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Chromatography Method Development For Impurity Analysis And Degradation

[1]Jaydeep De, [2]Parimeeta Chanchani

[1]Student, [2]Assistant Professor

[1&2]Department of Chemistry,

[1&2]Mansarovar Global University, Sehore, M.P., India

Abstract: The stability of the active pharmaceutical ingredients (API) i.e., 1, 1-bis(7-chloro-4-hydroxy-3-quinolyl)-ethane (AMQ Dimer) is a critical factor through which the safety and shell efficacy and life of pharmaceutical products is ensured. The HPLC method is developed to conduct a comprehensive physical and chemical degradation of the study and validating on the methods in accordance to International Council of Harmonization (ICH) guidelines using an acquity H-class gradient system with a runtime of 15 minutes and achieving the C18 Hypersil gold column (100 mm * 2.1 mm i.d. 1.9 µm). HPLC method have meticulously helped in analyzing the APIs and their impurities with high accuracy and reliability. The degradation studies have focused on the various factors impact on the drugs like stress conditions, temperature, humidity, light exposure, hydrolysis (acidic and basic conditions), oxidation and reduction to add a valuable pathways and formation of products under different stress conditions. The result has pointed on the elevated temperature, high humidity and light exposure and formation of specific degradation products. Therefore, instead of AMQ Dimer, Famotidine USP drug impurity is selected for future research. The validation method is confirmed with the developed HPLC method using the guidelines to present the comprehensive stability under the accuracy, precision, specificity, and robustness of the product development and pharmaceutical formulations. The LOD and LOQ for AMQ Dimer impurity have determined with 0.0197 ppm (0.012%) and 0.0598 ppm (0.15%) respectively which is significantly not following entire degrading practices. Therefore, the study on famotidine USP to be conducted for guiding on the strategies and packaging solutions which ensure that integrity and stability of the APIs are ultimately enhancing the drug safety and efficacy**.**

Index Terms **-** High performance Liquid Chromatography (HPLC), Active Pharmaceutical Ingredients (APIs), Impurity Profiling, Degradation studies, Stress conditions, method validation, ICH guidelines, Pharmaceutical Formulations and Drug safety and efficacy.

I. INTRODUCTION

In the present era, the lifestyle and environmental challenges poses a heavy impact on the human population which is dependent on the drugs. It has also ensured with the critical aspects of the drug quality which add on the safety and effectiveness of the crucial tasks. The quality assurance and control of pharmaceutical industry has included chemical formulation posing on a significant challenge for analyzing both active pharmaceutical ingredients (API) and finished product before administration to patients [1]. This can also focus on the active pharmaceutical ingredients to meet with the finished products for generating data through inadequate and result based analysis.

The reliable analytical procedure has crucially managed to statutory certification of medications and their formulation to regulatory bodies. The effective monitoring and control of the assay and contaminants have established by the test and safety by its impurities [2]. The analysis of contaminants in medicines is pressing a concern with the contemporary pharmaceutical analysis of the product with success of the medicine.

The rise in the public and media interest in drug safety to manage the impurity profile of pharmaceutical. It is critical with the solutions and various medications present a wide range of difficulties due to diverse nature and qualities [3]. The pharmaceutical dosage form like tablets, soft and hard gelatine capsules, and injections with the way to manage the medicine mode of action. It has also formulated a wide variety of excipients within release of drug in the body. The intended delivery method presents the drug and impurity extraction from formulation varies with the way to depend on the matrix chosen within the pharmaceutical industry and chemical laboratories in terms of speed, selectivity, cost, reproducibility, and accuracy of results [4]. It presents the solutions to the problems where the suitable analytical condition can be raised.

The present study focuses on the development of the HPLC method which use for the drugs and their impurities. It has also conducted physical and chemical degradation studies for understanding on how various stress conditions affecting APIs. The developed methods meet with the validation under the international council for harmonization (ICH) guidelines for precision, accuracy, specificity, and robustness.

II. IMPURITY PROFILE

Impurity profile in active pharmaceutical ingredients (API) have significantly influence the drug safety, quality, and efficacy. It has crucially managed the section where robust methods can utilize the impurity profiling during drug development and manufacturing [7]. It has outlined the section where the impurity for APIs and identification, quantification and controlling of the impurities meet the regulatory requirements. There are different types of the impurity including organic and inorganic impurity, elemental impurity. In organic impurity, the process related impurity arises from the synthetic process that include intermediate yet by-products and degradation products. It has certainly formed the breakdown of the API under various conditions such as light, temperature, heat, or pH changes [8]. The inorganic impurity has added on the solvents used during synthesis that are not completely removed. It has managed the residues of reagents, catalysts, or ligands to use the synthesis process. Elemental impurity has traced the heavy metals amounts like lead, cadmium, or mercury which can be introduced to the raw materials or equipment.

The 1, 1-bis(7-chloro-4-hydroxy-3-quinolyl)-ethane (AMQ Dimer) is a chemical compound which can interest on the pharmaceutical research to meet with the presence of an impurity in certain drugs [6]. It has compute on the essential profiling structure where purity and safety of pharmaceutical products has been essential. It has core structure of two quinoline unit with each quinoline unit substituents attached at 7th position of chlorine atom and 4th position of hydroxyl group making a linking ethane bridge at the 1-position of each quinoline.

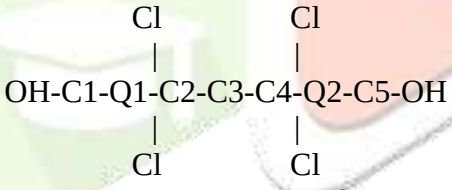

Figure 1: Chemical structure of AMQ Dimer

For the current study, the AMQ Dimer have certainly not degrade the methods of pharmacy. Therefore, the Famotidine (FAMT), 3-[2-(aminoiminomethyl) amino]-4-thiazolyl-methylthio]-N-(aminosulfonyl)-propanimidoamide is certainly focused in future parameter to meet with the requirement of the drug profile for degradation purpose.

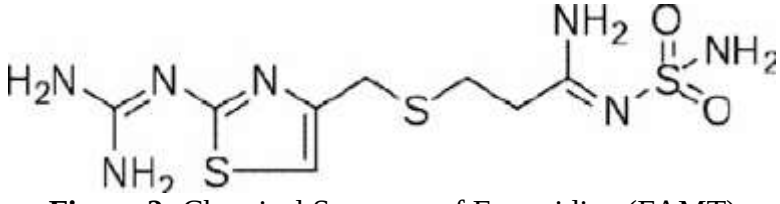

Figure 2: Chemical Structure of Famotidine (FAMT)

The empirical formula for the famotidine is C_8 $H_{15}N_7O_2S_3$ and molecular weight is 337.43. This product is white to pale yellow crystalline compound where the glacial acetic acid has practically not being sound with the methanol and water and insoluble in ethanol.

III. FORCED DEGRADATION STUDIES

The impurities of active pharmaceutical ingredients have set out the stable profile under various stress conditions [4]. It has critically developed a robust HPLC methods ensuring the drug safety and efficacy.
The forced degradation for the AMQ Dimer impurity has significantly quantified under the conditions and validating on the routine quality control of Amodiaquine hydrochloride [10]. This can indicate on the method sensitivity and suitability for traced impurity.

Table 1. Conditions for Forced Degradation Studies [5]

Degradation Type	Experimental Conditions	Storage Conditions	Sampling Time (days)
Hydrolysis	Control API (no acid or base)	40˚C, 60˚C	1,3,5
	0.1M HCl	40˚C, 60˚C	1,3,5
	0.1 M NaOH	40˚C, 60˚C	1,3,5
	Acid control (no API)	40˚C, 60˚C	1,3,5
	Base control (no API)	40˚C, 60˚C	1,3,5
	pH: 2,4,6,8		1,3,5
Oxidation	3% H_2O_2	25˚C, 60˚C	1,3,5
	Peroxide control	25˚C, 60˚C	1,3,5
	Azobisisobutyronitrile (AIBN)	25˚C, 60˚C	1,3,5
	AIBN control	25˚C, 60˚C	1,3,5
		25˚C, 60˚C	1,3,5
Photolytic	Light 1 × ICH	NA	1,3,5
	Light 3 × ICH	NA	1,3,5
	Light 3 × ICH	NA	1,3,5
Thermal	Heat chamber	60˚C	1,3,5
	Heat chamber	60˚C /75% RH	1,3,5
	Heat chamber	60˚C	1,3,5
	Heat chamber	60˚C /75% RH	1,3,5

2.1.1 ACID HYDROLYSIS

The exposure to the acidic conditions i.e., hydrochloric acid helps in the evaluation of attaining stability and degradation of the products of API [5].

2.1.2 ALKALI HYDROLYSIS

The alkali hydrolysis includes the basic conditions such as sodium hydroxide (NaOH) to evaluate the degradation behavior through treatment of API [5].

2.1.3 OXIDATIVE STRESS

The use of the oxidizing agents i.e., hydrogen peroxide to investigate on the API is susceptible to oxidation. The ICH Q1A (stress testing) have included the conditions for performing the forced degradation studies on drug substances and drug products. It has included the temperature for accelerated testing i.e., > 50^0C and humidity with 75% relative humidity and oxidation and photolysis of the stability in the testing of the solution [5].

2.1.4 PHYSICAL STRESS CONDITIONS

The API is elevated with the thermal degradation temperatures which help in attaining thermal stability. Another condition is of photostability, it has exposed the API to light and determined the sensitivity to light-induced degradation [11].

2.1.5 DEGRADATION OF PRODUCT IDENTIFICATION

The separation and detection of the products are highly separated with the use of developed HPLC method. It has employed techniques like mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy to identify the characterize the degradation products [11].

3.1 MATERIAL AND METHODS

The drug and impurities were synthesized with the characterized situations where the HPLC grade (methanol and acetonitrile) and sodium hydroxide with procure with the chromatographic separation to be carried out with the HPLC system consisting of pump, column oven, autosampler, detector and D-7000 system to acquisition to manage the chrom elite versions [15].

3.2 Chromatographic conditions

The mobile phase had two components where component A consist of the mixture of the HPLC grade in different proportion adjusted with the pH to manage the API drug. The component B consist of the mixture where aqueous solution of the HPLC grade would manage to filter and separate the components in the A and B with the situations that are performed with the flow rate conditions using a linear gradient it has certainly change the component variation in the wavelength detection and injection volume with certain µl [15].

3.3 Method Validation

Within the HPLC method, the validation method also adds on the reliability, reproducibility, and suitable range to intend on the purpose of drug detection. The validation process follows a International council for Harmonization (ICH) guidelines.

3.3.1 Specificity Determination

The selection of the method ensures that the API drug can be identified distinctly and quantify the impurities without interference from other components in the sample matrix. The use of the diode array detection (DAD) or MS to confirm the purity of the peaks corresponds on the API and impurities.

3.3.2 Determination of Robustness

The evaluation of the method reliability under small yet deliberate variations in the method parameters such as pH, flow rate and mobile phase composition has ensured on the methods that remains unaffected by the minor changes in analytical conditions [13].

3.3.3 Determination of Accuracy and Precision

In the assessment of the closeness measured with the values to true value is done by analyzing the concentration of API and impurities and comparing the results with the actual values [11]. While precision is evaluating the reproducibility of the method under different conditions where intra-day and inter day precision have involved repeated analysis of samples to calculate on the relative standard deviation (%RSD).

3.3.4 Determination of Linearity

After the experimental analysis, the calibration curve will be plotted. However, the linearity within the API and impurities concentration can be detected against the plotting of the calibration curve. It will utilize the linear regression analysis to determine the correlation coefficient (R2) to be close to 1 [12].

3.3.5 Determination of Limits of Detection (LOD) and Quantification (LOQ)

It has determined the smallest concentration of the analyte to reliably detect on the calculated way on signal to noise ratio (S/N ratio). Also, the lowest concentration has established the quantitatively measured with the acceptable precision and accuracy.

3.3.6 System Suitability Test (SST)

The routine system suitability tests are conducted to ensure the consistent performance of the HPLC system can do it for regular use. The parameters such as retention time, resolution, peak area, and theoretical plates are monitored [10].

IV. DISCUSSION

4.1 Discussion of Chromatographic Measures for Drug Profile

The stability of the active pharmaceutical ingredients (APIs) has ensured the formation of degradation products which are harmful. It has also highlighted on the therapeutic effects of reducing the drug through physical and chemical stress condition through which understanding how stress conditions affect APIs. This can subject to the elevated temperatures i.e., 400C and 600C in a controlled environment While the stability at low temperature can be assessed on -200C and 40C for APIs that require cold storage. The humidity conditions have exposed APIs to high moisture sensitivity within 75% RH to 90% RH [13]. It has set out the degradation rates to determine the extent of hydrolytic degradation. This can direct that stability in low humidity conditions is evaluated with the APIs in the sensitive yet desiccated manner. The APIs are exposed to the UV and visible light to stimulate on the sunlight effects and artificial lighting. The photodegradation products are focusing on the assessment of the light exposure [14].

1, 1-bis(7-chloro-4-hydroxy-3-quinolyl)-ethane (AMQ Dimer). Amodiaquine hydrochloride analytical literature did not mention this AMQ Dimer impurity before now. For quantitation, an Acquity H-class gradient method with a runtime of 15.0 min was devised; a C18 Hypersil gold column (100 mm x 2.1 mm i. d. 1.9 m) was utilised for chromatographic separation, and the method was verified for characteristics including accuracy, precision, linearity, and robustness [16]. For AMQ Dimer impurity, the limits of detection (LOD) and quantification (LOQ) were determined to be 0.0197ppm (0.012%) and 0.0598ppm (0.15%), respectively.

The initial HPLC method have added on the column C18 and mobile phase and detection parameters based on the chemical properties of the impurities and API. It has optimized on the high sensitivity and selectivity with the well-resolved peaks for API and impurities [17]. Within the degradation of the studies, the stability profile of APIs under the different stress conditions. Acid and alkali hydrolysis affected the APIs to showcase on the considerable stability. The thermal and light exposure have influenced degradation rates and proper storage conditions to highlight its importance in the developed practices. It has coupled with the Mass Spectrometry (MS) to provide detailed structural information on degradation of the products within the unknown degradation of compounds [18].

The HPLC method have focused on the ICH validation criteria where the accuracy and precision within the acceptable limits through %RSD values below 2%. The method has evaluated that specificity is high and no interference from degradation products [18]. The linearity is confirmed with the wide range of the concentration to correlated coefficient (R2) greater than 0.999. LOQ and LOD with the way to sufficiently low for detecting the trace impurities. In robust environment, the method can improve with the varied analytical conditions [17].

The HPLC method have added on the drug and impurity analysis to manage the reliable yet accurate way to meet with regulatory requirement. The degradation studies have included on the valuable insights for stable yet API for informing proper storage and handling practices.

The study concludes on the expanding the method to GC-MS for evaluation of other drugs and impurities and applying the methods to real-world pharmaceutical samples for further validation and shifting it to drug profile of Famotidine USP for further research in the direction.

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