
Chromatogram for Flow rate 0.8 mL/min-1



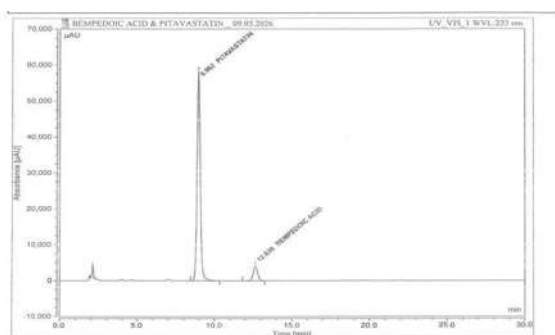
Chromatogram for Flow 0.8 mL/min-2



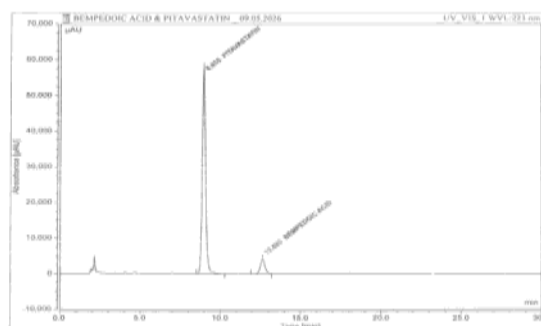
Chromatogram for WL 219 nm-1



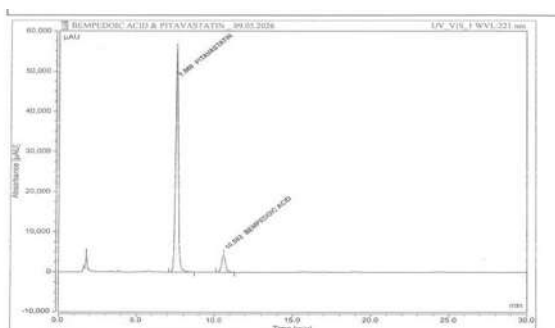
Chromatogram for WL 219 nm-2



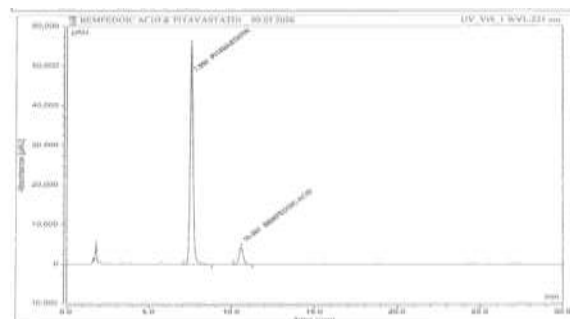
Chromatogram for WL 223 nm-1



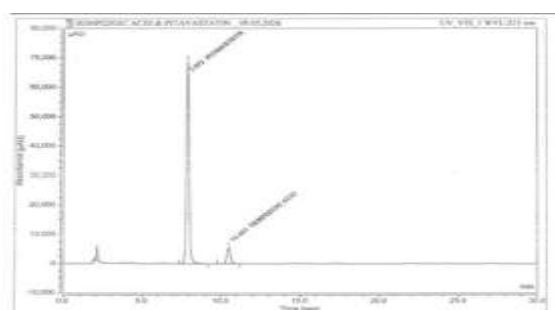
Chromatogram for WL 223 nm-2



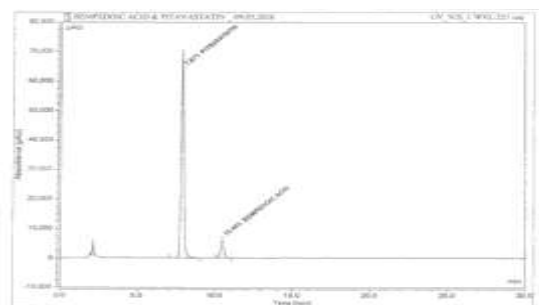
Chromatogram for Flow rate 1.2 mL/min-1



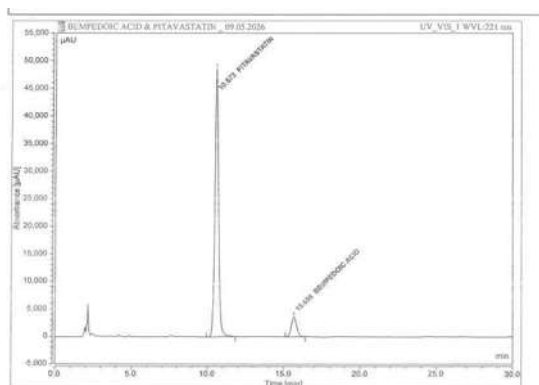
Chromatogram for Flow rate 1.2 mL/min-2



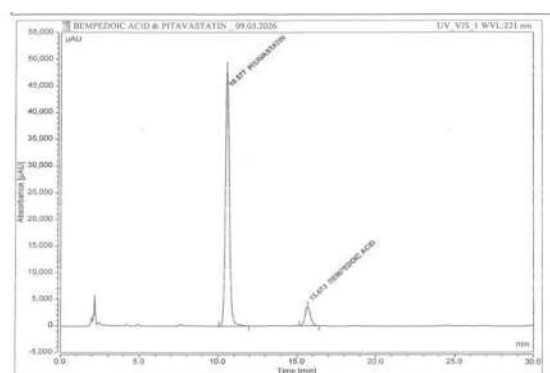
Chromatogram for Buffer 52.5% -1



Chromatogram for Buffer 52.5% -2



Chromatogram for Buffer 47.5% -1



Chromatogram for Buffer 47.5% -2

Fig 6.6: Robustness Chromatograms of Bempedoic acid and Pitavastatin calcium.

Table 6.6: Robustness assessment results for Bempedoic acid and Pitavastatin calcium

ROBUSTNESS FLOW RATE	BPA Area	BPA Rt	PTV Area	PTV Rt
FLOW0.8ML/MMT-1	111438	15.758	1033521	11.240
FLOW0.8ML/MMT-2	114469	15.765	1033161	11.242
WL-219NM-1	101508	12.648	828344	8.993
WL-219NM-2	103137	12.652	830877	8.987
WL-223NM-1	82070	12.630	854012	8.962
WL-223NM-2	81730	12.625	852612	8.955
FLOW1.2ML/MIN-1	76374	10.582	689646	7.560
FLOW1.2ML/MIN-2	76167	10.567	691137	7.550
BUFFER 52.5%-1	86697	15.658	825948	10.572
BUFFER 52.5%-2	86419	15.673	827658	10.577
BUFFER 47.5%-1	91152	10.483	833325	7.872
BUFFER 47.5%-2	88358	10.485	832934	7.873

Results

The robustness study demonstrated that minor variations in flow rate, wavelength, and buffer conditions produced no significant changes in the analytical results. Therefore, the developed method was considered robust and reliable under tested conditions.

6.1.7 Degradation studies

Degradation testing was performed to investigate the stability of Bempedoic acid and Pitavastatin

calcium under various degradation conditions. The resulting samples were examined using the developed analytical method.

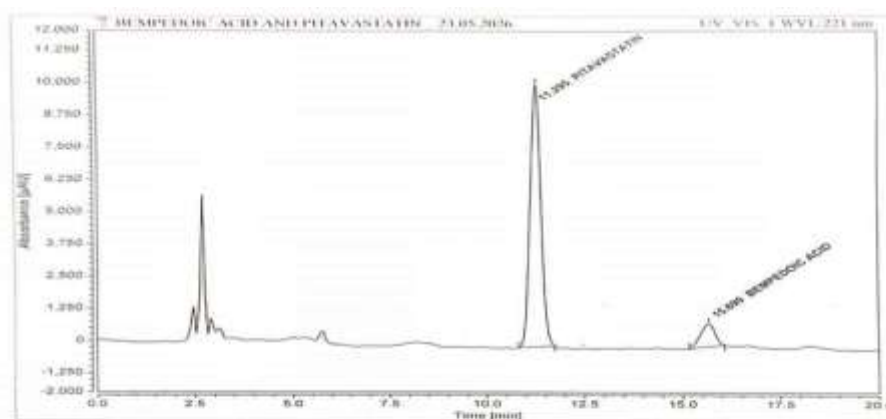
Photolytic Degradation - (254 nm, 1 day),

Thermal degradation – (105° C, 6 hours),

acid degradation – (0.1 N HCl, 105° C, 6 hours),

base degradation-(0.1 N NaOH, 105° C, 6 hours),

peroxide degradation – (20% H₂O₂, 105° C, 6 hours)



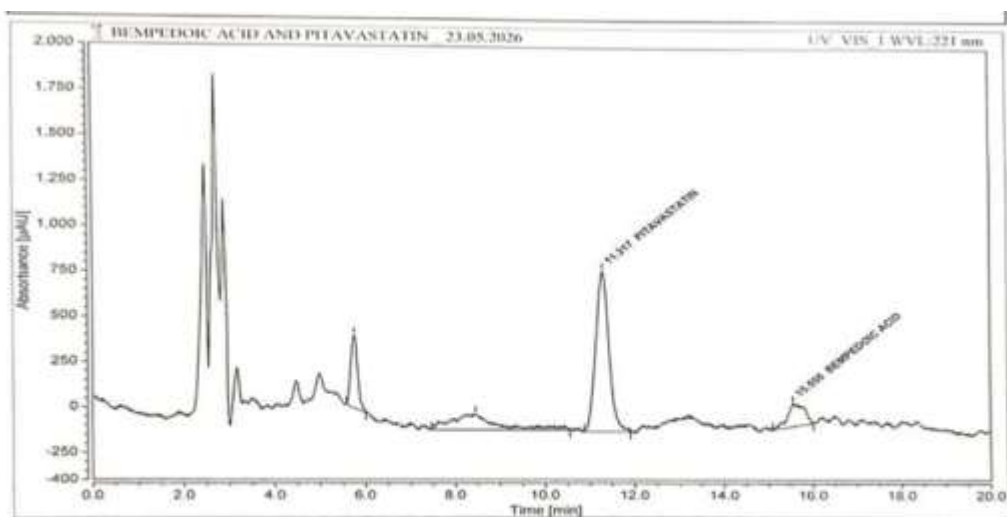
Peak No.	Peak Name	Ret. Time (min)	Area (a.u.)	Area %	Assessment (IUPAC)	Plate (IUPAC)	Resolution (IUPAC)
1	PITAVASTATIN	11.240	1033521	89.63	0.95	7419	8.0
2	BEMPEDOIC ACID	15.758	111438	9.37	0.87	7402	7.05

HPLC LOGBOOK NO CDTLHPLC-TF/03/AUG-01/2026-277

2-83

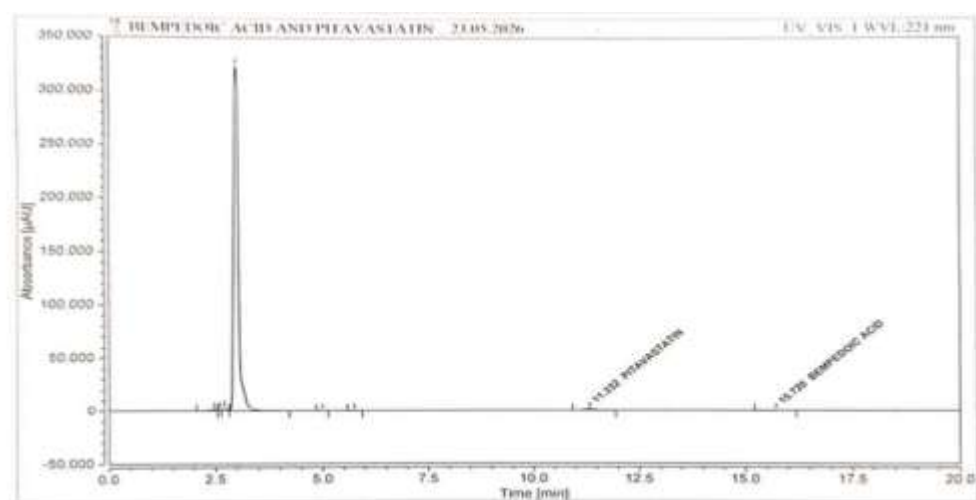
Photolytic degradation chromatogram of bempedoic acid and Pitavastatin calcium





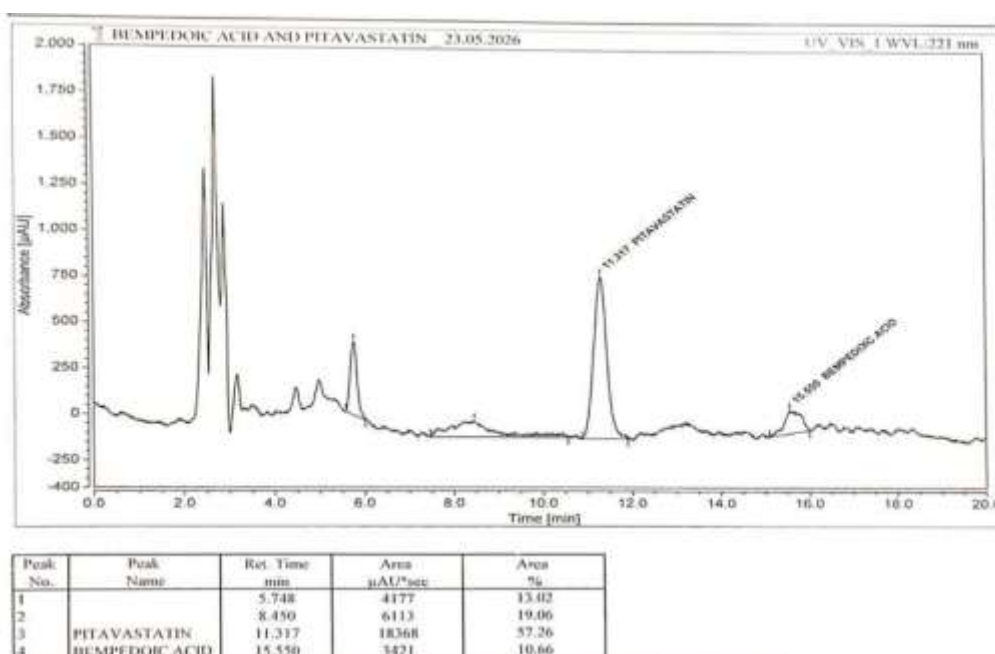
Peak No.	Peak Name	Ret. Time min	Area $\mu\text{AU}^2\text{sec}$	Area %
1		5.748	4177	13.02
2		8.450	6113	19.06
3	PITAVASTATIN	11.317	18368	57.26
4	BEMPEDOIC ACID	15.550	3421	10.66

Thermal degradation chromatogram of bempedoic acid and Pitavastatin calcium

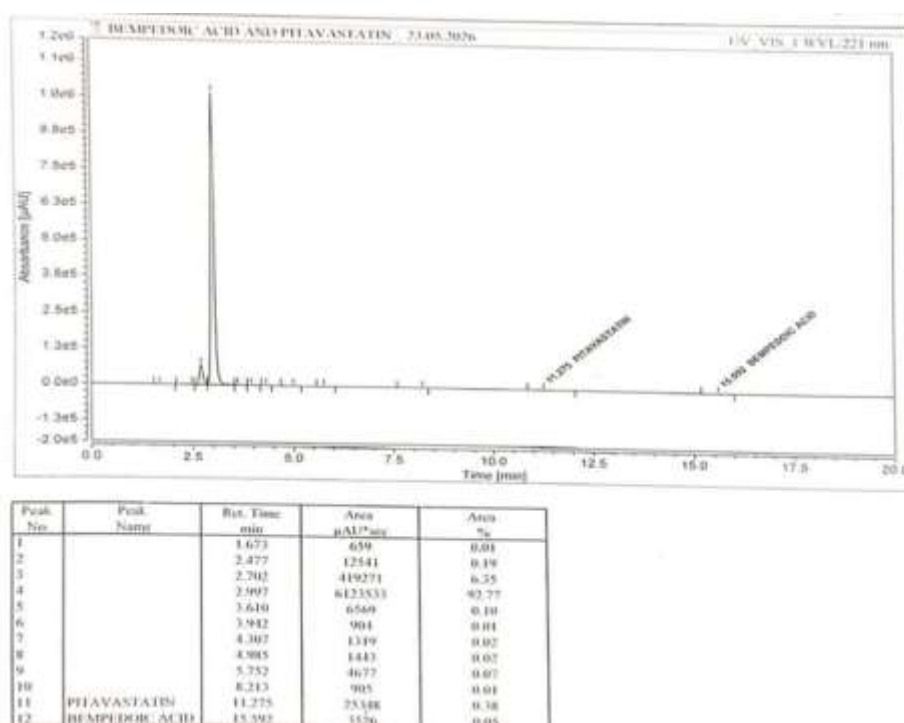


Peak No.	Peak Name	Ret. Time min	Area $\mu\text{AU}^2\text{sec}$	Area %
1		2.467	13490	0.52
2		2.590	1415	0.05
3		2.697	26803	1.03
4		2.958	2541983	97.25
5		4.990	1050	0.04
6		5.747	3113	0.12
7	PITAVASTATIN	11.332	22491	0.86
8	BEMPEDOIC ACID	15.729	3679	0.14

Acid degradation chromatogram of Bempedoic acid and Pitavastatin calcium



Base degradation chromatogram of Bempedoic acid and Pitavastatin calcium



H₂O₂ degradation chromatogram of bempedoic acid and Pitavastatin calcium.
Fig 6.7: Degradation Chromatograms of Bempedoic acid and Pitavastatin calcium

Table 6.7: Forced-degradation study results for Bempedoic acid and Pitavastatin calcium

DEGRADATION STUDY	BEMPEDOIC ACID		PITAVASTATIN CALCIUM	
	Area	Rt	Area	Rt
Photolytic Study	22628	15.690	195595	11.295
Thermal Studies	681423	15.875	119601	11.292
Acid Degradation	3421	15.550	18368	57.26
Base Degradation	3629	15.720	22491	11.332

H₂O₂ Degradation	3526	15.592	25348	11.275
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7. SUMMARY OF ANALYTICAL METHOD VALIDATION

Table 7.1: Summary Table

Validation parameter		Observed results	Acceptance criterion
Linearity interval (µg/mL)		BPA:72 -360 µg/mL and PTV:4 -20 µg/mL	Correlation coefficient (R) < 1*
correlation coefficient (R)		0.999	
Regression Slope (m)		6733.8	
Regression intercept (c)		961.27	
Linearity Regression equation (Y=mx + c)		y = 164756x +18161x	
Assay (% mean assay)		99.68%.	90-110%
System precision (%RSD)		0.3	NMT 2.0%
Method precision (%RSD)		0.2	NMT 2.0%
Accuracy (%recovery)		99.44%	98-102%
LOD		BPA:10.44807 µg/mL, PTV:10.43564 µg/mL	NMT 3
LOQ		BPA:31.6608 µg/mL, PTV:31.62314 µg/ML.	NMT 10
Robustness	FM	0.1	%RSD NMT 2.0
	FP	0.2	
	MM	0.4	
	MP	0.3	
	TM	0.9	
	TP	0.4	

8. CONCLUSION

A reliable RP-UPLC method was developed for the simultaneous estimation of Bempedoic acid and Pitavastatin calcium using a C18 column. Chromatographic separation was achieved with potassium dihydrogen phosphate buffer and acetonitrile (50:50, v/v) at 1.0 mL/min, with detection at 221 nm. The optimized conditions resulted in satisfactory of both analytes.

The method met the system suitability requirements and exhibited good linearity ($R^2 = 0.999$). The %RSD values for repeatability and intermediate precision were 0.2% and 0.5%, respectively. LOD values were 10.44807 and 10.43563, while LOQ values were 31.6608 and

31.62313 for the respective analytes. The assay of the marketed formulation showed 99.68% drug content. The developed method provided reduced retention and analysis times for Bempedoic acid and Pitavastatin calcium. Consequently, the procedure offers advantages such as simplicity, reduced analytical cost, and lower time requirements.

The validated method offered efficient analysis with reduced retention time and was suitable for routine quality- control testing. All elevated validation parameters complied with the application ICH requirements.



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