



Original Research Paper

A Comprehensive Review on Stability-Indicating Analytical Method Development for the Simultaneous Estimation of Metoprolol Tartrate, Amlodipine Besylate, and Chlorthalidone in Pharmaceutical Dosage Forms

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Abstract

Hypertension is one of the leading contributors to cardiovascular morbidity and mortality worldwide, necessitating the use of fixed-dose combination therapies to achieve effective blood pressure control and improve patient compliance. The combination of Metoprolol Tartrate (MT), Amlodipine Besylate (AMB), and Chlorthalidone (CTD) integrates complementary pharmacological mechanisms, making it a promising therapeutic option for the management of hypertension. The increasing use of this combination has created a need for reliable, sensitive, selective, and stability-indicating analytical methods for routine quality control, regulatory compliance, and stability assessment of pharmaceutical formulations.

This review comprehensively summarizes the published literature on analytical methods developed for the simultaneous estimation of Metoprolol Tartrate, Amlodipine Besylate, and Chlorthalidone, as well as related antihypertensive combinations. Emphasis is placed on reverse-phase high-performance liquid chromatography (RP-HPLC), ultra-performance liquid chromatography (UPLC), high-performance thin-layer chromatography (HPTLC), UV-visible spectrophotometry, and hyphenated analytical techniques. The review also discusses chromatographic optimization strategies, including column selection, mobile phase composition, buffer systems, detection wavelength, flow rate optimization, and system suitability parameters.

Furthermore, the article critically evaluates stability-indicating analytical methods with respect to forced degradation studies performed under acidic, alkaline, oxidative, thermal, photolytic, and humidity stress conditions in accordance with International Council for Harmonisation (ICH) guidelines. Method validation characteristics, including specificity, linearity, accuracy, precision, robustness, ruggedness, limits of detection and quantification, and solution stability, are comprehensively reviewed. Comparative evaluation of published methods highlights their analytical performance, advantages, limitations, and applicability for routine pharmaceutical quality control.

Recent advances in green analytical chemistry, Analytical Quality by Design (AQbD), design of experiments (DoE), and environmentally sustainable chromatographic approaches are also discussed to provide insight into modern analytical method development. The review identifies current research gaps, including the limited availability of dedicated stability-indicating methods for the simultaneous estimation of the Metoprolol Tartrate-Amlodipine Besylate-Chlorthalidone combination and the need for rapid, eco-friendly, and regulatory-compliant analytical procedures. Overall, this review serves as a valuable resource for researchers, academicians, and pharmaceutical analysts involved in the development, validation, and application of stability-indicating analytical methods for antihypertensive combination products.

Keywords

Metoprolol Tartrate; Amlodipine Besylate; Chlorthalidone; Stability-Indicating Method; RP-HPLC; Method Development; Analytical Method Validation; Forced Degradation Studies; ICH Guidelines; Pharmaceutical Analysis; Fixed-Dose Combination; Green Analytical Chemistry.

Graphical Abstract

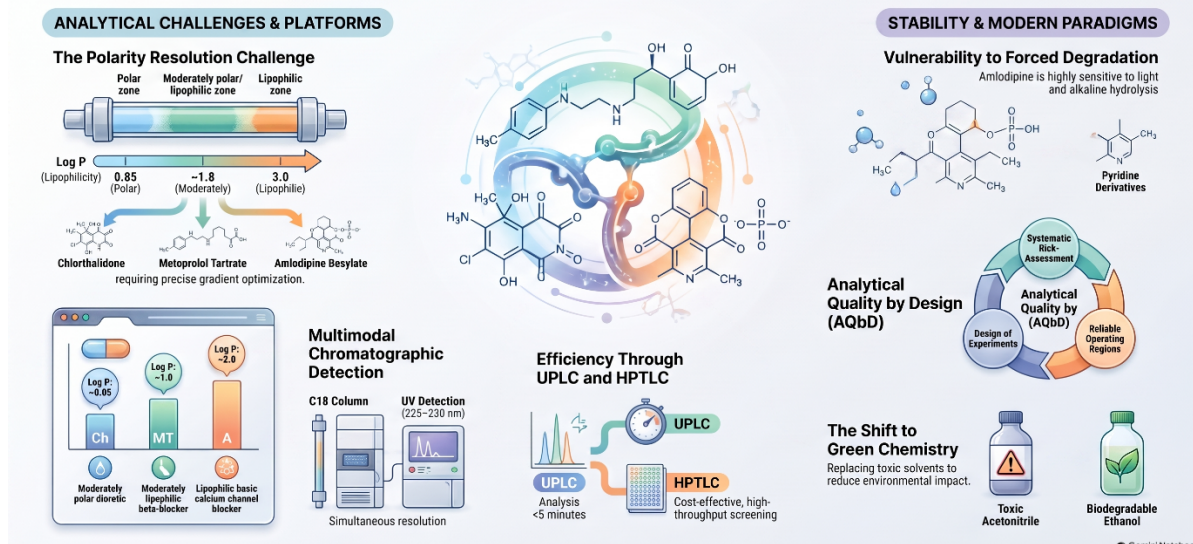
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Analyzing the Triple-Threat Hypertension Combo: MT, AMB, and CTD

Analytical challenges, stability profiles, and modern quality-control methodologies for Metoprolol, Amlodipine, and Chlorthalidone.



1. Introduction

Hypertension is one of the most prevalent chronic cardiovascular disorders worldwide and remains a leading risk factor for stroke, myocardial infarction, heart failure, chronic kidney disease, and premature mortality. According to global health reports, the increasing prevalence of hypertension has become a significant public health concern due to population aging, sedentary lifestyles, obesity, unhealthy dietary habits, and associated metabolic disorders. Effective blood pressure management is therefore essential for reducing cardiovascular morbidity and mortality, leading to the widespread use of antihypertensive medications either as monotherapy or, more commonly, as fixed-dose combination (FDC) formulations.¹

Among the available antihypertensive agents, metoprolol, a selective β_1 -adrenergic receptor blocker, is extensively prescribed for the treatment of hypertension, angina pectoris, cardiac arrhythmias, myocardial infarction, and heart failure. Metoprolol decreases heart rate, myocardial contractility, and cardiac output, thereby lowering blood pressure and reducing myocardial oxygen demand. It is commercially available as metoprolol succinate (extended-release) and metoprolol tartrate (immediate-release), both of which are widely incorporated into combination therapies because of their proven cardiovascular benefits and favorable safety profile.²

Chlorthalidone is a long-acting thiazide-like diuretic that acts by inhibiting sodium and chloride reabsorption in the distal convoluted tubule of the nephron. Compared with conventional thiazide diuretics, chlorthalidone demonstrates a longer elimination half-life and prolonged antihypertensive activity, making it one of the preferred first-line

diuretics in several international treatment guidelines. The complementary pharmacological actions of metoprolol and chlorthalidone provide superior blood pressure control, improved patient compliance, and reduced cardiovascular risk, which has encouraged the development of numerous fixed-dose pharmaceutical formulations.³

In recent years, several multidrug combinations containing metoprolol and chlorthalidone with other antihypertensive agents, including telmisartan, amlodipine, cilnidipine, benidipine, and efonidipine, have been introduced to optimize therapeutic efficacy and enhance patient adherence. The increasing complexity of these pharmaceutical formulations presents significant analytical challenges because the active pharmaceutical ingredients differ considerably in polarity, pKa, solubility, stability, and UV absorption characteristics. Consequently, the development of reliable analytical methods capable of simultaneous estimation of multiple components has become increasingly important for routine quality control, formulation development, dissolution testing, stability studies, and regulatory compliance.⁴

Analytical method development plays a fundamental role throughout the pharmaceutical product lifecycle, from early-stage formulation development to commercial manufacturing and post-marketing quality assurance. Regulatory agencies require validated analytical procedures that consistently demonstrate specificity, accuracy, precision, linearity, robustness, sensitivity, and reproducibility in accordance with International Council for Harmonisation (ICH) guidelines. Well-designed analytical methods ensure accurate quantification of active pharmaceutical ingredients, detection of impurities and degradation products,

and maintenance of product quality, safety, and efficacy during manufacturing and storage.⁵ Various analytical techniques have been reported for the simultaneous estimation of metoprolol, chlorthalidone, and their combination products. Among these, reverse-phase high-performance liquid chromatography (RP-HPLC) remains the most widely employed technique because of its excellent selectivity, sensitivity, reproducibility, and suitability for complex pharmaceutical matrices. Numerous RP-HPLC methods have been developed using different stationary phases, mobile phase compositions, detection wavelengths, and chromatographic conditions to achieve rapid and efficient separation of antihypertensive drug combinations. Recent investigations have also incorporated stability-indicating approaches capable of separating degradation products under forced degradation conditions, thereby supporting stability assessment and regulatory submissions.⁶ Besides chromatographic techniques, UV-visible spectrophotometry continues to be an economical and convenient analytical tool for routine pharmaceutical analysis. Techniques such as simultaneous equation methods, absorbance ratio methods, derivative spectrophotometry, ratio derivative spectrophotometry, and area under the curve (AUC) methods have been successfully applied for simultaneous estimation of metoprolol and its combinations with other antihypertensive drugs. Although spectrophotometric methods generally exhibit lower selectivity than chromatographic techniques, they remain attractive because of their simplicity, rapid analysis, minimal solvent consumption, and lower operational cost.⁷ More recently, researchers have focused on the development of green and sustainable analytical methodologies to reduce solvent consumption, minimize environmental impact, and improve laboratory safety. The adoption of eco-friendly chromatographic conditions, Analytical Eco-Scale, Green Analytical Procedure Index (GAPI), Analytical GREENness (AGREE), White Analytical Chemistry (WAC), and Blue Applicability Grade Index (BAGI) reflects the growing emphasis on environmentally responsible pharmaceutical analysis. Additionally, Analytical Quality by Design (AQbD) and Design of Experiments (DoE) approaches are increasingly being integrated into analytical method development to enhance robustness, optimize critical method parameters, and ensure lifecycle management of analytical procedures.⁸ Although numerous research articles describing analytical methods for individual or combined antihypertensive formulations have been published over the past decade, comprehensive reviews critically comparing chromatographic, spectrophotometric, densitometric, stability-indicating, and green analytical approaches remain

limited. Furthermore, rapid developments in sustainable analytical chemistry, updated ICH guidance, and AQbD-based method development warrant an updated and systematic evaluation of the available literature.⁹

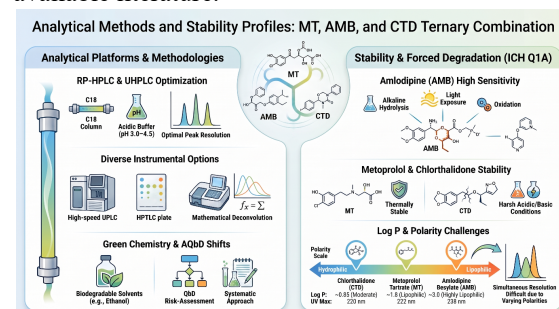


Figure 1. Ternary Drug Combination Stability Analysis

Therefore, the present review aims to comprehensively summarize and critically evaluate the published analytical methods for the simultaneous estimation of metoprolol, chlorthalidone, and related fixed-dose antihypertensive combinations. The review compares different analytical techniques, discusses method development strategies, validation parameters, chromatographic conditions, green analytical assessment tools, regulatory expectations, and current research trends while highlighting existing knowledge gaps and future opportunities for pharmaceutical analytical research.¹⁰

2. Physicochemical Properties

The selected antihypertensive drugs exhibit distinct physicochemical characteristics that significantly influence their chromatographic behavior and analytical method development. Variations in molecular weight, solubility, pKa, and lipophilicity affect retention time, peak shape, and separation efficiency during RP-HPLC analysis. A comparison of the important physicochemical properties of these drugs is presented in **Table 1**.¹¹⁻¹⁵

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3. Analytical Challenges

Simultaneous estimation of antihypertensive drugs by RP-HPLC is challenging because of their diverse physicochemical properties and varying chromatographic behavior. The major analytical challenges include ¹²:

- **Different polarity:** Large differences in polarity and lipophilicity result in varied retention times and difficult chromatographic separation.
- **UV overlap:** Similar UV absorption spectra complicate the selection of a common detection wavelength.
- **Stability issues:** Some drugs are susceptible to degradation under acidic, alkaline, oxidative, or photolytic conditions, requiring stability-indicating methods.
- **Simultaneous estimation:** Achieving adequate resolution and symmetrical peaks for multiple analytes in a single run requires careful optimization.

- **Matrix interference:** Excipients and degradation products may interfere with analyte peaks, affecting accuracy and specificity.

4. Regulatory Guidelines for Analytical Method Development

Analytical method development and validation for pharmaceutical formulations should comply with the guidelines established by the **International Council for Harmonisation (ICH)** to ensure the quality, safety, and efficacy of drug products. These guidelines provide standardized recommendations for stability studies, analytical method validation, impurity assessment, and lifecycle management of analytical procedures.¹³

The key regulatory guidelines relevant to stability-indicating analytical method development include:

- **ICH Q1A(R2):** Stability testing of new drug substances and products.
- **ICH Q2(R2):** Validation of analytical procedures, including specificity, linearity, accuracy, precision, detection limit, quantitation limit, robustness, and range.
- **ICH Q3B(R2):** Identification and qualification of degradation products in drug products.
- **ICH Q14:** Scientific and risk-based approach for analytical procedure development and lifecycle management.

Compliance with these guidelines ensures that the developed analytical method is reliable, reproducible, and suitable for routine quality control and regulatory submissions¹⁴.

Table 2. Key ICH Guidelines Relevant to Analytical Method Development

Guideline	Scope	Application
ICH Q1A(R2)	Stability Testing	Forced degradation and stability studies
ICH Q2(R2)	Method Validation	Validation of analytical procedures
ICH Q3B(R2)	Degradation Products	Identification and qualification of impurities
ICH Q14	Analytical Procedure Development	Risk-based method development and lifecycle management

5. Analytical Techniques for Simultaneous Estimation

Several analytical techniques have been reported for the simultaneous estimation of metoprolol, amlodipine, chlorthalidone, and other antihypertensive combinations. The choice of technique depends on the sensitivity, selectivity, accuracy, cost, and intended application.

The commonly reported analytical techniques include:

- **UV-Visible Spectrophotometry:** Simple, economical, and suitable for routine analysis; however, spectral overlap limits its application in complex mixtures.
- **RP-HPLC:** The most widely employed technique due to its excellent selectivity, precision, and ability to separate multiple analytes and degradation products.
- **HPTLC:** Offers rapid analysis with low solvent consumption and is suitable for routine quality control.
- **UPLC:** Provides higher resolution, shorter analysis time, and reduced solvent usage compared to conventional HPLC.
- **LC-MS/MS:** Highly sensitive and selective technique mainly used for impurity profiling, bioanalysis, and pharmacokinetic studies.

These analytical methods have been extensively applied for the estimation of antihypertensive drugs in bulk drugs, pharmaceutical dosage forms, and stability studies¹⁵.

Table 3. Comparison of Common Analytical Techniques

Technique	Advantages	Limitations
UV Spectrophotometry	Simple, rapid, economical	Poor selectivity due to spectral overlap
RP-HPLC	High accuracy, precision, stability-indicating	Higher operational cost
HPTLC	Fast analysis, low solvent consumption	Lower sensitivity than HPLC

UPLC	High resolution, shorter run time	Expensive instrumentation
LC-MS/MS	Excellent sensitivity and specificity	High cost and technical expertise required

6. RP-HPLC Methods for Simultaneous Estimation

Reverse-phase high-performance liquid chromatography (RP-HPLC) is the most widely employed analytical technique for the simultaneous estimation of antihypertensive drugs because of its high selectivity, sensitivity, accuracy, and reproducibility¹⁶. It is extensively used for routine quality control, assay determination, dissolution studies, and stability testing of pharmaceutical formulations¹⁷.

Most reported methods utilize **C18 reversed-phase columns** with mobile phases consisting of phosphate buffer and organic solvents such as acetonitrile or methanol¹⁸. Detection is commonly performed using UV or PDA detectors within the wavelength range of **220-240 nm**, while chromatographic conditions are optimized to achieve adequate resolution, symmetrical peak shape, and shorter analysis time¹⁹.

Recent studies have also incorporated **stability-indicating RP-HPLC methods**, capable of separating degradation products formed under stress conditions. Furthermore, the application of **Analytical Quality by Design (AQbD)** and green analytical chemistry has improved method robustness, reduced solvent consumption, and enhanced environmental sustainability²⁰.

Table 4. Summary of Reported RP-HPLC Methods

Drug Combination	Column	Mobile Phase	Detection (nm)	Application
Metoprolol + Telmisartan + Amlodipine	C18	Buffer: Acetonitrile	230	Assay & Validation

Metoprolol + Chlorthalidone	C18	Phosphate Buffer: Methanol	223	Stability - Indicating Method
Metoprolol + Telmisartan + Chlorthalidone	C18	Buffer: Acetonitrile	225	Simultaneous Estimation
Metoprolol Tartrate + Amlodipine + Chlorthalidone	C18	Buffer: Acetonitrile	225-230	Synthetic Mixture Analysis
Amlodipine + Chlorthalidone	C18	Buffer: Methanol	237	Assay & Quality Control
Olmesartan + Chlorthalidone + Amlodipine	C18	Buffer: Acetonitrile	230	Tablet Analysis

7. Stability-Indicating Analytical Methods

A stability-indicating analytical method is one that accurately measures the active pharmaceutical ingredient in the presence of degradation products, impurities, and formulation excipients. These methods are essential for evaluating drug stability during manufacturing, storage, and shelf-life studies²¹.

According to **ICH Q1A(R2)**, forced degradation studies should be performed under various stress conditions to demonstrate the specificity of the analytical method. Typical degradation conditions include²²:

- **Acidic hydrolysis**
- **Alkaline hydrolysis**
- **Oxidative degradation**
- **Thermal degradation**

- **Photolytic degradation**
- **Humidity degradation**

A robust stability-indicating RP-HPLC method should provide complete separation of the drug from its degradation products with acceptable system suitability parameters, including resolution, theoretical plates, and tailing factor²³.

Table 5. Common Forced Degradation Conditions

Stress Condition	Purpose	Expected Outcome
Acid Hydrolysis	Evaluate acid stability	Formation of acid degradation products
Base Hydrolysis	Assess alkaline stability	Drug degradation under basic conditions
Oxidative Degradation	Determine oxidative susceptibility	Oxidation products
Thermal Degradation	Evaluate heat stability	Thermal degradation products
Photolytic Degradation	Assess light sensitivity	Photodegradation products
Humidity Study	Evaluate moisture stability	Hydrolytic degradation

8. Method Validation According to ICH Q2(R2)

Analytical method validation is a critical step to ensure that an analytical procedure is suitable for its intended purpose. According to **ICH Q2(R2)**, validation demonstrates that a method consistently produces reliable, accurate, and reproducible results²⁴. For simultaneous estimation of antihypertensive drugs, validation confirms the method's capability to accurately quantify each analyte in the presence of excipients, impurities, and degradation products²⁵⁻³⁰.

The commonly evaluated validation parameters are summarized below.

8.1 Specificity

Specificity is the ability of an analytical method to accurately measure the analyte in the presence of impurities, degradation products, and formulation excipients. A stability-indicating method should provide complete separation of all analytes without interference.

8.2 Linearity

Linearity assesses the ability of the method to produce results directly proportional to analyte concentration within a specified range. It is generally evaluated by constructing calibration curves with multiple concentration levels and determining the correlation coefficient (R^2).

8.3 Accuracy

Accuracy expresses the closeness between the measured value and the true value. It is commonly determined by recovery studies at different concentration levels (80%, 100%, and 120%), with acceptable recoveries typically ranging from 98-102%.

8.4 Precision

Precision measures the reproducibility of analytical results under identical conditions. It is evaluated as:

- Repeatability (intra-day precision)
- Intermediate precision (inter-day precision)

The percentage relative standard deviation (%RSD) should generally be less than 2%.

8.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is the lowest concentration that can be detected but not necessarily quantified, whereas LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy. These parameters indicate the sensitivity of the analytical method.

8.6 Robustness

Robustness evaluates the reliability of the method after small deliberate changes in analytical conditions, such as flow rate, mobile phase composition, pH, column temperature, or detection wavelength.

8.7 System Suitability

System suitability tests verify the performance of the chromatographic system before sample analysis. Common parameters include:

- Retention time
- Theoretical plates
- Resolution
- Tailing factor
- %RSD of peak area

These parameters ensure that the chromatographic system is functioning properly and producing consistent results.

Table 6. Validation Parameters According to ICH Q2(R2)

Parameter	Purpose	Acceptance Criteria
Specificity	Absence of interference	No interfering peaks

Linearity	Response vs. concentration	$R^2 \geq 0.999$
Accuracy	Recovery study	98-102%
Precision	Repeatability & intermediate precision	%RSD < 2%
LOD	Detection capability	As per method
LOQ	Quantification capability	As per method
Robustness	Effect of small changes	No significant variation
System Suitability	Chromatographic performance	Meets predefined criteria

9. Comparative Evaluation of Published Analytical Methods

A variety of analytical methods have been reported for the simultaneous estimation of metoprolol, amlodipine, chlorthalidone, and related antihypertensive combinations. Most studies employed RP-HPLC due to its superior selectivity and reproducibility, while UV spectrophotometry and HPTLC were utilized for routine quality control because of their simplicity and lower cost³¹. Recent publications have also incorporated AQbD principles and green analytical chemistry to improve method robustness and sustainability.

The reported methods differ in terms of chromatographic conditions, mobile phase composition, detection wavelength, run time, and validation characteristics³². Nevertheless, almost all methods demonstrated satisfactory linearity, accuracy, precision, and robustness in accordance with ICH guidelines³³⁻³⁷.

Table 7. Comparative Summary of Selected Published Methods³⁸⁻³⁹

Ref.	Drug Combination	Technique	Major Finding
1	Metoprolol + Telmisartan + Amlodipine	RP-HPLC	Validated simultaneous estimation
3	Chlorthalidone +	RP-HPLC	Short analysis

	Metoprolol + Telmisartan		time with good resolution
4	Metoprolol + Chlorthalidon e	Stability- indicating RP-HPLC	Forced degradation study performed
5	Metoprolol + Telmisartan + Amlodipine	AQbD RP-HPLC	Green and robust analytical method
9	Olmesartan + Chlorthalidon e + Amlodipine	RP-HPLC	Tablet dosage form analysis
11	Metoprolol Tartrate + Amlodipine + Chlorthalidon e	RP-HPLC	Simultaneou s estimation in synthetic mixture
19	Chlorthalidon e + Metoprolol + Telmisartan	HPTLC	Rapid and economic analysis
20	Amlodipine + Chlorthalidon e	Stability- indicating RP-HPLC	Stability assessment in tablet dosage form

10. Research Gaps and Future Perspectives

10.1 Research Gaps

Although numerous analytical methods have been reported for the simultaneous estimation of antihypertensive drugs, several limitations remain. Most published studies focus on binary or ternary combinations involving **metoprolol**, **telmisartan**, **amlodipine**, and **chlorthalidone**, while comparatively fewer reports are available for **Metoprolol Tartrate-Amlodipine Besylate-Chlorthalidone** combinations. Furthermore, many reported methods emphasize assay determination without comprehensive forced degradation studies or impurity profiling⁴⁰.

Most RP-HPLC methods employ conventional optimization approaches, whereas only a limited number of studies have incorporated **Analytical**

Quality by Design (AQbD), **Design of Experiments (DoE)**, and **green analytical chemistry** concepts. Additionally, advanced analytical techniques such as UHPLC and LC-MS/MS remain underutilized for routine quality control of fixed-dose antihypertensive formulations⁴¹.

Therefore, there is a need for rapid, economical, environmentally sustainable, and stability-indicating analytical methods that comply with the latest ICH guidelines and are suitable for routine pharmaceutical analysis⁴².

10.2 Future Perspectives

Future analytical research should focus on the development of robust and eco-friendly methods capable of simultaneous estimation of multiple antihypertensive drugs with shorter analysis time and reduced solvent consumption. The integration of AQbD and DoE can improve method robustness and lifecycle management, while green chromatography can minimize environmental impact⁴³.

Emerging technologies such as UHPLC, LC-MS/MS, artificial intelligence (AI), machine learning, and chemometric tools offer new opportunities for rapid method optimization and impurity profiling. Adoption of these advanced approaches is expected to enhance analytical efficiency, regulatory compliance, and overall quality assurance in pharmaceutical analysis⁴⁴⁻⁴⁵.

11. Conclusion

The simultaneous estimation of antihypertensive drugs remains an important aspect of pharmaceutical quality control due to the increasing use of fixed-dose combination therapies. Among the available analytical techniques, **RP-HPLC** is the most widely preferred because of its excellent selectivity, sensitivity, precision, and suitability for stability studies. UV spectrophotometry and HPTLC continue to serve as simple and cost-effective alternatives for routine analysis.

The reviewed literature demonstrates significant progress in analytical method development; however, only a limited number of studies have reported comprehensive **stability-indicating methods** for combinations containing **Metoprolol Tartrate**, **Amlodipine Besylate**, and **Chlorthalidone**. Recent developments in AQbD, green analytical chemistry, and advanced chromatographic techniques have improved method robustness and sustainability, but further research is still required.

Overall, this review provides a concise summary of current analytical methodologies, validation practices, regulatory requirements, and future research directions. It is expected to serve as a useful reference for researchers and pharmaceutical analysts involved in the development and validation of analytical methods for antihypertensive combination products.

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Conflict of Interest

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All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Author Contributions

Dhruv Kaushikkumar Patel contributed to the conceptualization of the review, conducted the literature search, performed data curation and formal analysis of the published studies, prepared the original draft of the manuscript, and developed the tables and visual presentation of the review. Dr. Sejalben G. Patel contributed through supervision, methodology, validation, project administration, and critically reviewed and edited the manuscript to improve its scientific quality. Dr. Ujashkumar Shah contributed to the conceptualization of the study, provided overall supervision, scientific guidance, and critical review, revised the manuscript for important intellectual content, and gave the final approval of the version to be submitted for publication. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

Data Availability Statement

This review article is based exclusively on published literature. No new experimental data were generated or analyzed during this study. All data supporting the findings of this review are available within the cited references.

Ethical Approval

Not applicable. This review article does not involve human participants, animals, or clinical studies.

Informed Consent Not applicable.

Consent for Publication All authors have reviewed and approved the manuscript for publication.

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