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STUDY OF POST SHELF LIFE AND MARKETED TABLETS OF ANTIBIOTIC DRUG TABLETS BY RP-HPLC METHOD

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ABSTRACT

The quality, safety, and efficacy of pharmaceutical products are critically dependent on their stability and proper analytical evaluation. Antibiotic drugs, particularly Ciprofloxacin, are widely used in the treatment of bacterial infections and must maintain their therapeutic effectiveness throughout their shelf life. However, degradation due to environmental factors such as temperature, light, humidity, and pH can significantly affect drug potency, leading to reduced efficacy and potential safety concerns. In recent years, increasing attention has been given to the evaluation of pharmaceutical products beyond their labeled shelf life, especially due to the practical use of expired medications in certain conditions.This review focuses on the application of Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) as a reliable and widely accepted analytical technique for the estimation of antibiotic drugs in pharmaceutical formulations. The paper discusses various aspects of method development and validation in accordance with ICH guidelines (Q2(R1)), including parameters such as specificity, linearity, accuracy, precision, limit of detection, limit of quantification, robustness, and ruggedness. Additionally, the importance of forced degradation studies in the development of stability-indicating methods is highlighted, with emphasis on different stress conditions such as acidic, basic, oxidative, thermal, and photolytic degradation.

INTRODUCTION

Antibiotics have transformed modern medicine by providing effective means to prevent and treat bacterial infections that once caused high morbidity and mortality rates. Among these, fluoroquinolones stand as a widely used and potent class of synthetic antibacterial agents characterized by broad-spectrum activity, high oral bioavailability, and favorable pharmacokinetic properties. Antibiotic drug, a second-generation fluoroquinolone, has remained a drug of choice for many infections due to its high potency against Gram-negative bacteria and moderate activity against Gram-positive organisms. It acts by inhibiting bacterial DNA gyrase and topoisomerase IV, enzymes critical for DNA replication and transcription, leading to bacterial cell death. The increasing use of Antibiotic drug in clinical practice underscores the need for rigorous quality assurance and stability evaluation to ensure its safety and efficacy throughout its shelf life.

Antibiotic drug: Chemical and Pharmacological Overview

Antibiotic drug hydrochloride (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)- 3-quinolinecarboxylic acid) is a synthetic broad-spectrum antibacterial agent. It is a white to slightly yellow crystalline powder, sparingly soluble in water and methanol, and exhibits strong absorption in the ultraviolet region. Its molecular weight is 331.34 g/mol, and it has the molecular formula $C_{17}H_{18}FN_3O_3$. The structural features of Antibiotic drug include a fluorine atom at position 6, which enhances lipophilicity and antibacterial activity, and a piperazine ring at position 7, responsible for its broad Gram-negative coverage. Pharmacologically, Antibiotic drug exhibits concentration-dependent bactericidal activity. After oral administration, it is rapidly absorbed, achieving peak plasma concentrations within 1–2 hours. The bioavailability is approximately 70%, and the drug shows extensive distribution in body tissues and fluids. It is primarily excreted unchanged in the urine, with an elimination half-life of about 4 hours. Due to these characteristics, Antibiotic drug is used for urinary tract infections, respiratory tract infections, gastrointestinal infections, and skin infections.

Quality Assurance in Pharmaceutical Products

The quality, efficacy, and safety of pharmaceutical products are paramount in healthcare. The quality of a finished dosage form depends on several factors including raw materials, formulation design, manufacturing process, and storage conditions. Quality assurance and quality control (QA/QC) programs ensure that drugs conform to established specifications before release and remain within acceptable limits during their shelf life. Drug degradation can occur due to environmental factors such as temperature, humidity, light, and pH variations, which may lead to reduced potency, formation of toxic degradation products, and compromised therapeutic effectiveness.

In the context of Antibiotic drug, stability is particularly important because the drug contains functional groups prone to hydrolysis and oxidation. Prolonged storage, exposure to moisture, or improper packaging can lead to degradation, resulting in a decrease in assay values and altered dissolution characteristics. Hence, stability-indicating analytical methods are necessary to monitor the quality of marketed formulations and post-shelf life products.

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Post Shelf Life Evaluation and Its Significance

Shelf life represents the period during which a drug product maintains its labeled potency, purity, and quality under specified storage conditions. After this period, the manufacturer no longer guarantees full therapeutic activity. However, in practice, many drug formulations remain chemically stable beyond their labeled expiry dates, particularly if stored under optimal conditions. The evaluation of post-shelf life samples can provide valuable insights into the real-world stability of pharmaceuticals and help determine whether drugs remain safe and effective after expiry.

Several studies have demonstrated that many solid oral dosage forms retain substantial potency even after expiration. For instance, antibiotics like Antibiotic drug have shown degradation rates dependent on temperature and humidity but often maintain more than 90% potency under controlled conditions. Such findings are crucial for hospital pharmacies and regulatory authorities, especially in developing countries, where expired drugs are sometimes used during shortages. Nevertheless, any extension or post-shelf life use must be justified by validated stability studies to prevent therapeutic failure or adverse reactions.

Analytical Method Development and Validation

Accurate and reliable analytical methods are essential for quality control, stability testing, and regulatory compliance in pharmaceutical manufacturing. Among the various analytical techniques, High-Performance Liquid Chromatography (HPLC) has emerged as a powerful and versatile tool due to its precision, sensitivity, and reproducibility. Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is particularly suitable for analyzing Antibiotic drug because of its polarity and UV absorbance properties. The method allows for quantification of the active pharmaceutical ingredient (API) as well as its degradation products in both bulk and dosage form.

Method development involves optimizing parameters such as mobile phase composition, flow rate, column type, and detection wavelength to achieve efficient separation and accurate quantification. Once developed, the method must undergo validation in accordance with International Council for Harmonisation (ICH) guidelines, assessing parameters like linearity, precision, accuracy, specificity, robustness, and limit of detection (LOD).

Table 1: Typical Validation Parameters for RP-HPLC Methods (as per ICH Q2(R1))

Parameter	Description	Acceptance Criteria
Linearity	Ability to produce results proportional to concentration	$r^2 \geq 0.999$
Precision	Repeatability and reproducibility	%RSD ≤ 2.0
Accuracy	Closeness to true value	98–102% recovery
LOD/LOQ	Sensitivity of method	As per signal-to-noise ratio
Robustness	Effect of deliberate variations	No significant change in results

Validated RP-HPLC methods have been widely used to estimate Antibiotic drug in bulk drugs, tablets, and combined dosage forms *(18)*. These methods also serve to detect impurities and degradation products, ensuring that formulations meet pharmacopeial standards even after prolonged storage.

Stability-Indicating RP-HPLC Methods

A stability-indicating method is an analytical procedure capable of distinguishing the API from its degradation products. Such methods are crucial for studying the stability behavior of Antibiotic drug under stress conditions like heat, light, oxidation, acid, and base hydrolysis. The use of RP-HPLC for this purpose provides excellent separation efficiency and accurate quantification of degradation profiles. Forced degradation studies as per ICH Q1A(R2) guidelines help in identifying possible degradation pathways and ensuring the method's specificity.

Previous research has demonstrated the successful application of RP-HPLC for determining
Antibiotic drug and related substances in tablets, injections, and ophthalmic formulations. By comparing chromatograms of standard and stressed samples, one can determine the extent of degradation and the suitability of storage conditions. Such studies form the foundation for assessing the chemical stability of both marketed and post-shelf life formulations.

Marketed Formulation Analysis

The quality of marketed pharmaceutical products may vary depending on manufacturing practices, excipients used, and storage conditions during distribution. Regulatory bodies such as the WHO and CDSCO emphasize routine market surveillance and post-marketing quality evaluation. Variations in assay values or dissolution profiles among brands may indicate manufacturing inconsistencies or degradation during transport. Comparative RP-HPLC analysis of different marketed brands of Antibiotic drug tablets can reveal such differences and guide regulatory decisions.

Evaluating post-shelf life samples of these marketed formulations is equally important. It provides data on whether products maintain acceptable potency and purity beyond their expiry dates, reflecting the robustness of formulation design and packaging.

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These findings also help determine the need for improving storage guidelines or reformulating products for enhanced stability.

Instrumentation

The below mentioned instruments were used in order to analyze the formulated film coated tablets. The HPLC (Agilent 1260, Waldron, Germany) system having a pump (model; G7116A), model of autosampler (G7129A) and the column dimension was COSMOSIL C18 (250 cm × 4.6 mm) 5 µm (Paisley, UK) applied for the method development of simultaneous assay determination for CVA and CFX and dissolution determination of CVA. Moreover, the detector used was UV/VIS which operated at 220 nm. In addition, the used software was Agilent OpenLab (Version 3.2.0.0) in order to process and evaluate the data. Furthermore, for weighing of reagents, analytical balance revealing four digits on screen was used (Radwag, model: AS 220.X2, Poland) and sonicator (MRC, model: ACP-150H, India) was applied to dissolve the reagents. Similarly, for dissolution determination of CFX, UV-VIS spectrophotometer (Agilent Cary 60) was used.

Parameter for validation of HPLC method:

The validation parameter as per ICH guideline and USP are

1) Accuracy
2) Precision
3) Linearity
4) Limit of detection
5) Limit of quantification
6) Selectivity specificity
7) Robustness
8) Ruggedness

Validation Parameters for HPLC Method

1) Accuracy

Accuracy is the closeness of the analytical result to the true or accepted reference value. It shows how correctly the method measures the actual amount of drug present in the sample. In HPLC method validation, accuracy is commonly determined by **recovery studies**, where a known amount of standard drug is added to the sample at different levels such as **80%, 100%, and 120%**. The percentage recovery is then calculated. A good method should show recovery close to 100%, usually within acceptable limits.

2) Precision

Precision indicates the degree of agreement among repeated test results when the method is applied multiple times under the same conditions. It shows the reproducibility of the method. Precision is usually expressed as **%RSD**. It includes **repeatability** or intra-day precision, where the same sample is analyzed several times on the same day, and **intermediate precision**, where analysis is performed on different days, by different analysts, or using different instruments. Low %RSD indicates good precision.

3) Linearity

Linearity is the ability of the analytical method to give test results that are directly proportional to the concentration of analyte within a given range. In HPLC, different concentrations of standard solution are prepared and injected, and a calibration curve is plotted between concentration and peak area. The linearity is confirmed by calculating the regression equation and correlation coefficient. A correlation coefficient close to **1**, commonly $\mathbf{R^2 \geq 0.999}$, indicates good linearity.

4) Limit of Detection (LOD)

The limit of detection is the lowest amount of analyte in a sample that can be detected but not necessarily quantified accurately. It indicates the sensitivity of the method. LOD may be determined by signal-to-noise ratio or by using the standard deviation of response and slope of calibration curve.

5) Limit of Quantification (LOQ)

The limit of quantification is the lowest amount of analyte in a sample that can be quantitatively determined with suitable accuracy and precision. LOQ is important when the method is used to estimate very low concentrations of drug or impurities.

6) Selectivity / Specificity

Selectivity or specificity is the ability of the HPLC method to measure the analyte accurately in the presence of other components such as excipients, impurities, degradation products, or solvents. In tablet analysis, this parameter confirms that the Ciprofloxacin peak is not affected by tablet excipients or degradation peaks. It is evaluated by comparing blank, standard, sample, and degraded sample chromatograms. No interfering peak should appear at the retention time of the drug.

7) Robustness

Robustness is the capacity of the analytical method to remain unaffected by small deliberate changes in method conditions. These changes may include slight variation in flow rate, mobile phase composition, pH, column temperature, or detection wavelength. If the method gives consistent retention time, peak area, and assay results despite these small changes, it is considered robust. Robustness confirms reliability during routine laboratory use.

8) Ruggedness

Ruggedness is the reproducibility of the method under normal but variable laboratory conditions. It is checked by performing the analysis using different analysts, instruments, days, or laboratories. If the assay results remain consistent with low %RSD, the

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method is considered rugged. Ruggedness proves that the method can be successfully used in different practical conditions without affecting accuracy and precision.

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