Comparative Greenness Profiling of Analytical Procedures for the Concurrent Estimation of Amlodipine Besylate and Atenolol Using Click Analytical Chemistry Index (CACI)

Dhvani Prajapati[1], Priyanka Rohit[1], Dharti Prajapati[1], Dr. Ashok Akabari[2], Lalit Chaudhary[2], Pooja Mistry[2], Divya Solanki[2], Dr. Ketan Shah[3]

[1] *PG Scholar, Department of Pharmaceutical Quality Assurance, Shree Naranjibhai Lalbhai Patel College of Pharmacy Umrakh, Gujarat, India*
[2] *Professor, Department of Pharmaceutical Quality Assurance, Shree Naranjibhai Lalbhai Patel College of Pharmacy Umrakh, Gujarat, India*
[3] *Principal,* Shree Naranjibhai Lalbhai Patel College of Pharmacy Umrakh, Gujarat, India

Abstract: *Amlodipine (AML) and Atenolol (ATN) are widely used antihypertensive agents frequently formulated in combination therapy for improved blood pressure management. The present review summarizes and compares the analytical methods reported for the simultaneous estimation of AML and ATN, including UV spectrophotometry, HPTLC, HPLC, UPLC, and LC–MS/MS techniques. In addition to analytical performance, the environmental sustainability of these methods was evaluated using green analytical chemistry concepts and Click Analytical Chemistry Index (CACI)-based assessment. Spectrophotometric and HPTLC methods demonstrated superior environmental compatibility due to lower solvent consumption, reduced waste generation, and minimal energy requirements. HPLC and UPLC methods provided excellent accuracy, precision, and robustness, while LC–MS/MS techniques offered the highest sensitivity and selectivity for bioanalytical applications. Overall, the comparative evaluation highlights the importance of balancing analytical efficiency with environmental sustainability and supports the adoption of greener analytical approaches for the routine estimation of Amlodipine and Atenolol.*

***Key Words*: Amlodipine, Atenolol, Analytical Technique, Method Validation, CACI Assessment, Chromatographic Methods, Spectrophotometric Method.**

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1. INTRODUCTION

1.1. Hypertension, a major global health concern, affects approximately 1.28 billion adults worldwide, with a significant proportion remaining undiagnosed or inadequately managed (1–3). According to the World Health Organization (WHO), nearly 46% of hypertensive individuals are unaware of their condition, and only 42% receive appropriate treatment. Uncontrolled hypertension is a leading risk factor for cardiovascular diseases, stroke, and renal failure, necessitating effective therapeutic strategies to achieve optimal blood pressure control (1–3). Among the widely prescribed antihypertensive agents, amlodipine besylate (AML) and Atenolol (ATN) have gained clinical significance due to their complementary pharmacological actions (4). AML, a long-acting dihydropyridine calcium channel blocker (CCB), inhibits calcium ion influx into vascular smooth muscle and cardiac myocytes, leading to vasodilation, reduced peripheral resistance, and decreased myocardial oxygen demand. It is extensively used for managing hypertension, angina, and coronary artery disease. AML's IUPAC name is 3-Ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate, with a molecular formula of $C_{20}H_{25}ClN_2O_5$ and a molecular weight of 408.88 g/mol. It is slightly soluble in water but freely soluble in methanol and ethanol, with a pKa of 8.6 and a log P of 3.0, indicating moderate lipophilicity (5–7). Atenolol (ATN) is a selective β_1-adrenergic receptor blocker that exerts its antihypertensive effect by reducing heart rate, myocardial contractility, and cardiac output, thereby decreasing blood pressure and myocardial oxygen demand. It is commonly prescribed for the treatment of hypertension, angina pectoris, cardiac arrhythmias, and secondary prevention following myocardial infarction. The IUPAC name of atenolol is 2-{4-[2-Hydroxy-3-(propan-2-ylamino)propoxy]phenyl}acetamide, with a molecular formula of $C_{14}H_{22}N_2O_3$ and a molecular weight of 266.34 g/mol. Atenolol is freely soluble in water and exhibits low lipophilicity with a log P value of approximately 0.2 and a pKa of 9.6, contributing to its favorable pharmacokinetic and pharmacodynamic properties (8–11). The chemical structure of both drugs are shown in Fig. 1.

The growing clinical importance of the AML–ATN combination has created a demand for reliable analytical methods for their simultaneous estimation

in pharmaceutical dosage forms and biological matrices. Accurate quantification of both drugs is essential for quality control, stability studies, dissolution testing, pharmacokinetic investigations, and regulatory compliance. Consequently, numerous analytical techniques, including UV spectrophotometry, High-Performance Thin-Layer Chromatography (HPTLC), High-Performance Liquid Chromatography (HPLC), Ultra-Performance Liquid Chromatography (UPLC), and Liquid Chromatography–Mass Spectrometry (LC–MS/MS), have been developed and validated for their determination. Each technique offers distinct advantages regarding sensitivity, selectivity, accuracy, precision, analysis time, and cost-effectiveness(12-20).

In recent years, environmental sustainability has become an important consideration in pharmaceutical analysis. Conventional analytical methods often involve hazardous organic solvents, high energy consumption, and significant waste generation, which may adversely affect the environment. To address these concerns, Green Analytical Chemistry (GAC) principles have been increasingly incorporated into analytical method development(21-24). Furthermore, computational tools such as the Click Analytical Chemistry Index (CACI) have emerged as valuable approaches for assessing compatibility, sustainability, and overall suitability of pharmaceutical systems. CACI evaluates molecular and physicochemical characteristics, including molecular structure, polarity, hydrogen-bonding capacity, solubility, and stability-related parameters, providing valuable insight into formulation feasibility and interaction potential(25,26).

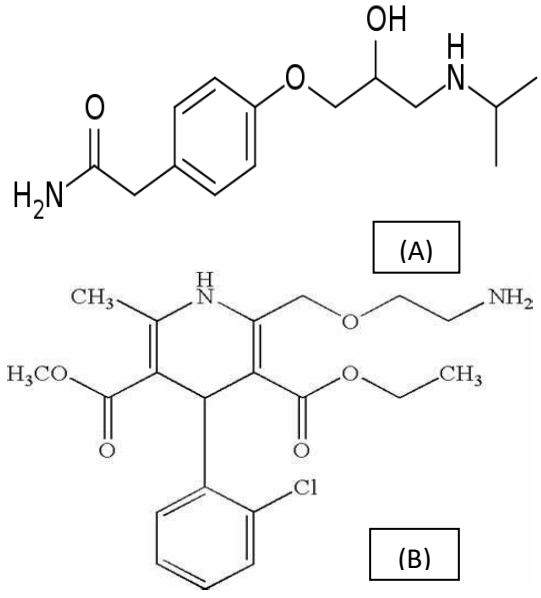

Figure. 1 Chemical Structure of (A) Atenolol (ATN) (B) Amlodipine Besylate (AML)

The CACI assessment of the Amlodipine–Atenolol combination indicates favorable compatibility between the two active pharmaceutical ingredients, supporting their successful incorporation into fixed-dose formulations(25,26). Therefore, a comparative evaluation of analytical methods used for the estimation of AML and ATN, together with CACI-based assessment and green analytical chemistry considerations, is essential for identifying reliable, environmentally responsible, and regulatory-compliant analytical approaches. This review aims to summarize the reported analytical methodologies for AML and ATN and critically evaluate their analytical performance, environmental sustainability, and compatibility characteristics.

This review aims to critically evaluate various analytical methods employed for the estimation of AML and ATN, with a focus on their greenness assessment published between 2010 and 2025. The objectives are to compare the efficiency, accuracy, and sustainability of different analytical techniques, assess their environmental impact, highlight recent advancements in green analytical chemistry, and propose future directions for developing greener, cost-effective, and regulatory-compliant methods. The novelty of this review lies in its comprehensive comparison of conventional and green analytical approaches, providing insights into sustainable pharmaceutical analysis. By supporting eco-friendly analytical techniques, this review contributes to reducing environmental pollution, enhancing regulatory compliance, and encouraging the adoption of green methodologies in the pharmaceutical industry. The findings of this review will be valuable for researchers, regulatory authorities, and pharmaceutical industry, paving the way for a more sustainable and responsible approach to drug analysis(13-19).

Methodology

To gain a comprehensive understanding of the analytical methods, physicochemical properties, and pharmacological attributes of AML and ATN, the authors conducted an extensive review of credible scientific literature and authoritative sources at both national and international levels. The collected information was systematically interpreted and concisely presented to elucidate the relevant characteristics(5-11,20-22).

Physicochemical properties of drugs

The physicochemical attributes of a drug substance are essential considerations in the design and

optimization of analytical methods. Parameters such as molecular weight, log P (partition coefficient), aqueous solubility, polarity, pKa, and environmental pH significantly influence chromatographic behavior and method performance. Table 1 provides a comparative overview of these properties for AML and ATN.

Table -1: Physicochemical properties for AML and IND (5-11,20-22)

Parameter	AML	ATN
Molecular Formula	$C_{26}H_{31}ClN_2O_8S$	$C_{14}H_{22}N_2O_3$
Molecular Weight (g/mol)	567.1	266.33
Log P	2.96	0.16-0.20
Protein Binding(%)	98%	6-16% bound in plasma. Atenolol binds to two sites on human serum albumin.
Dose	oral	Oral,IV
Time to peak concentration	6-8 hours	2-4 hours after oral dose
Half-Life	30- 50 hours	6-9 hours
Bioavailibility	Approximately 60-65%	Oral(50%)
Metabolism	Extensive hepatic metabolism	Minimal(5%)
pKa	9.0 (basic)	9.6 (basic)
Solubility	Slightly soluble in water ; Soluble in methanol and sparingly soluble in ethanol	Freely soluble in water ; slightli soluble in ethanol ; practically insoluble in chloroform and ether

In chromatographic separations, compounds with higher molecular weights and greater lipophilicity often exhibit longer retention times due to stronger interactions with the stationary phase. The log P value, reflecting the partition coefficient of the unionized form of a drug, indicates its hydrophobic or lipophilic character. Based on the reported values, ATN demonstrates greater lipophilicity than AML, which may influence its retention and elution order in reverse-phase chromatography.

Polarity, a key physicochemical trait, aids in selecting appropriate solvents and optimizing mobile phase composition during the development of methods for multicomponent drug analysis. Polarity arises from the electron distribution within chemical bonds—nonpolar compounds share electrons equally, while polar compounds exhibit unequal electron sharing. This affects not only chromatographic retention but also solubility behavior in different media.

The pKa of a drug molecule provides insight into its ionization potential across various pH levels. AML and ATN, with pKa values near physiological pH, may exist in both ionized and unionized forms depending on the mobile phase conditions, which in turn affects their retention profiles. Understanding these physicochemical and pharmacokinetic properties is crucial for the rational development of selective, reproducible, and stability-indicating bioanalytical methods for the simultaneous estimation of AML and ATN.

Analytical methods for AML

1.2. UV spectrophotometric methods of AML

UV spectrophotometric analysis of AML has been extensively explored as a simple, sensitive, and cost-effective alternative to chromatographic techniques for routine quality control. The drug exhibits a distinct absorption maximum between 237–239 nm, which is consistently reported across multiple studies as the ideal wavelength for quantitative determination. Öztürk and Kadıoğlu et al. developed a direct UV spectrophotometric method using methanol as a solvent, demonstrating excellent linearity in the range of 2–17 µg/mL, with recovery values close to 100% and %RSD below 2%, indicating high precision and accuracy (22). Khdadad et al. developed reagent-assisted spectrophotometric techniques involving oxidation followed by complex formation with crystal violet, enhancing selectivity in the visible region when excipient interference was present (23). Likewise, Bachhav et al. validated a straightforward UV-Visible spectroscopic method following ICH Q2(R1) parameters, showing strong linear correlation ($R^2 > 0.999$), satisfactory accuracy (98–102%), and acceptable sensitivity through LOD and LOQ evaluation (24). Overall, these studies collectively confirm that UV spectrophotometry provides a robust, reproducible, and environmentally benign analytical tool for estimating AML in pure form and pharmaceutical formulations, particularly suitable for laboratories requiring rapid and economical assays.

1.3. Chromatographic methods for AML

Literature review revealed that, many researchers has been developed HPLC and UPLC methods for the estimation and stability assessment of AML, using quality by design strategies and green analytical principles. Kasagić-

Vujanović et al. developed a rapid and consistent hydrophilic interaction liquid chromatographic (HILIC) method for the quantitative estimation of AML and its related impurities (D, E, and F). Using a Box–Behnken design and Derringer's desirability function, they optimized the chromatographic conditions, achieving optimal separation on a ZORBAX NH_2 column (250 × 4.6 mm, 5 µm) with an acetonitrile–ammonium acetate buffer (pH 4.0) system (90.5:9.5 v/v) at 230 nm detection (25).

Gigopulu et al. presented a green and robust RP-HPLC method utilizing ethanol as an eco-friendly solvent for determining related substances of AML in film-coated tablets. The optimized conditions—RP-select B column (250 × 4.0 mm, 5 µm), mobile phase of sodium dihydrogen phosphate buffer (pH 4.0) and ethanol (60:40 v/v)—successfully separated impurities D and F with high selectivity, confirmed through forced degradation. The method satisfied Analytical Eco-Scale and AGREE assessment metrics, proving its compliance with Green Analytical Chemistry principles (26).

Ibrahim et al. developed an environmentally benign UPLC method for the quantitative estimation of AML, applying a 3^2 full factorial design to evaluate the influence of methanol percentage and column temperature. The optimized chromatographic conditions (60% methanol, 25°C) produced sharp and symmetrical peaks with retention at 5.37 ± 0.21 min and a resolution of 2.17 ± 0.07. The method demonstrated high sensitivity (R^2 = 0.994), excellent reproducibility (%RSD < 2%), and a low HPLC-EAT environmental impact value (2.91), indicating sustainability and analytical efficiency (27).

Qasim et al. developed a simple, precise, and stability-indicating RP-HPLC method for AML in bulk and tablet formulations using a mobile phase of water and methanol (50:50 v/v) and an Eclipse XDB-C18 column (250 × 4.6 mm, 5 µm). A linear range of 5–25 µg/mL (R^2 = 0.994) with %RSD < 0.8% at a detection wavelength of 360 nm was achieved. Forced degradation under acidic, alkaline, oxidative, thermal, and photolytic stress confirmed its capability to detect degradation products, validating its suitability for stability studies and routine quality control (28).

Jeelani et al. developed a rapid and reliable stability-indicating HPLC method for the estimation of AML and its related impurities in bulk drug. The separation was performed on a core-shell C18 column (100 × 4.6 mm, 2.6 µm) using a gradient system of methanol and 0.4% ammonium hydroxide, with detection at 237 nm. The method effectively resolved AML and seven impurities within 15 minutes, exhibiting stable peak shape under high-pH conditions. Forced degradation under acid, base, oxidative, thermal, and photolytic stress confirmed method specificity with clear separation of degradants. Validation parameters including linearity, precision, accuracy, and robustness established its suitability for routine quality control analysis of AML drug substance (29).

Furthermore, Gajić et al. reported a validated stability-indicating RP-HPLC method for quantifying AML in the presence of impurity D in both tablets and irradiated samples. Separation was achieved on a ZORBAX Eclipse XDB-C18 column using a mobile phase of acetonitrile: methanol: triethylamine solution (15:35:50 v/v/v, pH 3.0) at 273 nm. The method exhibited linearity from 10–75 µg/mL (R^2 = 0.999), with LOD and LOQ of 0.2 µg/mL and 0.66 µg/mL, respectively. Photostability studies revealed pseudo-first-order degradation kinetics with a half-life of 38.4 days under UV and 43.3 days under visible light, confirming adequate photostability in Alu/PVC/PVDC blister packaging (30).

Collectively, these studies highlight the development of HPLC and UPLC methods for AML—from conventional stability-indicating techniques to greener, design-optimized analytical procedures. Each method meets ICH Q1A(R2) and Q2(R1) validation criteria, ensuring precision, accuracy, and robustness. Furthermore, the developed methods support the growing emphasis on environmental sustainability and analytical efficiency in pharmaceutical quality control.

1.4. Analysis methods for ATN

Several analytical methodologies for ATN have yielded significant advancements in precision, selectivity, and applicability across pharmaceutical and biological matrices.

Patel et al. (2023) developed a sensitive UV–visible spectrophotometric method for the quantitative determination of atenolol in tablet formulations. The method exhibited excellent linearity within the concentration range of 5–30 µg mL^{-1} (R^2 > 0.999), with recovery values between 98.5% and 101.2% and relative standard deviation (%RSD) below 2%. The simplicity, rapidity, and cost-effectiveness of the method make it suitable for routine quality control analysis of atenolol-containing pharmaceutical products (31).

Kumar et al. (2024) developed and validated a stability-indicating RP-HPLC method for the determination of atenolol in bulk drug and pharmaceutical dosage forms. Chromatographic separation was achieved using a C18 column with a mobile phase consisting of phosphate buffer and methanol under isocratic conditions. The method demonstrated excellent linearity (R^2 > 0.999), precision (%RSD < 2%), and robustness. Forced degradation studies confirmed its ability to effectively separate atenolol from its degradation products, establishing its suitability for stability assessment and routine quality control applications (32).

Ahmed et al. (2023) reported an LC–MS/MS method for the simultaneous quantification of atenolol in human plasma.

The method exhibited high sensitivity with a lower limit of quantification in the nanogram range and excellent selectivity without significant matrix interference. Validation studies confirmed satisfactory accuracy, precision, recovery, and stability, making the method appropriate for pharmacokinetic and bioequivalence investigations (33)

Collectively, these studies highlight that recent electrochemical and chromatographic developments have significantly improved the accuracy, speed, and environmental compatibility of ATN determination. They also demonstrate the field's shift toward integrated impurity profiling, enantiomeric differentiation, and matrix-specific quantification, aligning with modern regulatory and quality assurance requirements.

1.5. Analytical methods for AML and ATN in combination

Pharmaceutical analysis encompasses a broad spectrum of techniques aimed at quantifying active pharmaceutical ingredients (APIs) or detecting impurities and degradation products within various matrices. Accurate and validated analytical methods are indispensable across all phases of drug development, from formulation and stability studies to quality control and regulatory submissions.

Several analytical methodologies have been reported in the scientific literature for the quantitative and qualitative estimation of AML and ATN. These include spectroscopic techniques such as UV-visible spectrophotometry, chromatographic approaches like HPLC and high-performance thin-layer chromatography (HPTLC), as well as hyphenated methods such as LC-MS and LC-MS/MS.

A summary and classification of the published analytical methods for both drugs are illustrated in Table 2, highlighting the diversity of techniques employed for their analysis.

1.6. Spectroscopic Methods for AML and ATN

Spectroscopy refers to the study of how electromagnetic radiation interacts with matter, typically as a function of wavelength or frequency. This interaction provides critical insight into the structural, electronic, and quantitative aspects of chemical substances. Traditionally, spectroscopy focused on the interaction of photons with molecules, but the scope has now extended to encompass the behavior of charged particles (electrons, protons, ions) and their energy transitions upon collision with other particles. Owing to its simplicity, rapidity, and non-destructive nature, spectroscopic analysis remains a cornerstone in the development of analytical techniques for pharmaceutical compounds (34,35).

AML and ATN , owing to their UV-absorbing chromophores, have been widely analyzed using various UV-visible spectrophotometric methods. These methods are cost-effective, require minimal sample preparation, and provide reliable quantitative data for bulk and formulation analysis.

Most of the spectrophotometric assays for these drugs employ methanol as the solvent of choice due to its favourable properties, such as high polarity, excellent solubilizing capability for both polar and non-polar drugs, and a low UV cutoff (~205 nm), which ensures minimal background interference in the UV range. Typically, the analytical wavelength range used for detecting AML and ATN spans 220–290 nm, depending on the structural features and the nature of the UV transitions.

Numerous researchers have proposed diverse spectroscopic methods for the simultaneous estimation of AML and ATN. These include classical techniques such as the absorbance ratio (Q-analysis), dual wavelength (DW) method, area under the curve (AUC) methods, as well as advanced computational techniques like first derivative ratio spectrophotometry, Vierodt's method, and the ultraviolet absorbance correction method. Table 2 summarizes the reported spectrophotometric techniques developed for the simultaneous quantification of AML and ATN(25-29,42-44).

1.7. HPLC Methods for AML and ATN

Table 2 summarizes the reported High-Performance Liquid Chromatography (HPLC) methodologies for the analysis of amlodipine besylate (AML) and atenolol (ATN), highlighting the widespread use of chromatographic techniques for their simultaneous estimation in pharmaceutical formulations and biological matrices. HPLC remains one of the most reliable, precise, and reproducible analytical techniques for quantitative pharmaceutical analysis owing to its excellent separation capability, sensitivity, and suitability for routine quality control applications(30-33,45-48).

The literature reveals that acetonitrile, methanol, and aqueous buffer systems are the most frequently employed mobile phase components in HPLC methods developed for AML and ATN. Acetonitrile is commonly selected because of its low viscosity, high elution strength, and compatibility with UV detection, whereas methanol is preferred for its favorable solvent properties and cost-effectiveness. Buffer systems including phosphate, acetate, and orthophosphoric acid buffers are used to maintain appropriate pH conditions, improve peak symmetry, and enhance analyte stability during chromatographic separation. These mobile phase combinations facilitate efficient separation of AML and ATN from excipients, impurities, and degradation products, thereby ensuring accurate quantification.

A review of published HPLC methods demonstrates considerable diversity in chromatographic conditions.

Various stationary phases have been employed, including Kromasil C18, Phenomenex Luna, Inertsil ODS, Hypersil BDS, Eclipse XDB, and Waters Symmetry columns, with dimensions generally ranging from 150–250 mm and particle sizes between 3 and 5 µm. Mobile phases typically consist of mixtures of acetonitrile or methanol with aqueous buffers adjusted to pH values between 2.5 and 6.0. Flow rates commonly range from 0.8 to 1.5 mL/min, while UV detection wavelengths are generally selected between 230 and 240 nm to provide adequate sensitivity for both analytes.

Reported linearity ranges for AML and ATN extend from low microgram-per-milliliter concentrations to approximately 150 µg/mL, reflecting the versatility of HPLC methods for assay determination, dissolution studies, content uniformity testing, and bioanalytical applications. Retention times vary according to chromatographic conditions, with amlodipine generally eluting between 2.5 and 8.0 min and atenolol between 2.0 and 7.0 min. These variations demonstrate method-specific optimization aimed at improving resolution, reducing analysis time, and enhancing analytical efficiency.

Several stability-indicating HPLC methods have been reported for AML and ATN, demonstrating effective separation of the drugs from their degradation products formed under acidic, alkaline, oxidative, thermal, and photolytic stress conditions. Such methods comply with ICH guidelines and are widely employed for stability assessment and shelf-life evaluation. Recent advances also include Analytical Quality by Design (AQbD)-based HPLC method development, wherein critical method parameters are systematically optimized using experimental design approaches. These strategies improve method robustness, precision, and reproducibility while facilitating regulatory compliance(35,36,45-48).

Overall, the diverse range of HPLC methods reported for amlodipine besylate and atenolol highlights the flexibility, accuracy, and reliability of chromatographic techniques in pharmaceutical analysis. The incorporation of stability-indicating protocols, AQbD-assisted optimization, and environmentally conscious chromatographic practices has further strengthened their applicability in routine quality control, stability studies, and pharmaceutical research.

1.8. High Performance Thin Layer Chromatography (HPTLC) methods for AML and ATN

High Performance Thin Layer Chromatography (HPTLC) is an advanced planar chromatographic technique widely employed in pharmaceutical analysis due to its simplicity, cost-effectiveness, high sample throughput, and low solvent consumption. HPTLC has gained considerable importance in the simultaneous estimation of amlodipine besylate (AML) and atenolol (ATN), particularly in routine quality control, stability studies, and dosage form analysis. A comprehensive review of the literature reveals several validated HPTLC methods reported for the simultaneous determination of AML and ATN in combined pharmaceutical formulations. Table 2 summarizes the reported HPTLC techniques developed for both drugs(35,49-52).

Most HPTLC methods utilize precoated aluminium plates coated with silica gel 60 F254 as the stationary phase owing to their excellent reproducibility and separation efficiency. Various mobile phase systems have been reported for achieving optimal resolution between AML and ATN, commonly comprising mixtures of toluene, methanol, ethyl acetate, chloroform, ammonia, and triethylamine in different proportions. These solvent systems provide compact, symmetrical, and well-resolved chromatographic bands suitable for quantitative analysis.

Detection is generally performed using densitometric scanning in the UV region, with reported analytical wavelengths ranging from 230 to 240 nm. Several studies have reported simultaneous detection at 237 nm, which corresponds to the absorption maxima of amlodipine and provides adequate sensitivity for atenolol determination. The reported linearity ranges generally extend from 100–600 ng/spot for AML and 200–1200 ng/spot for ATN, demonstrating the applicability of HPTLC for quantitative analysis over a broad concentration range.

The characteristic Rf values reported for AML and ATN typically range between 0.30–0.45 and 0.55–0.75, respectively, depending on the chromatographic conditions employed. The significant difference in Rf values ensures satisfactory separation and minimizes interference from formulation excipients and degradation products. Validation studies conducted according to ICH guidelines have demonstrated acceptable accuracy, precision, specificity, robustness, and recovery for these methods(35,49-52).

Overall, HPTLC methods provide reliable, economical, and environmentally favorable alternatives to conventional chromatographic techniques for the simultaneous estimation of AML and ATN. Their reduced solvent consumption, rapid analysis time, and ability to analyze multiple samples simultaneously make them particularly suitable for routine pharmaceutical quality control and stability assessment.

1.9. Hyphenated Techniques

Liquid Chromatography coupled with Tandem Mass Spectrometry (LC–MS/MS) and Ultra-Performance Liquid Chromatography coupled with Tandem Mass Spectrometry (UPLC–MS/MS) have emerged as highly sensitive and selective analytical techniques for the determination of amlodipine besylate (AML) and atenolol (ATN) in biological matrices and pharmaceutical formulations. These hyphenated techniques combine the separation efficiency

of liquid chromatography with the superior detection capabilities of mass spectrometry, enabling accurate quantification at trace concentration levels(18,19,37,53-55).

Several bioanalytical studies have reported the simultaneous determination of AML and ATN in human plasma using LC–MS/MS methods. Sample preparation generally involves protein precipitation or liquid–liquid extraction using methanol or acetonitrile, followed by chromatographic separation on short C18 columns. These methods exhibit excellent selectivity, low limits of detection and quantification, and wide linearity ranges suitable for pharmacokinetic, bioavailability, bioequivalence, and therapeutic drug monitoring studies(37,53-55).

The application of UPLC–MS/MS has further enhanced analytical performance by reducing analysis time, improving peak resolution, and increasing sensitivity. Modern bioanalytical methods for AML and ATN typically achieve run times below 5 minutes while maintaining high accuracy and precision in accordance with international regulatory guidelines. Furthermore, the use of isotopically labeled internal standards and advanced sample preparation procedures has significantly improved method robustness and reproducibility.

Collectively, LC–MS/MS and UPLC–MS/MS techniques represent powerful analytical tools for the simultaneous determination of AML and ATN, particularly in biological matrices where high sensitivity and selectivity are essential. Their growing application reflects the increasing demand for rapid, reliable, and highly sensitive analytical methods in pharmaceutical research, clinical pharmacokinetics, and therapeutic drug monitoring.(37)

Table -2: Analytical method for estimation of AML and ATN

Title	Description	Reference
UV Spectrophotometric Method Development and Validation for Amlodipine Besylate in Bulk and Tablet Dosage Form	**λmax:**360nm **Linearity:** 5.40 µg/ml **Solvent:** Ethanol	52
Ultra-Violet Spectrophotometric Method for Estimation and Validation of Amlodipine in Bulk and Tablet Formulation	**λmax:**355nm **Linearity:** 10-50 µg/ml **Solvent:** Methanol /Water	53
Method Development, Validation and Stability Study of Amlodipine.In Marketed Formulation by UV Spectrophotometric Method	**λmax:**360nm **Linearity:** 6-80 µg/ml **Solvent:** Methanol	54
Analytical method development and validation of Amlodipine besylate in tablet dosage form	**Column:** C18 Column **Particle Size:** 5 µm **Detector:** UV Detector **λmax:** 237nm **Mobile Phase:** 15v Acetonitrile : 35vmethanol: 50v Buffer pH 3.0 **Flow Rate:** 1.0ml/min **Retention Time:** 12.3 min **Linearity:** 35-105 µg/ml	55
Development and Application of RP-HCPL Method of Oral Formulations Containing Amlodipine besylate	**Column:** C18 Column **Particle Size:** 5 µm **Detector:** UV Detector **λmax:** 265.0 nm **Mobile Phase:** 0.01M Sodium Dihydrogen Phosphate: Acetonitrile (8:2:1v/v/v), adjusted to pH 5.5 With Phosphoric Acid **Flow Rate:** 1.0 min^{-1} **Retention Time:** 6.10min **Linearity:** 10-50µg/ml	56
Development and validation of RP-HPLC method for quantitative analysis of Amlodipine besylate in pure and pharmaceutical formulations	**Column:** C18 Column **Particle Size:** 5µm **Detector:** UV Detector **λmax:** 232nm **Mobile Phase:** 0.025M Phosphate Buffer (pH3.7) : Acetonitrile (57:43v/v)	57

	Flow Rate: ±0.1ml/min **Retention Time:** 8.02min **Linearity:** 10-50µg/ml	
Validation of assay method of amlodipine in tablets by liquid chromatography	**Column:** Ascentis C18 **Particle Size:** 5µm **λmax:** 237nm **Mobile Phase:** Acetonitrile R-0.1 % solution of trifluoroacetic acid R (40:60) **Flow Rate:** 1.0ml/min **Detector:** UV Detector l	58
Simultaneous determination of amlodipine and bisoprolol in rat plasma by a liquid chromatography/tanden mass spectrometry method and its application in pharmacokinetics study	**Column:** Diampnsil C18 **λmax:** 237nm **Mobile Phase:** methanol: water: formic acid (75:25:0.01, v/v/v) **Flow Rate:** 0.3 ml/min **Linearity:** 20-60 µg/ml **Detector:** UV Detector	59
Analytical method development and validation of RP-HPLC and HPTLC method for the simultaneous determination of amlodipine besylate and celecoxib in bulk and pharmaceutical dosage forms	**Stationary phase:** Nucleosil C18 **λmax:** 238nm **Mobile Phase:** Acetonitrile pH adjusted to 8 ± 0.1 with triethylamine **Flow Rate:** 1.2 ml/min **Linearity:** 2.0 – 28.0 µg/ml **Detector:** UV Detector	60
HPTLC simultaneous determination of amlodipine, atorvastatin, rosuvastatin and valsartan in tablets using phenyl -	**λmax:** 365nm **Stationary phase:** phenyl modified Aleppo bentonite (Ba phenyl) **Linearity: 0.50-10.0** µg/ml	61
modified Aleppo bentonite	**Mobile phase**: Acetonitrile: Buffer 45:55, v/v at pH 6.0 **Rf value**: 0.27 **Minimum concentration**:0.5 µg/sport **RSD%:** 4.0 %	
Development and validation of spectrophotometric method for determination of atenolol in pure material and pharmaceutical dosage	**λmax**: 225nm **Linearity:**5-40µg/ml **Solvent:** Methanol **Correlation coefficient**: 0.9979	62
Method Development and Validation of Atenolol Drug by Spectrophotometric and HPLC Technique in Forensic Application	**λmax**: 225nm **Linearity:**5-40µg/ml **Solvent:** Methanol **Correlation coefficient**: 0.9979	63
Method development and validation of atenolol using two HPLC system	**Column:** Inertsil (ODS -3 250mm ×4.6mm, 5 µm siloxane) **Detector:** UV /PDA (Photo diode array detector) **λmax:** 276 nm **Flow rate:** 1 ml/min **Mobile phase:** organic solvent of acetonitrile and water (v/v) **Retention time:** 1.5 min **Linearity =** 2-10 µg/ml	64
HPLC Method for Determination of Atenolol in Human Plasma and Application	**Column:** Stainless steel merck 250 C8 column, 5µm **Mobile phase**: Acetonitrile: methanol: 0.02 M	65

to a Pharmacokinetic Study in turkey	phosphate buffer (Ph 5) (20:20:60) **Detector: UV** detector **λmax:** 226 nm **Flow rate:** 1 ml/min **Linearity:** 0.05- 10.0 µg/ml **Correlation coefficient**: 0.99 **Retention time**: 1.76 min	
RP-HPLC method development for the determination of atenolol related substance in bulk drug	**Column:** C18 **λmax:** 226nm **Detector:** UV detector **Mobile phase**: 1.0 g of octane -1-sulphonic acid sodium salt and 0.4 g of tetra -n- butyl ammonium hydrogen sulphate in a mixture of 20 volumes of tetrahydrofuran, 180 volumes of 804 g/l solution of methanol and 800 volumes of phosphate and PH adjusted to 3 ± 0.2 using dilute ortho – phosphoric acid **Linearity:** 3.2 µg/ml **Retention time:** 8.0 min	66
RP-HPLC method development and validation for simultaneous estimation of Cilnidipine, Atenolol and Chlorthalidone	**Column:** Hypersil-keystone C18 under isocratic condonation **Mobile phase**: Methanol and triple distilled water (80/20 v/v) having PH 7 **Flow rate:** 1.0ml/min **λmax:** 225 nm **Retention time:** 5.36 min **Detector:** UV **Linearity:** 6-36 µg/ml **Correlation coefficient**: 0.999	67
Simultaneous analysis of losartan potassium, atenolol and hydrochlorothiazide in bulk and in tablets by high-performance thin - layer chromatography	**Stationary phase:** Silica gel plates **Mobile phase:** Toluene: methanol: triethylamine (6.5: 4: 0.5) v/v **λ max:** 274 nm **Rf value:** 0.43 **Linearity range:** 250 - 1250ng per band **Correlation coefficient**: 0.999 **Detector**: UV	68
Validated HPTLC Method for Simultaneous Estimation of Atenolol and Aspirin in Bulk Drug and Formulation	**Stationary phase**: Aluminum plates precoated with silica gel 60 F254 **Mobile phase:** n-butanol: water: acetic acid (8:2:0.2 v/v/v) **λ max:** 235 nm **RF value:** 0.23 ± 0.02 **Linearity range:** 100µg /ml **% RDS:** 0.68 - 1.14 **Detector:** UV	69
Simultaneous HPTLC analysis of atenolol indapamide in tablets formulations	**Stationary phase:** Aluminum plates precoated with silica gel 60 F254 **Mobile phase:** toluene: ethyl acetate: methanol: ammonia (5: 3: 3: 0.1) v/v **λ max**: 229 nm **Rf**: 0.27 **Linearity**: 100µg/ml **Detector**: UV	70
Photodegradation studies on Atenolol by liquid chromatography	**Column**: Ace C18 **Mobile phase:** TEA acetate (pH 4; 0.01 M)– acetonitrile 96:4) Phosphate buffer solutions (pH 7.4: 0.01 M), ammonium acetate buffer (pH 7:0.01 M) and triethylammonium (TEA) acetate buffer	71

	Detector: UV	
Quantitative determination of atenolol in dried blood spot samples by LC–HRMS: A potential method for assessing medication adherence	**Column:** Ascentis express C18 **Mobile phase:** Methanol: water (60: 40, v/v) **Flow rate:** 0.2 ml/min **Linearity:** 25 µg/ml **Detector:** UV	72
Determination of atenolol, chlorthalidone and their degradation products by TLC-densitometric and chemometric methods with application of model updating	**Column =** Silica gel 60 F254 **Mobile phase:** Chloroform: methanol: ethyl acetate: ammonia solution (75: 28: 2: 1.6 by v/v) **λ max: C**227nm **Linearity;** 32 – 40 µg/ml **RF value**: 0.04 **Detector**: UV	73
Simultaneous TLC - Densitometric analysis of atenolol and lercanidipine hydrochloride in tablets	**Column =** Silica gel 60 F254 **Mobile phase:** Chloroform: methanol: ethyl acetate: ammonia solution (75: 28: 2: 1.6 by v/v) **λmax: C**227nm **Linearity;** 32 – 40 µg/ml **RF value**: 0.04 **Detector**: UV	74
Gas chromatography/mass spectrometry method for determination of atenolol in rabbit plasma	**Concentration rage**: 15 – 250 µg/ml **RSD % slop:** 4.81 **Column:** HP- 5 MS column with 0.25 µm film thickness **Flow rate**: 1 ml/min **Injector & Detector temperatures**: 280 °C **Carrier gas:** N – methyl – N – (trimethylsilyl) trifluoroacetamide (MSTFA)	75
Simultaneous determination of atenolol and amlodipine using second derivation spectroscopy	**λ max:** Amlodipine =264 nm Atenolol = 264 nm **Linearity:** Amlodipine = 5.0 – 45.0 µg/ml Atenolol= 5.0 – 50.0 µg/ml **Solvent:** Distilled Water	76
UV- absorbance difference method for simultaneous estimation of atenolol and amlodipine besylate in combined dosage forms	**λ max: Amlodipine =**265 nm and 278 nm Atenolol = 209 nm and 244 nm **Linearity:** Amlodipine = 0.35 µg/ml Atenolol= 20 µg/ml **Solvent:** Ethanol	77
A new method development and validation of dual wavelength UV Spectro Photometric method for Simultaneous estimation of Atenolol and Amlodipine besylate in combined dosage form	**λ max:** Amlodipine =263 nm and 277nm Atenolol = 230 nm and 242 nm Mixture: 263 nm and 277nm 230 nm and 242 nm **Linearity:** Amlodipine = 1-6 µg/ml Atenolol= 5-30 µg/ml **Correlation coefficient**: Amlodipine = 0.9987 Atenolol= 0.9983 **Solvent:** Methanol	78
Simultaneous estimation of atenolol and amlodipine besylate as A.P.I and in tablets dosage forms by vierodt's method using UV spectrophotometer	**λmax:** Amlodipine =238 nm Atenolol = 273 nm **Linearity**: Amlodipine = 4-32 µg/ml Atenolol= 20-200 µg/ml **Correlation coefficient:** Amlodipine = 0.9932 Atenolol= 0.9991 **Solvent:** Distilled water	79

Simultaneous estimation of atenolol and amlodipine besylate in tables formulations by vierodt's method using UV spectrophotometry	**λ max**: Amlodipine =239.6 nm Atenolol = 224.6 nm **Linearity:** Amlodipine = 4-32 µg/ml Atenolol= 4-28 µg/ml **Correlation coefficient**: Amlodipine = 0.9932 Atenolol= 0.9991 **Solvent:** Distilled water	80
RP-HPLC Method for the Simultaneous Estimation and Validation of Amlodipine Besylate and Atenolol in Bulk and Tablet Dosage Form in Biorelevant Dissolution Medium (Fassif)	**Column:** C18 Column, kromasil C18 **Detector:** PDA (photodiode array detector) **λ max:** 250nm **Mobile Phase:** Acetonitrile: Potassium di hydrogen phosphate solution (0.01M, pH 3.0 adjusting with Ortho phosphoric acid): Methanol (15:30:55) **Flow Rate:** 1.0 ml/min **Retention Time:** Amlodipine = 2.589 min Atenolol = 3.711 min **Linearity:** Amlodipine = 25-125 µg/ml Atenolol= 5-25 µg/ml **LOQ:** Amlodipine = 0.004 µg/ml Atenolol= 0.015 µg/ml **LOD:** Amlodipine = 0.001 µg/ml Atenolol= 0.005 µg/ml	81
RP-HPLC method development and validation for the simultaneous estimation of amlodipine and atenolol in bulk and Tablet dosage forms	**Column:** C18 Column, BDS C18 **Detector:** UV detector **λ max:** 213nm **Mobile Phase:** Phosphate buffer (1gm of Sodium dihydrogen phosphate and 2gm of Di Potassium hydrogen phosphate in 1000ml ml of water); pH 5(adjusted with Ortho phosphoric acid) Methanol and Acetonitrile in the ratio of 40: 30: 30(v/v) **Flow Rate:** 1.0 ml/min **Retention Time:** Amlodipine = 2.540 min Atenolol = 5.950 min **Linearity:** Amlodipine = 6-14 µg/ml Atenolol= 60-140 µg/ml **LOQ**: Amlodipine = 1.22 µg/ml Atenolol= 15.94 µg/ml **LOD**: Amlodipine = 0.40 µg/ml Atenolol= 5.26 µg/ml	82
RP-HPLC method development and validation for the simultaneous estimation of amlodipine and atenolol in bulk and Tablet dosage forms	**Column:** Intertsil C18 Column **Detector:** UV detector **λmax:** 225 nm **Mobile Phase:** Buffer: Acetonitrile: Methanol (4:3.5:2.5, v/v/v/). The buffer prepared with Triethyl amine and adjusted pH to 3.0 **Flow Rate:** 1.0 ml/min **Retention Time:** Amlodipine = 5.97 min Atenolol = 2.23 min	83

	Linearity: Amlodipine = 5-15 µg/ml Atenolol= 50-150 µg/ml **Correlation coefficient**: Amlodipine = 0.9998 µg/ml Atenolol= 0.9992 µg/ml	
Development and validation of RP-HPLC for simultaneous estimation of atenolol and amlodipine in tablet dosage forms	**Column:** C18 Column **Detector:** UV detector **λmax:** 237 nm **Mobile Phase:** Buffer: Acetonitrile: Methanol (35:10:55, v/v/v/). The buffer prepared with ammonium acetate and sodium pentane sulphonate and adjusted pH to 3.0 with phosphoric acid **Flow Rate:** 1 ml/min **Retention Time:** Amlodipine = 5.0 min Atenolol = 1.67 min **Linearity**: linear over the concentration range of 80%to 120% µg/ml	84
Simultaneous estimation of amlodipine and atenolol in human plasma: a sensitive LC-MS/MS method validation and its application to a clinical PK study	**Column:** C18 column **Detector:** UV detector **λmax:** 230 nm **Mobile Phase:** f 0.01% formic acid in water and methanol: acetonitrile (6:4, v/v) at a ratio of 30:70 (v/v) **Flow Rate:** 0.5 ml/min **Linearity:** Amlodipine = 50-8000 µg/ml Atenolol= 10-800 µg/ml	85
	LOQ: Amlodipine = 50 µg/ml Atenolol= 10 µg/ml	
Simultaneous determination of atenolol and amlodipine in tablets by high-performance thin-layer chromatography	**Column:** Merck HPTLC plates (0.2 mm thickness) precoated with 60F254 silica gel on aluminum sheet **Detector:** UV detector **λ max:** 230 nm **Mobile Phase:** methylene chloride: methanol: ammonia solution (25% NH) (8.8:1.3:0.1; v/v) **Flow Rate:** 1 ml/min **Retention Time:** Amlodipine = 0.75 min Atenolol = 0.33 min **Linearity**: 10-500 µg/ml	86

2. CACI(Click Analytical Chemistry Index)

Is an online computational tool used in pharmaceutical research to predict the compatibility between drug molecules or between a drug and excipients. It helps researchers evaluate whether two compounds can be successfully combined in a formulation without significant interaction problems.

The CACI system analyzes molecular and physicochemical properties such as:

- Molecular structure
- Molecular weight
- Hydrogen-bonding ability
- Polarity
- Solubility characteristics
- Chemical reactivity
- Stability-related parameters

Based on these properties, the software generates a compatibility score ranging from poor to excellent compatibility. A higher score indicates a greater likelihood that the substances can coexist in a pharmaceutical formulation without causing instability or degradation.(87-93)

Importance of CACI in Pharmaceutical Research

- Predicts drug–drug compatibility.
- Assists in fixed-dose combination development.
- Reduces the need for extensive experimental screening.
- Helps identify potential formulation challenges early.

- Supports preformulation and Quality-by-Design (QbD) studies.
- Saves time and development costs.(87-91)

Computer-Aided Compatibility and Interaction (CACI) is a computational tool used to evaluate the compatibility and interaction potential of pharmaceutical ingredients during drug formulation development. The system employs molecular descriptors, physicochemical properties, and predictive algorithms to assess the suitability of combining active pharmaceutical ingredients (APIs) and excipients in a dosage form.

CACI provides an efficient and cost-effective approach for preformulation studies by identifying potential compatibility issues before extensive laboratory experimentation. The analysis helps formulation scientists predict possible interactions that may affect the stability, efficacy, safety, and quality of pharmaceutical products. By utilizing computational techniques, CACI reduces development time and minimizes the risk of formulation failure.

The CACI methodology evaluates various parameters including molecular structure, chemical reactivity, physicochemical characteristics, hydrogen-bonding potential, polarity, solubility behavior, and interaction tendencies. Based on these factors, the software generates a compatibility score that indicates the likelihood of successful formulation development. Higher scores generally represent better compatibility and lower risks of undesirable interactions.(87-92)

In pharmaceutical research, CACI is widely applied during the development of fixed-dose combinations, tablet formulations, capsule formulations, and other drug delivery systems. The information obtained from CACI analysis assists researchers in selecting suitable formulation components, optimizing manufacturing processes, and improving product stability.(94-96)

The use of CACI supports a Quality-by-Design (QbD) approach by providing scientific evidence for formulation decisions. As a result, it serves as a valuable tool in modern pharmaceutical development, contributing to enhanced product quality, reduced development costs, and improved regulatory compliance.

The CACI assessment of the Amlodipine–Atenolol combination indicates favorable compatibility between the two active pharmaceutical ingredients. The analysis suggests that the drugs can coexist in a combined dosage form without significant physicochemical incompatibilities that could adversely affect product quality, stability, or therapeutic performance. The molecular properties of both drugs support their suitability for formulation development and fixed-dose combination products.

From a formulation perspective, the combination demonstrates acceptable stability characteristics under normal storage conditions. The assessment shows a low probability of undesirable interactions that could result in degradation, loss of potency, or reduced efficacy. The compatibility profile indicates that both drugs can maintain their individual pharmacological activities when incorporated into the same formulation.

The therapeutic significance of this combination is substantial because the two drugs target different pathways involved in blood pressure regulation. Amlodipine primarily reduces vascular resistance, while Atenolol decreases cardiac workload. The combined action leads to enhanced blood pressure control compared with monotherapy and may improve patient compliance by reducing pill burden.

Furthermore, the compatibility analysis supports the feasibility of manufacturing the combination using conventional pharmaceutical processes. The favorable compatibility characteristics suggest that formulation development can proceed with minimal risk of instability-related issues. This contributes to improved product quality, longer shelf life, and reliable therapeutic performance.

Overall, the CACI evaluation confirms that the Amlodipine–Atenolol combination possesses desirable compatibility, stability, and formulation characteristics. The findings support its use as a fixed-dose combination for the effective management of hypertension and associated cardiovascular conditions, while also providing a strong basis for further formulation optimization and experimental validation.

Advantages of the Amlodipine–Atenolol Combination

- Improved control of blood pressure through dual mechanisms of action.
- Reduced risk of cardiovascular complications associated with hypertension.
- Better patient compliance due to combination therapy.
- Good compatibility and stability profile.
- Suitable for fixed-dose tablet formulation.
- Reduced dosing frequency and enhanced therapeutic effectiveness.

Table 3: Greenness score of analytical methods of AML and ATN

Name of Method	AGREE SCORE	Ref.
UV Spectrophotometric Method		(37)

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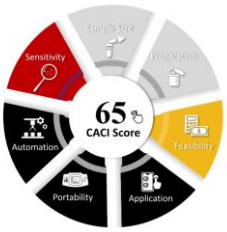
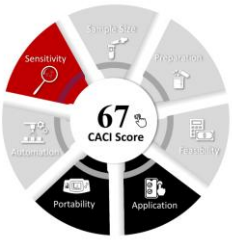
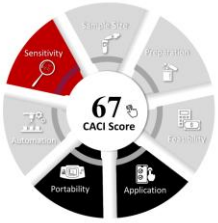
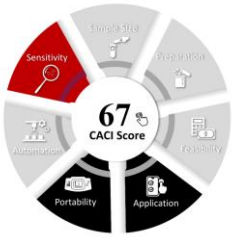
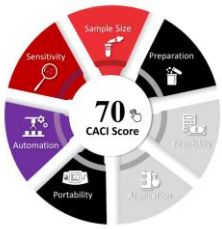
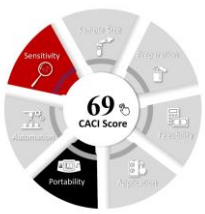
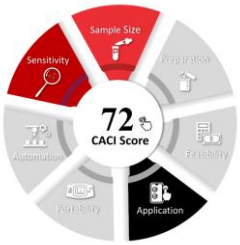
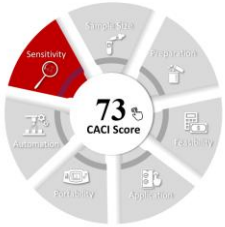
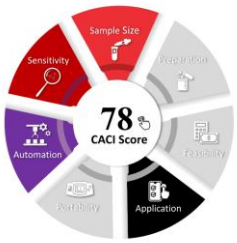
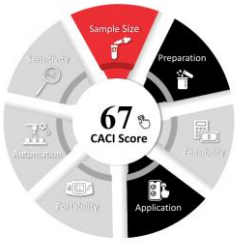
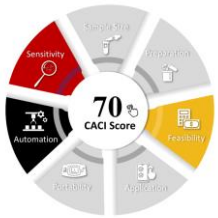
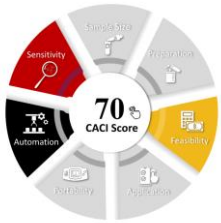
Ultra-Violet Spectrophoto metric Method		(38)
HPLC Method		(39)
RP-HCPL Method		(40)
LC-MS/MS method		(41)
LC-MS/MS method		(42)
HPTLC Method		(38)

HPTLC Method		(40)
UV Spectrophoto metric Method		(43)
UV Spectrophoto metric Method		(44)
HPLC Method		(45)
HPLC Method		(46)
HPTLC Method		(47)

HPTLC Method		(48)
HPTLC Method		(49)
Liquid Chromatography Method		(50,51)
Liquid Chromatography Method		(52)
Gas Chromatography Method		(53)
UV Spectrophotometric Method		(54,55)
UV Spectrophotometric Method		(56)
RP- HPLC method		(57)
RP- HPLC method		(58)
LC–MS/MS method		(59)
HPTLC - Method		(36)

Comparative CACI Evaluation of Analytical Methods for Estimation of Amlodipine and Atenolol

The growing emphasis on sustainable analytical chemistry has encouraged the evaluation of analytical methods using computational greenness assessment tools. Among these, the Computer-Aided Compatibility Index (CACI) provides a systematic approach for assessing the environmental compatibility and sustainability of analytical procedures.

The CACI evaluation considers various factors including solvent consumption, reagent toxicity, energy requirements, waste generation, operational safety, and overall environmental impact.

Various analytical methods have been reported for the estimation of Amlodipine (AML) and Atenolol (ATN), including UV spectrophotometric, HPTLC, RP-HPLC, UPLC, and LC–MS techniques. Comparative CACI assessment revealed notable differences in their environmental compatibility profiles. UV spectrophotometric methods demonstrated the most favorable CACI performance owing to their minimal solvent consumption, simple instrumentation, low energy requirements, and limited waste generation. These characteristics contribute to excellent environmental compatibility and sustainability.

HPTLC methods also exhibited favorable CACI scores due to reduced solvent usage and the capability to analyze multiple samples simultaneously. The lower consumption of organic solvents and relatively simple operational procedures further enhance the environmental performance of HPTLC techniques. Consequently, these methods represent an attractive alternative for routine pharmaceutical analysis.

In contrast, conventional RP-HPLC methods showed moderate CACI performance. Although HPLC provides excellent analytical accuracy, precision, and robustness, the frequent use of organic solvents such as methanol and acetonitrile contributes to increased environmental burden. The generation of solvent waste and higher energy consumption associated with chromatographic systems may negatively influence the overall compatibility score.

UPLC methods demonstrated improved CACI characteristics compared with traditional HPLC techniques. The reduction in analysis time, solvent consumption, and waste generation significantly enhances environmental sustainability while maintaining high analytical performance. These advantages make UPLC a greener chromatographic alternative for the simultaneous estimation of Amlodipine and Atenolol.

Among the evaluated methods, LC–MS and LC–MS/MS techniques generally exhibited lower CACI performance despite their exceptional sensitivity and selectivity. The requirement for high-purity solvents, sophisticated instrumentation, elevated energy consumption, and complex operational procedures contributes to a greater environmental impact. Therefore, although these methods provide superior analytical capability, their sustainability profile is comparatively less favorable.

Overall, the comparative CACI evaluation indicates that spectrophotometric and HPTLC methods possess superior environmental compatibility, whereas chromatographic techniques offer enhanced analytical performance. UPLC represents a balanced approach by combining high analytical efficiency with improved sustainability characteristics. The findings emphasize the importance of integrating environmental considerations into analytical method selection and support the adoption of greener analytical practices for the estimation of Amlodipine and Atenolol.

3. RESULTS AND DISCUSSION

The comparative analysis confirmed that each analytical technique possesses distinct advantages depending on the intended application. HPLC and UPLC methods provide superior analytical reliability, UV and HPTLC methods offer greater environmental sustainability, and LC–MS/MS techniques provide unmatched sensitivity. The favorable CACI assessment further supports the suitability of the Amlodipine–Atenolol combination in fixed-dose formulations and highlights the importance of integrating analytical efficiency with environmental responsibility in pharmaceutical analysis.

4. Conclusion

This review provides a comprehensive overview of the analytical methods reported for the simultaneous estimation of Amlodipine (AML) and Atenolol (ATN) in pharmaceutical formulations and biological matrices. Various techniques, including UV spectrophotometry, HPTLC, HPLC, UPLC, and LC–MS/MS, have been successfully applied for their determination, each offering distinct advantages in terms of sensitivity, selectivity, accuracy, precision, and cost-effectiveness. UV spectrophotometric and HPTLC methods were found to be simple, economical, and environmentally sustainable, making them suitable for routine quality control analysis. HPLC and UPLC methods demonstrated excellent analytical performance and robustness, while LC–MS/MS techniques provided superior sensitivity and selectivity for pharmacokinetic and bioanalytical studies. Furthermore, the CACI assessment indicated favorable compatibility between AML and ATN, supporting their development as fixed-dose combination formulations. Overall, the findings emphasize the importance of selecting analytical methods that not only ensure reliable quantification but also align with Green Analytical Chemistry principles. Future research should focus on developing greener, faster, and more efficient analytical approaches that maintain high analytical quality while minimizing environmental impact.

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