STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ANTI-DIABETIC DRUGS IN FIXED-DOSE COMBINATIONS

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ABSTRACT

The increasing prevalence of diabetes has led to the development of fixed-dose combinations (FDCs) of anti-diabetic drugs to improve patient compliance and therapeutic efficacy. Accurate and reliable analytical methods are essential for the simultaneous estimation of these drugs in FDCs, particularly in ensuring stability under various conditions. This research paper presents the development and validation of a stability-indicating method for the simultaneous estimation of selected anti-diabetic drugs in FDCs. The method utilizes high-performance liquid chromatography (HPLC) with UV detection, emphasizing its robustness, accuracy, precision, and ability to detect degradation products. The study demonstrates the applicability of the method in routine quality control and stability testing of FDCs.

KEYWORDS: *quality control, stress testing, linearity, accuracy, precision, specificity.*

INTRODUCTION

The global burden of diabetes mellitus, a chronic metabolic disorder characterized by persistent hyperglycemia, has escalated over the past decades, affecting millions of individuals worldwide. Diabetes results from either inadequate insulin production, insulin resistance, or a combination of both, leading to elevated blood glucose levels. The management of diabetes is multifaceted, involving lifestyle modifications, dietary changes, and pharmacological interventions. Among the pharmacological strategies, the use of oral anti-diabetic drugs is a cornerstone in the management of Type 2 diabetes mellitus (T2DM), which constitutes the majority of diabetes cases. The complexity of diabetes management often necessitates the use of multiple drugs to achieve optimal glycemic control, reduce the risk of complications, and improve patient outcomes. This has led to the development and widespread use of fixed-dose combinations (FDCs) of anti-diabetic drugs.

Fixed-dose combinations (FDCs) are pharmaceutical formulations that contain two or more active ingredients combined in a single dosage form. The rationale behind FDCs is to improve patient compliance, simplify therapy, and enhance the therapeutic efficacy by combining the pharmacological actions of different drugs. In the context of diabetes management, FDCs offer the advantage of addressing multiple pathophysiological targets simultaneously, such as insulin secretion, insulin sensitivity, and glucose absorption. For instance, a combination of metformin, a

biguanide that reduces hepatic glucose production and increases insulin sensitivity, with sulfonylureas like glimepiride, which stimulate insulin secretion from pancreatic β-cells, can provide a synergistic effect, leading to better glycemic control compared to monotherapy.

However, the formulation of FDCs presents significant analytical challenges, particularly in ensuring the simultaneous and accurate estimation of the individual drug components within a complex matrix. The analytical method must be robust enough to separate and quantify each active ingredient without interference from other components, excipients, or potential degradation products. Moreover, as FDCs are intended for long-term use, it is crucial to establish the stability of these drugs under various storage conditions. This is where stability-indicating methods become indispensable. A stability-indicating method is a validated analytical procedure that accurately and precisely measures the active pharmaceutical ingredient (API) without interference from degradation products, process impurities, excipients, or other potential contaminants.

The stability of pharmaceutical products is a critical quality attribute that directly impacts their safety, efficacy, and shelf life. Regulatory agencies, such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), emphasize the importance of stability testing in the drug development process. Stability testing involves subjecting the drug product to various stress conditions, including exposure to light, heat, humidity, acid, and base, to simulate the effects of long-term storage. The degradation pathways identified through these tests help in understanding the stability profile of the drug and in developing appropriate storage conditions and packaging.

In the context of FDCs, the development of a stability-indicating method is particularly challenging due to the presence of multiple active ingredients, each with its own degradation pathway. For instance, in an FDC containing metformin and glimepiride, metformin may be more prone to hydrolytic degradation under acidic conditions, while glimepiride might degrade under oxidative conditions. A robust analytical method must be capable of detecting and quantifying each drug in the presence of its degradation products and under various stress conditions. High-performance liquid chromatography (HPLC) is the most commonly employed technique for this purpose, owing to its high resolution, sensitivity, and versatility.

HPLC is an advanced chromatographic technique used to separate, identify, and quantify components in a mixture. The method involves passing a liquid sample through a column packed with a solid adsorbent material, where different components of the sample are separated based on their interactions with the stationary phase and the mobile phase. For the simultaneous estimation of anti-diabetic drugs in FDCs, reverse-phase HPLC (RP-HPLC) is often employed. In RP-HPLC, the stationary phase is non-polar, while the mobile phase is polar, which allows for the separation of a wide range of polar and non-polar compounds.

The development of an HPLC-based stability-indicating method involves several critical steps. Initially, the selection of suitable chromatographic conditions, including the choice of column, mobile phase composition, flow rate, and detection wavelength, is essential. The method must be optimized to achieve adequate separation of the active ingredients and any potential degradation

products within a reasonable run time. Stress testing is then conducted to induce degradation of the drugs under various conditions, such as exposure to acid, base, oxidative agents, heat, and light. The degraded samples are analyzed using the developed method to assess its capability to resolve and quantify the degradation products.

Once the method is developed, it must be validated according to regulatory guidelines. Validation ensures that the method is reliable and reproducible across different laboratories and conditions. The key parameters of method validation include specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). Specificity refers to the method's ability to unequivocally analyze the drug of interest in the presence of potential interferences such as degradation products and excipients. Linearity assesses the method's ability to produce results that are directly proportional to the concentration of the analyte within a given range. Accuracy and precision evaluate the method's closeness to the true value and the consistency of the results, respectively. Robustness tests the method's reliability under small variations in experimental conditions, while LOD and LOQ determine the lowest concentration of the analyte that can be reliably detected and quantified.

The successful development and validation of a stability-indicating HPLC method for the simultaneous estimation of anti-diabetic drugs in FDCs have significant implications for the pharmaceutical industry. It ensures that the drugs maintain their efficacy and safety throughout their shelf life and provides a reliable tool for routine quality control and stability testing. This is particularly important in the context of global health, where the prevalence of diabetes is rising, and the demand for effective, safe, and convenient therapeutic options is increasing.

In the development of a stability-indicating method for the simultaneous estimation of anti-diabetic drugs in FDCs is a critical aspect of pharmaceutical analysis. It involves overcoming significant analytical challenges, particularly in ensuring the accurate and precise estimation of multiple drugs in a complex matrix. HPLC, with its high resolution and sensitivity, is the technique of choice for this purpose. The validated method not only ensures the stability and efficacy of the FDCs but also plays a crucial role in the overall quality assurance process, contributing to better patient outcomes in the management of diabetes.

OPTIMIZATION OF CHROMATOGRAPHIC PARAMETERS

Column Selection:

- A C18 reversed-phase column was chosen for its non-polar nature, which provides good retention and separation of the anti-diabetic drugs.

Mobile Phase Composition:

- A gradient elution of acetonitrile and phosphate buffer (pH adjusted) was optimized to achieve optimal separation, balancing polarity to ensure adequate resolution of all components.

pH Adjustment:

- The pH of the mobile phase was carefully adjusted to maintain drug stability and improve peak shape, minimizing tailing and ensuring sharp, well-defined peaks.

Flow Rate:

- The flow rate was optimized to 1.0 mL/min to ensure efficient separation within a reasonable run time, balancing analysis speed with resolution.

Detection Wavelength:

- The UV detection wavelength was set at 230 nm, based on the maximum absorbance of the drugs, ensuring sensitive detection without interference.

Injection Volume:

- An injection volume of 20 µL was selected to provide a sufficient sample load for accurate quantification while preventing overloading of the column.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

LOD Determination:

- The Limit of Detection (LOD) is the smallest concentration of the analyte that can be detected but not necessarily quantified. It was determined using the signal-to-noise ratio (S/N), where an S/N ratio of 3:1 is typically considered indicative of LOD. LOD values were calculated for each drug component to ensure that even trace levels could be detected during analysis.

LOQ Determination:

- The Limit of Quantification (LOQ) is the smallest concentration of the analyte that can be quantitatively measured with acceptable precision and accuracy. An S/N ratio of 10:1 was used to calculate the LOQ. The LOQ values were established to ensure reliable quantification of low concentrations of the drugs in the fixed-dose combination, even in the presence of excipients or degradation products.

Validation:

- Both LOD and LOQ were validated to ensure they meet the regulatory guidelines, ensuring the method's sensitivity and accuracy for low-concentration analysis in routine quality control and stability studies.

CONCLUSION

The developed HPLC method is a robust, accurate, and precise stability-indicating method for the simultaneous estimation of selected anti-diabetic drugs in fixed-dose combinations. It is well-suited for routine quality control and stability testing of FDCs, providing reliable results even in the presence of degradation products. This method can be adapted for other FDCs, contributing to the quality assurance of anti-diabetic therapies.

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