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Validation of Analytical Methods for Fluconazole: A Review

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A B S T R A C T

Fluconazole concentration was quantitatively determined using an RP-HPLC and a UV spectrophotometric assay technique that were designed and verified. A quick, accurate, easy and selective UV spectrophotometer technique has been created to estimate the amount of fluconazole. This study aims to create and validate a dissolution test for fluconazole, an antifungal medication employed in treating superficial, skin and mucocutaneous infections triggered by Candid species. Spectrophotometric analysis was performed at the optimal absorption wavelength of 260.8 nm in water. The methods have been successfully applied to the determination of Fluconazole. The UV method enables quick and cost-effective quantification of the drug. The method developed was validated in accordance with ICH guidelines, covering aspects such as linearity, precision, accuracy, robustness, and the limits of detection (LOD) and quantification (LOQ). That is why a review is required which can make all the research at one platform in the form of this review paper. That is why a review is required which can make all the research at one platform in the form of this review paper.

Keywords: Keywords: Fluconazole, UV spectrophotometric, RP-HPLC

INTRODUCTION

Ken Richardson discovered one of the most important anti-fungal drug fluconazole (Diflucan).

History of Fluconazole

Fluconazole was patented by **Pfizer** in 1981 in the United Kingdom and came into commercial use in 1988 Fischer and Ganellin (2010). Patent expirations occurred in 2004 and 2005, Whalen et al (2018).

Fluconazole is a synthetic antifungal agent belonging to the group of triazoles. This drug is structurally related to the antifungal agents that are imidazole derivative. Drug was approved by FDA in the United States on January 9, 1990. Fluconazole is a hygroscopic, crystalline powder that dissolves easily in methanol and slightly soluble in water. It possesses advantageous pharmacological characteristics, such as a relatively extended half-life and the capability for both oral and parenteral administration. It is available in tablet, suspension, parentral and gels for treatment of local, systemic fungal infection and deep organ candidiasis.

It functions as a fungistatic drug by blocking the fungal cytochrome P450 enzyme 14α-demythylase and stopping lanosterol from being converted to ergosterol, which is a crucial part of the fungal cytoplasmic membrane, and the consequent build-up of 14α-methyl sterols.

IUPAC name of fluconazole-**2-(2,4-Difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol**

Formula: $\mathbf{C_{13}H_{12}F_2N_6O}$

Molar mass: **306.277 g·mol^{-1}**

Medical use

- ✓ Treatment for localized Candida infections of the vagina ("yeast infections"), as well as infections in the throat and mouth·
- ✓ Some systemic Candida infections, such as bloodstream, kidney, or joint infections, in individuals with robust immune systems. When treating active infections in patients with weakened immune systems, as well as infections affecting the heart or central nervous system, other anti fungals are typically chosen.
- ✓ Preventing Candida infections in individuals with compromised immune systems, including those receiving chemotherapy for cancer and becoming neutropenic, as well as transplant recipients, patients with severe HIV infections, and premature infants.

- ✓ Used as a secondary treatment option for cryptococcal meningoencephalitis, a fungal infection affecting the central nervous system.

Pharmacology:

Pharmacodynamics

Fluconazole, like other antifungals in the imidazole and triazole class, inhibits the fungal cytochrome P450 enzyme 14α-demethylase. Fungal demethylase is far more sensitive to fluconazole than is mammalian demethylase activity. This inhibition prevents the conversion of lanosterol to ergosterol, an essential component of the fungal cytoplasmic membrane, and subsequent accumulation of 14α-methyl sterols. Fluconazole is primarily fungistatic, however, it may be fungicidal against certain organisms in a dose-dependent manner, specifically Cryptococcus.

Pharmacokinetics

Fluconazole is nearly entirely absorbed after two hours after oral dosage. Bioavailability is not significantly affected by the absence of stomach acid. Concentrations measured in the urine, tears, and skin are approximately 10 times the plasma concentration, whereas saliva, sputum, and vaginal fluid concentrations are approximately equal to the plasma concentration, following a standard dose range of between 100 mg and 400 mg per day. Fluconazole has a zero order elimination half-life; metabolism accounts for just 10% of its elimination, with the remaining 90% coming from sweat and urine excretion. Patients with impaired renal function will be at risk of overdose. It is a white, crystalline powder that is soluble in alcohol and very slightly soluble in water when in bulk powder form.

Adverse Effect of Fluconazole.

- Headache
- Stomach pain
- Diarrhoea
- Feeling or being sick (nausea or vomiting)
- Skin rash

Serious side effects

- ✓ Serious side effects are uncommon and happen in less than 1 in 100 people.
- ✓ The white of your eyes turn yellow, or your skin turns yellow although this may be less obvious on brown or black skin, or if you have pale poo and dark pee- these can be sighs of liver problems.
- ✓ You bruise more easily or get infections more easily – these can be signs of a blood disorder
- ✓ You have a faster or irregular heartbeat.
- ✓ Fluconazole can cause a serious heart problem.
- ✓ Liver problems--loss of appetite, stomach pain (upper right side), tiredness, itching, dark urine, clay-colored stools, jaundice (yellowing of the skin or eyes).
- ✓ Changes in your sense of taste.

Serious allergic reaction

It happens rarely but it's possible to have a serious allergic reaction (anaphylaxis) to Fluconazole. Possible to reduce metabolism and raise the concentration of any drug that is metabolized by these enzymes. Its *cans* infections. Fluconazole may increase the serum concentration of Erytpossible impact on the QT interval also raises the possibility of cardiac arrhythmia when taken in conjunction with other medications that also lengthen the QTinterval. Berberine has been shown to exert synergistic effects with fluconazole even in drug-resistant *Candida albihromycin*.

Analytical Method

Development and validation of analytical methods are crucial to the discovery, development and production of pharmaceutical products formulated with multiple drugs. These products, commonly called combination products, aim to address unmet patient needs by combining the therapeutic effects of multiple drugs into a single product. Pharmaceutical analysis is crucial to bulk drug and pharmaceutical formulation quality assurance and control.

Historically, chemistry-driven pharmaceutical research has been crucial to the advancement of pharmaceutical development, with guidance from pharmacology and clinical sciences. The fields of chemistry, pharmacology, microbiology, and biochemistry have all contributed to the field of drug development, setting a new standard whereby new pharmaceuticals are now developed through collaboration between biologists and chemists rather than just chemists' creative ideas. When a novel drug molecule is discovered that has therapeutic potential for treating, preventing, or curing disease, the process of developing new drugs begins. The synthesis and characterization of such molecules which are also called active pharmaceutical ingredients (APIs). The R&D process includes drug discovery and development, as well as the drug molecule, or active pharmaceutical ingredient (A.P.I.).

Pharmaceutical analysis is crucial to bulk drug and pharmaceutical formulation quality assurance and control. The need for innovative analytical techniques in the pharmaceutical industries has increased due to the industry's rapid growth and medication production across the globe. Analytical method development has become the basic activity of analysis. The development of better analytical tools and methods has resulted in shorter analysis times, more accuracy and precision and lower analysis costs. In quality control laboratories, analytical techniques are employed to guarantee the identification, purity,safety, effectiveness, and performance of pharmaceutical products. Regulatory bodies are emphasizing analytical procedures in production more and more.

Modern pharmaceutical analysis needs the following requirements.

1. The analysis should take a minimal time and should be economical.

2. The accuracy of the analysis must accept the guidelines of Pharmacopoeia.

3. The chosen method should be precise and selective.

Validation

The word validation derived from the Latin validus, which means "strong," and denotes that something has been demonstrated to be accurate, valuable, as well as up to quality. According to the International Organization for Standardization, validation is the process of ensuring through analysis and the presentation of accurate information that the specific conditions for a certain intended use are satisfied.

The documented proof that offers a high level of assurance to a desired outcome with predetermined conformity is the validation process. In the pharmaceutical industry, the word "validation" is widely used. The term "valid or validity" refers to something that is "legally defined". The validation concept was first proposed by the Food and Drug Administration (FAD) in the mid-1970s to improve the quality of pharmaceutical products. Since a wide variety of procedures, methods or activates are validated to check and improve their quality.

Validation parameters

- Accuracy
- Precision
- Repeatability
- Robustness
- Specification
- Linearity
- Range
- Detection limit
- Quantitation limit

Accuracy: The closeness of a measured value to a standard or known value is referred to as accuracy.

Precision: The term "precision" describes how closely two or more measurements measure up.

Repeatability: The capacity of a method to produce similar results for several preparations of the same material is known as repeatability.

Robustness: Robustness refers to a method's ability to deal with minor, intentional changes in its parameters.

Specification: A specification is a set of predetermined standards and the test procedures that go along with them that should be used for analysis.

Linearity: The capacity of an analytical method to produce test findings that are directly proportionate to the concentration (amount) of analyte in the sample, within a specified range, is known as linearity.

Range: The interval between the higher and lower concentrations (amounts) of analyte in the sample (containing these concentrations) for which it has been shown that the analytical procedure has a suitable degree of linearity, accuracy, and precision is known as the methodology's range.

Detection limit: the lowest concentration of analyte in a sample that is detected but may not be precisely quantified.

Quantization limit: The lowest concentration of analyte in a sample that can be quantitatively identified with a suitable precision and accuracy is known as the quantitation limit of a particular analytical process.

Fluconazole Drug: A Review

In Rege et. al.(1992) demonstrated that fluconazole, an oral active antifungal medication, is clinically advantageous for the maintenance treatment of cryptococcal meningitis. For fluconazole, a sensitive gas-liquid chromatographic assay with electron capture detection was created. This method excluded the need for the chromatographic column to be pretreated and only needed a single extraction step. The assay had a 0.999 correlation coefficient and was linear from 0.1 to 20 p.g/ml. Less than 9%o separated the intraassay and interassay coefficients of variance. The average difference between the measured and goal values was only 8%. The recovery rates from extractions varied from 87 to 106%. Three AIDS patients with cryptococcal meningitis taking 200 mg of fluconazole per day had steady-state plasma fluconazole levels (mean + standard deviation) ranging from 8.95 1.32 to 11.41 + 0.63 ug/ml. These values fell within the expected range for this dose rate based on prior research. In one patient receiving 400 mg/day, the ratio of plasma to cerebrospinal fluid fluconazole concentration was 0.73 at steady state, which was in line with previous studies. Later on Hülsewede and Dermoumi (1996) worked on Fluconazole levels in serum were measured using a well diffusion bioassay employing a strain of Candida pseudotropicalis and an HPLC method with a reverse phase apparatus, an isocratic mobile phase, and 1-phenyl-1,2 ethanediol as internal standard. The range of 1 mg/L to 30 mg/L can be determined for fluconazole using both approaches. We found that there was a r = 0.89 correlation between the two approaches. For clinical routine work, both techniques are helpful in tracking the serum level of fluconazole.

Nouf M. Aloudah et. al. (2005) worked on HPLC assay of fluconazole and itsapplication to patientswith early septic shock. This study outlines an accurate, straightforward and precise HPLC approach for measuring fluconazole in plasma samples with sufficient sensitivity to track patients experiencing early-stage septic shock for a month. Using an HPLC system with a UV absorbance detector (set at 262 nm), the separation was carried out.

Kim et.al.(2007) also worked on sensitive and precise HPLC-UV method available for measuring the amount of fluconazole (FLA) in human plasma. Dichloromethane was used to do a one-step liquid-liquid extraction (LLE) of FLA from plasma in order to prepare the sample. The internal standard that was employed was phenacetin. FLA and phenacetin had chromatographic retention durations of 4.6 and 8.3 minutes, respectively. 0.05 μg/mL was the lower limit of quantification (LLOQ), and the chromatograms showed no signs of interference. By assessing the developed HPLC-UV method's intra- and inter-day precisions and accuracy in a linear concentration range of 0.05 to 10.00 μg/mL, it was validated. The developed technique was effectively utilized in a bioequivalency investigation that involved giving three × 50 mg FLA capsules and a single 150 mg FLA tablet orally to healthy male volunteers from Korea.

In order to quantify the amount of fluconazole in human plasma Dgither et.al .(2009), a straight forward reversed phase high performance liquid chromatography test was created, verified, and the stability of fluconazole was examined. After separation on a Nova-Pak C18 cartridge at room temperature, spectrophotometric detection of fluconazole and acetophenetidin (internal standard) was made (retention period 5.7 and 11.8 minutes, respectively). The mobile phase was supplied at a flow rate of 1.0 ml/minute and consisted of a combination of 0.01 M phosphate buffer (pH 7.0) and acetonitrile (75: 25, v: v). The Amicon Centrifree technology was utilized to deproteinize the plasma samples. There was no evidence of interference from eleven regularly used medicines or blank plasma. 0.1 μg/ml was the detection limit. Within the range of 0.2 (lower limit of quantitation) to 12 μg/ml, calibration curves showed a linear trend (R2 ≥0.9987). The intra- and inter-run coefficients of variation and bias were, respectively, less than 8.6% and less than 9.2%, and less than 16.5% and less than 11.5%. Acetophenetidin and fluconazole had mean extraction recovery rates of 83% and 90%, respectively. After 5 hours at room temperature (≥94%), 10 weeks at −20°C (103%), and 3 cycles of freezing at −20°C and thawing at room temperature (≥87%), fluconazole remained stable in the plasma. Fluconazole remained stable in deproteinized samples for 48 hours at −20°C (≥96%) and 16 hours at ambient temperature (≥100%). The fluconazole stock solution (1 mg/ml in methanol/phosphate buffer 10:90) was stable for three weeks at −20°C (≥90%) and six hours at ambient temperature (≥99%).

Later Al-Rimawi (2009)A straightforward liquid chromatographic technique that indicates stability was created and tested for the examination of fluconazole and its related compounds (A, B, and C) in capsule formulations. This investigation used liquid chromatography using a reversed-phase C18 column and a UV detector set to 260 nm. The method of isocratic elution was applied with a 40:60, v/v methanol and water mixture. The USP requirements for new assay determination methods, which cover accuracy, precision, specificity, linearity, and range, were followed in the validation of this new method. The present protocol exhibits strong linearity within the Fluconazole dosage range of 0.05-0.15 mg/ml. The method's accuracy is at 99.3%. The relative standard deviation of the replicates indicates 0.61%, which indicates the accuracy of this procedure. In accordance with USP specifications for the quantitative assessment of contaminants, which include accuracy, precision, linearity and range, selectivity, and Limit of quantitation (LOQ), the same method for the analysis of substances related to fluconazole was also validated. This approach allows the low-concentration detection and quantification of these contaminants due to the low limit of quantification (LOQ) of the associated chemicals. Meshram et. al. (2009) discovered Tinidazole, a member of the 5-nitroimidazole group, and fluconazole, an oral active triazole antifungal drug, can be simultaneously estimated using a quick and sensitive reversed-phase high-performance liquid chromatographic technique in a combined dose tablet. Using 0.05 M potassium dihydrogen phosphate buffer (pH 3.25, corrected with orthophosphoric acid) and acetonitrile (82:18, v/v) as the mobile phase at a flow rate of 1.5 mL/min with detection at 210 nm, chromatographic separation was performed on an ODS Hypersil C18 column. The asymmetry factors, with a total run time of fewer than 7 minutes, are 1.26 ± 0.07 for tinidazole and 1.36 ± 0.04 for fluconazole. For fluconazole and tinidazole, the calibration curves were linear and fell between 6 and 14 μg/mL and 80 and 190 μg/mL, respectively. The method's linearity, accuracy, precision, and specificity were all confirmed. For

fluconazole and tinidazole, the mean recovery is 99.65 ± 0.84 and 99.34 ± 0.70, respectively. The procedure's usefulness is confirmed through its implementation on a market formulation that underwent many stressful situations. There are two well-resolved possible breakdown products of tinidazole when exposed to alkaline stressful conditions. The two target medicines and degradation products were effectively isolated by the approach. There isn't any chromatographic interference seen.

Wu et. al. (2009) measure fluconazole in human plasma, an LC-MS/MS technique was created that is both sensitive and quick. Protein precipitation methods were applied to 70 microliters of plasma. Acitonitrile and water in 0.1% formic acid was used as a gradient mobile phase to achieve chromatographic separation on a C18 column. Using an electrospray ionization source, fluconazole and its internal standard tagged with deuterium were monitored in positive mode. The technique was thoroughly verified within the 0.01 to 10 µg/mL range. Precision within the day ranged from 2.84% to 10.8% and between 5.27% and 11.5%, respectively. For fluconazole, the procedure recovery efficiency varied between 98.6% and 104.4%. There were negligible matrix effects and no carryover. The ability to maintain fluconazole in blood at room temperature for up to 72 hours ensured that the quantities of the drug in scavenged blood specimens could be used for model development for newborn PK study. In a fluconazole pharmacokinetic experiment, this approach has been applied to fifty-five preterm and term infants under the age of ninety days. This approach has the potential to be a useful tool for therapeutic drug monitoring of fluconazole to maximize pediatric antifungal therapy due to its quick sample preparation cycle and reduced quantitation limit. AUC/MIC >50 for the majority of Candida species with a MIC90 of less than 8 µg/mL could be attained with the use of TDM and a pharmacometric technique to determine the ideal fluconazole dosage regimen for newborns and early infants.

Few years later Pothana Sadasivudu et. al. (2009) worked on the development and validation of UV and RP-HPLC methods of fluconazole analysis in pharmaceutical solid dosage forms. For the purpose of quantifying fluconazole in pharmaceutical solid dosage forms such as capsules, uncoated tablets, and dispersible tablets, an RP-HPLC and a UV spectrophotometric assay method were created and verified. Using 0.1M HCl as a solvent, the UV technique was performed at 260 nm. The HPLC and UV techniques demonstrated linearity within the range of 1 to 100 µg/mL and 50 to 400 µg/mL respectively. For every dose type, the HPLC approach proved to be exact and accurate. For the examination of fluconazole only in capsule dose form, the UV technique and HPLC had a strong correlation.

Work on different UV-spectrophotometric techniques are developed and validated for the measurement of fluconazole in bulk and solid dose forms was done by Amit Singh et. al. (2011). Fluconazole (FLZ), an antifungal medication, can be determined in raw material and tablet form using an easy-to-use, sensitive, and precise UV spectrophotometric technique. Phosphate buffer (PB), stomach fluid simulant (HCl), vaginal fluid simulant (VFS), and phosphate buffer saline (PBS) at pH 1.5, 4.2, 6.8, and 7.4, respectively, are the four distinct simulated media in which the medication exhibits maximum absorption at 261 nm. In the drug concentration range of 10–100 µg/mL, Beer's law is followed. For several media, including HCl, VFS, PB, and PBS, the limits of detection have been determined to be 2.24, 1.49, 1.42, and 1.19 µg/mL, while the limits of quantification are 6.82, 4.50, 4.29, and 3.63 µg/mL, in that order. The techniques have been effectively used to measure FLZ in tablets and bulk medications. According to ICH guidelines, results are statistically validated. The UV approach allows for the quick and affordable measurement of drugs in tablet and bulk dose forms.

Shanmugusundaran et. al. (2011) performed on A precise and practical high-performance liquid chromatography (HPLC) method has been developed for analyzing Fluconazole and Tinidazole in their combined tablet dosage form. The analysis utilized a reversed-phase Kromasil stainless steel C18 column (250 x 4.6 mm, 5 µ), with Acetonitrile as part of the mobile phase mixture. The mobile phase, consisting of a 55:45% v/v mixture of Water and Acetonitrile, was deployed in a low pressure gradient mode at a flow rate of 1 ml/min. The injection volume was set at 20µl. Fluconazole and Tinidazole had retention times of 2.5 and 3.1 minutes, respectively. The method demonstrated linearity in detecting concentrations ranging from 10 to 50µg/ml for both Fluconazole and Tinidazole. The method was found to be applicable for determination of the drug in tablets.

Sousa et .al. (2011) discovered sensitive and accurate HPLC-UV technique for the measurement of fluconazole (FNZ) in human plasma has been developed. FNZ was extracted from plasma using liquid–liquid extraction (LLE) and ethyl acetate to create the sample. The internal standard utilized was nevirapine (NVP). FNZ and NVP had chromatographic retention durations of 3.4 and 5.7 minutes, respectively. There were no interferences seen in the chromatograms, and the lower limit of quantification (LLOQ) was set at 0.5 µg/mL. The intra-day and inter-day precisions and accuracies of the HPLC-UV method were assessed in a linear concentration range of 0.5 to 8.0 µg/mL in order to validate it. The technique was created, verified, and successfully used in bioequivalence trials in which healthy male volunteers from Brazil were given a single 150 mg FNZ pill orally.

Then Yanamandra et. al. (2011) discovered Reverse Phase Ultra Performance Liquid Chromatography (RP-UPLC) technique was used in a novel way to develop and validate a fast and selective analytical method for the simultaneous separation and analysis of azithromycin, fluconazole, and secnidazole in human serum, their pharmaceutical dosage forms, and raw materials. Technologically speaking, the developed analytical UPLC method outperforms conventional HPLC in terms of speed, resolution, solvent consumption, and analysis cost. The separation was eluted in reverse phase mode for 10 minutes, and ultra violet detection was performed at 210 nm. A gradient program employing 0.002M Na2HPO4 and acetonitrile as organic solvent led to an efficient separation on a BEH C18 sub-2-µm UPLC column. Benzophenone served as the internal benchmark. It was discovered that there were more than 4.5 resolutions between azithromycin, fluconazole, and secnidazole. The active ingredient in the medication was removed from the tablets by diluting it with a 50:50 v/v mixture of methanol and acetonitrile. Secnidazole, fluconazole, benzophenone, and azithromycin had linear calibration graphs. Excellent recoveries were demonstrated by the approach for all medicines in bulk and prepared goods. When kept in a refrigerator between 2 and 8 oC, it was discovered that the test solution remained stable in the diluent for 72 hours. The International Conference on Harmonization (ICH) guidelines for linearity, accuracy, precision, specificity, and robustness were met by the validated UPLC method, which has been successfully applied to pharmaceutical formulations because no chromatographic interferences from the tablet excipients were detected. To the best of our knowledge, no other publication has included the validated reverse phase analytical method used in this inquiry for the simultaneous separation and quantification of azithromycin, fluconazole, and secnidazole utilizing UPLC technology.

Kai Bin Liew et. al. (2012) worked on development and application of simple HPLC-UV method for fluconazole quantification in human plasma. A quick, easy, and high-performance liquid chromatography technique was provided for measuring fluconazole in human plasma. Reversed phase C-18 column with UV detection at 260 nm was used to perform the separation. The buffer-acetonitrile (80:20, v/v) mobile phase was made up of glacial acetic acid to get the pH down to 5.0. For fluconazole, the mean recovery was 102.8% ± 5.53, while for tinidazole, it was 78.27% ± 1.97. Tinidazole was stable in stock solution for 8 hours. Fluconazole and tinidazole had retention times of 8.3 and 6.7 minutes, respectively, and the sample ran for fewer than ten minutes overall.

Development and validation of dissolution test for fluconazole capsules by HPLC and derivative UV spectrophotometry done by Josilene Chaves Ruela Correa et. al. (2012), in this work developing and validating a dissolve test for fluconazole, an antifungal used to treat cutaneous, cutaneomucous, and superficial infections resulting from Candida species, in capsule dosage form is the aim of this work. Methods using UV-FDS (UV first derivative spectrophotometry) and HPLC were used for quantitative assessment. In accordance with ICH and USP guidelines, the established HPLC and UV-FDS procedures for the antifungal evaluation were selective and satisfied the requirements for an acceptable and validated approach. Cost, simplicity, apparatus, solvents, speed, and application to varying demands should all be considered for regular analysis applications.

Hetal Jebaliya et. al. (2013) performed their research oncomparative validation study of fluconazole by HPLC and UPLC with forced degradation study. The most basic stability signifying phase reversal. The development and validation of isocratic HPLC and UPLC technologies has allowed for the determination of fluconazole in bulk and solid pharmaceutical dosage forms.

Lotfy et. al. (2013) high performance liquid chromatographic method (HPLC) that is straightforward, precise, accurate, and indicates stability has been developed for the detection of fluconazole (FLZ) in the presence of the degradation products that are produced during the stress degradation study. FLZ was exposed to hydrolysis by acids, alkalis, and neutrals as well as oxidation, photolysis, and thermal breakdown. Under heat stress, extensive deterioration was seen in the oxidative medium. On a C-18 column, the drug was successfully separated from degradation products by employing phosphoric acid 0.5% v/v: acetonitrile (80:20% v/v) as the mobile phase. The detector was set at 261 nm, and the flow rate was 1.5 mL min−1. It was discovered that FLZ and its primary oxidative breakdown product had retention durations of 5.389 and 2.729 minutes, respectively. For fluconazole, linearity was found in the 0.5–50 μg/ml range. It was discovered that the recovery percentage of fluconazole was 99.91 ± 0.74. The technique can be utilized as a stability-indicating approach because it successfully extracts fluconazole from the products of its oxidative destruction. The kinetics of fluconazole oxidative degradation, which was discovered to follow a zero-order reaction, was also investigated using the suggested methodology. The reaction rate constant (k) was 2.91 × 10−8 mole/min, and the t1/2 was 21.66 minutes.

H Jebaliya et. al. (2013) worked on the development and validation of straightforward, stability-indicating isocratic HPLC and UPLC methods for determining fluconazole in bulk and solid pharmaceutical forms have been successfully completed. For HPLC analysis, a SunFire C18 column (250 × 4.5 mm, 5 μm particle size) was utilized, while UPLC analysis employed a BEH C18 column (100 × 2.1 mm, 1.7 μm particle size). The mobile phase was composed of Methanol:Water (70:30) for HPLC and Methanol:Water (55:45 v/v) for UPLC, with isocratic flows maintained at 1 mL/min for HPLC and 0.30 mL/min for UPLC. Detection for both the HPLC and UPLC systems was carried out at 211 nm, with the column oven temperature set at 30°C, which provided optimal elution. The injection volumes were 2 μL for HPLC and 20 μL for UPLC, respectively.

Fluconazol method validation by RP-HPLC for determination in biological skin matrices, credit goes to Alessandra C. Ayub et.al. (2007**).** In their work the systemic treatment of superficial mycoses involves the use of the bis-triazole antifungal fluconazole (FCZ). Approximately 16% of patients experience the difficulty of drug interactions and the occurrence of adverse reactions, given the medication's numerous benefits against systemic fungal infections. Validation of the FCZ approach using reversed-phase high-performance liquid chromatography in the linear range of 2 to 32 μg/mL makes it appropriate for use in biological matrices following topical penetration investigations. The technique is evaluated in a simulated FCZ alcoholic solution that is applied to skin extracts during Franz cell in vitro permeation investigations.

A Singh et. al. (2014) worked on article introduces a novel reverse phase-high performance liquid chromatography (RP-HPLC) method for measuring the bis-triazole antifungal agent fluconazole (FLZ) across various simulated biological fluids: gastric fluid simulant (GFS, pH = 1.5), vaginal fluid simulant (VFS, pH = 4.2), topical simulated media-phosphate buffer (PB, pH = 6.8), blood plasma simulant-phosphate buffer saline (PBS, pH = 7.4), and in methanol. The RP-HPLC analysis was conducted using a octadecyl silane (ODS-3) Hypersil C18 column (250 mm × 4.6 mm × 5 μm), with a mobile phase of water (pH 5.2, adjusted with orthophosphoric acid) and acetonitrile (80:20, v/v), at a flow rate of 2.5 mL/min and detection at 260 nm. The calibration curve for FLZ was linear between 0.2–40 μg/mL, 0.1–40 μg/L, 0.2–40 μg/mL, 0.1–40 μg/mL, and 0.2–50 μg/mL for GFS, VFS, PB, PBS, and methanol, respectively. The validation of this method with regards to linearity, precision, accuracy, and specificity met the guidelines set forth by the International Conference on Harmonisation (ICH) and the United States Food and Drug Administration (USFDA).

Yadav Anandkumar et. al. (2015) performed the development and validation of a UV spectroscopic method for the estimation of fluconazole. They states that, a quick, easy, accurate, and selective UV spectrophotometer technique has been created to estimate the amount of fluconazole. The maximal absorption wavelength in water for the spectrophotometric measurement was 260.8 nm. The developed method's linearity, precision, accuracy, robustness, LOD, and LOQ were all validated in accordance with ICH guidelines.

S Manzoor et. al. (2015) worked on the research outlines a straightforward and logical approach to creating a molecularly imprinted polymer (MIP) designed for the extraction of fluconazole from its sample. The MIP was effectively synthesized using methacrylic acid as the functional monomer, ethylene glycol dimethacrylate as the crosslinker, and acetonitrile as the porogenic solvent, with fluconazole serving as the template molecule. This was achieved through a non-covalent approach. The non-imprinted polymer (NIP) was created using the same synthesis procedure as the MIP, except without including the template molecule. Scanning electron microscopy, infrared spectroscopy, thermogravimetric analysis, and nitrogen Brunauer–Emmett–

Teller measurements provided insights into the structural and morphological features of both MIP and NIP. The efficacy of MIP as a sorbent was showcased by using it in solid phase extraction cartridges to isolate fluconazole from commercial capsule samples via an offline analytical method. Fluconazole quantification was achieved using UPLC–MS, yielding a detection limit (LOD) of approximately 1.63 x 10^-10 mM. Additionally, a high recovery rate of approximately 91-110% (n=9) was recorded. The selectivity of the MIP for fluconazole was assessed by comparing its binding affinity to structurally similar analogues, miconazole, tioconazole, and secnidazole, with percentage recoveries of 51%, 35%, and 32%, respectively.

S Navees et. al. (2015) worked on A straightforward, precise, rapid and cost-effective UV spectrophotometric method has been developed for the estimation of fluconazole. The UV method offers a straightforward, quick, and cost-efficient approach for quantifying fluconazole in capsule form only, with its maximum absorbance observed at 210 nm. This study proves to be highly beneficial, offering a simple and precise method for the easy determination of Fluconazole.

UK Sarker et.al. (2019) worked on the study aimed to detect and quantify the impurities present in both the fluconazole bulk drug and the FLUNAC™ capsules (150 mg).The study was conducted using High-Performance Liquid Chromatography (HPLC) equipped with a diode array detector. The total impurity levels were found to be 0.368% in the fluconazole bulk drug and 0.392% in the capsule form, respectively.

U K Sarker et. al. (2020) worked on This study aimed to ascertain the types and quantities of impurities present in both the fluconazole bulk drug and the FLUNAC™ capsule (150 mg). Methodology involved the use of High-Performance Liquid Chromatography (HPLC) equipped with a diode array detector. The mobile phase consisted of acetonitrile and water in an 85:15 ratio, with a flow rate set at 0.7 mL/min, and detection carried out at a wavelength of 260 ± 1 nm. Findings revealed the presence of two impurities, one previously identified as impurity A and the other remaining unidentified, in both the fluconazole bulk drug and the FLUNAC capsule (150mg). The overall impurity levels were found to be 0.368% in the bulk drug and 0.392% in the capsule. The study concluded that the total impurity content in both forms was below 1%, deeming it acceptable.

VSH Araujo et.al .(2021) worked on Vulvovaginal candidiasis represents a significant public health challenge, showing a high rate of occurrence in women. Presently, there's a notable rise in the detection of Candida species. resistant to existing treatments, highlighting the urgency in finding new therapeutic options. There is documented evidence of the synergistic effects of curcumin with fluconazole. Nevertheless, curcumin's high lipophilicity demands the employment of drug-delivery systems to fully harness its benefits, with nanostructured lipid carriers being among the promising options. As of now, there isn't a validated high-performance liquid chromatography (HPLC) method available in the literature for the simultaneous determination of fluconazole and curcumin. Consequently, this study has formulated and validated an HPLC method for the concurrent measurement of fluconazole and curcumin, both encapsulated in nanostructured lipid carriers. This method adheres to the validation standards set by the International Conference on Harmonization (ICH) – specifically the Q2 (R1) guidelines – and the Food and Drug Administration's (FDA) Guidance for Bioanalytical Method Validation. The developed method was utilized to assess the encapsulation efficiency and the amount of curcumin and fluconazole encapsulated within nanostructured lipid carriers. This method demonstrated selectivity, precision, accuracy, and robustness in the simultaneous analysis of both drugs. It facilitated the measurement of how effectively curcumin and fluconazole were encapsulated and the quantity of each drug loaded into the nanostructured lipid carriers.

K A Kamalapurkar et. al. (2021) worked on A new straightforward, precise, quick, and economical UV spectrophotometric technique has been formulated and validated for evaluating fluconazole in both its bulk form and as a tablet. Fluconazole exhibited its highest absorbance at 260 nm within the UV spectrum (200-400 nm). The method demonstrated linearity within the 20-100µg/ml range, with an excellent regression coefficient ($R^2 = 0.997$). A % RSD value of less than 2 signifies the precision of the procedure. The % RSD for inter-day and intra-day precision was recorded at 1.883 and 0.345, respectively. Recovery percentage was observed to be within the range of 98.12 – 102%. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 6.5637µg/ml and 19.89012µg/ml, respectively. The proposed method underwent validation for linearity, accuracy, precision, sensitivity, and robustness following ICH guidelines. The outcomes demonstrate that this method is suitable for the routine analysis of Fluconazole in both bulk and tablet forms.

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