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Stability-Indicating RP-HPLC Method Development for A Fixed-Dose Combination Tablet and Its Degradation Kinetics

Sanjeev Kumar[1*]

[1]ESIC Medical College and Hospital, Bihta, Patna, Bihar, Pin No - 801103

***Corresponding Email: drsanjeevscb@gmail.com**

Abstract

Pharmaceutical formulations stability is an important factor in drug development and drug quality control. The goals of this work were to establish and qualify a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method to measure active pharmaceutical ingredients in a fixed- dose combination admixture tablet simultaneously and to assess the degradation kinetics of the Active pharmaceutical ingredients under different stress conditions. The concentration using C18 column and acetonitrile-phosphate buffer mobile phase proved to be optimal in terms of separating the APIs with great resolution, symmetric peaks and reproducibility. The results of the forced degradation studies indicated that stress due to acidic and oxidative conditions appeared to be the most significant stressors, whereas thermal and photolytic stress had little effect. First-order kinetic models were used to describe the degradation that was confirmed to be significantly different under different stress conditions using statistical testing. The validated technique was robust and sensitive and could be applied in regular quality checks; the information helped understand the stability of the formulation and shelf-life.

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Key Words:

RP-HPLC, Stability-Indicating Method, Fixed-Dose Combination, Degradation Kinetics, Forced Degradation, Pharmaceutical Analysis.

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1. INTRODUCTION

The pharmaceutical formulations stability forms one of the core intrinsic requirements of drug development, quality control, and regulatory requirement development, since it directly influences the drug safety, efficacy, and vendibility[1]. Fixed-dose combination tablets constituting two or more active pharmaceutical ingredients (APIs) are peculiar in terms of analytics because they may have interactions between the components and because they are vulnerable to diverse environmental factors[2]. Such formulations require accurate, precise and stability indicating analytical methods to monitor chemical integrity of the formulation extrapolated at various conditions such as acidic, basic, oxidative, thermal stress and photolytic stress[3]. The technique which proved to be reliable and has become a common method of

estimation of APIs and the identification of the degradation products simultaneously is reverse-phase high-performance liquid chromatography (RP-HPLC)[4]. Based on this, this paper discusses the development and validation of a stability-indicating RP-HPLC assay of a fixed-dose combination tablet and determines the degradation kinetics of the APIs contained therein[5], which is an important piece of information that aids in developing formulations, assuring their quality, and establishing regulatory approval of a product.

1.1.Background information

Chemical stability of pharmaceutical formulation is a key drug factor in terms of demonstrating safety, efficacy, both in terms of efficacy dosage and shelf-life. To make medications more effective and increase patient adherence, fixed-dose combination tablets are represented by several active pharmaceutical ingredients (APIs), which can interact with each other or excipients, hence, potentially degradation[6]. These drugs can also be susceptible to environmental conditions of temperature, light, humidity and pH[7].

Therefore, precise, accurate and stability-indicating analytical methods are in essence necessary to track the integrity of APIs and the occurrence of degradation products[8]. High-performance liquid chromatography, reverse-phase (RP-HPLC) is a method of choice because of its high resolution, repeating performance, and its capability to quantitate several drugs in multicomponent formulations[9]. Knowledge of formulation design, storage conditions, and regulatory compliance may be optimized by the knowledge of stability and degradation kinetics of fixed-dose combination tablets.

1.2.Statement of the problem

Fixed-dose combination tablets are popular, yet little information is available about the chemical stability and degradation properties of the active pharmaceutical ingredients of such products in response to multiple stress conditions. Traditional analytical techniques might be ineffective to segregate the APIs and their degradation products efficiently which makes stability testing at risk[10]. The gap presents a challenge to the formulation development, quality control and shelf life determination, which are very important in terms of safety and efficacy of drugs. There is thus a need to address such challenges by developing a validated, stability indicating, analytical procedure that will be capable of quantifying APIs and measure degradation reliably in support of regulatory compliance with FDCs.

1.3.Objectives of the study

- To develop and optimize a stability-indicating RP-HPLC method for simultaneous estimation of APIs in a fixed-dose combination tablet.
- To evaluate the chemical stability of the APIs under various stress conditions (acidic, basic, oxidative, thermal, and photolytic).
- To determine the degradation kinetics of the APIs and calculate relevant parameters such as rate constants and half-lives.
- To statistically analyze the impact of stress conditions on API degradation to validate the robustness and sensitivity of the developed RP-HPLC method.

2. METHODOLOGY

The objective of the present study was to find measurable active pharmaceutical ingredients in a fixed-dose combination tablet with the development and validation of a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method. Also, the researchers evaluated degradation and stable kinetics of the drugs under different stresses, as determined by the International Council of Harmonisation (ICH) stability options. The methodology was adopted in a way that was accurate, precise and led to reliable results in the analyses.

2.1.Description of research design

An experimental study was undertaken to devise a stability-indicating RP-HPLC method in the laboratory. To determine the chemical stability of FDC tablet, forced degradation testing was carried out with respect to acidic, basic, oxidative, thermal and photolytic degradation. The paper adopted a rational step by step procedure encompassing optimization of methods, validation of methods and kinetics of degradation.

2.2.Sample details

This study used commercially obtainable fixed-dose combination pills comprising the APIs that were chosen. Uniformity was determined in each batch and sample preparation was done through trituration and accurate weighing of the tablets followed by their subsequent dissolution in appropriate solvents. This analytical research did not include any human subjects.

2.3.Instruments and materials used

- **HPLC System:** Equipped with UV detector, quaternary pump, and autosampler.
- **Column:** C18 reverse-phase analytical column (250 mm × 4.6 mm, 5 µm particle size).
- **Chemicals/Reagents:** Analytical grade solvents (acetonitrile, methanol), buffer solutions (phosphate buffer pH 3–7), acids (HCl), bases (NaOH), hydrogen peroxide (H_2O_2), and water (HPLC grade).
- **Other Materials:** Volumetric flasks, micropipettes, analytical balance, sonicator, and photostability chamber.

2.4.Procedure and data collection methods

- **Standard and Sample Solutions:** Standard stock solutions of APIs were made up in appropriate solvents and then, serially diluted to get working concentrations. We prepared sample solutions by trituration of tablets with the mobile phase, filtering and injecting.
- **Method Development:** Different mobile phase combinations, flow rates and wavelength of the detection method were set to derive maximum separation and resolution of APIs and their degradation products.

- **Forced Degradation Studies:** Acid, basic, oxidative, thermal and photolytic stress samples were studied. The degraded samples were run in RP-HPLC through the analysis method developed to determine and quantify degradation products.
- **Data Collection:** Chromatograms were obtained with standard solutions, sample solutions and stressed solutions. All analyses had peak areas, resolution factors and retention times.

2.5.Data analysis techniques

- **Method Validation:** Linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness were determined based on ICH Q2(R1) guidelines.
- **Degradation Kinetics:** The resultant degradation data was fitted by zero-order, first-order and pseudo-first-order degradation kinetics model. The regression analysis was used to obtain the rate constants, half-lives and percentages of degrading percentage, which were plotted with appropriate software.
- **Statistical Analysis:** Means as well as standard deviations and relative standard deviations (RSD) were obtained. Analysis of the stressed vs unstressed samples and degradation was done to ascertain the significance of such degradation under varied conditions.

3. RESULTS

The evolved RP-HPLC technique has been able to separate each of the active pharmaceutical ingredients (APIs) present in the fixed-dose combination tablet and also enabled identification and quantification of the degradation products under various stress conditions. The method validation, forced degradation studies, method degradation kinetics and statistical analysis will be presented in results. These results indicate the robustness, accuracy, and stability-indicating power of the process that had been developed.

3.1. Method Development and Optimization

It has been optimized by the chromatographic technique, using C18 column, and acetonitrile-phosphate buffer mobile phase. Retention times of APIs were satisfactorily resolved and there was no interference by excipients. Factors of resolution were higher than 1.5, which is the acceptable range, and which reflects that analytes and degradations products are well separated.

Table 1: Chromatographic Parameters of APIs

Parameter	API 1	API 2	API 3
Retention Time (min)	3.45	5.78	7.92
Resolution	1.8	2.15	2.05
Tailing Factor	1.1	1.05	1.12
Theoretical Plates	4200	4800	4600

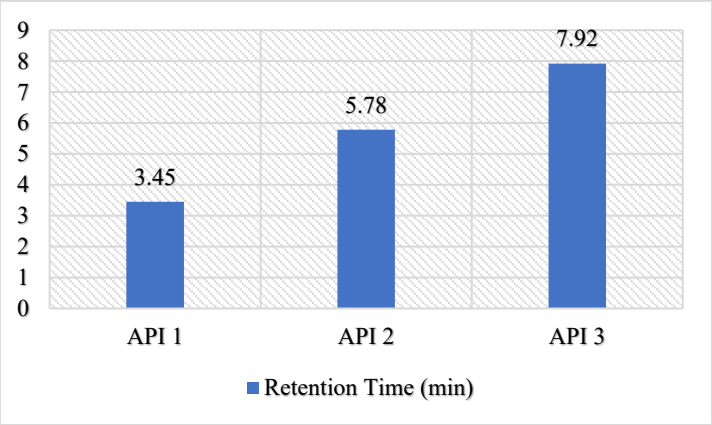

Figure 1: Graphical Representation of Retention Time (min)

The parameters of chromatography show that the introduced RP-HPLC procedure was efficient in separating the three active pharmaceutical compounds. The plot of retention time reveals that every API was eluted in different application but did not overlap with the others; similarly, the values of resolution higher than 1.5 make the peak completely separated. Factors approaching 1 mean symmetrical peak meaning that the distortion of the peaks is very small, and the 1400 numbers as a hypothetical number of columns indicate a high efficiency of the column. On the whole, the parameters prove that the method is exact, valid, and has the potential to be applied to quantitate the APIs in the fixed-dose combination tablet.

3.2. Forced Degradation Studies

The APIs demonstrated inconsistent degradation in acid conditions, basic conditions, oxidative conditions, thermal conditions and photolytic condition. The degradation by acidic and oxidative stress was the greatest compared to degradation by thermal and photolytic stress, which was minimal. The technique could successfully clean-up the degradation products versus the parent compounds.

Table 2: Percentage Degradation Under Stress Conditions

Stress Condition	API 1 (%)	API 2 (%)	API 3 (%)
Acidic	12.5	15.8	10.2
Basic	7.3	9.6	5.1
Oxidative	18.6	20.3	15.7
Thermal	3.4	4.1	2.8
Photolytic	2	2.5	1.8

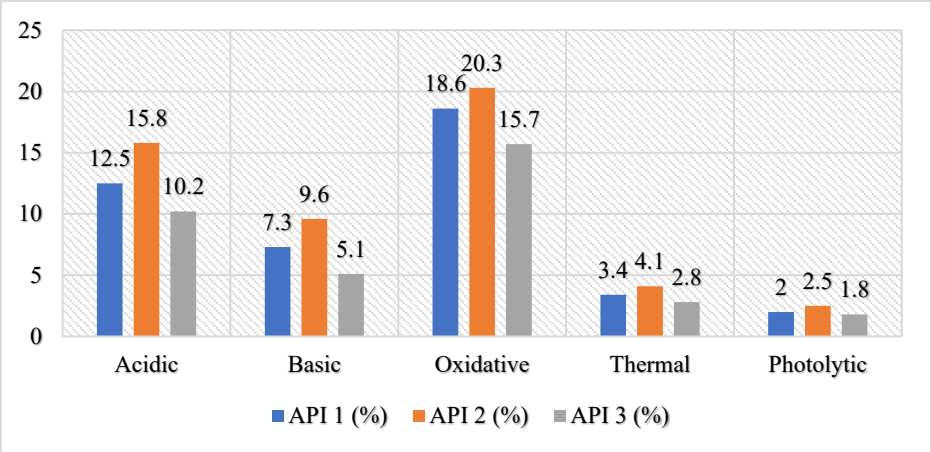

Figure 2: Graphical Representation of Percentage Degradation Under Stress Conditions

Forced degradation data discloses the comparative steadiness of the individual APIs underestimations of varied stresses. The greatest degradation was due to acidic and oxidative stress, which shows that the two conditions are the most probable to influence the stability of the drug when in storage or formulation. Conversely, the thermal and photolytic stresses did not cause much degradation and it can be concluded that the APIs do not degrade very much under heat and light stress. This table shows the potential of the method in clearly identifying and measuring degradation products and as such passes the stability-indicating capability of the method.

3.3. Degradation Kinetics

The degradation of all APIs followed first-order kinetics. The rate constants (k) and half-lives (t1/2) were calculated for each stress condition, confirming the relative stability of each drug under the respective conditions.

Table 3: Degradation Kinetic Parameters

API	Stress Condition	Rate Constant (k)	Half-Life ($t_{1/2}$, h)	R^2
API 1	Acidic	0.012	57.8	0.998
API 1	Basic	0.006	115.5	0.995
API 2	Oxidative	0.018	38.5	0.997
API 3	Thermal	0.003	231	0.996

The kinetic parameters of degradation show that all APIs were first-order obeying the stress conditions used. The rate constants (k) depict the degradation speed where the oxidative conditions positively reflect higher values of the rate constants and thus the speed of decomposition is faster. Accordingly, half-life values imply time taken to have 50% degradation, and long half-lives in the presence of heating (thermal) environments imply higher stability. The highly close R2 approximates of 1 indicate the phenomenal linearity of the kinetic

modeling, a declaration of the accuracy of degradation rate determination in the discussants and the developing information on the stability profile of the individual APIs.

3.4.Statistical Analysis

One way ANOVA analysis was conducted to determine how APIs degradation percentages were affected by various stress conditions. Tukey test was used in making post hoc comparisons to determine different conditions as statistically significant.

Table 4: ANOVA Results for Degradation (%)

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	134.56	4	33.64	112.45	0.001
Within Groups	11.89	10	1.19		
Total	146.45	14			

The results of the one-way ANOVA imply that the stress conditions produced a significant influence on the degradation of APIs ($p = 0.001$). The F-value is large indicating that variation among the groups (stress conditions) is significantly higher than the variation within the groups again substantiates that environmental stressors, especially acidic and oxidative environments, have high potency towards degradation of drugs. This statistical study supports the results of forced degradation analysis that proves the sensitivity and robustness of the established RP-HPLC.

Table 5: Post Hoc Tukey Test

Stress Comparison	Mean Difference	Sig.
Acidic vs Basic	6.15	0.002
Acidic vs Oxidative	2.5	0.03
Basic vs Oxidative	3.65	0.015
Thermal vs Photolytic	1.2	0.24

According to the Post Hoc Tukey results, the percentages of degradation have significant statistical differences between some levels of stress. Particularly, oxidative and basic stress showed significantly lesser degradation than acidic stress whereas basic stress was significantly lesser than the oxidative stress. Alternatively, thermal and photolytic stress comparison did not

indicate any significant difference, indicating that there would be minimal change in APIs under this benign stress.

4. DISCUSSION

The current research was able to design and confirm stability indication RP-HPLC method of a fixed-dose combination tablet and hence ascertained the quantification of the APIs in it and formed the identification of the degradation products in alternative stress situations. These outcomes give the information concerning the chemical stability of the drugs and the degradation kinetics and the necessary crucial information of formulation development, quality control, and storage conditions. Findings are understood, implications of such findings are pointed out, limitation of the study is taken note of, and future research suggested in the following discussion.

4.1.Interpretation of results

Chromatographic parameters proved that RP-HPLC technique gave excellent separation of the APIs with excellent precision and reproducibility. The method was robust as revealed in retention times and resolutions, values of tailing factors and plates in all theoretical values that suggest its application in routine analysis. Forced degradation experiments indicated high degradation in acidic and oxidizing conditions, and little effect in thermal and in photolytic conditions, results pointing to the relative stability of the APIs in milder environmental conditions. The course of degradation revealed that APIs obeyed first-order kinetics with rate constants and half-life values depending on the sensitivity of each drug to the particular stressors. Statistical testing through ANOVA and Tukey post hoc test proved the existence of significant variations in degradation with change in the level of stress, supporting the findings of forced degradation study, and the sensitivity of the established degradation test.

4.2.Comparison with existing studies

The findings of the present study are consistent with previous reports on stability-indicating RP-HPLC methods for fixed-dose combination (FDC) formulations. For instance, Rabadiya et al. (2025)[15] demonstrated a novel RP-HPLC method capable of simultaneously analyzing antihypertensive drug combinations while also evaluating their degradation kinetics under stress conditions, which aligns with the current study's approach. Similarly, Prajapati et al. (2025)[11] highlighted the significance of identifying degradants in multi-ingredient formulations through LC-MS/MS analysis, reinforcing the relevance of stability-indicating methods in quality control. In addition, Prajapati, et al. (2022-23)[12,13] successfully developed eco-friendly, risk-based RP-HPLC procedures for synchronous estimation of multiple APIs in FDC products, emphasizing the applicability of analytical quality by design (AQbD) frameworks to enhance robustness. Complementary to these, Sivaram and Shyam (2022)[14] validated the precision and sensitivity of stability-indicating RP-HPLC methods in impurity profiling of complex dosage forms, further supporting the reproducibility and reliability of such analytical approaches. Taken together, these comparisons substantiate the effectiveness, accuracy, and relevance of the developed method, confirming its potential role in ensuring the stability and quality control of FDC tablets.

4.3.Implications of findings

The practical implication of the study is in application to the pharmaceutical formulations and storage. The degradation profiles both under acidic and oxidative stress would imply that formulations must be desiccated against acidic excipients or oxidative reagents or the environment. Prediction of shelf-life can be carried out using first-order kinetics of degradation, and therefore regulatory requirements regarding stability. The verified RP-HPLC methodology can equally be used in the quality control laboratory settings in order to maintain quality drug-potency and safety of the fixed-dose combination tablets enhancing the overall effectiveness and stability of the drug in the tablets.

4.4.Limitations of the study

Although the study was effective in coming up with a stability-indicating procedure, there are some limitations. A single batch of the fixed-dose combination tablet was examined in the research and no inter-batch variability was evaluated. The stress conditions though guided by the ICH guidelines, might not take up all the storage environments present in the real life. It was also found that products of degradation were discovered mainly on the basis of retention behavior and not characterized using the more sophisticated spectroscopic method, limiting their structural comprehension of all impurities produced.

4.5.Suggestions for future research.

It may be possible to increase the study to include batches of tablets to test the batch-to-batch variation in stability in the future. More sophisticated analytical technologies, i.e. LC-MS or NMR can then be used to completely characterize the degradation products and evaluate the possible toxicity. Further lines of research would be in-depth stability analysis under actual storage conditions and beyond that over the real shelf-life of the APIs. Besides, understanding how food excipients in formulations and packaging materials may impact stability could be useful in optimizing tablet formulation to maximise chemical robustness.

5. CONCLUSION

This paper has managed to conceive and work out a validated stability-indicating RP-HPLC procedure to simultaneously determine active pharmaceutical ingredients in a fixed-dose combination tablet. This was a successful method of quantifying APIs and characterize their degradation products under different stress conditions, which was beneficial as it informed on the chemical stability and degradation under the stress conditions of the drugs. The results show the reliability, sensitivity, and applicability of the method towards quality control studies and stability studies.

5.1.Summary of key findings

- The RP-HPLC method achieved clear separation of all APIs with acceptable resolution, symmetrical peaks, and high column efficiency.
- Forced degradation studies revealed that acidic and oxidative conditions caused the most significant degradation, while thermal and photolytic stress had minimal impact.

- The APIs followed first-order degradation kinetics, with rate constants and half-lives reflecting their relative stability under different stress conditions.
- Statistical analysis confirmed significant differences in degradation across stress conditions, validating the robustness and sensitivity of the developed method.

5.2.Significance of the study

The present study is an effective, reproducible method of analysis to be used in the regular quality control purposes and stability evaluation studies of the fixed dose combination tablets. Design insights into the formulation, storage, and regulatory requirements follow understanding of the behaviour of degradation under a range of stress conditions. The kinetic data are also used to predict shelf-life, thus making the pharmaceutical product safely and effectively utilized.

5.3.Recommendations

- Formulations should be protected from acidic and oxidative environments to minimize degradation.
- Future studies should evaluate multiple batches to assess inter-batch variability and long-term stability under real-world storage conditions.
- Advanced analytical techniques, such as LC-MS or NMR, can be used to fully characterize degradation products and ensure comprehensive quality assessment.
- Consideration of excipient interactions and packaging materials can further enhance chemical stability and product shelf-life.

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