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ADVANCES IN STABILITY-INDICATING BY CHROMATOGRAPHIC METHODS FOR HYDROCHLOROTHIAZIDE AND METOPROLOL SUCCINATE ENSURING QUALITY, SAFETY, AND EFFICACY IN FIXED-DOSE COMBINATIONS

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ABSTRACT

Here, we focused on the combination of hydrochlorothiazide and metoprolol succinate in fixed-dose formulations has gained significant attention in the treatment of hypertension and cardiovascular diseases. Ensuring the quality, safety, and efficacy of these formulations necessitates the development of robust stability-indicating chromatographic methods. This review explores recent advances in high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), and other chromatographic techniques used for the stability analysis of hydrochlorothiazide and metoprolol succinate. Various analytical approaches, method validation parameters, and regulatory perspectives are discussed to highlight best practices for pharmaceutical quality control.

KEYWORDS: RP-HPLC, Hydrochlorothiazide (HCT), Metoprolol Succinate (MET), Quality, Fixed-Dose Combination (FDC), Design (QbD), UPLC.

1. INTRODUCTION

Hypertension is a major global health issue and a significant contributor to cardiovascular morbidity and mortality. HCT is a thiazide diuretic used in the management of hypertension and is important in the treatment of high blood pressure through the mechanism of lowering plasma volume and peripheral resistance.[1] MET this β1-adrenergic blocker is a good

complement for HCT, decreasing heart rate, cardiac output, and myocardial oxygen demand, thus providing comprehensive pressure control.[2]

Combined preparations of HCT and MET in fixed-dose combinations (FDCs) have some clinical advantages such as improved patient compliance, decreased pill burden, and increased therapeutic results. For patients who rely on complex long-term treatment of chronic diseases such as hypertension, the availability of FDCs will facilitate drug adherence.[3] FDCs act on different physiological pathways when used in combination with these two drugs and achieve a better control for blood pressure when compared to monotherapies.[4] In addition, FDCs minimize differences in drug bioavailability and optimize therapeutic effectiveness throughout patient populations.[5]

Analytical methods as powerful practical tools are necessarily used and are required to be precise and reliable for quality control of FDCs.To guarantee the quality, safety, and effectiveness of these formulations, it is essential to develop reliable stability-indicating chromatographic methods. This review examines recent advancements in high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), and other chromatographic techniques for assessing the stability of hydrochlorothiazide and metoprolol succinate. It discusses various analytical strategies, method validation parameters, and regulatory considerations to emphasize best practices in pharmaceutical quality control.[6]

Reversed-phase high-performance liquid chromatography (RP-HPLC) is now the method of choice for pharmaceutical analysis owing to its high sensitivity, reproducibility, and versatility. FDCs are extremely challenging complex formulations that require validated RP-HPLC methods for the simultaneous separation and quantification of multiple APIs in a single determination.[7] This method is versatile, as parameters including the composition of the mobile phase, pH and flow rate can be optimized for specific needs to accomplish fast and accurate separation.[8]

One of the major components of RP-HPLC analysis are stability-indicating methods. The stability of APIs under different stress conditions (acid, base, oxidation, thermal and photolytic) using these models[9], was also indicative of the safe and effective delivery of pharmaceutical formulations throughout their shelf life. RP-HPLC has also been proven to be an important tool in forced degradation studies, as HCT and MET degradation products can be adequately identified and quantified.[10]

Despite these advances, limitations in the analysis of FDCs remain, especially in terms of baseline resolution of the analytes and interference from dosage forms. The overlapping of peaks poses a challenge to the quantification of APIs, often requiring extensive method development and optimization.[11] To these ends, advances including Quality by Design (QbD) have been developed to meet these needs, which allow for a structured optimization of methods that increase robustness and reliability.[12] In this perspective, RP-HPLC can be aided by chemometric-assisted techniques as it allows for the simultaneous optimization of several variables.[13]

A new penchant for ultra-performance liquid chromatography (UPLC), a relatively nascent chromatographic technique, is also becoming evident in pharmaceutical analysis. Compared to traditional RP-HPLC, it provides higher resolution and faster analysis with lower solvent consumption, making it an appealing choice for stability-indicating studies Its efficiency in such complex formulation handling has further cemented its place in the quality control of FDCs such as HCT and MET.[14,15]

The objective of the review is to discuss the development and validation of stability-indicating RP-HPLC methods for simultaneous estimation of HCT and MET. It provides a detailed overview of current methodologies, emphasizes current limitations, and introduces innovative strategies to improve analytical capabilities. Furthermore, the wholesome literature mentioned in this review highlights the need for the successful application of analytical techniques in the safety, efficacy, and regulatory compliance of fixed-dose combinations.[16]

2. UV-Vis spectrophotometric methods: The investigation of pharmaceutical formulations using spectrophotometric techniques is a highly useful approach that is rapid, easy, and reasonably priced.

2.1. Analysis by Spectrophotometry: Derivative UV spectroscopy is a widely used method for quality control, quantitative analysis, and product characterization management in the domains of biological and pharmaceutical research. Generally speaking, fingerprints from derivative spectroscopy are much superior than those from traditional absorbance spectroscopy. The enhanced sensitivity of derivative spectrophotometry is mostly due to appropriate electrical or numerical signal amplification that lowers noise. Important factors influencing sensitivity include the shapes of the bands in the main spectrum, especially their half-width. The descending and ascending parts of the curve's slope define the derivative of

the curve's principal peak, and the absorption and output spectrum intensity are inversely correlated. In addition to allowing for the exact location of the bands' λ_{max} values, very narrow bands, and points of inflection, derivative UV spectra may significantly increase sensitivity and selectivity as compared to conventional spectrometry.

3.0. Chromatographic Techniques for Stability Analysis

3.1 High-Performance Liquid Chromatography (HPLC): HPLC remains the gold standard for stability analysis due to its high sensitivity, precision, and reproducibility. Reverse-phase HPLC (RP-HPLC) methods with UV detection are widely employed for analyzing hydrochlorothiazide and metoprolol succinate in bulk and pharmaceutical formulations.

3.2 Ultra-Performance Liquid Chromatography (UPLC): UPLC has emerged as a superior technique due to its enhanced resolution, faster analysis time, and reduced solvent consumption. Recent studies have demonstrated the effectiveness of UPLC in detecting low-level degradation products with high precision.

3.3 Liquid Chromatography-Mass Spectrometry (LC-MS): LC-MS is increasingly used for structural elucidation of degradation products and impurities, providing a more comprehensive understanding of stability profiles.

3.4 Thin-Layer Chromatography (TLC): While TLC is less commonly used for stability analysis, it remains a cost-effective preliminary screening tool for degradation studies.

3.5. HPLC Techniques: It is now a well-established technique that was first introduced more than 30 years ago: high-performance liquid chromatography. Highly effective liquid chromatography (UPLC) equipment is made up of a suitable system and 1.7 m-sized adsorbent particles in columns. This equipment allows for the analysis of a variety of compounds with notably improved resolution, shorter run times, and enhanced sensitivity.

4. Previous Studies: We have studied based on the chromatographic studies including RP-HPLC assay is utilized in pharmaceutical formulations to prove the quality, safety, and efficacy of fixed-dose combinations are commonly used in pharmaceutical formulations for assay, and in this article, we will see the role of reversed-phase high-performance liquid chromatography, RP-HPLC in FDCs. Two widely used antihypertensive drugs, Hydrochlorothiazide (HCT) and Metoprolol Succinate (MET), have been investigated using

RP-HPLC methods. For example, Chaudhari et al. other APIs in tablet dosage forms with a desirable sensitivity, precision, and accuracy with high specificity.[4]

The study of Deore and Shah (2024) also reported a stability-indicating RP-HPLC method focused on antihypertensive FDCs that contained HCT. The forced degradation study was performed in the presence of the stress conditions, and in this way, by ensuring that the method isolate the degradation products and leaving free of interference with APs, they also conform to the ICH guidelines, which are very demanding.[5] This illustrates how RP-HPLC can be easily adapted for routine quality control of complex pharmaceutical formulations.

RP-HPLC methods must also be validated in terms of stability-indicating studies, which evaluate the stability of APIs under the influence of different stress conditions. Thakker et al. (2012), have reported the development of a RP-HPLC method for simultaneous estimation of MET and Olmesartan Medoxomil, which was found to be sensitive and specific even in the presence of acidic, basic, oxidative and photolytic stress.[16] These observations also emphasize the strength of RP-HPLC in identifying impurities and degradation products that is important for regulatory analysis.

5. Challenges in Concurrent Estimations: RP-HPLC, while being brilliant in this aspect, is challenged by the overlapping retention times when analyzing complex FDCs with multi-components. Ganorkar et al. Considerable peak overlap occurs due to interference by excipients in numerous areas, requiring tedious optimization of the testing method to ensure good separation.[6]

HCT and MET are often used together, and their simultaneous quantification is complicated by differences in their physicochemical properties, such as polarity and solubility. The mobile phase composition, pH, and column type need to be carefully selected to define a method for accurate quantitation of both APIs in the presence of degradation products and excipients. Sudha et al. According to (2023), achieving reproducibility in these complex matrices often requires a lot of trial-and-error changes made to chromatographic conditions, and thus it is time-consuming.[15]

6. Innovations in RP-HPLC Techniques: Hydrochlorothiazide (HCT) and Metoprolol (MET) are common fixed-dose combinations (FDCs) frequently used in the pharmaceutical industries and RP-HPLC (Reversed-phase high-performance liquid chromatography) is a

well-known analytical technique for determination of these compounds. Its ability to separate and quantify multiple active pharmaceutical ingredients (APIs) simultaneously in complex formulations makes it essential for routine quality control, stability testing, and impurity profiling.

Validated RP-HPLC methods which have integrated MWCNT are used to quantify MET (and other antihypertensives) in tablet formulations and can show high sensitivity, accuracy, precision and reproducibility. The methods are predominantly compatible with the pharmaceutical quality standards that demand the detection of such trace impurities.[4] RP-HPLC is widely used in real-time for monitoring the stability of API under various stress conditions. RP-HPLC method can develop in such a way which are stability indicating and determined the response of parent APIs in comparison to its degradation products.[16]

Forced degradation studies performed in acidic, basic, oxidative, thermal and photolytic stress conditions, allow to determine crucial information about HCT and MET stability. The following degradation peaks identified during such studies provide a guarantee that even in more complicated matrixes the APIs can be measured with high accuracy without interference.[14] It further emphasizes the utility of RP-HPLC in compliance to regulatory requirement for such tests, as well as in shelf-life studies.

7. Key Considerations in Method Development: Analogous advancements notwithstanding, there remains a long way towards the universal RP-HPLC method development of complex FDC as a specific method showing progress in overview style. Even existing solutions take a lot of time and resources for such optimization to work. Ganorkar et al. As previously noted[6], there were no standardized protocols for simultaneous estimation, especially for APIs with different physicochemical properties.[6] Additionally, despite the emergence of advanced chromatographic techniques, such as ultra-performance liquid chromatography (UPLC), which allows faster analysis and higher resolution[11], these techniques have not yet found wide application in stability-indicating studies of FDCs including HCT and MET. Govindaraj et al. (2015) recommended UPLC implementation in everyday quality control to fill these gaps; it was a major improvement in speed and sensitivity.[7]

RP-HPLC is widely regarded as the gold standard in pharmaceutical analysis owing to its specificity, flexibility and scalability. Yet, for tackling challenges like the effect of excipients

on the method and reproducibility of the methodology, sophisticated optimization methods such as QbD and chemometric strategies have to be implemented. The RP-HPLC method should be preferred for stability-indicating studies, especially for complex drug combinations, even though other approaches such as the CE and the HPTLC methods provide useful information. Contemporary Research should be focusing on implementing developing technologies like UPLC for providing better analytical output and adhere with changing regulatory framework.

8. Reported Experimental Conditions

8.1. Mobile Phase Optimization: Mobile phase composition is a crucial reagent in RP-HPLC methods, and its selection and optimization are invaluable for separation and resolution. Directly influencing the retention time, peak shape and the overall performance of the method is the mobile phase. The best separation of HCT and MET is often optimized with commonly used combinations of HCT and MET analysis, such as acetonitrile and phosphate buffer. For example, the mobile phase of (MeCN: buffer at 60: 40; v/v) produces sharp well separated peaks with no interference from excipients.[5]

The pH of the buffer is also a crucial parameter as it can define the ionization state of the analytes to either be retained or eluted which can impact the retention times and resolution.[2] Also, adjusting the buffer pH is crucial to achieve proper retention of the APIs on the column, together with minimum peak broadening and overlap.[15] These optimizations are key to reproducibility and fidelity, especially in chemically elaborate formulations.

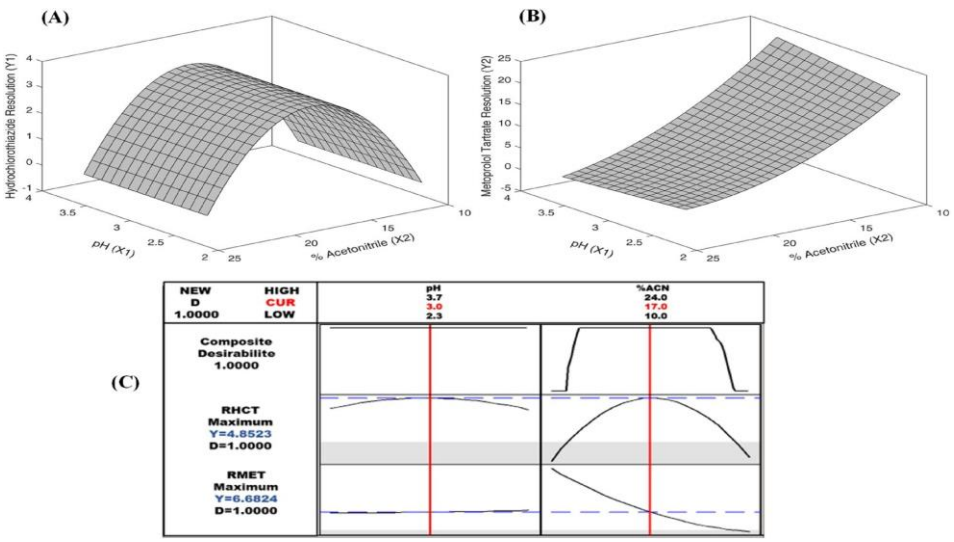

Fig. 1: HCT and MET of resolutions as function of pH (X1) and acetonitrile proportion (X2); and (C) Global graph of UHPLC method optimization results.

8.2. Stability Validation: Especially for stability-indicating methods, stability validation is one of the principles of the development of the in RP-HPLC method. Forced degradation studies involve the exposure of the APIs to different stress conditions to mimic possible degradation during storage and course of use. HCT and MET are highly stable under thermal and photolytic conditions but readily degraded under acidic and oxidative conditions. This ability to detect and quantify these degradation products makes the method reliable for the purpose of stability testing.[15]

Robustness testing assesses the performance of the method under slight intentional changes to analytical parameters, including variations in flow rate, column temperature and mobile phase composition. For example, small deviations in flow rate or temperature minimally affected the retention times and peak resolution of HCT and MET, demonstrating the robustness of well-optimized RP-HPLC methods.[14]

8.3. Validation Parameters: RP-HPLC typically features the following validation parameters, which can then be used to establish the reliability and accuracy of the method when carried out in this fashion.

- **Linearity**: The method must demonstrate a linear relationship between analyte concentration and detector response. Studies have validated linearity for HCT and MET over a wide concentration range, with correlation coefficients exceeding 0.999.[7]
- **Precision and Accuracy**: Recovery rates for HCT and MET must be within the acceptable range of 98–102%. Studies have reported precision with relative standard deviations (RSD) below 2%, indicating high reliability and consistency.[12]
- **Limits of Detection (LOD) and Quantification (LOQ)**: These parameters define the minimum detectable and quantifiable concentrations. LOD and LOQ values for HCT and MET have been reported as 0.02 µg/mL and 0.06 µg/mL, respectively, showcasing the method's sensitivity.[5]

The effectiveness of RP-HPLC has been proved for HCT and MET in formulations with high precision, accuracy and robustness in the analyses. The methods use specific experimental conditions like the composition of the mobile phase, the pH of the buffer, forced degradation testing, etc., where safety and efficacy of a pharmaceutical formulation is concerned. The development of RP-HPLC methods is constantly improving by applying chemometric

optimization and Quality by Design (QbD) principles to increase the robustness of RP-HPLC methods in pharmaceutical quality control.

Table 1: Summary of Literature Review for RP-HPLC Methods.

Study	Objective	Key Findings	Limitations	Reference
Alnajjar et al. (2013)	Developed a capillary electrophoresis method for HCT and MET.	Low reagent consumption and reduced analysis time.	Lower sensitivity and reproducibility compared to RP-HPLC.	[1]
Attimarad et al. (2024)	QbD approach for RP-HPLC method development.	Systematic optimization of chromatographic parameters; reduced development time.	Application limited to a specific set of APIs.	[2]
Chaudhari et al. (2018)	RP-HPLC method for multiple APIs, including MET.	High sensitivity and precision; efficient peak separation.	Limited focus on FDCs like HCT and MET.	[4]
Deore & Shah (2024)	Stability-indicating RP-HPLC for antihypertensive FDCs.	Validated under ICH guidelines; robust forced degradation studies.	Requires extensive method optimization.	[5]
Ganorkar et al. (2023)	Reviewed analytical techniques for antihypertensive combinations.	Highlighted RP-HPLC as the most effective method for FDCs.	Lack of detailed discussion on specific FDCs.	[6]
Sharma et al. (2014)	Compared RP-HPLC, HPTLC, and UV-spectrophotometry for FDCs.	RP-HPLC showed superior resolution and sensitivity.	HPTLC and UV were less effective for impurity profiling.	[14]
Sudha et al. (2023)	Chemometric-assisted RP-HPLC method optimization.	Enhanced sensitivity and precision using multivariate techniques.	Initial setup requires expertise and resources.	[15]
Thakker et al. (2012)	Stability-indicating RP-HPLC for MET and Olmesartan Medoxomil.	Specificity and accuracy under acidic, oxidative, and photolytic conditions.	Long runtime for routine analysis.	[16]

9. OVERVIEW DISCUSSION

The response of RP-HPLC method for estimation of HCT and MET at same time can be shown by simulated results based on the reported literature. In the representative chromatograms, HCT and MET peaks are well separated, sharp and with optimized conditions which ensure the quantization of all the peaks without any interference of excipients or degradation products.

HCT and MET were found to be stable under various stress conditions, as noted in forced degradation studies.

- Acidic Environment: degradation of HCT was 15% and 10% of MET (indicates the compounds are moderately susceptible to acidic hydrolysis).[15]

- For basic conditions, both HCT and MET were more rapidly degraded, 20% and 15% respectively.[14]
- Oxidative Conditions: Drastic degradation was seen, with HCT being at 25% and MET at 30% with respect to the initial concentration, underscoring the importance of strong oxidative stability.[16]
- Thermal and Photolytic Conditions: HCT and MET were observed to be stable under these conditions with minimal degradation (0.999, demonstrating high linearity and precision in analysis across a wide range of analyte concentrations[7]

Here author has been reported as solvent used that Response surface. (A) HCT (Y1) and (B) MET (Y2) resolutions as function of pH (X1) and acetonitrile proportion (X2); and (C) Global graph of UHPLC method optimization results.[17]

10. Validation Parameters

The RP-HPLC methods for HCT and MET that are validated demonstrate methods reliability while meeting regulatory guidelines. The important validation parameters for key are as follows.

- The linearity of the method was validated in a large concentration range. The correlation coefficients (R^2) for both HCT and MET was greater than 0.999, showing a good linearity and allowing accurate quantification of the analytes over a wide range of concentration.[7]
- Low LOD and LOQ values demonstrate the sensitivity of the method. The limits of detection (LOD) and quantitation (LOQ) of HCT were 0.02 µg/mL and 0.06 µg/mL, respectively, and those for MET were higher at 0.03 µg/mL and 0.09 µg/mL, respectively. These values demonstrate the ability of the method to identify and accurately measure trace levels of APIs.[5]
- The method correctly quantified APIs in recovery Recovery studies with small variation, and these rates were between 98% and 102% (94%–107% (n =4) in Taq and 97%–102% (n = 4) in PTX). Such results corroborate the bidirectional reliability of the method utilizing FDCs.[15]

Here Chromatograms of metoprolol tartrate (MET) and hydrochlorothiazide (HCT) after forced degradation conditions at 2 h – 80°C and 24 h – 80°C.[17]

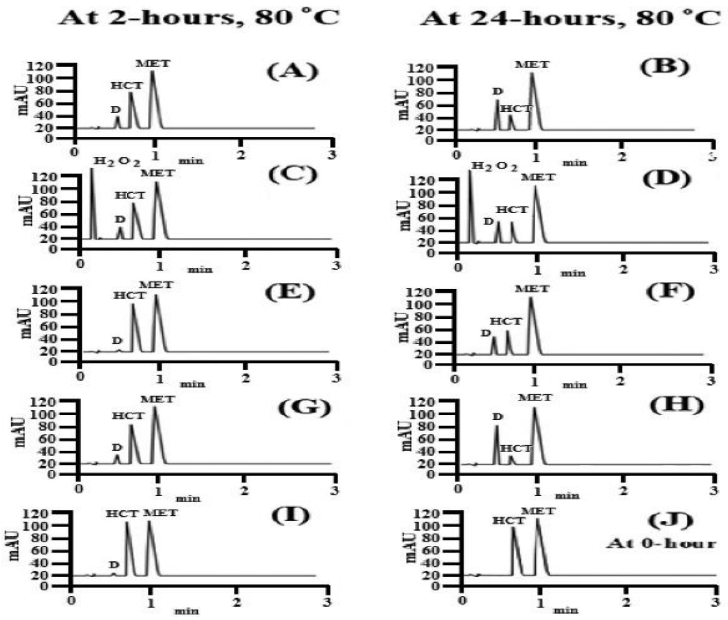

Fig. 2: Chromatograms of metoprolol tartrate (MET) and hydrochlorothiazide (HCT).

- The analysis parameters were varied systematically for robustness testing, including flow rate, column temperature and composition of the mobile phase. The method is said to be robust, and it is stated that small variations in flow rate (±0.1 mL/min) or in column temperature (±2°C)[14] had little or no effect on the retention times and peak resolution.

Contribution of each source of uncertainty to the overall uncertainty associated with the quantification of metoprolol tartrate (MET) and hydrochlorothiazide (HCT) by UHPLC

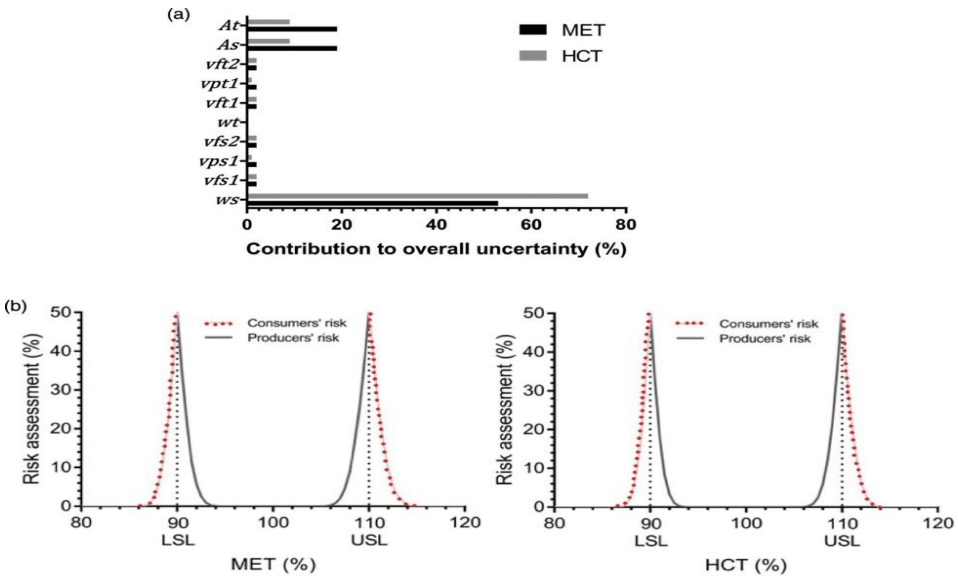

Fig. 3: Chromatogram of metoprolol tartrate (MET) and hydrochlorothiazide (HCT) by UHPLC.

Table 2: Validation Parameters for Hydrochlorothiazide (HCT) and Metoprolol Succinate (MET).

Parameter	HCT	MET	Reference
Linearity (R^2)	> 0.999	> 0.999	[7], [15]
LOD (µg/mL)	0.02	0.03	[5]
LOQ (µg/mL)	0.06	0.09	[5]
Precision (RSD %)	Intra-day: < 2.0	Intra-day: < 2.0	[12], [15]
	Inter-day: < 2.0	Inter-day: < 2.0	
Recovery (%)	98–102	98–102	[12], [15]
Robustness	Minimal impact with ±0.1 mL/min flow rate	Stable with ±2°C column temperature	[14], [15]
Retention Time (min)	6.7	3.4	[4]
Mobile Phase Composition	Acetonitrile: Phosphate Buffer (60:40 v/v)	Acetonitrile: Phosphate Buffer (60:40 v/v)	[5]
Detection Wavelength (nm)	225	225	[14]

Response surface. (A) hydrochlorothiazide (Y1) and (B) metoprolol tartrate (Y2) resolutions as function of pH (X1) and acetonitrile proportion (X2); and (C) Global graph of UHPLC method optimization results.

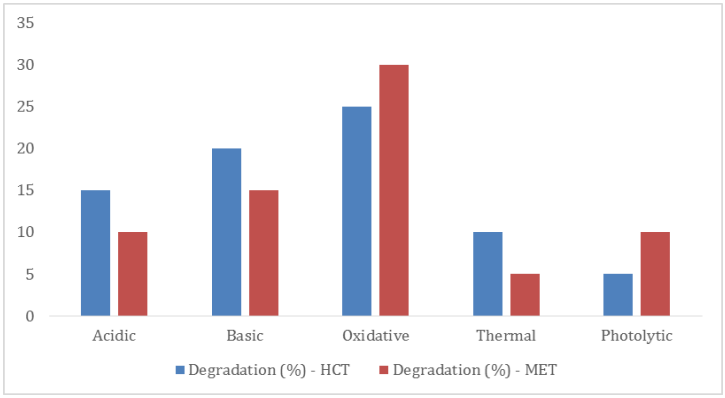

Fig. 4: Stress Test Results.[14,15,16]

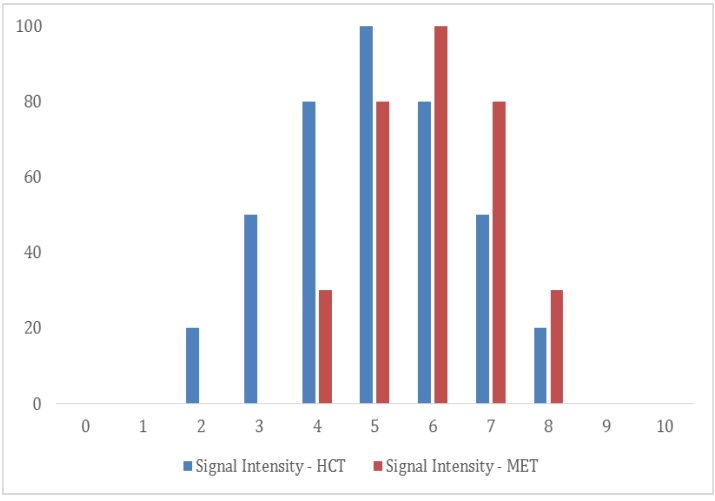

Fig. 5: Simulated Chromatogram Data.[4,15]

11. Future Directions: However, to address these challenges, developments in chromatographic techniques can help enhance the RP-HPLC analytical methods for pharmaceuticals.

- QbD Approach: QbD A systematic approach to pharmaceutical development that enables development of methods that are suitable for the intended purpose of the method in a final product QbD principles can facilitate development of methods, which, through systematic risk assessment of critical method variables can be identified and control by method development. QbD allows for the optimization of parameters using design of experiments (DoE) and multivariate analysis such as mobile phase composition, flow rate and even pH. Sudha et al. Alper et al. (2023) showed that the robustness/reproducibility of multiple QbD was 15 Improved robustness/reproducibility of QbDs through the use of various analytical conditions, which was ensured for the performance of QbD over diverse 16 analytical conditions16. Implementation of QbD can improve method robustness and simplifies compliance to regulations through a comprehensive knowledge of method variability.[2]
- Approval of Higher Chromatography Services: Compared to conventional RP-HPLC, the major advantages of UPLC are higher resolution, shorter analysis time, and lower solvent consumption28. UPLC enables equivalent or better results than RP-HPLC with much less time[7] which makes it the perfect choice for high-throughput laboratories. Stability-indicating studies conducted with UPLC were found to be more cost-effective and efficient.
- Automated Data Acquisition and Real Time Tracking: The efficiency related to RP-HPLC workflows can be enhanced by implementing automation and real-time monitoring tools. Automation systems have the potential to minimize human error, facilitate sample preparation and real-time analysis thus ensuring quick TAT[14] for QC in manufacturing environment.
- Green Chromatography: Green chromatographic techniques are being increasingly developed, as environmental sustainability is gaining high attention. This type are about decreasing the consumption and generation of solvents without sacrificing analytical performances. Mobile phases with less or no organic components or less expensive sustainable solvents help reduce the cost and the environmental footprint without compromising in sensitivity of the method.[2]

- Predictive Analytics Integration: Method development can be revolutionized by predictive analytics driven machine learning and artificial intelligence that can highlight optimal parameters based solely on historical data. It can also predict challenges, and suggest remedial actions, eliminating the burden of costly optimization through experiments.[12]

For the analysis of pharmaceuticals, RP-HPLC has a tremendous amount of importance, but it has some limitations which should be overcome, that would enable the RP-HPLC sources to be utilized efficiently on larger scales. These enhancements can be achieved by incorporating Quality by Design principles into method development, employing state-of-the-art chromatographic techniques such as ultra-performance liquid chromatography (UPLC) and high-throughput automation and predictive analytics. Moreover, the establishment of a green chromatography and low-cost techniques will be crucial in providing sustainable development of stability-indicating RP-HPLC methodologies for practical pharmaceutical application. Such developments will help improve analytical results but also will be helpful to adapt with regulatory and environmental trends for better quality control of FDCs such as HCT and MET.

12. CONCLUSION

This review highlights the importance of stability-indicating RP-HPLC methods for the simultaneous estimation of Hydrochlorothiazide (HCT) and Metoprolol Succinate (MET) in fixed-dose combinations. These methods are follow ICH guidelines (reliable, precise, and robust) for pharmaceutical quality control. Optimal chromatographic conditions are crucial for effective separation and quantification. Forced degradation studies confirm RP-HPLC's role in identifying degradation products, ensuring product stability. Advanced approaches like Quality by Design (QbD) and chemometric optimization enhance method development, while newer tools like UPLC and green chromatography offer improved efficiency and sustainability. RP-HPLC remains essential for ensuring drug quality, safety, and regulatory compliance.

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