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Various Analytical Development Techniques for Ofloxacin - A Review

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ABSTRACT
Ofloxacin is an antibiotic used to treat both gram-positive and gram-negative microorganisms. Various analytical methods like chromatographic, and spectrofluorimetric, spectrophotometric, UPLC, HPLC. Simple, fast, and dependable spectrophotometric methods for determining Ofloxacin in bulk and pharmaceutical dosage forms were developed. In terms of mean values and standard deviations, there was no significant difference in the proposed approaches performance. The developed method was validated for various parameters as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, limit of detection, limit of quantitation, ruggedness, robustness. Hence it is concluded that the assay method is found to be valid in terms of reliability, precision, accuracy, linearity and range, repeatability, intraday and interday precision, reproducibility and hence it is suitable for routine quality control assay of the drug in pure forms and pharmaceutical formulations.

KEYWORDS: Ofloxacin, HPLC, UPLC, RP-HPLC, Spectrophotometry, Spectrofluorimetry, Validation.

I. INTRODUCTION:

Ofloxacin is a synthetic broad-spectrum antibacterial antibiotic. Ofloxacin is a fluorinated carboxy quinolone with the chemical formula 7-fluoro- 2- methyl-6- (4- methyl piperazine- 1- yl)-10-oxo-4-oxa -1-azatricycol trideca-5-tetraene-11-carboxylic acid (fig. 1)Ofloxacin is a gram-positive and gram-negative antibiotic that is used to treat urinary tract infections, conjunctivitis, gonorrhoea, and respiratory tract infections[(1)]. It is an antibiotic with a broad spectrum of activity against both Gram-positive and Gram-negative bacteria. It inhibits cell division by blocking DNA gyrase, a type II topoisomerase, and topoisomerase IV[(2,3)], an enzyme required to segregate duplicated DNA. Fluoroquinolones disrupt DNA replication by inhibiting an enzyme complex known as DNA gyrase. This can also have an impact on mammalian cell replication. Some congeners of this drug class, in particular, have high activity not only against bacterial topoisomerases, but also against eukaryotic topoisomerases, and are hazardous to cultured mammalian cells and in vivo tumor models. Although the quinolone is extremely toxic to mammalian cells in culture, the mechanism of its cytotoxicity is unknown. Quinolone-induced DNA damage was documented for the first time in 1986[(4)].

Several analytical techniques, including microbiology, capillary electrophoresis, atomic absorption spectrometry, voltammetry[(5,6)], potentiometry and conductometry, polarography, chemiluminescence spectrometry, HPTLC, HPLC, RP-HPLC, spectrofluorometry, and spectrophotometry, have been used to determine ofloxacin[(7,8)]. The assay procedure described in these pharmacopoeias employs non-aqueous titration to estimate ofloxacin. As a result, an attempt has been made to develop new Zero, First, and Second Order Spectrophotometric methods for estimating Ofloxacin in bulk and pharmaceutical formulations with good accuracy, simplicity, precision, and economy [(9)]. The fluoroquinolone (quinolone) class of chemotherapeutic drugs is regarded as a last-resort treatment for life-threatening bacterial infections [(10)].

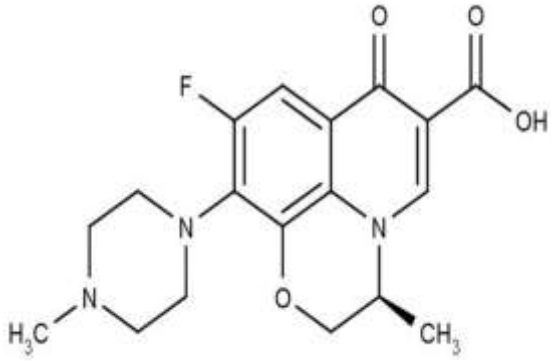

Fig. 1. Structure of Ofloxacin

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SPECTROPHOTOMETRIC METHOD:

The determination of the fluoroquinolone antibiotic Ofloxacin (OFL), in both pure form and pharmaceutical formulations, quick, easy procedures using spectrophotometry were devised. Methanol was used to produce the sample and standard solution mixtures **(11).** The foundation of this procedure is the creation of an ion-pair complex between the acid dye and the basic medication (OFL). The ion-pair complex OFL's absorption spectra were measured in the 350–600 nm region. The complex uses chloroform as its solvent, and its maximum absorbance is at 430 nm. Investigated are the consequences of the analytical parameters. With a correlation coefficient of R^2 of 0.9998, Beer's law was followed in the concentration range of 0.434 to 11.564 g/ml. Between 98.61 and 101.61% of ofloxacin was recovered on average **(12).** The performance of the suggested methods in terms of mean values and standard deviations did not differ noticeably. The devised techniques were effectively used to calculate the concentration of ofloxacin in pharmaceutical formulations (11). The suggested approach has the advantages of being more sensitive for determining the examined drug in pure form and pharmaceutical formulations, as well as being comparatively simple, quick, cost-effective, and free from auxiliary reagents when compared to the existing visible spectrophotometric methods. In contrast to the previously described spectrophotometric approaches mentioned above, the proposed method does not require time-consuming experimental steps like heating. This method is relative immunity from interference by common diluents and excipients in quantities significantly greater than those seen in pharmaceutical formulations is its most appealing aspect. The statistical characteristics and recovery data demonstrate the method's excellent precision and accuracy in addition to its robustness and toughness. The examined substance could therefore be tested regularly for quality using the established method in both pure form and pharmaceutical preparations**(12).**

HIGH PRESSURE LIQUID CHROMATOGRAPHIC METHOD (HPLC)

The quantitative measurement of ofloxacin in bulk and commercial formulations has been devised and confirmed using a straight forward, call environmentally friendly reverse phase chromatographic approach. Hypersil silica C_{18} (250 mm 4.6 mm, 5 particle size) was used as the column in the devised technique, and water and methanol were used as the mobile phase. Using a PDA detector, the mobile phase is passed at a flow rate of 1–5 ml/min, and the eluted solution is monitored between 270–320 nm. The assay procedure is linear for concentrations between 5 and 30 g/ml. 0.9998 is the corelation coefficient. The developed method's average recovery percentage is determined to be between 98% and 100%.The fact that the excipients in the formulation did not interfere with the process also suggests that the devised approach is accurate. The only mobile phases they use are methanol and water. Solvents used in the developed process are safe for the environment. The percentage RSD value of precision analysis is < 2% It shows that reproducibility guidelines have been applied to the established method. According to the ICH criteria, the validated procedure was carried out. It complies with research on robustness, linearity, accuracy, and precision. Parameters for validation are within the limitations (13).

The purpose of determining the amount of ofloxacin in eye drops, an easy-to-use, quick-to-validate HPLC approach was created. The mobile phase was an Acetonitrile:Buffer mixture in a 35:65 v/v ratio, and the column was a Thermo separation Products C_8 (250 cm 4.6 mm i.d., 5 m) column. Using a UV detector, the detection range was discovered to be 290-315 nm. To verify the system's applicability, specificity, precision, linearity and range, accuracy, ruggedness, and resilience, validation parameters were used. Over the concentration range of 50-300 g/ml, the technique was linear. The RSD values for intra- and inter-day variance were determined to be less than 0.8%, indicating good precision. Recovery studies using the usual addition method were conducted to evaluate the accuracy of the method. The average percent recoveries gained being less than 105% on average indicates that the procedure is accurate. The ability of an analytical procedure to be unaffected by minute changes in method circumstances is known as robustness. The HPLC assay created for ofloxacin qualification was carried out in mobile phase. Many factors, including best condition, linear relationship with coefficient of correlation, robustness, accuracy, reproducibility, and precision were taken into consideration when evaluating the method. According to the specificity studies' findings, there were no contaminants because there was no interference with ofloxacin's peak. The process was discovered to be quick. So, it can be concluded that

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the suggested procedure will be extremely accurate and precise in normal laboratory analysis [14].

SPECTROFLUORIMETRIC METHOD

The determination of ofloxacin at nanogram concentration in bulk and its formulation has been created using the quick, sensitive, and affordable spectrofluorimetric approach. At a buffer pH lower than 2, the relative fluorescence intensity of ofloxacin was measured at 295 nm for excitation and 485 nm for emission. Relative fluorescence intensity = 1369 x concentration (in ng/ml) - 166.1 with regression coefficient (r^2) = 0.9998 was found to have a linearity range of 200-1400 ng/ml. According to the USP and ICH recommendations, the approach underwent testing and validation for a number of criteria. The process is accurate, precise, and reproducible with a low relative standard deviation < 2%., according to the results. Moreover, the recovery rate was effective. This analytical technique can be used to determine the fluoroquinolones in different dose forms, such as ofloxacin, using spectrofluorimetry. For the bioanalytical determination of ofloxacin in blood samples, this approach can be further extended. The suggested approach is quite straightforward and does not call for laborious medication processing or extraction steps. The techniques have good accuracy and precision and a wider linear range. As a result, the data presented in the manuscript for the determination of ofloxacin in its pure and dosage form by spectrofluorimetric method show that the proposed method is accurate, precise, linear, and selective and offers benefits of reagent availability and stability, less time consumption, and high sensitivity. As a result, it can be expanded for ofloxacin routine analysis in the pharmaceutical industry, hospitals, and research laboratories [15].

ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY(UPLC)

A method for simultaneously estimating ofloxacin in tablet dose form using RP-UPLC with stability indicators was created and verified. Using a Waters C_{18} Acquity UPLC BEH (100 mm 2.1 mm, 1.7 m) column with a mobile phase made up of water, acetonitrile, and triethylamine at a ratio of 85:15:0.1 v/v, the separation was accomplished under optimal chromatographic conditions. PDA detection at 300 nm was used to perform an isocratic elution at a flow rate of approximately 0.5 ml/min at ambient oven temperature. The retention time for Ofloxacin is < 5 min respectively. Acidic, alkaline, oxidative, photolytic, and thermal conditions all led to the degradation being noticed. With ofloxacin, the linearity was discovered to be between 50 and 300 g/ml. For Ofloxacin, the percent recoveries were discovered to be between 99% and 100% below average. The values were confirmed to be within the limits after the procedure had been validated in accordance with ICH guidelines. Therefore, it was determined that the suggested method was straightforward, linear, accurate, exact, stability indicating, robust, and particular. These findings show that the procedure is resilient, accurate, exact, specific, easy, and time-saving. It can be used to regularly check the quality of marketed dosage forms [16].

VALIDATION METHOD:

Recovery studies were performed to investigate the accuracy and precision of the proposed approach by adding a known amount of standard drug solutions of ofloxacin to pre analyzed tablet solution. The generated solutions were then analyzed using a method that was proposed.

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Fig. 2. VALIDATION PARAMETERS

The ability of an analytical method to produce test results that are directly proportional to the concentration of analyte in sample within a certain range is referred to as linearity. The range of an analytical method is the range of analytes that have been proved to be determined within a reasonable level of precision, accuracy, and linearity. Dilutions of 5 - 30 µg/ml OFL were made, and absorbance was measured at 302 and 289 nm for each dilution. The degree of agreement among individual test results when the method is applied repeatedly to several samplings of homogeneous samples is defined as an analytical method's precision. It was expressed as a coefficient of variation and provides an indication of random error results (CV). In each case, the correlation coefficient was calculated. Accuracy is the proximity of the method's test results to the true value. To test the precision, 20 pills were weighed and powdered before being analyzed. Recovery studies were conducted by adding standard drug to the sample at three different concentration levels, namely 80%, 100%, and 120% of the actual amount, while taking into account the percentage purity of added bulk drug samples. Repeatability of method was determined by six times 5- 30 µg/ml of drug solution, measurement of peak areas was performed and from the peak areas the % RSD was determined. The variance of results within the same day (intra-day) and between days (inter-day) was investigated. Intra-day precision was calculated by analyzing OFL three times on the same day at 302 nm and 289 nm. The inter-day precision was determined by evaluating both medicines separately once a day for two days at 302nm and 289 nm. Intra-day studies were carried out by making dilutions of 5 - 60 µg/ml of OFL and measuring their absorbance at 301 nm and 289 nm after 3 hours. Inter-day studies were carried out by making dilutions of 5 - 60 µg/ml of OFL and measuring their absorbance at 302 nm and 289 nm on the first and second days, respectively. Another analyst tested the absorbances, and the results were reviewed using the t-test to ensure repeatability [17].

II. CONCLUSION

The application of a form ion pair complexation reaction with acid dye for the

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measurement of a fluoroquinolone antibiotic in pure form and pharmaceutical formulation is described in this research. The proposed method, like the visible spectrophotometric, spectrofluorimetric, HPLC, UPLC, and RP-HPLC methods, has the advantages of being relatively simple, quick, cost-effective, free of auxiliary reagents, and more sensitive for determining the studied drug in pure form and pharmaceutical formulations. Furthermore, unlike some other medications, the proposed approach does not require time-consuming experimental steps such as heating. This study discussed the statistical parameters and recovery data for the specific drug and presented several analysis methods. As a result, the numerous procedures employed for the specific medicine were successful, and the validation results were concluded in this report for future reference.

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